UNIVERZA V LJUBLJANI | VF Veterinarska fakulteta

International Summer School for Veterinary Students and Postgraduates

FROM SPERM AND OOCYTE TO OFFSPRING: THE JOURNEY OF CANINE AND FELINE REPRODUCTION

Maja Zakošek Pipan

From Sperm and Oocite to Offspring: The Journey of Small Animal Reproduction

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This manual has not undergone professional proofreading. Consequently, there may be typographical errors, grammatical mistakes, or other inconsistencies. We appreciate your understanding and apologize for any inconvenience this may cause.

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Authors

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Magi Casal graduated from the Faculty of Veterinary Medicine, University of Zürich, Switzerland in 1984, worked as an associate pathologist until 1986, and obtained her Master's in Virology/Vaccinology from the University of Bern in Switzerland in 1989. She then worked in Small Animal Reproduction from 1988 until 1991, when she went to the School of Veterinary Medicine, University of Pennsylvania in Philadelphia to absolve a residency in Medical Genetics, Reproduction, and Pediatrics. After finishing the residency, she went to graduate school and received her PhD in virology/gene therapy from the University of Pennsylvania in 1999. After a one year postdoc in the hematology (stem cells) at the University of Illinois in Chicago, she became a faculty member in 2000 and has been a full professor since 2018.

Stefano Romagnoli

Stefano Romagnoli graduated in Veterinary Medicine from the University of Pisa in 1982. He was a Fulbright Fellow at the University of Minnesota (1984-86), where he completed a Master of Science Degree in Theriogenology. He then returned to Italy as an Assistant (1987) and Associate Professor (1991) at the Faculty of Veterinary Medicine of the University of Pisa, and then joined the Faculty of Veterinary Medicine of the University of Padova as a Full Professor in 2001.

He is a Diplomate of the European College of Animal Reproduction, has co/authored more than 70 papers in refereed journals and more than 300 papers in total including non-refereed journals, book chapters, scientific abstracts, editorials and lay publications. His research interests originally focused on ruminant reproduction, but he subsequently switched to small animal reproduction which has been the object of his clinical and research activity for the last 30 years. His scientific activity has a special focus on reproduction in cats, as he has served as President of the Italian Feline Practitioners Association (1993-1999) and the European Society of Feline Medicine (2000-2006).

He is keen in developing international relationships fostering collaborative research projects across countries and continents and advising foreign graduates. He has also been President of the European Board of Veterinary Specialisation (2008-2010), President of the European College of Animal Reproduction (2011-2014) and has served as Secretary of the European Association of Establishments for Veterinary Education (2004-2008). During his involvement with EBVS, EAEVE and ECAR he has developed an interest for quality assessment of undergraduate and postgraduate education in the veterinary medical field and has been a team member or Chairperson or Coordinator of teams of experts assessing quality of undergraduate education in 14 veterinary schools in Europe and Asia. He is the current (2019-2027) President of the Italian Society for Animal Reproduction.

Magdalena Schrank

After graduation from the University of Veterinary Medicine Vienna (AT), she moved to Italy to obtain her Ph.D. degree in Veterinary Science at the University of Padua (IT). Having been raised in a household of dog breeders, the clinical and the scientific aspect of reproduction medicine in small animals has always been a special interest of gher. At the moment she is working in the Emergency Service of the Veterinary Teaching Hospital of the University of Padua and as an external Professor.

Tanja Švara

Tanja Švara graduated at the Veterinary Faculty University of Ljubljana (SLO) and immediately afterwards began training in the field of veterinary pathology, which she completed with an M. Sc. and a Ph.D. She also obtained the title of national specialist in veterinary pathology. For many years she passed on her knowledge to students in practical clases of pathology, and for the past few years she has been a lecturer in pathology and cytopathology. Cytopathology and pathology of the reproductive organs of dogs and rabbits are areas of particular interest to her. Her bibliography includes 68 peer-reviewed journal articles.

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Tanja Plavec

Tanja Plavec graduated as a Doctor of Veterinary Medicine from the University of Ljubljana, Slovenia in 2003. She continued with a multi-disciplinary PhD program in Biomedicine at the University of Ljubljana, while working as a veterinarian at the Small Animal Clinic of the Veterinary Faculty, University of Ljubljana. She was focusing on research in the antioxidant status of oncologic patients under the mentorship of Prof. Dr. Janoš Butinar. During that time she spent a year at the Hofheim Veterinary Clinic.

She was awarded her PhD in 2008 and after that she was involved in teaching the students surgical skills until June 2018. In 2018 she proceeded her professional path at the Hofheim Veterinary Clinic, where she was working especially in veterinary oncology and surgical oncology under guidance of Dr. Martin Kessler, Dipl ECVIM-CA (Onc) and Dr. Jarno Schmidt, Dipl ECVIM-CA (Onc). Since November 2021 Tanja is again a member of Small Animal Clinic at the Veterinary Faculty of Ljubljana, and is responsible for soft tissue and oncologic surgery.

Tanja Plavec is also an active member of the European Society of Veterinary Oncology and Veterinary Society of Surgical Oncology and reviewer and author of research and professional papers in oncology and surgery. Her main research interest is surgical oncology, multimodality treatment in oncology, surgery of the urogenital system and perianal region. In her free time Tanja is a passionate mountaineer and a photographer, where she is accompanied with her loyal Tibetan terrier Lajk.

Maja Zakošek Pipan

After receiving her degree in veterinary medicine in 2005, she began working at the Clinic for the Reproduction at the Veterinary Faculty of Ljubljana. In 2009 -2010 she had the privilege to join a wonderful team of clinicians and researchers at the Veterinary School of the University of Pennsylvania for 18 months, where she worked under the guidance of Dr. Margret L. Casal. Faced with the highest level of professionalism, this exceptional experience encouraged her to actively pursue with alternate route of specialization in small animal reproduction medicine, which she completed in 2018. During that time, she continued to learn from worldwide reputable specialists and scientists, and she spend a lot of time in Philadelphia and at one of the biggest reproduction centers for small animals in France – Alford Veterinary School and at the University of Padova under the guidance of prof. dr. Stefano Romagnoli. In 2019 she also published a book about taking care of a neonatal dogs and cats. She is the author of many original studies in the field of small animal reproduction.

Reviewers Notes

Prof. dr. Slobodanka Vakanjac

Achieving success in small animal reproduction requires a thorough understanding of the normal reproductive processes and potential problems that may arise. This manual, contributed to by international experts, explores the physiological foundations of fertility and reproduction, and offers strategies for addressing common practice issues.

The manual thoroughly examines male and female infertility in dogs and cats, discussing natural mating and artificial insemination techniques for both species. Detailed sections on pregnancy, parturition, and dystocia lead into a comprehensive guide on neonatal care, including the management of congenital problems in puppies and kittens.

This practical manual is an extensive resource designed to assist with diagnosing and treating reproductive disorders in small animals. It serves as an invaluable reference for veterinary surgeons, students, nurses, and anyone involved in managing the breeding of dogs and cats.

Prof. dr. Slobodanka Vakanjac (vakanjac@vet.bg.ac.rs)

Prof. dr. Janko Mrkun

This manual on the reproduction of small animals is a commendable contribution to the field and provides a comprehensive and insightful overview of this subject. The authors have done an excellent job in selecting relevant chapters that cover the entire spectrum of reproductive processes in small animals, from basic physiology to advanced reproductive technologies.

Each chapter is carefully crafted and presents the information in a clear and concise manner that is informative for novices and experienced professionals alike. Of particular note is the literature review, which covers a wide range of studies and sources and provides a comprehensive understanding of current research and developments in the field.

The authors' expertise is evident in their ability to distill complex scientific concepts into understandable and engaging content. They strike a balance between detailed explanations and practical applications, making the material both educational and applicable to real-world scenarios.

The inclusion of chapters on neonatology is also particularly valuable. These sections provide important insights into the care and management of neonatal animals, covering both common challenges and advanced care techniques. This addition ensures that the book serves as a holistic guide covering the entire reproductive cycle from conception to the early stages of life.

The book also excels in discussing ethical considerations and welfare implications, an aspect that is often overlooked in reproductive studies. This balanced approach ensures that readers are not only informed about the scientific aspects, but are also encouraged to think critically about the wider implications of their work.

Overall, the manual is characterized by its comprehensive coverage, clear presentation, and relevance to current issues in small animal reproduction and is a must for undergraduates, graduate students, and veterinarians studying reproduction. It is not only educational, but also encourages further research and innovation in the field. I congratulate the authors on such a valuable and insightful manual.

Prof. dr. Janko Mrkun (janko.mrkun@vf.uni-lj.si)

Editor Acknowledgment

I would like to express my deepest gratitude to Dr. Mateja Stvarnik, Jernej Gačnikar and Mateja Bogataj for their invaluable help in the preparation of the summer school program, which also led to the creation of this study manual for veterinary students.

My sincere thanks go to all the authors (Margeret L. Casal, Stefano Romagnoli, Magdalena Schrank, Nataša Šterbenc, Tanja Plavec, Tanja Švara) who not only provided their materials but also gave insightful presentations during the Summer School. Their expertise and dedication greatly enriched the learning experience and content of this guide. Their commitment to sharing knowledge and fostering a collaborative environment was truly inspiring.

Special thanks are also due to the reviewers- (Slobodanka Vakanjac and Janko Mrkun) who promptly and carefully reviewed all the content. Your attention to detail and constructive feedback have ensured the high quality of this guide.

Finally, I am deeply grateful to my family, who spent much of their vacation time supporting me. Their love, patience and encouragement were essential in making this project a success. I could not have done it without you.

Thank you all for your dedication and support.

Sincerely yours,

Maja zakošek Pipan

1 DISTINCTIVE CHARACTERISTICS OF REPRODUCTIVE CYCLES IN FEMALE DOGS AND CATS

Magdalena Schrank

The cat is not a small dog

Dogs and cats are two of the most frequently kept pets and share a good amount of similarities. Regardless, differences between cats and dogs become quite obvious once reproductive physiology and in particular the oestrus cycle is discussed. The queen is to be considered a seasonally polyestrous species with induced ovulation and does not differ in main characteristics from its wild ancestors. The dog on the other hand is a non-seasonal, monoestrus breeder with spontaneous ovulation and its reproductive physiology changed in some aspects greatly with domestication. The difference in ovulation between cats and dogs is of great importance as it determines the hormonal pattern in specific stages of the estrus cycle. These specific differences are important not only for the occurrence of pathologies but influence also how, when and with which drugs the estrus cycle may be influenced. The following manuscript aims to illustrate the basics of the physiological estrus cycle in cats and dogs, its differences and possible methods to interfere with the physiological process.

1.1. The queen

Cats are considered to be seasonally polyestrus. Season depends on the length of day making them long-day breeders. In European countries, this usually means, that during the winter months, cats enter a non-cyclic stage until day length increases again. Countries with fewer differences regarding daylight length throughout the year may encounter feline populations that reproduce all year round. Studies have shown that a stable exposure of 14 hours of artificial light for at least 2 months may induce and maintain cyclic activity whereas a reduction to 8 hours will evoke a period of anestrus. If light exposure is increased to 24 hours cyclicity will be maintained yet the number of heat cycles will decrease (two per month with 14 hours of light compared to one per month with 24 hours of light) (Leyva et al., 1989). Although cats will not enter a phase of anestrus in case of 14 hours of light exposure overall pregnancy rates may still decrease in what may be considered winter months. Ovulation in the queen is induced by mating. Regardless, spontaneous ovulation may occur in one in three queens and the probability increases with increasing body weight (Binder et al., 2019).

Up until today, no clear scientific explanation is given on why some queens ovulate spontaneously and others don't. It has been described that queens in the presence of an intact male tend to ovulate more frequently spontaneously compared to females living without intact males. In group-housed queens, spontaneous ovulation without the presence of an intact male has been observed in up to 22% whereas this percentage increased to up to 57% once an intact male has been introduced (Gudermuth et al., 1997).

The estrus cycle of the queen may be generally divided into proestrus, estrus and interestrus. A diestrus may be present in two distinct forms which differ not only in length but also in clinical, behavioral and cytological presentation. In cases of ovulation without fecundation, a shortened luteal phase takes place which may last up to 40 days. In the case of ovulation followed by fecundation, the luteal phase lasts for the duration of the pregnancy (around 63-65 days). Anestrus is dependent on individual and environmental factors but may last several months.

During proestrus, which lasts usually less than 24 hours, vulvar swelling, vocalization and typical rolling on the floor or rubbing against furniture, objects or the owner are present. In this short period, the

female is not receptive to the male. Cytologically this period presents with less than 50% of cornified cells and intermediate cells. Proestrus is rarely observable in queens.

Estrus lasts between 5-7 days typically and vocalization and rubbing increase. The female frequently takes a "praying" position with lordosis, anterior limbs lowered and elevation of the back with the tail either held high or laterally. Although such behavior is observed in the majority of cats research has shown that only 8% of individuals show these patterns at the first day of estrus, with an increase to 80% by the fourth and 100% by the sixth day. During this period the female will accept the male. Estradiol concentration increases to levels of >20 pg/ml. Growing follicles increase to 2-3mm in diameter and are numerous on both ovaries (between three and six follicles on each ovary). Cytologically the amount of cornified cells increases to over 75% and large intermediate cells may be observed. An increase of cornification nearing 100% as known and observed in the canine species is rare in the cat. Regardless cornification cells often still have a clearly visible nucleus. If the queen is mated or in cases in which spontaneous ovulation occurs the follicular phase is shortened. Furthermore mating before adequate follicular maturation may result in a non-ovulatory cycle regardless of the inducing stimulus through mating.

Interestrus is a phase seen only in cases ovulation (either spontaneous or induced) does not occur. It lasts for around 8-9 days and dominant follicles undergo atresia following a decrease in estradiol concentration. At this point, behavioral patterns connected to the estrus and mating disappear. A new wave of follicular growth begins and proestrus is expected to start again at the end of the short interestrus.

Once ovulation occurs the queen enters diestrus. During this phase corpora lutea (CL) may be visible in abdominal ultrasound. Progesterone concentration increases around the fourth day after mating (Verstegen et al., 1993). Progesterone is the dominant hormone in this period and prevents the exhibition of estrous behavior. In the case of diestrous without fecundation, the CL regresses around day 40 allowing cyclicity resumption. In the luteal phase of pregnant queens, CL and elevated progesterone levels remain until shortly before parturition.

Anestrus is the most variable phase depending on individual and environmental factors. In general, as soon as the days become shorter cyclic activity decreases until a complete arrest until daylength increases again in spring.

Ovulation in itself depends not only on the stimulus of copulation but also on the intensity and the timing of mating and occurs within the first 36 hours after mating. A high number of matings in a short period in the middle of estrus increases the probability of successful ovulation. Research has shown that only one out of twelve cats ovulated following a single mating on the first day of estrus, whereas four out of twelve ovulated after a single mating on the fourth day of estrus. If the intensity of stimulus is evaluated queens which were mated three or more times between the first and the third day of estrus were more likely to ovulate (ten out of twelve cats) (Wildt et al., 1980). As a general rule, four or more matings are required to arrive at the sufficient release of luteinizing hormone. The LH surge peaks at around 2 hours after mating and values remain elevated for the following 8-12 hours (Tsutsu et al., 2004).

Spontaneous ovulation is not the norm in cats, yet it may occur way more frequently than initially believed. The exact hormonal pathway and influencing factors remain largely unknown to this day. The correlation with increasing body weight may indicate the importance and influence of energy storage and the quantity of adipose tissue on ovarian and follicular activity and behavior. Furthermore, females living alone with their owners were more prone to spontaneous ovulation compared to animals living with other females and/or castrated tomcats. Age appears to have little effect on this particularity of feline reproductive physiology.

Once the physiology of the feline cyclic activity is understood conclusions may be drawn also regarding the onset of puberty in queens and influencing factors. It has been described that queens enter puberty at around 8-10 months of age yet it appears that the length of day is of great importance. The photoperiod influences the hypothalamus-pituitary gland-gonadal axis through melatonin. During the seasons with short day length melatonin secretion is increased and ovarian function is thereby reduced. Because cats are polyestrous and the month of birth of kittens is therefore quite variable, so is the interval between birth and first estrus of female kittens. A range of 181 to 560 days between date of birth and onset of first estrus has been described with a mean of 345±9 days. In kittens born between March and June mean interval was described as 343±9.5 days. For female kittens born between July and October instead, the interval between birth and first estrus was significantly shorter with a mean of 242±6.3 days. In a smaller percentage of kittens born between July and October, it has been described that the individuals did not enter the first heat in the first year following their birth but only in the second one resulting therefore in a significantly longer interval with a mean of 519.2±5.8 days (Tsutsu et al., 2004). These findings have been contradicted in the literature with other studies not observing any influence of the month of

birth on the onset of puberty (Romagnoli et al., 2004). Regardless, researchers agree that the first heat occurs with increasing day length yet remains very variable in countries in which day length does not vary particularly throughout the seasons.

Throughout the literature on feline reproductive physiology reports frequently contradict each other due to the fact, that the feline shows a high variability in presentation depending on their environment, latitude of their home, activity level, individual components and others.

The influence of melatonin on cyclic activity and onset of puberty has been and is still taken advantage of to delay puberty and or decrease cyclic activity in adult female cats. Studies have shown that melatonin administration had a more reliable effect when administered to suppress estrus compared to individuals in which puberty should be postponed. Furthermore, effects seem to be dependent on individual factors as well as on the way of administration (subcutaneous implant, orally administered drugs) (Schäfer-Somi, 2017). GnRH agonist implants are further used in influencing the feline estrus cycle and are used with the scope to delay puberty onset, induction of estrus and induction of anestrus.

1.2. The bitch

Overall, it may be said that bitches are monoestrus, non-seasonal, polytocous animals with spontaneous ovulation, yet there are many more differences with the queen's cycle and a lot of individual differences between bitches.

Spontaneous ovulation results in a luteal phase also in the case of non-mated estrus similar or slightly longer in length compared to the ones after mating. The following anestrus is very variable in length depending on breed but also depending on the individual itself. Interestrus intervals are described to last between 5-12 months but may be even longer. The typical interestrus interval is described as 6-7 months which is the length typically known by and communicated to the owner of an intact bitch. Although there are breed and individual variabilities the interestrus interval is not influenced by the presence or absence of pregnancy due to the physiological luteal phase in non-pregnant bitches. The onset of puberty is also quite variable and occurs at an age of 6-14 months and is correlated with size with large and giant breeds entering puberty later than small and medium-sized breeds.

The estrus cycle of the bitch is divided into 4 phases (proestrus, estrus, diestrus and anestrus). The length of each of these phases is very variable with a range of 5-20 days of proestrus (follicular phase; rise of estrogen), 5-15 days of estrus (decline of estrogen and initial luteal phase), 50-80 days of diestrus (luteal phase with high progesterone levels) and anestrus (hormonal "pause" until onset of next cycle) of 80-240 days. This variability renders owner communication and education difficult.

Bitches show endocrinological similarities with humans, rodents and other mammals. Ovulation occurs in response to an abrupt LH-surge at the end of proestrus followed by an elevation of LH and FSH of 1–3 days and 1–4 days, respectively. Ovulation occurs 48–60 h after LH surge (Concannon, 2011; Beccaglia et al., 2016). In bitches maturation of oocytes happens in the distal uterine tubes around 2 days after ovulation. The increase in progesterone is measurable in blood samples and is part of the procedure of determining ovulation and optimal mating timing. Ovarian activity and luteal phase are not influenced by photoperiod and even hysterectomy doesn't influence these processes (Olson et al., 1984). The persistence of the CL and the continuous progesterone production results in what is commonly known as false- or pseudopregnancy. Non-pregnant bitches may show symptoms similar to bitches near birth and the period shortly after birth with an increase in mammary activity, nesting behavior, changes in appetite and in some cases depression due to the absence of pups.

Proestrus is characterized by vulvar swelling accompanied by serosanguinous vulvar discharge and increased licking of the genital area. Although Proestrus length has been reported to be very variable it lasts on average 9 days during which symptoms progressively increase. Cytology obtained by vaginal smear shows vaginal epithelial proliferation and an increase in cornification. Overall epithelial cell profile is initially dominated by parabasal cells (accompanied by varying numbers of erythrocytes and neutrophils), and changes to being dominated by small intermediate squamous cells, large intermediates, and large cornified cells. Percentage of cornified cells increase to 98–100%.

In vaginoscopy, the mucosa appears edematous, changes color from pinkish to white, with serosanguinous fluid presence and deepening vaginal folds that become more prominent in both axes (Jeffcoate and Lindsay, 1989). The bitches behavior towards the male changes slowly from initial aggression to indifference and playfulness. During the follicular phase 2-8 follicles may be visible as 3mm large structures on each ovary

Serum estradiol increases during proestrus to reach peaks of 40–120 (mean 70) pg/ml (□150–450, mean 255 pmol/l). Proestrus ends with the onset of receptive behavior typically occurring 0.5–3 days after the peak in estradiol and within a day of the preovulatory LH surge. Endocrinologically proestrus is considered concluded with the preovulatory LH surge. The LH surge is the critical factor in selecting the ovulatory wave of follicles.

Estrus lasts in the majority of cases around 9 days during which bitches seek males and accept mounting attempts. The sharp decline of estrogen:progesterone ratio results in initial wrinkling and crenulation of the vaginal mucosa in vaginoscopy within the first 24 hours after LH surge. Behavioral estrus may differ importantly among bitches, with bitches accepting males already at the beginning of estrus; the same may be said for reflexes such as the presentation of the vulva and/or flagging of the tail. Estradiol levels decline after the peak value in late proestrus. Progesterone on the other hand increases rapidly after the LH surge to values of 1-3 ng/ml (3-6 nmol/l). Further increase may be slower and a plateau may be encountered for 1-3 days. A study has shown that estrus behavior is more intense in bitches in which estrogen withdrawal was induced and progesterone was administered concomitantly to the withdrawal (Concannon et al., 1979). The canine pattern of luteal growth and slow regression is not dissimilar to that observed in hysterectomized individuals of polyestrous species. Although progesterone levels are a good and valuable method to monitor ovulation, ovarian ultrasound can also determine the time of ovulation with considerable accuracy, based on the transient 1–2 day marked increase in echogenicity of previously anechoic follicles at ovulation, followed by a return of anechoic structures (Reynaud et al., 2006).

During diestrus estrous-behavior starts to cease and is considered to last until evidence of the ongoing luteal phase becomes minimal. At the end of diestrus serum progesterone levels decline below 2 ng/ml (~6.4 nmol/l) (Concannon, 2011; Kutzler et al., 2003; Veronesi et al., 2002). Knowledge about the first day of diestrus (D1) may be useful for the determination of the day of birth.

During canine anestrus the absence of overt evidence of ovarian activity is observable and it is believed that it lasts for a minimum of 7 weeks after progesterone declines below 1–2 ng/ml. During this phase physiologic endometrial repair takes place. The apoptotic index and percent of degenerated epithelial cells in the endometrium are high during the mid-luteal phase, low in early anestrus and absent by day 120 (Chu PoYin et al., 2002). Estradiol, LH and progesterone concentrations reach basal levels. LH has been reported to increase sporadically in a pulsatile manner at intervals of 7-18 hours. The reason for the variability in anestrus length is not clear. Suspected involved factors are the proximity to other bitches, the presence of a dominant female within the pack and the social interactions with them and subliminal effects of an endogenous circannual cycle. Certain breeds seem to present a sensibility to photoperiods (e.g., autumn breeding in basenji dogs) yet are rare and therefore the dog's cyclicity is considered non-seasonal. Induction of heat and contraception are an important part of research in and applied canine reproduction. Administration of low concentrations of progesterone via subcutaneous implants may result in cycle suppression. Oral administration of megestrol acetate is used and different protocols are available. All progestins suppress ovarian cyclicity in dogs by preventing the increase in LH pulsatility resulting in proestrus. Down-regulation of LH and FSH by continuous GnRH-agonist administration provides good and reversible estrus prevention. Induction of premature proestrus and estrus has been accomplished with highly variable success rates using estradiol, FSH, FSH plus LH, or eCG. Follicle luteinization, ovulation failure, short luteal phases or failed implantation are frequently encountered complications. Dopamine agonist administration for 7–30 days in anestrous lower prolactin levels often resulting in premature but normal proestrus, including normal pre-proestrus increases in LH pulsatility (Spattini et al., 2007). Pulsatile GnRH administration may induce proestrus which progresses into a spontaneous fertile estrus. Over 80% of bitches in anestrus will rapidly enter an induced proestrus in response to physiological doses of GnRH administered regularly or constantly but it has been shown that constant administration results more frequently in fertile estrus in late compared to early anestrus. The opioid-antagonist naloxone has been reported to cause acute rises in LH, especially in late anestrus. This knowledge is useful and important in cases in which veterinarians and breeders search to prepone a fertile estrus.

Reference list and suggested readings

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2 REVIEW OF PATHOLOGY OF NON-PREGNANT FEMALE REPRODUCTIVE SYSTEM

Tanja Švara

2.1 Anatomy and histology of female genital system

The ovaries are paired organs located in the dorsal abdominal cavity, caudal to the kidneys. Each ovary is suspended from the abdominal wall by the mesovarium (Aspinall, 2011). The part of the mesovarium almost completely surrounds the ovary and forms an ovarian bursa, a pocket-like, fat-covered structure with a small slit or opening on the medial side that connects the interior of the bursa to the peritoneal cavity (Buerglet CD, 1997; Foster, 2017).

The ovary of the bitch is oval in shape and about 1 to 1.5 cm long (König HE, 2006) and has mulberry-like appearance (Buergelt CD, 1997). The ovary is covered with an outer epithelial layer that is of mesothelial origin. The epithelial cells vary from simple squamous to distinctly cuboidal or columnar. Beneath the epithelium is a capsule of the ovary (formerly *tunica albuginea*), a layer of dense fibrous tissue. The bitch also has invaginations of the surface epithelium into the capsule of the ovary; they are called subsurface epithelial structures (SES), and structures called granulosa cells rests (sin. granulosa cell islands or granulosa cell cords), which are aggregates of granulosa cells in a tubular arrangement (Foster, 2017).

The ovarian parenchyma is subdivided into cortex (parencyhmatous zone) and medulla (König HE, 2006; Foster, 2017). The cyclic ovary should have a polycavitary appearance on the cut surface, it contains follicles and corpora lutea in various stages of development and regression, stromal connective tissue and blood vessels (Buerglet CD, 1997; König HE, 2006). The mature follicles of a bitch measure between 5 and 8 mm (Buerglet CD, 1997), and the medulla contains large blood vessels, lymphatic vessels, nerves and loose connective tissue as well as remnants of the mesonephric tubules, the so-called rete ovarii (Foster, 2017).

The oocytes develop in the follicles, with each follicle containing individual oocytes. The follicles are named according to their stage of development: primordial, primary, secondary and tertiary follicles (Graafian follicle). The primordial follicle is lined with a single-layered follicular epithelium, the granulosa cells, which are flat. The primary follicle is lined with a layer of cuboidal granulosa cells. The secondary follicle has several layers of granulosa cells surrounding the oocyte. A fluid-filled space (*antrum*) forms in the tertiary follicle and at one end of the follicular cavity there is a hillock (*cumulus oophorus*) containing the maturing oocyte. In close contact with the oocyte is a clear membrane, the zona peluccida, which is surrounded by a layer of radially arranged granulosa cells, the corona radiata. With further maturation, the tertiary follicle becomes a Graafian follicle, which finally ruptures and releases the oocyte (König HE, 2006).

Ovulation occurs after the rupture of the follicle. Ovulation occurs through the outer surface of the ovary and the oocyte is caught by the infundibulum of the uterine tube. The space that appears after ovulation fills with blood and then with luteal cells, forming the *corpus haemorrhagicum* and *corpus luteum*. Follicles that do not ovulate become atretric (Foster, 2017).

The uterine (Fallopian) tube (sin. oviduct) conducts the oocytes from the ovary to the uterine horn (Aspinall, 2011) and consists of four segments: the infundibulum, the ampulla, the isthmus and the urethrotubal junction (uterine ostium), which are supported by mesosalypinx (König HE, 2006; Foster, 2017). Fertilisation normally takes place in the isthmus (König HE, 2006).

The uterus of the bitch is bicornuate, with two uterine horns and one uterine body (Foster, 2017). The uterus is supported by the broad ligament or mesometrium, which is continuous with the mesovarium and mesosalpinx. The wall of the uterus consists of three layers:

- endometrium with columnar epithelium, glandular tissue and capillaries,
- myometrium with smooth muscle fibres,
- mesometrium, which is part of the visceral peritoneum (Aspinall, 2011).

During the oestrus cycle, the endometrium undergoes drastic changes; during the luteal phase, there is marked hyperplasia and hypertrophy, which dramatically increases the diameter and weight of the uterus (Schlafer, 2012).

The cervix separates the external genitalia from the uterus. The cervix of the bitch has no transverse folds as in ruminants and sows. The cervix of the bitch opens on the dorsal aspect of the cranial vagina (Foster, 2017).

The vagina extends from the cervix to the external urethral orifice. It consists of longitudinal folds which dilate to allow the foetus to exit during parturition. The vagina is lined with stratified squamous epithelium, which changes in response to the hormones of the oestrus cycle (Aspinall, 2011).

The vestibule is a continuation of the vagina that runs from the external urethral orifice to the outside at the vulva. Its structure is similar to that of the vagina, but the walls are not ridged by longitudinal folds (Aspinall, 2011).

The vulva marks the external opening of the tract and is located in the perineum, ventral to the anus, below the tail. It consists of two vertical labiae joined dorsally and ventrally with the vulval cleft between them. Immediately inside the ventral part of the cleft is the clitoris, which consists of cavernous erectile tissue. Normally, the labia are held tightly together to prevent the entry of infection, but in the bitch during proestrus and oestrus, the vulva enlarges and is slightly relaxed (Aspinall, 2011).

2.2 Disorders of sexual development (DSD)

The classification of DSD is very complex and is based on the complete description of the anomaly, sex chromosome type, presence of SRY, gonad type, tubular genitalia and external genital phenotype (Schlafer and Foster, 2016). DSDs are divided into three categories: sex chromosome DSDs, XY DSDs and XX DSDs. If the sex chromosome is unknown, the DSD is classified according to the gonadal type; there are gonadal dysgenesis, testicular DSD, ovarian DSD and ovotesticular DSD (Foster, 2017).

The term DSD is now preferred, replacing the previously used terms intersex, hermaphroditism, sex reversal and others, which are now avoided.

Abnormal sexual development commonly results in abnormal external genitalia, which are partially female and partially male. The spectrum of changes ranges from nearly normal-appearing females, with grossly normal ovaries and uteri to male genitalia with small genitalia, perhaps hypospadias and gonads in the scrotum that grossly look as testis but histologically contain ovarian tissue. The normal-appearing testes may be attached to the complete and well-formed uterine horns (Schlafer and Foster, 2016).

2.3 Pathology of the ovary

2.3.1 Development anomalies of the ovary

Ovarian agenesis is the complete absence of an ovary or ovaries. In bilateral agenesis, the tubular genitalia may be absent; if they are present, they are underdeveloped. This condition is extremely rare (Schlafer and Foster, 2016).

Duplication of the ovarian tissue is very rare but possible (Schlafer and Foster, 2016). It arises by two mechanisms: the ovaries develop separately or splitting in two already developing ovaries. The former is theoretically possible and is often used to explain cases in which incompletely spayed bitches return to oestrus (ovarian remnant syndrome) (Foster, 2017).

Ovarian hypoplasia represents ovary or ovaries that are smaller than normal at the time when oestrus should occur. The bitches have a lack of estrus activity, or they never develop an oestrus. Hypoplastic ovaries occur in DSD (Foster, 2017).

Ectopic adrenal tissue. Small light brown nodules representing ectopic adrenal tissue are occasionally found within the mesovarium within a few centimetres of the ovary. The nodules are composed of adrenal cortex tissue (Schlafer and Foster, 2016).

Ovarian cysts (OC) are cystic structures that arise from various ovarian and extraovarian structures. There are about 16 types of ovarian and extraovarian cysts, and to differentiate between the different types, more detailed examination of localization, estimation of ovarian steroid hormone concentration, histopathology, and special stains are required (Buerglet CD, 1997; Schlafer and Foster, 2016; Knauf et al., 2018). Expanded cysts lose their structure and the arrangement of the cell lining changes under the pressure of the cyst fluid, making it difficult to determine the origin of the cyst (Akihara et al., 2007).

Cystic ovarian disorders account for approximately 80% of ovarian pathology in dogs (Dow, 1960). The most common types of intraovarian cysts in bitches are follicular cysts, cysts of subsurface epithelial structure (SES) and cystic rete ovarii (Knauf et al., 2018; Sasidharan et al., 2021). Many bitches with ovarian cysts display no signs, but persistent follicular cysts are hormonally active and can lead to an increased risk of developing cystic endometrial hyperplasia-pyometra and have the potential to induce hypoestrogenism (Sasidharan et al., 2021).

2.3.2 Neoplastic conditions of the ovary

Primary ovarian tumours are divided into three categories according to the origin of the neoplastic cells:

- tumours of the surface celomic epithelium and the SES (epithelial tumours): papillary and cystic adenomas, papillary adenocarcinomas,
- sex cord stromal tumours: granulosa-theca cell tumours, thecoma or luteoma,
- germ cell tumours: dysgerminomas, teratomas.

The most common primary ovarian tumours in bitches include sex cord stromal tumours and carcinomas (Foster, 2017).

Very rarely, metastases from other primary sites occur in the ovaries. In bitches, mammary carcinomas can metastasize to other organs, including the ovaries (Schlafer and Foster, 2016).

Sex cord-stromal tumours arise from the sex cord and/or follicles, and most tumours have regions with a combination of cells of the granulosa cell, theca cells, luteal cells, Sertoli cells, or interstitial endocrine cells phenotype. Granulosa cell phenotype usually predominates, which is why most of them are called granulosa cell tumours (GCT) (Foster, 2017). Sex cord stromal tumours are more common in older animals but can occur in animals of any age and even in very young animals (Agnew and MacLachlan, 2016). GCT are unilateral or bilateral with have a smooth surface. They can be solid, cystic or polycystic, with cysts ranging in size from a few millimetres to several centimetres (Agnew and MacLachlan, 2016). Histologically, the neoplastic cells resemble granulosa cells and form solid sheets, cords, trabeculae or nests. Within the follicular structures are multiple layers of cells that resemble granulosa cells, with palisading at the periphery (Agnew and MacLachlan, 2016). Call-Exner bodies, rosettes of neoplastic cells around a central deposit of eosinophilic proteinaceous material, can be seen in some GCT and, when present, are a useful diagnostic feature (Agnew and MacLachlan, 2016). Most of sex cord stromal tumours produce anti-Müllerian hormone, oestrogens, androgens, and/or inhibin, and the latter can be immunohistochemically stained in most of these tumours. Prolonged oestrus and pyometra may occur in bitches with GCT (Foster, 2017). Metastasis in bitches is rare. Metastasis can occur to the regional lymph nodes, via the blood to a variety of organs or, rarely, by implantation in the abdominal cavity (Agnew and MacLachlan, 2016).

Epithelial tumours often occur in the bitch and they arise from SES. They are often multifocal and bilateral (Foster, 2017). Grossly, the affected ovary is enlarged and multinodular and has a cystic or villous, shaggy appearance. The cut surface typically has multiple cysts containing thin yellow to cystic, multinodular brown fluid, that are located between solid regions. Carcinomas may also appear as proliferating cauliflower-like growths that protrude from the surface of the ovary and invade neighbouring structures. Both adenomas and carcinomas of the ovary usually consist of arboriform papillae that protrude into the lumen of cystic cavities, and they are sometimes further subclassified as papillary (papillary adenoma or carcinoma) or cystic (cystadenoma or cystadenocarcinoma) (Agnew and MacLachlan, 2016). Most of ovarian epithelial tumours stain for cytokeratin 7 (Foster, 2017).

There are no clearly defined criteria to distinguish benign from malignant tumours. Metastasis, obvious vascular invasion, and extension of the tumour into adjacent structures such as the ovarian bursa or peritoneum are unequivocal signs of malignancy. If these are not present, ovarian carcinomas are identified by their larger size, the presence of foci of necrosis and haemorrhage, cellular atypia and the

tendency for the neoplastic cells to pile up on one another, mitotic count and, in particular, stromal invasion. Carcinoma may also exfoliate and disseminate through the peritoneal cavity as carcinomatosis (Agnew and MacLachlan, 2016) or metastasize to lymph nodes and other organs (Foster, 2017), although data on the frequency of metastasis are lacking.

2.3.3 Ovarian remnant syndrome (ORS)

ORS is a condition characterised by clinical signs related to functional residual ovarian tissue that remain in the abdominal cavity after ovariohysterectomy (OVH) (Ball et al., 2010). Small pieces of ovarian tissue are thought to be left behind due to surgical error (Schlafer and Foster, 2016), but ectopic ovarian tissue (extra ovarian tissue) or duplication of ovarian tissue may also be responsible for a small number of cases of ORS (Foster, 2017). The clinical signs in ORS mimic proestrus and oestrus, and the interval between OVH and diagnosis can range from 1 to 120 months (Ball et al., 2010). The syndrome can be easily recognised by serial vaginal cytology, hormonal studies, ultrasound examinations and exploratory surgical examinations. Due to the hormone-producing ovarian remnants, a so-called stump pyometra can develop (Hagman, 2018), and neoplastic transformation can occur in the ovarian remnants, leading to tumours (McEntee, 2002; Ball et al., 2010).

2.4 Pathology of the uterine (Fallopian) tubes

A primary pathology of the uterine tubes is uncommon. Hydrosalpinx and salpingitis usually develop secondary to disease of the uterus (Schlafer and Foster, 2016).

Hydrosalpinx is a condition in which fluid accumulates in one or both uterine tubes. The uterine tube is distended, uniformly or irregularly with clear, watery mucus that fluctuates. The tubes are thin-walled and tortuous. Hydrosalpinx is the result of obstruction of the uterine tube and may be congenital or caused by chronic inflammation (Schlafer and Foster, 2016).

Salpingitis, an inflammation of the uterine tubes in bitches, is rare, usually suppurative and associated with metritis/pyometra (Gelberg and McEntee, 1986). It is usually bilateral and not detectable macroscopically. In mild salpingitis, only the mucosa is affected, whereas in more severe inflammation, the loss of epithelium in the mucosal folds leads to fusion of the mucosal folds and the formation of intramucosal cysts (Schlafer and Foster, 2016); however, in the bitch, these cysts may also result from the accumulated, entrapped secretions of the lining epithelium (Gelberg and McEntee, 1986).

2.5 Pathology of the uterus

2.5.1 Abnormality of position or location

Torsion of the uterus is defined as a twisting of the uterus or uterine horn perpendicular to its long axis (Gowda et al., 2019), but the horns can also twist around the other horn so that they intertwine. Various causes of uterine torsion include jumping, running, or rolling during excessive play, premature uterine contraction in late pregnancy, foetal physical activity, partial abortion, hereditary weakness or variations in the length and mobility of the ovarian and uterine ligaments, and the use of oxytocin (Gowda et al., 2019). Uterine torsion in the bitch is uncommon, and in almost all cases, torsion occurs in the pregnant uterus, but can also occur with pyometra, hydrometra, endometrial polyps, and endometrial tumours (Schlafer and Foster, 2016). Unilateral torsion is more likely to occur in bitches (Gowda et al., 2019). Twisting of more than 180° leads to congestion and oedema of the uterus, oedema of the placenta and foetal death. The uterine wall is friable and prone to rupture (Schlafer and Foster, 2016). Uterine torsion is a life-threatening condition in the bitch (Gowda et al., 2019).

Prolaps of the vagina, cervix and/or uterus is only exceptionally in bitches. Usually, prolapse occurs immediately or up to 48 hours after delivery of the last neonate (Jadhao et al., 2020). Uterine prolapse can occur spontaneously or follow forced extraction in dystocia (Gupta Ajay, 2006). Uterine inertia, dystocia and an oversized fetus are among the causes of this condition (Jackson, 2004). Prolapse may be complete, with both horns protruding from the vulva, or limited to the uterine body and one horn (Gupta Ajay, 2006; Jadhao et al., 2020); sometimes intestine and urinary bladder may also be present within prolapsed uterus (Schlafer and Foster, 2016). (The pathological sequel of prolapse include congestion and oedema of the prolapsed organs, followed by haemorrhage, necrosis, and sepsis or gangrene (Schlafer and Foster, 2016), so it should be considered an emergency situation (Jadhao et al., 2020).

Prolapse of the vagina may occurs during proestrus and oestrus when under the influence of oestrogen, an oedematous swelling of the vaginal mucosa can develop (Alan et al., 2007). Rarely, vaginal prolapse can be caused by vaginal tumours or as a result of previous trauma (Arbeiter and Bucher, 1994; Williams et al., 2005).

Rupture of the uterus may occur spontaneously but is usually a result of obstetrical manipulations. Most ruptures occur in the uterine body near the pelvic brim. Mucosal ruptures are of minor importance, while complete ruptures are often fatal, either due to bleeding, the spread of uterine inflammation to the peritoneum or the displacement of retained membranes into the abdominal cavity. Most ruptures occur in uteri that are devitalized due to torsion or prolonged dystocia (Schlafer and Foster, 2016).

2.5.2 Pathology of the endometrium

Endometrial hyperplasia is widespread in the bitch. It appears in two forms: generalized cystic endometrial hyperplasia (CEH) and pseudoplacentational endometrial hyperplasia (PEH). In both forms, endometrial secretions often accumulate in the lumen of the uterus, and in PEH, cellular debris from superficial necrosis is also present, which can be mistaken for a pyometra. In both forms, these exuberant endometrial secretions likely contribute to the development of bacterial infections and associated inflammation (endometritis), which can develop into a pyometra – this association has been recognized for years and is referred to as CEH–pyometra syndrome (Schlafer, 2012; Schlafer and Foster, 2016).

Cystic endometrial hyperplasia can affect a single or a few glands or glands along segments of the endometrium, and sometimes the whole endometrium is affected. Cystic dilated endometrial glands can be up to 1 cm in size. Progesterone plays the major role in the induction of endometrial hyperplasia in the bitch, but even here the endometrium response to progesterone depends on oestrogen priming. Oestrogens bind to the oestrogen receptors present in the endometrial cells and act on these cells to induce the synthesis of intracellular receptors for progesterone. Many cases of CEH develop after the use of long-acting progestational compounds to delay the onset of oestrus. In some cases of CEH, ovarian tumours, particularly sex cord-stromal tumours and papillary cystadenocarcinomas, and paraovarian cysts are present (Schlafer and Foster, 2016). The accumulation of secretions can lead to an accumulation of mucoid fluid in the uterus – mucometra (Schlafer and Foster, 2016).

Noncystic endometrial hyperplasia is not macroscopically recognizable, except as a thickening of the endometrium. The thickening is due to an increase in the size and number of glands in the endometrium. The epithelial cells are hypertrophied with clear cytoplasm. In woman endometrial hyperplasia is a precancerous lesion, whereas it does not in the bitch (Schlafer and Foster, 2016).

Canine pseudoplacental endometrial hyperplasia in the dog is a form of hyperplasia in which a very characteristic segmental hyperplasia develops, consisting of localized proliferation of the endometrium that closely resembles the implantation sites of pregnancy. An unopened uterus may have one or more ovoid distended areas that resemble pregnancy sites. Prior the opening the uterus the differential diagnoses include endometrial polyp, chronic pyometra, leiomyoma, or foetal mummification (Schlafer and Foster, 2016). Segmental endometrial hyperplasia can occur in virgin bitches and is frequent part of a pseudopregnancy. The histologic features of PEH are characteristic – the hyperplastic endometrium may appear as a broad-based polypoid mass extending into the uterine lumen, or it may form a continuous band of protruding tissue (Schlafer and Foster, 2016).

Adenomyosis is the presence of endometrial glands and stroma between the muscle bundles of the myometrium, particularly near the cervix, and is not common in bitches (Schlafer and Foster, 2016; Foster, 2017). In some cases it is a malformation, in other cases it results from hyperplastic overgrowth of the endometrium. Adenomyosis, as it occurs in domestic animals, shares features with endometriosis in menstruating primates (Schlafer and Foster, 2016; Foster, 2017).

Endometrial polyps are pendulous lesions composed of abundant connective tissue stroma and dilated endometrial glands. They arise from the endometrium and can be very large, up to several centimetres (Foster, 2017) and causing the distension of the uterine horn(s) that can be confused with tumours of the myometrium, chronic pyometra, pregnancy, or sites of foetal resorption or segmental cystic endometrial hyperplasia. Endometrial polyps may be solitary or multiple, and their shape is adapted to the uterine lumen. They can be observed protruding through the cervix into the vagina and sometimes even out of the vagina(Schlafer and Foster, 2016). Their aetiology is unknown, but they usually occur with CEH (Foster, 2017).

Hydrometra and mucometra are accumulations of thin or viscous fluid in the uterus associated with endometrial hyperplasia or due to congenital or acquired obstruction of the lumen of the uterus, cervix

or vagina. Grossly there is uniform or segmental dilation of the uterus. If the hydrometra or mucometra persists over time, the endometrium becomes atrophic (Schlafer and Foster, 2016 Foster, 2017).;

Uterine serosal inclusion cyst are small cysts on the surface of the uterus that are occasionally observed in pluriparous bitches. Thin-walled cysts measuring from a few millimetres to two centimetres or more, containing a clear watery fluid, form from small folds of the peritoneum that adhere to each other and form pieces of serosal mucosa in which secreted fluid slowly accumulates (Schlafer and Foster, 2016).

Pseudopregnancy is a condition where an intact or spayed bitch shows clinical signs typical of the peri- and post-partum period of pregnancy, despite the bitch is not being pregnant (Root et al., 2018). The condition is an exaggerated form of a normal physiological process. Every intact bitch has a prolonged luteal phase of oestrus, called physiologic or covert pseudopregnancy. Some dogs develop an exaggerated response called overt pseudopregnancy, which often occurs six to eight weeks after oestrus (Foster, 2017; Root et al., 2018). The mechanism is poorly understood, but prolactin or its receptors play a role. Overt pseudopregnancy develops in dogs with elevated prolactin concentrations or dogs that have an increased sensitivity to prolactin. Hyperprolactemia occurs in response to visual stimuli that show the presence of surrogate neonates results in mammary gland development, lactation, and maternal behaviour. Uterine changes may include the formation of PEH and mucometra (Foster, 2017).

2.5.3 Inflammatory diseases of the uterus

Pyometra is an acute or chronic suppurative infection of the uterus with accumulation of pus in the uterine lumen. It occurs relatively frequently in the bitch and usually affects older animals, especially those that are not bred. Most cases of pyometra in the bitch are associated with endometrial hyperplasia. It usually develops a few weeks after oestrus (Schlafer and Foster, 2016).

In less advanced cases, the uterus may be slightly enlarged, with mild endometrial hyperplasia and inflammation, while in more advanced stages there is a remarkable distension of the uterine horns, which may occupy most of the abdominal cavity. The distension of the uterine horns may be symmetrical or asymmetrical, uniform or ampulla-like, as in the mid-pregnancy uterus. The cervix is completely or almost completely closed. The uterine wall is friable, and rupture or perforation with secondary peritonitis is common (Schlafer and Foster, 2016). The appearance of the exudate in the uterine horns depends on the aetiology (Schlafer and Foster, 2016). The most common bacteria in pyometra are *E. coli* (faecal isolates and urinary tract pathogens), but other normal vaginal microorganisms or urinary tract pathogens (*Staphylococcus* sp., *Streptococcus* sp., *Klebsiella* sp., *Pseudomonas* sp., *Proteus* sp., *Hemophilus* sp., *Pasteurella* sp., *Serratia* sp.) can also be isolated (Schlafer and Foster, 2016). The uterine mucosa is irregularly thick, necrotic and ulcerated with multifocal irregular superficial haemorrhages, and in other portions it is hyperplastic, dull white and dry with small cysts. Histologically, remarkable hyperplasia of the endometrium and progestational proliferation can be seen in almost all cases (Schlafer and Foster, 2016). Lesions outside the genital tract include widespread extramedullary haematopoiesis and immune complex glomerulopathy (Foster, 2017).

Pyometra is a medical emergency that requires immediate treatment (Xavier et al., 2023).

2.6 Pathology of postpartum uterus

“ is a postpartum pathology of unknown cause. In a normal bitch, according to some authors (Schlafer and Foster, 2016) overt uterine bleeding usually stops 7-10 days after parturition, according to other authors (Foster, 2017) in 1 to 6 weeks, and placentation sites normally remain visible until 12 weeks after parturition (Orfanou et al., 2009). The bitches affected by subinvolution of placental sites may bleed for several weeks or months. The blood loss can lead to anaemia and death. Grossly, there are ellipsoidal enlargements of the uterine horns in the areas of placentation; the affected areas are haemorrhagic and irregularly thickened. Histologically, large cells interpreted as syncytial triphoblast cells persist into the involutionary period; these cells can grow infiltratively into the myometrium and in some cases cause perforation of the uterus (Schlafer and Foster, 2016). The uterus is prone to ascending infections, endometritis and open pyometra (Foster, 2017).

2.7 Pathology of the cervix, vagina, and vulva

2.7.1 Cyst of the vagina or vulva

Only a few cases of cysts at this site have been reported in intact dogs. Cysts in the vagina or vulva occur as cystic dilatations of Gartner's duct, from the urogenital tract and from remnants of the mesonephric duct (Sanchez Jimenez et al., 2019).

Gartner's duct are remnants of the embryonic mesonephric (Wolffian) ducts located on both sides of the vaginal floor. The cysts may be isolated or the whole duct may be dilated and tortuous (Schlafer and Foster, 2016).

Bartholin glands are not present in dogs, so cysts originating from these glands should not be included in the list of differential diagnoses (Sanchez Jimenez et al., 2019).

2.7.2 Ruptures of the vagina and vulva

Ruptures of the vagina and vulva are rare and are usually acquired as parturient injuries (Prassinos et al., 2010). The outcome depends on whether lacerations become infected and, if so, with which organism (Schlafer and Foster, 2016). Possible outcomes include cellulitis, abscess, gangrene or peritonitis, cicatrisation of the wall and subsequent stricture (Schlafer and Foster, 2016).

2.7.3 Swelling of the vulva

Swelling of the vulva is a physiological response to oestrogen and occurs mainly during the oestrus. The swelling may persist due to exposure to exogenous (topical oestrogen cream in the home) or endogenous oestrogens (due to cystic follicular disease or granulosa cell tumour) (Schlafer and Foster, 2016; Ivaldi et al., 2022).

2.7.4 Vaginal hyperplasia

Vaginal hyperplasia is an exaggerated response of the vaginal mucosa to oestrogens during the proestrus–oestrous phase of the cycle that can protrude through the vulvar labia (Bucci et al., 2022). The protrusion of hyperplastic vaginal mucosa can be complicated by involvement of the urethra and trauma to the exposed tissues (Post et al., 1991)

2.7.5 Inflammatory diseases of the vagina and vulva

Granular vulvitis (vaginitis) occurs in most domestic animals, including dogs. Grossly, papular eruptions occur exclusively on the vulvar mucosa. Histopathologically, the papules are characteristically composed of subepithelial lymphoid aggregates (lymphoid follicles) (Schlafer and Foster, 2016).

2.7.6 Neoplastic and tumour-like lesions of the uterus, cervix, vagina, and vulva

Smooth muscle tumours (leiomyoma, leiomyosarcoma)

Leiomyomas are the most common tumours of the tubular genitalia in bitches. They arise from smooth muscle cells in the wall of the uterus, cervix or vagina. They may be solitary, but sometimes also multiple. Most smooth muscle tumours in bitches are benign, regardless of their histological characteristics. They frequently occur in association with ovarian follicular cysts or oestrogen-producing ovarian tumours and often with endometrial hyperplasia, and mammary gland hyperplasia; they may regress after spaying. Genital smooth muscle tumours may grow up to 10-12 cm in size, but most of them are not invasive. They grow as nodular or elliptical masses or as bulbous polyps in the lumen of the genital organs. They are not encapsulated but are well demarcated, light brown to white in colour and can be easily shelled out. On the cut surface they have watered-silk appearance, while their colour depends on the amount of muscle and connective tissue. Leiomyosarcomas are invasive, have more mitoses and frequently contain areas of necrosis. Histologically, smooth muscle tumours are composed of whirling bundles of neoplastic cells that resemble smooth muscle cells (Schlafer and Foster, 2016; Foster, 2017).

Canine transmissible venereal tumour (TVT) (Sticker's sarcoma) is a contagious tumour that occurs in both sexes on the external genitalia (Ujvari et al., 2017). It is transmitted during coitus by the transfer of intact neoplastic cells that grow like a graft (xenograft). The morphology and immunohistochemical characteristics of the neoplastic cells suggest a histiocytic phenotype (Ujvari et al., 2017). TVT is reported worldwide, and the tumours is more common where dogs are allowed to run free (Schlafer and Foster, 2016; Setthawongsin et al., 2022). TVT is more common in tropical and subtropical regions and is uncommon in North America and northern and central Europe, although occasional cases have been reported in imported dogs (Setthawongsin et al., 2022). The tumours can be located in the genital area

and extragenital sites such as the rectum, skin, oral and nasal cavities, and eyes. They appear as firm, friable, brownish, ulcerated, nodular or polypoid masses. In bitches, TVT may spread directly to the cervix, uterus and uterine tubes. Although metastasis is uncommon, TVT can spread to regional lymph nodes, skin and subcutaneous tissue, but spontaneous regression can occur in less than six months (Schlafer and Foster, 2016). Cytologic diagnosis is of great value for easy and rapid on-site diagnosis (Setthawongsin et al., 2022). The aspirates yield a large number of round cells with round nuclei and single or multiple prominent nucleoli. The nuclei are located eccentrically. The cells have moderate amounts of pale blue cytoplasm, often containing multiple punctate vacuoles. Mitotic activity is often high. Inflammation (plasma cells, lymphocytes, macrophages and neutrophils) may be present (Solano-Gallego and Masserdotti, 2023).

Carcinomas of the endometrium and cervix are rare in domestic animals. Since CEH is common in dogs, but carcinoma is rare, there does not appear to be a correlation between the two (Schlafer and Foster, 2016).

Primary skin tumours that occur in the perineal region frequently involve also affect the vulva (Schlafer and Foster, 2016).

Metastatic (secondary) tumours of the tubular genitalia are rare. Serosal implantation occur in peritoneal carcinomatosis (Schlafer and Foster, 2016).

Vaginal polyps are common in older, usually intact bitches. They are often solitary, have a diameter of up to several centimetres and are grossly indistinguishable from leiomyoma (Foster, 2017).

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3 UNDERSTANDING PREGNANCY PHYSIOLOGY: MONITORING, MANAGEMENT, AND PREDICTING PARTURITION

Magdalena Schrank

Understanding pregnancy physiology is of great importance and allows identification of problems at the earliest moment possible. Neonatal mortality in cats and dogs is already high which makes monitoring of pregnancy and fetal development as well as correct management immensely important. Pregnancy diagnosis is most commonly performed using ultrasound examination. Endocrine pregnancy tests are of little use in small animal reproduction due to their low accuracy and the fact that they are applicable at a time of gestation at which ultrasound examination is already possible.

A lot of researchers have studied methods to predict parturition date in order to be able to program elective cesarean at the correct date. Fetal measurements have been shown to be indicative of the date of parturition, yet knowledge of the ovulation date remains the most accurate and most frequently used parameter. Measurement of progesterone concentration during the last days of pregnancy has a high accuracy in predicting the onset of parturition yet it was shown that a single measurement is of little use.

3.1 Physiology of pregnancy

In the bitch progesterone is solely produced by the corpus luteum (CL) (Concannon, 2011). Due to spontaneous ovulation pregnant and non-pregnant females show nearly equal progesterone profiles during the diestrus period, yet differences have been reported after day 25 of diestrus. Concomitantly to high progesterone levels decrease in hematocrit has been reported which may arrive at 40% at term of pregnancy and may remain at these levels for 1-2 months after parturition. Prolactin increases as well by day 32 of pregnancy. This increase occurs simultaneously with a detectable increase in plasma concentration of relaxin around day 25-27. During the second half of pregnancy, prolactin may be considered the main luteotrophic hormone (Johnson, 2008). Relaxin in the bitch is considered entirely of placental origin, peaks mid-pregnancy, remains then elevated until term, and declines at parturition. Increased insulin resistance has been reported and makes pregnancy in diabetic bitches high-risk pregnancies (Concannon, 2009). An increase in fibrinogen, other coagulation factors, and c-reactive protein need to be taken into account when interpreting blood exams of pregnant bitches. In the canine species, fertilization occurs in the uterine tubes and the fertilized ova develops into morulae before entering the uterus (Concannon, 2011). Migration of embryos within the uterus from day 12-17 after LH surge results in an equal distribution within both uterine horns. By day 22-23 after LH surge implantation is completed (Concannon, et al. 2001; Reynaud et al. 2006). Although progesterone concentrations decrease initially slowly, a sharp decline is observed within 48 hours of whelping (Concannon, 2011). This sharp decrease in progesterone concentration is a result of an acute increase of prostaglandin F2□; a process that does not occur in non-pregnant luteal phases. An exception is singleton pregnancy, wherein progesterone may or may not decrease to <2 ng/mL at term. Parturition is possible due to the sharp decline of progesterone and the concomitant loss of inhibitory effects on the uterus together with the increase in prostaglandin and oxytocin levels which promote uterine activity (ER, 1999). Cortisol levels increase and are an important factor for the initiation of parturition.

In the queen, development of the first 3-4 days takes place in the oviducts before migration into the uterine horns. Implantation occurs around day 12-16 after first mating (England and Heimendahl, 2010).

Progesterone secretion during pregnancy usually prevents the occurrence of estrus. When the queen stops lactation and it is still breeding season, a brief period of interestrus will follow until she enters the next estrus; outside of the breeding season the queen will enter anoestrus. This estrous period usually starts around 10-15 days after weaning. In some queens, estrus may be seen even while nursing 10-12 days after parturition (England and Heimendahl, 2010). The first mating after parturition is often not fertile due to incomplete uterine involution. Spontaneous or induced follicular growth with estrous behavior and mating have been described during pregnancy, suggesting that the ovaries are responsive to gonadotropins during gestation in this species (England and Heimendahl, 2010). Prolactin concentrations increase in the pregnant queen around 25-35 days after first mating arriving at plateau levels at around day 50 of pregnancy (Lopate, 2012). Prolactin, similar to the dog has an important luteotropic role and in case of suppression will result in pregnancy loss (England and Heimendahl, 2010; Verstegen, 1998). Progesterone is produced by the CL and levels follow a similar pattern as was described in the bitch yet peak values of progesterone may be measured as early as 11 days after first mating (Schmidt et al. 1983). Removal of the gonads during pregnancy will result in a sharp decline of serum progesterone concentrations within 24-48 hours after surgery resulting in either reabsorption or abortion depending on which day of pregnancy ovariectomy is performed. Relaxin is suspected to have a similar role as prolactin being a luteotrophic factor during pregnancy. Its concentrations increase from day 20-30 onward (England and Heimendahl, 2010; Verstegen, 1998).

3.2 Pregnancy length

Pregnancy length in the bitch is usually calculated from the day of ovulation and in these cases is described as lasting 63±1 days (Concannon, 2011; Mir et al., 2011). In queens pregnancy length has been reported to be around 63-65 days if calculated from first mating (Johanson, 2022). In cases in which the ovulation date of bitches is unknown, breeders frequently use the day of mating as a reference point, yet in cases of multiple matings on following days, each breeder has their own opinion from which mating day they will start calculating. This method of determining pregnancy length is very unreliable and, in these cases, pregnancy length has been described as 57-70 days (Schrank et al., 2022). In the queen determination of optimal breeding timing is rarely performed for different motives. Vaginal smear is an important part of determining optimal breeding timing in the bitch, yet the manipulation in queens if performed repeatedly and with a certain intensity may cause ovulation (England and Heimendahl, 2010). Furthermore, cat breeders organize mating differently than dog breeders, since multiple matings in the queen increase the probability of ovulation. In dogs no effect of the number of matings on the probability of pregnancy is observable. In the queen instead, multiple matings provide more stimuli which increase the probability of ovulation and therefore the probability of pregnancy. Queens are therefore usually for more consecutive days in the males' vicinity to allow these repeated matings. Research has shown that pregnancy length is influenced by litter size in some dog breeds (Eilts et al., 2005; Gavrilovic et al., 2008) whereas others report no significant effect (Tsutsui et al., 2006). Although such findings are interesting, before interpretation and comparison of the data the method of calculation of pregnancy length has to be considered.

3.3 Pregnancy diagnosis

Pregnancy may be diagnosed in early stages through abdominal palpation in both bitches and queens. At palpation, a structure similar to a pearl necklace may be encountered which represents the early stages of gestational sacks and the concomitant enlargement of the uterus. At this stage areas between the gestational sacks are well defined; a characteristic that is lost with continuous growth of the embryo/fetus. Although palpation may be considered the method for early pregnancy diagnosis, it has to be considered that if too much pressure is applied damage may be done and in inexperienced hands, the pearl necklace structure of the uterus may be confused with the presence of fecal matter in the colon. Furthermore, palpation in large or giant breeds is extremely difficult considering that the structures in question are quite small relative to the dam's size. The most reliable tool for pregnancy diagnosis in the bitch and the queen is ultrasound examination. It has been reported that the conceptus may be visualized as early as 15 days after ovulation in the bitch although cardiac activity is measurable at around day 22 (England and Heimendahl, 2010). In the queen fluid-filled gestational sacks may be visualized as early as day 11 after mating, the embryo at around day 14, and cardiac activity at around day 15 (England and Heimendahl, 2010). Ultrasound examination has further the advantage of providing information on fetal growth, viability, and early diagnosis of problems such as empty gestational sacks or pathologic accumulation of fluid in the uterus (England and Russo, 2006). Spherical, anechoic structures surrounded

by a well-defined hyperechoic wall are visible as well as the embryo inside. The size and appearance depend on the day of gestation. At day 24-28 fetal structures are visible quite well and cardiac activity can be measured. The cardiac frequency of fetuses is well above the mothers ranging from 200-250 beats/min. Fetal anatomy may be clearly visible around day 40-50. The presence or absence of fetal cardiac activity, homogenous growth, corpuscular or otherwise echoic structures within the amniotic fluid, and abnormal or lack of fetal growth are important indicators and should be evaluated in all fetuses if possible. Pregnancy can be confirmed by abdominal palpation, diagnostic imaging, and by detecting the hormone relaxin in blood (Johnson, 2008). Abdominal palpation is easily and quickly performed. Although this is the most subjective method of pregnancy diagnosis, it is a reliable method for those skilled in palpation. Uterine enlargement caused by pregnancy cannot be accurately differentiated from uterine enlargement caused by some other process, such as pyometra, based on abdominal palpation findings alone (England and Russo, 2008; Beccaglia and Luvoni,2006). Skeletal calcification is sufficient at around day 45 in the bitch and day 40 in the queen after LH surge and mating, respectively, and allows determination of the number of pups and/or kittens by abdominal radiography (England and Heimendahl, 2010). Radiography is rarely used for pregnancy diagnosis, yet it may be considered useful not only to determine the number of pups/kittens but allows also an estimate of their size and allows measurements of head diameter in relation to the pelvic canal of the bitch. Endocrine pregnancy tests similar methods for early pregnancy diagnosis are a well-researched subject and solutions are sought after. Due to the particular hormonal pattern in case of pregnancy in humans and other mammals, early pregnancy diagnosis using either blood or urine is possible. In cats and dogs, due to their particularities in estrus cycles and post-ovulation hormonal patterns, early pregnancy diagnosis other than palpation is difficult and unreliable and therefore (although used in research) very rarely applied. Relaxin may be used for pregnancy diagnosis at around day 21 after breeding but is a more sensitive indicator from day 30 on as its levels are different in pseudo-pregnant or non-pregnant bitches (Concannon, 2011 et al.). At this moment in gestation ultrasound examination is possible, reliable, and gives information on the fetal development, viability, and presence of possible complications and may therefore be considered as the superior method. In the cat relaxin is a pregnancy-specific hormone and is detectable during gestation until placental expulsion (Stewart and Stabenfeldt,1985). Its detection is possible either in urine or serum. Commercial relaxin assays have been used in studies and have been described as reliable 28 days after mating. Although reliable it poses the same problem as relaxin measurements in the bitch as its application is possible in a stage of pregnancy in which ultrasound examination gives more reliable and overall, more information on the presence or absence of pregnancy and its physiological progress. A significant elevation of prolactin may be measured in pregnant bitches and research is conducted on its usefulness for pregnancy diagnosis in the canine species. Measurement of progesterone levels is not useful to determine pregnancy. In queens, the failure to return into estrus by day 45 may be an indicator of pregnancy, yet at this point pregnancy most probably has already been confirmed or excluded by ultrasound examination (England and Heimendahl, 2010).

3.4 Pregnancy management

Pregnancy management is of great importance. Many different factors have to be taken into consideration and although it is up to the breeder to apply this knowledge, it is up to the veterinarian to inform them to increase the probability of physiological and stress-free parturition in both bitches and queens (Johnson, 2008). First and foremost, the environment needs to be addressed. An area/room/kennel should be dedicated to birth and the period until weening. This area should be easy to sanitize and should be separated from other domestic animals and/or factors such as noise, children, and similar. Furthermore, breeders should be able to control environmental temperature, and airflow (e.g., open windows) should be avoided. An adequate environmental temperature of around 30 degrees Celsius has been indicated. Temperature has to be then adapted to the stage of the neonatal period as pups slowly reach the ability to maintain a stable body temperature independently of their surroundings. Adequate temperature control may be achieved by a heat source either heating the room in itself or via heat sources that apply heat to a certain area of the whelping box (e.g., infrared heating lamps). In case heating lamps are used it is very important to follow instructions regarding the correct distance between the pups and the lamp to ensure adequate heating and avoid burns. The whelping box in itself has to be of the correct size, made of correct materials, and bedding within should be provided to allow nest-building behavior. It is generally considered useful to provide the bitch with access to the whelping box days before parturition especially in primiparous bitches (Johnson, 2008). Although bitches (especially primiparous ones) often search for the presence of their owners, cats tend to isolate themselves. The whelping box of the queen should also have a top cover to give the queen the sensation of a den. Not all queens tolerate the presence or help of humans during the parturition process. Throughout pregnancy and especially

towards the end monitoring of behavioral patterns, appetite, and body weight is of great importance and the owner has to be informed of the early signs of beginning parturition (e.g., nesting, increased respiratory rate, agitation). Ultrasound examination of the pregnant bitch in late stages of pregnancy may be useful in cases of bitches of advanced age, litters with a single pup, or in cases of pathological findings during the first ultrasound. In cases of physiological pregnancy in adult bitches of good health and without signs of complications the usefulness of repeated ultrasound examinations needs to be evaluated taking the stress that may be caused into consideration. Correct nutrition during pregnancy and the lactation period is of great importance and has been reported to influence early embryonic survival, litter size, birth weight, and neonatal survival (Kelley, 2002; Scantlebury et al., 2001; Wright-Rodgers et al., 2005; Chew et al., 2001; Kuhlman and Rompala, 1998). Especially in the last days of pregnancy and the lactation period correct and adequate assumption of calcium is of great importance to prevent the occurrence of eclampsia.

Owners should have alerted a veterinarian at the beginning of parturition to allow fast intervention in case of dystocia.

3.5 Determining day of birth

Progesterone assays are frequently used to determine date of parturition. The abrupt decrease of progesterone to values under 2 ng/ml (around 6.4 nmol/l) in the last 24 hours before parturition is measurable (Concannon, 2011; Johnson, 2008; Kutzler et al., 2003). At parturition, serum progesterone levels are lower than 1 ng/ml (Johnson, 2008; Kim et al, 2007; Veronesi et al., 2002; Concannon et al., 1974; DeCramer et al., 2019). Measurement of progesterone performed only once before parturition has been reported to be of low diagnostic value (Rota et al., 2015). Rectal temperature has been described as a possible indicator of parturition. In most bitches a drop in rectal temperature of around 1 degree Celsius or more may be observed 12-24 hours before parturition (Johnson, 2008; Rota et al., 2015; Michel et al., 2011; Verstegen-Onclin, 2008). Reliability of this parameter is still in doubt but nevertheless a value frequently considered by both veterinarians and breeders. Ultrasound examination in late stages of labor allows measurements of various parameters, both fetal and maternal, which are considered indicators for the parturition date. Fetal parameters taken most frequently into consideration are crown-rump length (CRL), body diameter (BD), biparietal diameter (BP), and kidney length. Measurements should be performed in at least two fetuses in both uterine horns (Alonge et al., 2016; Sridevi, 2013; Beccaglia and Luvoni, 2012). Considering the vast differences in body size and body weight of dog breeds, accuracy of these parameters may be influenced (Alonge et al., 2016). Formulas created specifically for different weight classes mean to correct this bias. CRL may be measured only until day 45 after LH peak and the highest accuracy is reported on day 30 of pregnancy (Kutzler et al., 2003; Luvoni and Beccaglia, 2006; England et al., 1990). BP may be measured as early as day 30 of pregnancy (Kutzler et al., 2003). Accuracy of BP is reported to be good during the 5th and 6th week but has been considered a reliable parameter up until week 8 of pregnancy (Beccaglia and Luvoni. 2012). During the last week instead, accuracy decreases and BP should therefore not be used to determine the correct date for an elective cesarean section (Beccaglia and Luvoni. 2012; De Cramer and Nöthling, 2018). Kidney length is a rather new parameter but is strongly correlated to gestational age (Gil et al., 2018). It appears that the best period that gives the accuracy, sensitivity, and specificity is between 15 to 11 days before parturition. Fetal maturity is of great interest and may be determined by evaluation of gastrointestinal (GI) tract motility and kidney development (Gil et al., 2018; Gil et al., 2015). Although GI tract motility may be visible as early as 9-13 days before parturition a long observational period is needed to accurately diagnose its presence. Duration of observational period decreases and may be easily and rapidly detected between day 1-4 before parturition (Gil et al., 2015). Changes in the kidney's appearance during the last 5 days before parturition are not indicative of imminent parturition yet when GI tract motility is visible, renal development may be considered completed (Gil et al., 2018). Kidney cortical thickness, medullary thickness, and cortico-medullary thickness ratio have been investigated and it is reported that these parameters are proportional to the number of days left until parturition (Siena G, Milani, 2021). Lung maturity may easily be considered one of the most important requirements for extra-uterine survival. Fetal lung echogenicity has been analyzed using mean grey level assessment, which shows a rapid increase of echogenicity from days 49 to 56 and a plateau phase at 57–63 days post ovulation (Banzato et al., 2017). The lung-to-liver ratio of mean grey level was proposed as an accurate parameter for fetal lung maturity evaluation, with 83% specificity and sensitivity (Banzato et al., 2017), due to its significant decrease during the last week before parturition.

Fetal heart rate (FHR) is monitored routinely during abdominal ultrasound examination from first pregnancy diagnosis on (Lopate et al., 2018; Smith, 2007; Gil et al, 2014; Beccaglia et al, 2016). Fetal cardiac activity is considered normal when FHR is over 220 bpm. Values between 180 and 220 bpm may indicate

moderate fetal distress. If FHR reaches values under 180 bpm fetal distress may be considered severe and a further decrease to values of under 160 bpm requires immediate intervention (Sridevi, 2013; Gil et al, 2014; Beccaglia et al, 2016; Vieira et al., 2020). This parameter is therefore considered the most important indicator of the necessity of an emergency cesarean section. Studies have reported that fetuses with FHR of under 130 bpm should be delivered within 1-2 hours and that mortality rates increase drastically in cases of FHR <100 bpm (Johnson, 2008; Smith, 2007). Temporary decreases and increases of FHR have been described from day 5 before parturition onwards (Gil et al, 2014; Giannico, 2016). Single measurements of FHR may therefore cause concern. It is advised to perform repeated measurements of as many pups of the litter as possible to properly assess fetal health.

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4 NORMAL PREGNANCY AND PARTURITION IN DOGS

Maja Zakošek Pipan

4.1 Gestational length

Gestation length in the dog varies considerably depending on whether it is calculated from a single breeding (57- 72 days), from an LH surge (64-66 days), from ovulation (62-64 days) or from the onset of cytological diestrus (D1) (56-58 days). The relative timing of the most important events in the canine pregnancy is shown in Table n°1.

A pregnancy length different from 63 days indicates that breeding occurred either before (duration of pregnancy 64 days or longer) or after (duration of pregnancy 62 days or shorter) ovulation. This is an important piece of information for future breedings. Events relative to the most important hormones have been studies mostly in Beagles. Data on other breeds are lacking and extrapolating results from breed to breed may not necessarily be correct at all times.

4.2 Canine embryos

Canine embryos enter the uterus as zygotes or morulae around day 8-9 after ovulation. For the first 1-2 days they move actively up and down the uterine horn in which they arrived, and then for the remaining 2 days they migrate to and from the opposite horn mixing with the other embryos (Shimizu et al., 1990). Implantation starts around day 13-15 after ovulation. The canine placenta is endotheliochorial and zonary, with blood accumulation and extravasation in the marginal areas; blood components are observed as green and brown borders of the placenta and are thought to be important for foetal nutrition.

Because of the type of placentation, in small animals only 5-10% of the total immunoglobulin provided by the bitch is transferred through the endotheliochorial placenta to the pup. Therefore, the majority of passive immunity is derived through colostrum.

Table 1: Some of the most relevant reproductive event of the canine pregnancy timed as relative to the LH peak, ovulation and onset of cytological diestrus (D1). Adapted from Concannon and Lein, (1989)

Reproductive event	Days from the LH peak	Days from ovulation	Days from D1
Onset of proestrus	-25 to -3	variable	variable
Vaginal cornification1	-1 to +7	-4 to +4	-10 to -2
Onset of oestrus	-4 to +5	-7 to +2	-13 to -4
LH peak	0	-2 to –3	-8 to -9
Maximum fertility	-1 to +6	-3 to +4	-3 to -10
Ovulation2	+2 to +3	0	-5 to -7
Fertilization	+4 to +6	+2 to +4	-2 to -5
D1	+8 to +9	+5 to +7	0
Behavioral diestrus	+10 to +14	+7 to +11	+1 to +5
Zygotes enter uterus	+11 to +12	+8 to +9	+2 to +3
Attachment	+16 to +18	+13 to +15	+7 to +9
Ultrasound diagnosis possible	+19 to +22	+16 to +19	+10 to +13

Abdominal palpation possible	+20 to +25	+17 to +22	+11 to +16
Foetal heartbeats	+22 to +25	+17 to +22	+11 to +16
Pregnancy anemia	+25 to +30	+22 to +27	+16 to +21
Radiopaque fetal skeleton	+44 to +46	+41 to +43	+35 to +37
Luteolysis and hypothermia	+63 to +65	+60 to +62	+55 to +57
Parturition	+64 to +66	+62 to +64	+56 to +58

4.3 Pregnancy diagnosis

4.3.1 Abdominal palpation

Abdominal palpation is a simple way to assess pregnancy status, and it can be fairly accurate if performed between 25 and 35 days of gestation. Manual palpation is best performed using the hand as a sort of a "gate" using the following technique:

- Grasp the ventral aspect of the abdomen and then close your hand around it
- Place the thumb and 4 fingers as dorsally as possible, ideally right below the spinal cord and try to touch (pressing through the abdomen of the dog) the tip of your thumb with the tip of your middle finger
- Pull your hand ventrally letting abdominal viscera pass through your thumb and middle finger
- The uterus is felt as the only organ with a fibrous-like consistency, as intestinal loops have little if any consistency and cannot be palpated normally

This manoeuvre can be repeated in 3-4 different points of the abdominal cavity starting from close to the pelvic inlet and moving cranially, checking for presence of embryonic vesicles. At 25-30 days embryonic vesicles are felt like ping-pong balls and can easily be counted. After day 35 they increase in size and start elongating thus becoming more difficult to identify and count. Manual palpation can be difficult in obese or large size bitches especially if only one pup is present. Also, care should be taken to be as gentle as possible as well as to avoid repeatedly palpating the same bitch, as this may cause an increased rate of embryonic/fetal resorption, especially if unexperienced people do it.

4.3.2 Radiographs

On X-ray, uterine enlargement can be observed as early as day 21 after ovulation. Calcification of the fetal skeleton occurs from day 45 on, with selected areas of the skeleton becoming calcified later in the course of pregnancy (see the paper on Parturition Monitoring). Although the amount of radiation used in routine X-ray studies is minimal and highly unlikely to cause damage to foetuses, it is better to avoid performing unnecessary radiographs in pregnant animals. An increased risk of neoplasia or hemopoietic alterations in Beagle pups is reported following the use of very high doses of X-rays during experimental studies (Nold et al., 1987). The only practical use of radiography in pregnant bitches and queen is to determine litter size as well as size of the whole foetus (in case of large singletons or foetal monsters) or the fetal skull to assess the risk of dystocia especially in bitches with a history of difficult parturition.

4.3.3 Ultrasonography

Ultrasonography is a relatively unexpensive and very accurate tool to assess pregnancy, its only drawback being a lack of accuracy in counting the number of foetuses. A tendency to both underestimate as well as overestimate litter size has been reported (England and Allen, 1990) although overestimation may be due also to foetal resorption. When using 7.5 to 10.0 MHz transducers foetal and extrafoetal structures can be identified with accuracy. Canine gestational sacs can be detected as anechogenic structures around day 18 post-ovulation (Figure 1), and embryonic heartbeat can be detected around day 23. Despite the possibility to make a very early pregnancy diagnosis using ultrasound, clients are normally adviced to come in for pregnancy diagnosis at 30 days, as false negatives are common when performing an ultrasonography prior to 28 days, and the trust a client may have on his/her veterinarian may suffer when this happens Between day 27 and 31 post-ovulation the embryo shape becomes bipolar and limb buds can be detected. Stomach and bladder are observed on days 29-33 and 31-35, respectively. Fetal movement are

evident around day 32-34, and the fetal skeleton appears as a hyperechoic structure on day 29-33. The last organ to become visible on ultrasound is the bowel around day 57-63 of gestation. Clinical evidence suggests that the onset of fetal bowel movements coincides with the completion of fetal maturation, and some authors use this as a parameter to decide when to perform a C-section. However, this issue is still controversial, therefore the observation of fetal bowel movements should not be the only criteria, and daily fetal movement and fetal heart rate should also be monitored and evaluated. Among extrafoetal structures, the diameter of the inner chorionic cavity (ICC) is a rather accurate way to stage the canine pregnancy until day 30-35. After that time selected foetal structures should be evaluated, with head and trunk diameters being the most accurate (England and Allen, 1990).

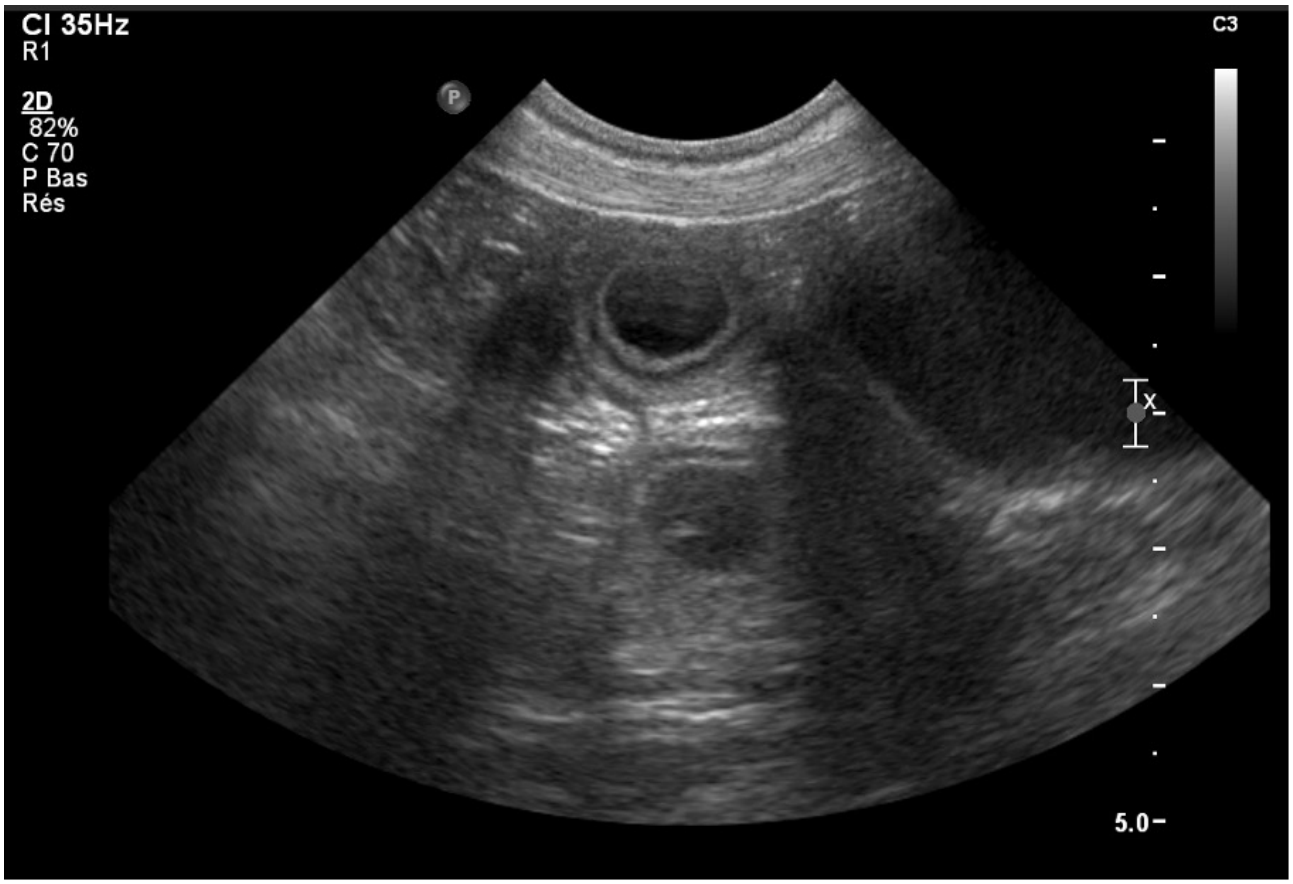

Figure 1: Confirmed pregnancy on day 18 after ovulation

4.3.4 Acute phase proteins

Acute phase proteins such as C-reactive proteins, haptoglobin, acid glycoprotein, ceruloplasmin and fibrinogen increase as soon as placentation starts, due to the inflammatory reaction that takes place at the endometrial level when the trophoblast starts eroding it. Fibrinogen is produced by the liver and is found at values of 100-150 mg/dl in normal dogs, and rises to values of 250-300 mg/dl around day 25-28 post-ovulation. A value of 300 mg/dl at 28 days is considered 100% accurate in diagnosing pregnancy in the bitch. Haptoglobin and ceruloplasmin are bound to iron and haemoglobin, respectively. Haptoglobin is normally found at values of 35-50 mg/dl, and in pregnant animals increases to values of 75-100 mg/dl around day 18-20 after ovulation (Romagnoli et al., 2005). In healthy females, an increase in acute phase proteins is considered a good indirect indicator of implantation, and can be used in practice as an aid in pregnancy diagnosis (or to rule out pregnancy). Obviously, a pre-breeding sample must be drawn to make the test accurate; also, a rise in acute phase proteins could be due to a variety of other factors associated to inflammatory reaction anywhere in the organism, including endometrial inflammation due to a pyometra.

4.4 Hormones of pregnancy

Maintenance of pregnancy in the bitch and queen depends on ovarian secretion of progesterone for the entire length of gestation. The ovaries are the only source of progesterone, as demonstrated by the fact that ovariectomy is inevitably followed by abortion at any stage of gestation. The canine feto-placental unit has the possibility to metabolize small amounts of exogenous progesterone, but no progesterone-synthesizing activity has been demonstrated. Corpora lutea secrete progesterone based on stimuli provided for by the pituitary, initially with Luteinising Hormone (LH), then with LH and prolactin (Figure 2). The luteotrophic action of LH is present at all stages of the canine gestation, even towards the end of pregnancy when serum progesterone concentrations start to decline. Such a decline is probably due to luteolytic factors which override the luteotrophic action of LH itself.

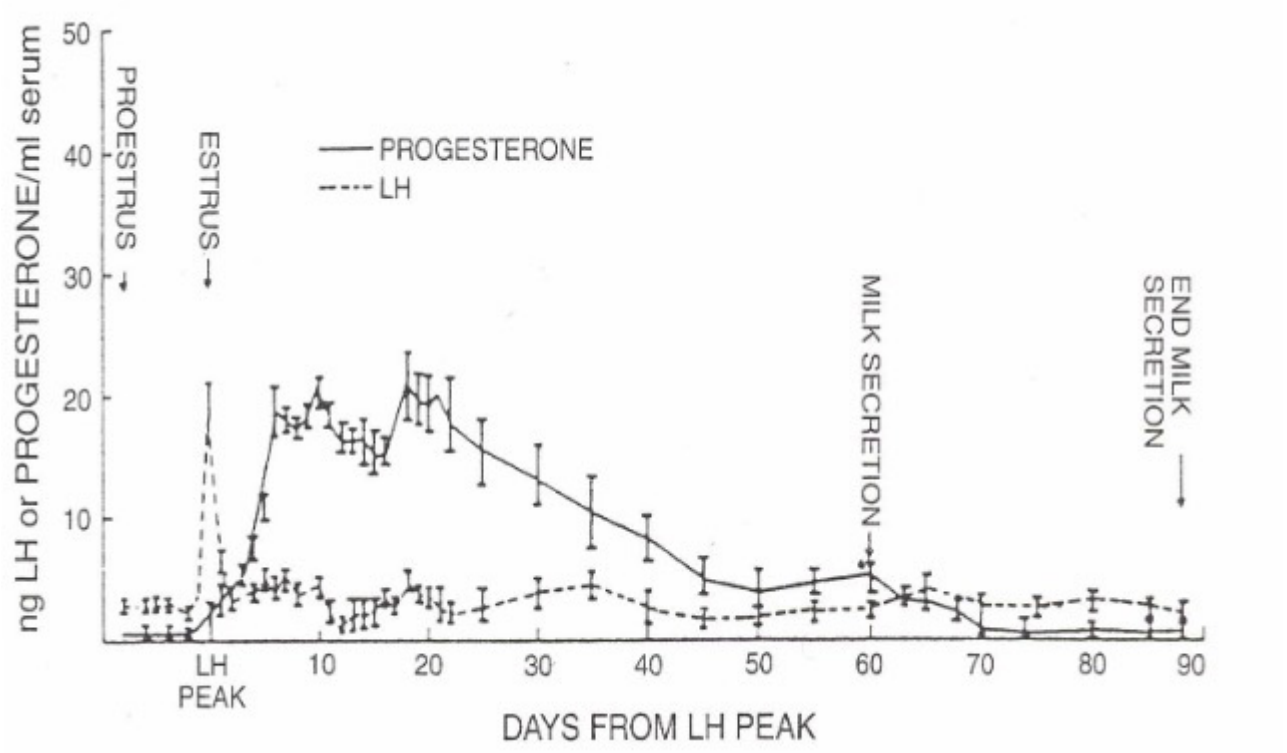

Figure 2: Hormonal correlates of the canine pregnancy and onset and end of postpartum milk secretion. Prolactin I secreted from day 25 onwads, and plays a role both in mammary development as well as in supporting luteal function. Author: Olson et al., 1984b

4.4.1 Progesterone

Progesterone concentrations are similar in pregnant and non-pregnant diestrous bitches. Therefore, assaying progesterone cannot be used as test for diagnosing pregnancy. Differences in serum progesterone concentrations between pregnant and non-pregnant beagle dogs have been observed, although these are not significant and therefore of no practical use (Smith and McDonald, 1974; Concannon et al., 1975; Concannon et al., 1977). A peak in serum progesterone concentration in the bitch occurs around the 3rd week of pregnancy to levels of 15-80 ng/ml, after which P4 levels fluctuate between 10 and 20 ng/ml until the end of pregnancy or until day 50-80 in non- pregnant bitches. The relative amount of progesterone secreted by pregnant bitches with a large litter has not been compared to the amount secreted by bitches with a small litter, although a difference is likely to occur depending on the number of active corpora lutea present. The number of corpora lutea should be equal to the number of foetuses except if foetal loss or embryo splitting (resulting in birth of twins) occur. The lack of clinical data on serum progesterone concentrations in pregnant bitches of breeds other than the Beagles as well as in middle age to older bitches makes it difficult to manage cases of infertility, especially cases of hypoluteoidism when exogenous progesterone supplementation is necessary. In the bitch or queen there is no regulatory effect of the endometrium on luteal function as is known in other species. Hysterectomized bitches cycle normally and have normal luteal phases (Olson et al., 1984a), which implies that canine and feline CL lifespan is pre-programmed (9 weeks in the bitch, 25-45 days in the queen). In both species prostaglandin F2a is secreted by the endometrium at the end of pregnancy (Olson et al., 1984b), and its absence in hysterectomized bitches does have any effect on duration of diestrus. Prostaglandin F2a administered

in luteolythic doses will cause in both species a decrease in serum progesterone such as what happens prior to parturition.

4.4.2 Prolactin

Prolactin increases during the second half of diestrus, after day 25 post-LH peak, and reaches concentrations which are much higher in pregnant than in non pregnant bitches. Prolactin-lowering drugs such as cabergoline, bromocriptine and metergoline cause a dose-dependant decrease in serum prolactin as well as progesterone concentrations (Okkens et al., 1991). Serum LH does not always decrease following treatment with an antiprolactinic drug, therefore its mechanism of action in supporting progesterone secretion form the corpora lutea remains not totally clear. In non-pregnant bitches prolactin concentrations are low (<2.0 ng/ml) during most of diestrus, perhaps increasing in those bitches demonstrating clinical signs of pseudopregnancy. Prolactin values tend to increase during the last week of pregnancy to about 40 ng/ml, to reach values of approximately 100 ng/ml during the last 1-2 days prior to whelping, peaking at values slightly above 100 ng/ml during the 1-2 days after parturition (Concannon et al., 1978). Prolactin increases in response to suckling by pups.

4.4.3 Estrogens

Estrogens are reported to show no difference between pregnant and non-pregnant bitches after estrus (Concannon et al., 1975). However, if specific estrogens are considered estradiol and estrone demonstrate a specific pattern of secretion. Estradiol is low in pregnant bitches and high in non-pregnant ones until around the 5th week post- ovulation. Conversely, during the same period estrone is low in non-pregnant bitches and high in pregnant ones (Chakraborty, 1987). In many other species estrone is secreted by the feto-placental unit. However, the source of estrone secretion (whether the feto-placental unit or the adrenal) has not been investigated in the pregnant bitch.

4.4.4 Relaxin

Relaxin is a key hormone of pregnancy both in the bitch and queen, as it rises between the third and fourth week of gestation being secreted by the placenta, peaks at about 6.0 ng/ml 2-3 weeks prior to parturition and declines within a few days following placental detachment or hysterectomy. Its role is to cause relaxation of the birth canal, softening of the perineum and softening of the nipples to facilitate suckling from the neonates. Relaxin is non- detectable in males, in non-pregnant females and during stages other than pregnancy or the puerperium. It remains high in pregnant bitches ovariectomized provided that they are treated with a progestational drug (Tsutsui and Stewart, 1991). Relaxin concentrations may vary in bitches of different breeds, as Labrador retrievers were observed to have higher levels than Beagles (Steinetz et al., 1987). Commercial kits for rapid determination of canine relaxin as a pregnancy test have developed over the last decade, but have not been very successful as the time at which pregnancy can be diagnosed is not early enough to give an advantage over the use of ultrasound. Still, if available a relaxin kit can be of help to establish if foetal death and resorption have occurred in a risk pregnancy.

4.5 Peculiarities of feline pregnancy

Ovulation occurs 24-27 hours after breeding and pregnancy length can be therefore be accurately calculated based on first occurrence of mating. Fertilized ova reach the uterine horn 3-4 days post-ovulation, and implantation occurs around 12-13 days after ovulation. Length of feline gestation is approximately 63 days (range 52-74 days) and does not appear to be influenced by age of the queen, parity, number of kittens per litter, mean weight of kittens born, weight gain during pregnancy or genetic background (Munday and Davidson, 1993).

Serum P4 concentrations increase above the 2.0 ng/ml threshold level about 3-4 days after mating, peak at values >15 ng/ml between day 11 and 30 of pregnancy, and remain above threshold until day 62 (Verstegen et al., 1993a). Parturition occurs approximately 24-48 hours after luteolysis. Early work reporting that ovariectomy could be done after day 45 of gestation without causing abortion (because of placental production of P4) was challenged from experiments done in Belgium where queens were showed to abort 7-10 days following ovariectomy performed at day 45 of gestation. Recent data suggest that placental secretion of progesterone in (pregnant) queens may occur at midpregnancy although probably such P4 secretion decreases in late term queens (Gultiken et al., 2018). As in dogs, prolactin is considered a major luteotrophic factor in the feline pregnancy, and anti-prolactin compounds such as cabergoline has been shown to be able to lyse the feline corpus luteum causing abortion as early as day 30 of gestation, whereas the luteolytic action of prostaglandin F2a in the queen is evident only after day 30 to 40.

Cat breeders generally do not have pregnancy diagnosed on their queens on a regular basis. Occasionally queens with a history of infertility may be brought in to the veterinarian for a uterine ultrasound around 20-25 days post- breeding, a time when manual palpation of the abdomen easily allows to detect foetal vesicles of 2.5 cm diameter. Radiography and ultrasound can be used for pregnancy diagnosis as early as day 17 after breeding, while calcification of the fetal mandible, cranium, scapula, humerus, femur, vertebral bodies and ribs can be observed as early as day 38 after breeding. Radiographic identification and measurement of crown-rump length has been used to estimate fetal age: feline fetuses measure approximately 10.5 cm at 50 days, 12 cm at 55 days and 14.5 cm at parturition.

4.6 Care of the pregnant female

Good pregnancy management starts before the beginning of proestrus, at a time when vaccination or immunization protocols should be checked and updated. In proestrus health status should be assessed with a clinical exam which should include palpation of the mammary glands as well as of the abdominal organs. In bitches with a history of infertility a blood sample should be drawn to perform a complete blood count and serum biochemistry in order to assess baseline values of blood cells and serum proteins/enzymes. Pregnancy status should be assessed during the 5th week in normal, healthy females, while it should be anticipated to the 4th week in females with a history of infertility, as P4 supplementation may be needed already at implantation time. Also, if not pregnant a bitch with a history of cystic endometrial hyperplasia might be treated with prostaglandins or with a progesterone antagonist to spare her uterus from an excessive progestational stimulation.

Pregnant females should not be exposed to foreign animals as they can be potential source of infection with viruses against which the female may not be immunized. A pregnant female displaying signs of disease should be seen by a veterinarian as soon as possible. If possible, anything that can be performed at the animal's premises such as clinical exam, sample collection (feces, urine, blood etc.) or remote miometrial monitoring should be done there rather than bringing the female to the veterinary clinic.

During pregnancy, bitches and queens should do a moderate physical exercise to maintain a good body condition and muscle tone, which will help having a normal parturition process. Vaccinations should be avoided after mating unless it is specifically requested by the vaccination protocol that the injection be done during pregnancy, such as with the Canine Herpes Virus (CHV) vaccine, which should be administered during late estrus and then repeated again 2 weeks prior to parturition. Modified live virus feline panleukopenia vaccines are teratogenic in the queen and their use in pregnant queens should be avoided. The use of griseofulvin during pregnancy in the queen causes congenital malformations in kittens such cleft palate, exencephaly, hydrocephalus, spina bifida, cyclopia and anophtalmia, atresia ani and atresia coli, and abnormalities of the heart (Scott et al., 1975).

A good maintenance diet should be fed from week 1 through 5. The commonest mistake made by unexperienced pet owners during this time, whether they prepare home made food or not, is overfeeding because they think that increased food intake is necessary already at an early stage of pregnancy. However, food intake should not be increased during the initial stages of pregnancy as only less than 1/3 of foetal growth occurs during the first 2/3 of gestation. Fetal size increase rapidly during the last 1/3 of pregnancy. Therefore, during the 6th week the amount of food should be increased of about 30%. During the last 1-2 weeks of gestation the female should be gradually switched to a growth/lactation type diet, which should be maintained also during lactation. Whenever litter size is large (and especially in small size bitches), the enlarged uterus will compress the stomach thus making ingestion of large quantities of food difficult. Therefore, a very concentrated food (>3.6 kcal/kg food dry matter) should be used in small amounts to be fed several times daily to compensate for reduced stomach capacity. In pregnant bitches, transient periods of reduced appetite may be observed especially during or just before labor stage 1 as well as during the expulsion phase. Periods of anorexia earlier in pregnancy should be looked at with caution especially if the pregnant female stops eating completely for more than half a day, as severe ketoacidosis leading to pregnancy toxaemia can be induced experimentally in the bitch by fasting (Abitbol, 1981). Periods of reduced food intake are described in pregnant bitches around the 3rd week of pregnancy, a time when implantation is taking place.

In the bitch and queen, calcium requirements increase in late gestation and during lactation. However, such increased requirements do not justify providing extra amounts of calcium and vitamin D in the diet, as this has been associated in other species with a higher risk of developing hypocalcemia and eclampsia during early lactation. Similar studies have not been conducted in the bitch. Considering the scarcity of data and the fact that bitches of different breeds might have different requirements in terms of mineral supplementation, there is no need to add calcium or vitamin D especially if a properly balanced diet is

used. Also, in the bovine a low blood pH has been associated with a reduced ability of parathyroid hormone to stimulate bone calcium release, and the use of blood acidifiers such as dietary anions reduces the incidence of milk fever. The relationship between blood pH and bone metabolism has not been studied in the bitch. From day 54-55 on owners should be instructed to measure rectal temperature on their bitches at least 3 times daily and plot the temperature data on graph paper. A clinical exam during the last week of pregnancy, including an X-ray of the abdomen, is advisable in bitches with a history of dystocia, or if the owner is particularly anxious to know about litter size in case it is her/his first time in assisting parturition of a bitch or a queen.

4.7 Using drugs in pregnancy

The most delicate period of the canine and feline pregnancy is the first month during which organogenesis takes place. Prior to day 20-22 following ovulation (when implantantion occurs and placental development starts) canine embryos are surrounded by "uterine milk", a protein endometrial secretion which is in homeostatic equilibrium with the blood compartment, i.e. any substance that arrives in the bloodstream reaches the endometrium. Therefore, use of any substance during this time carries the potential risk of harming fetal development even though there is no risk associated for the mother. After placental development fetuses become more resistant to toxic insults. Although no real "placental barrier" exist, most substance cannot reach the placental circulation unless they are present in high concentration and for a long time in the bloodstream. However, any drug that reaches the fetal circulation must be metabolised by the fetal kidney (in carnivores the fetal liver is not metabolically active) which in itself might threaten fetal survival. Aspirin, dexamethasone, bromocriptine, carbaryl, estradiol benzoate and cypionate, prostaglandin F2a and antiestrogen drugs are widely described as capable of causing embryonic/fetal death in the dog. The effect of various drugs on the canine pregnancy is reported in details by Papich (1989). Table n° 2 shows a brief summary of drugs which have been either tested in pregnant dogs and cats and proven safe or used in pregnant laboratory animals and pregnant women without any side effect.

Table 2: Drugs which have been either tested in pregnant dogs and cats and proven safe or used in pregnant laboratory animals and pregnant women without any side effect.

CATEGORY OF DRUGS	ACTIVE PRINCIPLES SAFE FOR USE IN PREGNANT BITCHES AND QUEENS
Antibiotics	Ampicillin, amoxicillin, carbenicillin, cephalosporins, clindamycin, cloxa- and dicloxacillin, hetacillin, lincomycin, neomycin, oxacillin, penicillin G, ticarcillin
Antimicotics	Miconazole (for topical use only)
Antiparasitics	Diethylcarbamazine, fenbendazole, mebendazole, ivermectin, piperazine, praziquantel, bunamidine, pyrantel, thenium
Anticancer drugs	None
Anesthetics	Lidocaine, naloxone
Gastrointestinal drugs	Antacids, sucralfate
Cardiovascular drugs	Digitalis
Anticonvulsivant drugs	None
Muscle relaxants	None
Endocrine drugs	None

4.8 Summary

Canine and Feline Pregnancy: Key Points

Canine Pregnancy:

- **Gestation Length**: Ideally 63 days; deviations indicate misalignment between ovulation and breeding.
- **Zygote Development:**
  - Zygotes/morulae reach the uterus: 8-9 days post-ovulation.
  - Implantation begins: 13-15 days post-ovulation.

- **Pregnancy Diagnosis:**
  - Manual palpation: Accurate between 25-35 days of gestation.
  - Ultrasonography: Recommended around 30 days of gestation.
  - X-ray: Used at the end of pregnancy to determine litter size and in specific dystocia cases.
- **Progesterone (P4):**
  - Source: The ovary is the only source.
  - Luteal P4 secretion:
    - Stimulated by LH in the first half of pregnancy.
    - Stimulated by prolactin in the second half.
  - P4 levels: No significant difference between pregnant and non-pregnant diestrous females.
  - P4 assay: Not a reliable pregnancy test in dogs.
- **Relaxin:** A pregnancy-specific hormone produced by the placenta; relaxes the birth canal, maternal perineum, and nipples.

Feline Pregnancy:

- **Gestation Length:** 52-74 days from mating.
- **Progesterone (P4):**
  - Serum P4 rises to >2.0 ng/ml 3-4 days after mating, confirming ovulation.
  - Prolactin: Major luteotrophic factor during the second half of pregnancy.
- **Pregnancy Diagnosis:**
  - Ultrasonography: Recommended around 25 days of gestation.
- **Pregnancy Care:**
  - Vaccination: Avoid unless the vaccine is necessary during pregnancy.
  - Health Precautions: Prevent exposure to external animals that may carry abortifacient microorganisms.
  - Exercise: Moderate physical activity is important.
  - Diet: Increase by about 30% from the 6th week; switch to a pup growth or lactation diet from parturition and throughout lactation.
  - Appetite: Lack of appetite is normal during parturition but should be taken seriously at other times due to the risk of ketoacidosis.

General Pregnancy Care:

- **Temperature Monitoring in Dogs:**
  - Measure rectal temperature three times daily from day 55 post-breeding.
  - Parturition typically begins within 24 hours following a temperature drop of 0.8-1.0 °C.
- **Medication**: Avoid drug use during pregnancy unless strictly necessary or if the drug is specifically required during pregnancy.

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5 NORMAL PARTURITION AND ADDRESSING DYSTOCIA: DIAGNOSIS, MEDICAL INTERVENTIONS

Maja Zakošek Pipan

The normal parturition in dogs is usually predictable and relatively smooth. In a healthy bitch, labor usually begins about 63 days after ovulation. The birthing process is divided into three phases: The first phase involves dilation of the cervix and mild uterine contractions, the second phase involves active delivery of the puppies, and the third phase involves expulsion of the placenta. Most dogs are able to deliver their puppies without complications, with each puppy being born within 30 to 60 minutes of the onset of active labor.

However, complications can occur, known as dystocia or difficult labor. Dystocia can be caused by a variety of factors, such as the puppies being too large, uterine inertia, or improper positioning of the puppies. It is a serious condition that requires immediate veterinary intervention to ensure the safety of the mother and her puppies. Signs of dystocia include prolonged labor, visible distress in the mother and failure to deliver puppies despite strong contractions.

Early detection and treatment of dystocia is crucial. Veterinary assistance may include manual manipulation, administration of drugs to stimulate labor or, in severe cases, surgical intervention such as a cesarean section. Understanding the normal birthing process and potential complications allows breeders and veterinarians to provide timely and effective care to ensure a healthy outcome for the mother and her litter.

5.1 Normal Canine Pregnancy:

5.1.1 Fertilization

Fertilization occurs in the oviduct. Sperm cells enter the oviducts within 25 seconds of breeding. They do however, need around 7 hours to capacitate, before they are ready to fertilize. Sperm cells can live up to 7-9 days within the bitch's uterus. The embryo enters the uterus as a morula day 8 to 12, and implantation occurs day 14 to 16. It is possible for a litter to be sired by more than one male (superfecundation) if she was bred by more than one male over her fertile period.

5.1.2 Gestation length

Gestation length = 62 to 64 days from ovulation. Due to variability in the time of ovulation compared to time of standing behavior in the bitch and the longevity of sperm in the female reproductive tract, gestation length from a single breeding can range from 58 (bred late) to 71 days (bred early).

5.1.3 Placentation

Dogs form an endotheliochorial zonary placenta with marginal hematomas containing the green pigment uteroverdin.

5.1.4 Pregnancy endocrinology

Progesterone must be present to maintain pregnancy. All progesterone is produced by the corpus luteum (CL) in dogs. Therefore, the bitch needs ovaries for progesterone production and maintaining pregnancy. A minimum of 2 ng/ml is needed to keep the bitch pregnant. If progesterone falls below this concentration, then the pregnancy will be lost. Progesterone is overall higher in pregnancy than in diestrus, although the overlap in the concentrations is too great to use for pregnancy diagnosis. Progesterone,

after rising throughout estrus, peaks at 35-40 ng/ml around 15-25 days of diestrus. Progesterone slowly declines during the last week of pregnancy (or 1 week prepartum). During the last week of pregnancy there is an abrupt decline in progesterone concentration.

Prolactin is luteotrophic in the pregnant bitch. It is needed to maintain the corpora lutea (CL), even though it is very low in the beginning of diestrus. The prolactin concentration is inversely related to the progesterone concentration. As progesterone falls, prolactin rises. Therefore, prolactin rises starting at midpregnancy. Prolactin is at a higher concentration in pregnant, than nonpregnant bitches and higher in overt pseudo pregnancy than diestrus without overt pseudo pregnancy.

Relaxin is a pregnancy specific hormone that comes from both the placenta and ovaries; however it is primarily of placental origin.

5.1.5 Physiologic changes

In late pregnancy, the bitch will have significantly higher total body water and blood volume. Pregnant bitches often appear slightly anemic (PVC 30-31%) and may have dilution of the albumin and globulin levels (*hypoalbuminemia, hypoglobulinemia*)

5.1.6 Nutrition

Pregnant dogs should be *fed a high quality puppy food throughout gestation*. If premium food is fed, no additional supplements are necessary and in fact, some may be harmful. The bitch should be fed her normal volume of food for the first 40 days and then should be allowed to feed free choice, monitoring body condition score and adjusting feed intake as necessary. One reason for this recommendation is the smaller gastric volume due to the amount of space occupied by the feti in the abdomen which requires smaller and more frequent meals at a time of high metabolic energy demands. Bitches will occasionally go off feed around three weeks of pregnancy. This cause is unknown and they typically will begin eating normally within a few days thereafter.

5.1.7 Drug Therapy

Great caution should be used when giving drugs to pregnant bitches, even topically, as they may have abortogenic or teratogenic effects. This also includes nutritional supplements. Bitches should not be vaccinated with adjuvented or modified live vaccines during pregnancy (this includes the common vaccines DA2PP and Rabies).

5.2 Pregnancy Diagnosis

Because bitches maintain their CL and have high progesterone during the entire long diestrous period, progesterone can NOT be used as a diagnostic test of pregnancy. Assessment of the physical changes of pregnancy is also unreliable as many dogs will go through an overt pseudopregnancy or “false pregnancy”.

5.2.1 Palpation

Abdominal palpation should be performed from 21 to 28 days after ovulation. After this point, the gestational sacs begin to elongate and the individual fetal units can no longer be felt. After approximately 50 days, the fetal bodies and movement can be felt through the abdomen. Accuracy is poor in a tense or obese animal.

5.2.2 Ultrasound

Can be performed easily after 21 days post ovulation. It is possible to evaluate earlier, however, remember that implantation is late in the dog (14-16 days) and so much earlier than 21 days is very difficult and often unreliable. Fetal heartbeats are visible after day 22 of gestation. Fetal heart rate and movement can be used to assess fetal viability. This is NOT an accurate method to count the number of puppies. This is the earliest and most accurate way to evaluate pregnancy in the bitch with the added advantage of accessing fetal viability. Can also be used to estimate gestational age.

5.2.3 Relaxin

Relaxin is a hormone made by the fetal placenta that can be measured in serum beginning around day 21 or gestation. Accuracy is good after 28 days of gestation. A commercial assay is available (Witness - Synbiotics).

5.2.4 Radiography

Less than 42 days from ovulation you can visualize an enlarged uterus but cannot differentiate pregnancy from uterine disease. You can first see calcification days 42 to 45 from ovulation (variable number of days from breeding). Late in gestation, radiography is the best predictor of fetal number, and can be used to estimate fetal age and to assess. for fetal death. Signs of fetal death include gas within or around fetus, collapse of the axial skeleton, overlap of cranial bones and/or failure of skeleton to calcify or fetus to grow; lack of signs of fetal death does not imply viability.

PREGNANCY EVENTS IN DAYS POST OVULATION

- Fertilization (2-5 days)
- Embryo attachment to uterus (14-16 days)
- Earliest that vesicles visible on ultrasound (17-21 days)
- Fetal swelling first palpable in uterus (20-22 days)
- **Fetal heartbeat visible on ultrasound (22-23 days)**
- Best time to palpate begins (21 days)
- **Relaxin test positive (28 days)** possible to get + at 21 days
- Uterine swelling visible on radiographs (28-30 days)
- Palpability of individual uterine swellings reduced (32-35 days)
- Calcification begins (42 days)
- **Earliest radiograph diagnosis of pregnancy (45 days)**
- Fetal pelvis visible on radiograph (51-55 days)
- Fetal teeth and toes visible on radiograph (57-61 days)
- **Whelping (62-64 days)**

5.2.5 Miscellaneous

Acute phase proteins (fibrinogen), measurement of serum prolactin after challenge with naloxone – these have been investigated but are not commercially available.

5.3 Physiology of Parturition

In bitches, the hormone progesterone, which is essential for the maintenance of pregnancy, is produced by the corpus luteum. Interestingly, progesterone levels do not differ significantly between pregnant and non-pregnant bitches. Progesterone levels peak between 15 and 80 ng/ml around the 20th to 30th day of gestation and gradually decrease thereafter. To maintain pregnancy, the progesterone level must remain above 2 ng/ml.

It is assumed that labor is triggered by the fetus. The maturation of the fetal adrenal glands leads to the release of cortisol, which subsequently increases ovarian estrogen secretion around days 45 to 60 of pregnancy. Estrogen promotes the expression of genes encoding proteins associated with myometrial contractions and increases the release of prostaglandins from the utero-placental complex, mainly from trophoblast cells. Prostaglandin E2 (PgE2), which has luteolytic properties, causes the involution of the corpus luteum and a decrease in circulating progesterone levels. As a result, progesterone levels fall rapidly from 4-10 ng/ml to around 2 ng/ml within 12 to 24 hours towards the end of pregnancy. It is assumed that this drop in progesterone triggers an increase in prolactin levels, which leads to lactation. Labor usually begins 24 to 48 hours after this sharp drop in progesterone levels.

Prostaglandin F2α is crucial for increasing the sensitivity of the myometrium to oxytocin, releasing calcium from intracellular stores to facilitate smooth muscle contractions and softening the cervix. Oxytocin, released from the posterior pituitary gland in response to increased cervical pressure, further stimulates myometrial contractions. This process begins when cervical pressure activates sensory pathways that signal the hypothalamus, resulting in neural impulses that trigger the posterior pituitary gland to release oxytocin, increasing uterine contractions.

5.3.1 Normal Parturition

* Stage I = **cervical dilation**. This occurs secondary to increased estradiol and prostaglandins, and decreased progesterone, uterine contractions and the pressure of the fetus at the cervix. *Contractions are*

not visible. The bitch is restless, pants and may vomit. Signs should increase in frequency and severity. The duration of this stage is variable but typically 6-12 hours.

* Stage II = **expulsion of fetuses**. The cervix should be fully dilated. The length of this stage is variable, 6 to 12 hours on average. Abdominal contractions are strong and coordinated. The chorioallantoic sac may be seen first, then the pup with or without the covering of the amniotic sac. Puppies may present cranially or caudally. You ordinarily see passage of a neonate every 30 to 60 minutes. You should see passing of the first pup within 4 hours of labor onset, and the bitch should deliver pups at least every 2 hours thereafter. The bitch should tear away the amniotic sac and lick the neonate to stimulate respiration.

* Stage III = **expulsion of placentas**. Placentas usually pass 5 to 15 minutes after each pup is born. During the birthing process, the bitch licks the placenta and start eating the foetal membranes, the remains of the placenta and also tears the umbilical cord, reducing the contamination of the nest and avoiding predators. The bitch may eat the placentas; they have no known physiological value, and may cause vomiting and diarrhea. If the placenta is retained within the uterus, it can predispose the bitch to metritis.

Devices are now commercially available to breeders for at home labor monitoring. Whlepwise™ (Veterinary Perinatal Specialties Inc., Wheat Ridge, CO) is a commercial company that offers the rental of fetal heart rate monitors and tocodynamometry (monitors to detect uterine contractions).

5.3.2 The Post Partum Period

Owners will occasionally request a “clean out shot” of oxytocin following completion of labor. It is typically unnecessary if the puppies are nursing adequately and there is appropriate milk let down. Nursing stimulates natural oxytocin release. However, an injection of oxytocin will not create any additional concerns, if requested. The most important issue is that the bitch and the puppies are evaluated immediately after whelping to confirm all puppies have been born, the bitch is healthy, there were no problems during whelping, and that there are no obvious congenital abnormalities in the neonates (eg. cleft palate).

5.3.2.1 Uterine involution

Normal uterine involution takes approximately 60 days and complete uterine healing requires a total of 120-150 days. Administration of oxytocin to promote uterine contraction post-partum is only necessary if the pups are stillborn or not nursing.

5.3.2.2 Lochia

Lochia - normal vaginal discharge present for up to 3 weeks after whelping. Lochia should be sero-mucoid and odorless, ranging in color from pale brown to pale green. Lochia may be slightly hemorrhagic.

5.3.2.3 Rectal temperature

A slight increase in rectal temperature (up to 39.2°C) is also normal during the first 1-3 days post whelping as long as the bitch is acting normally and the puppies are nursing well and are content.

5.4 Dystocia

Definition – Dystocia is defined as an inability to expel the fetus(es) from the uterus or birth canal

Dystocia comes from a Greek word and it means “dys” = difficult, “tokos” = birth

5.4.1 Risk Factors and Classification of Dystocia

Dystocia, or difficult labor, is more commonly seen in certain breeds of dogs and cats. Brachycephalic breeds, such as Bulldogs and Pugs in dogs, and Persians and British Shorthairs in cats, as well as dolichocephalic breeds like Siamese and Cornish Rex cats, are particularly prone to dystocia. This higher incidence is often due to congenitally narrowed birth canals and size mismatches between the mother and her offspring.

Other risk factors for dystocia include having a very small or very large litter, advanced maternal age, and underlying metabolic diseases that cause uterine inertia. Although there is a lack of studies specifically examining single-fetus pregnancies, it is hypothesized that the necessary hormonal signals to initiate labor may be insufficient in these cases, leading to delayed or absent labor signs and complications.

Dystocia can be categorized based on the presentation of the patient or the underlying cause. Unlike traditional methods derived from large animal studies, a new classification approach based on clinical presentation in small animals is proposed. The presentation of dystocia in patients can be classified into four main categories, while the underlying causes can be divided into maternal and fetal origins. Maternal

causes account for about 13% of dystocia cases, fetal causes for 37%, and small litter size for 17%. In approximately one-third of cases, the cause remains undetermined even after surgical intervention.

Most fetal causes of dystocia are due to malpresentation. This can include issues with how the fetus is positioned in the birth canal, such as anterior, posterior, or transverse presentation; the alignment of the fetal spine relative to the dam's pelvis; and the positioning of the fetus's limbs and head. Although up to 40% of canine fetuses are born in a posterior presentation, which is normal, complications arise from transverse presentations, anterior presentations with retained forelimbs, posterior presentations with retained hindlimbs, or neck malformations.

5.4.2 Causes

5.4.2.1 Maternal

*** Obstruction of passage**

- Pelvis - congenital or acquired
- Vagina - hypertrophy, neoplasia, prolapse, developmental
- Vulva - "infantile"

*** Abnormality of uterine function**

- Obese, debilitated, poorly exercised, too many pups, calcium and/or glucose depletion
- Primary inertia = no second stage of labor seen, requires C-section
- Secondary inertia = second stage starts but does not progress as uterine muscle fatigues. May be obstructive so be cautious with ecbolic agents.

*** Abnormality of pregnancy**

- Fetal fluid disorder
- Herniation or torsion of uterus

5.4.2.2 Fetal

*** Obstruction of passage**

- Relative oversize = birth canal too small
- Absolute oversize = pup too big, birth canal normal

*** Developmental abnormality**

- e.g. fetal monster, hydrocephalus, lymphedema, other malformations

*** Abnormal presentation, position, posture**

- Presentation = relation of long axis of pup to birth canal.

Cranial and caudal are normal.

- Position = relation of fetal vertebral column to birth canal
- Posture = disposition of head and limbs

Determination of dystocia

Guidelines for examination/intervention during whelping:

- Green discharge before the birth of the first pup
- 30 minutes of strong contractions with no pups delivered.
- 2-3 hours of weak and infrequent expulsive efforts failing to produce a pup.
- 4 of more hours between pups.
- Obvious problem (pup hanging out etc.)

Questions to ask breeder/owner:

- Obvious malpresentation?
- First stage > 12 hours, second stage weak and intermittent > 2 to 3 hours, second stage hard > 30 minutes, > 2 hours between pups?
- Abnormal vulvar discharge? Pus, frank hemorrhage, green fluid (denotes placental separation)
- Rectal temperature decline > 24 hours ago?
- Signs of labor not progressing?
- Systemic illness in bitch?
- Prolonged gestation?
- High risk pregnancy? (Previous pelvic trauma or dystocia)

Upon Presentation to the Veterinary Hospital:

Dystocia is a Medical Emergency and so, obtain as complete history as possible or have a nurse or assistant help in obtaining a history while the patient is assessed.

*History

- Previous disease or trauma
- Breeding dates
- History of previous whelping
- Pups and progress at this whelping
- Temperature drop data
- Treatment thus far

Fetal Heart Rate:

- > 180 bpm = normal
- 160 -180 bpm = mild to moderate fetal stress
- < 160 bpm = severe stress to fetus need immediate intervention in attempt to save puppy and consider emergency C-section.

* Physical examination

- General examination
- Abdominal palpation
- **Digital vaginal examination**
- Lateral abdominal radiographs
- Uterine monitors and/or ultrasonography to access fetal viability

* Diagnostics should consist of blood biochemistry and hematology, blood gas (including ionized calcium), and electrolytes. Assessing serum progesterone is warranted if whelping date has been exceeded and the female fails to demonstrate signs of active labour. Radiography is warranted to confirm pregnancy, ensure parturition has finished, or to assess for rare abnormalities such as gas in the uterus, or a fetus in transverse presentation.

Sonography is the modality of choice for dystocia. Ultrasound can diagnose fetal distress via assessment of fetal heart rate. Normal fetal heart rate should be in the range of 200 – 220 beats per minute. Fetal bradycardia is a sign of fetal distress and warrants intervention. If fetal heart rate is below 180 beats per minute, intervention may be indicated.

*** Veterinary intervention is necessary if any of the following conditions are present:**

- An obstruction is detected through a vaginal exam, radiography, or sonography.
- The bitch or queen has not initiated labor, and progesterone levels are below 2 ng/mL.
- The bitch or queen shows signs of systemic illness.
- The fetal heart rate is bradycardic, defined as below 160 to 180 beats per minute, at term.
- There is suspicion of uterine rupture or torsion.

Stabilize the bitch first!

If she is not doing well, the puppies are in grave danger as well.

Medical Management of Dystocia

The medical management of dystocia is a topic of debate, primarily due to the mixed outcomes associated with the use of ecbolics like oxytocin, which are often contraindicated and can lead to poor results. In bitches, medical management has a success rate of around 30%, and identifying suitable candidates for this approach is challenging (see Box 3).

When opting for medical management, it is crucial to address any electrolyte or acid-base imbalances before administering calcium or glucose supplements. If low ionized calcium levels are detected, 10% calcium gluconate can be administered intravenously at a dose of 0.2 ml/kg. This should be given as a constant rate infusion over several minutes while monitoring heart rate and rhythm via ECG and thoracic auscultation.

Blood glucose deficits should be corrected using a solution of 0.25 to 0.5 g/kg glucose diluted 1:2 with 0.9% NaCl, administered over 10 minutes as a constant rate infusion. If the bitch meets the criteria for medical management, oxytocin can be given at a dose of 0.5 to 2 IU subcutaneously or intramuscularly. Oxytocin should be used alongside calcium gluconate, as it induces smooth muscle contractions while calcium gluconate enhances the strength of these contractions. However, the repeated use of oxytocin is controversial, and if medical management does not induce parturition, surgical intervention becomes necessary.

Treatment of Dystocia

1. Manipulative Methods

- Feathering
- Digital manipulation
- Use of instruments

2. Pharmacologic Agents

- Oxytocin: An ecbolic that stimulates uterine contractions. It should not be used in cases of obstructive dystocia or if the cervix is not open. The recommended dose is 2 to 5 IU at intervals of 20 to 30 minutes, not exceeding 3 to 4 doses if ineffective.

Weight in kilograms (kg)	Oxytocin dose (IU)
< 5kg	0.25 IU
5-10 kg	0.5 -1 IU
10-30kg	1-3 IU
> 30kg	3-5 IU

If there is no response to the initial oxytocin injection, progressively higher doses may be used, with an **upper limit of 5 IU**.

DO NOT USE OXYTOCIN WITHOUT FIRST CONFIRMING THERE IS NO OBSTRUCTION!

If given with obstructive dystocia, uterine tetany, premature placental separation with resultant fetal hypoxia and death, and uterine rupture can occur.

* **Calcium** – Bitches may be uterine tissue depleted of calcium even if blood ionized calcium levels are not low. Consider administration of calcium (see Eclampsia).

* **Glucose** –Bitches often stop eating prior to whelping. Monitoring Blood glucose and considering supplementation, especially if intravenous fluids are being administered may give additional energy during delivery.

In summary, while medical management can be an option for treating dystocia, it requires careful candidate selection and monitoring. If medical methods fail, prompt surgical intervention is essential to ensure the safety of the mother and her offspring.

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6 CESAREAN SECTION – TIPS AND TRICKS

Tanja Plavec

The period of parturition and immediately after birth, is of the most delicate times for owners, especially if things do not go according to plan. Dystocia or difficult parturition is rare, occurring in around 5-6% of all pregnancies in bitches (Pretzer, 2008; Johnson, 2009), or just 2% if you exclude the most at-risk breeds where the incidence is highest (Bergström et al., 2006). In cats dystocia occurs in 5.8% of litters and range from 0.4% (large colony, mixed breeding) to 18.2 in Devon rex cats (Gunn-Moore and Thrusfield, 1995). Dystocia is also more common in older animals.

In this lecture we will look at the three pillars for a safe and effective cesarean section, i.e. correct planning, rapid execution and effective immediate care of the puppie and their assessment.

6.1 Type of caesarean section: elective vs. emergency CS

6.1.1 Emergency CS

Cesarean section (CS) is indicated in cases of dystocia (emergency CS), which may be caused by inadequate or insufficient uterine contractions that cannot be augmented by medication, inadequate positioning of the puppies in the birth canal, dead puppies, structural changes in the birth canal, and the detection of fetal stress on ultrasound (Smith, 2007). In approximately 60-80% of dystocia cases in bitches and queens surgical intervention is required. The determination if surgery is necessary is based primarily on the condition of the dam, progression of labor and fetal heart rate (Traas, 2008).

6.1.2 Elective CS

Elective (or planned) CS is recommended in bitches with a history of dystocia and emergency caesarean section, in bitches with fewer than three fetuses and in breeds where the fetuses have large heads or the mother's pelvis is not wide enough (Boxers, Bulldogs, Pugs, Boston Terriers, Scottish Terriers) (Bergström et al., 2006; Smith, 2007). Some specialists also recommend a prophylactic caesarean section for bitches with more than eight fetuses, as in these cases there is a high risk of complete unresponsiveness of the uterus. This avoids an emergency CS, increases the likelihood of survival of the puppies and allows the bitch to recover more quickly (Smith, 2007; Cain and Davidson, 2023).

Due to the increased incidence of dystocia in certain breeds, breeders are increasingly opting for elective CR. This avoids the exhaustion of the bitch from vaginal delivery, resulting in an easier and quicker recovery from the procedure and also increases the likelihood of puppy survival. As breeders are increasingly interested in elective CS, it is essential to know the pros and cons of elective or emergency CS. Although CS is a safe procedure, it can be associated with a number of complications. The most common of these are severe bleeding, infection and reduced survival rate for mother and puppies. Also, endometritis is slightly more common in bitches with emergency CS (Smith, 2007).

6.2 Timing of the cesarean section

6.2.1 Emergency CS

In dystocia the foremost indication for CS is a reduction in fetal heart rate (HR), indicating fetal stress. While fetal HR between 150 and 170 beats/minute indicates moderate to severe fetal stress, a HR of less than 150 beats/minute is considered an emergency and CS should be performed as soon as possible. Beware that a brief reduction in HR may occur due to passage of uterine contraction over a fetus.

Therefore, any fetus with a low HR should be monitored for 30-60 seconds or reassessed 1-2 minutes later (Smith, 2007).

6.2.2 Elective CS

In an ideal situation, the clinician will know the date of the LH surge as a result of progesterone concentrations measured during the breeding management process. Since the interval between ovulation and whelping is relatively consistent at 63 ± 1 days, planned CS can be safely performed after day 63 after the LH surge. There are some breed-specific variations for gestational length, including Cavalier King Charles Spaniels expected due date earlier at 62 ± 2 and Greyhounds later at 68 ± 1.5 days (both measurements are days from LH peak), this needs to be considered when planning the elective CS (Cain and Davidson, 2023). Further, CS can be scheduled when the progesterone concentration reaches 2 ng/mL at the end of the gravidity. Clients may be instructed to measure and record rectal temperatures two or three times daily and to request the CS when the rectal temperature is 37.2°C or lower (Smith, 2007). The temperature usually drops by 1.1 - 1.7°C from 6 - 18 hours before birth, and can drop to 35°C in small breeds, to 36°C in medium breeds and to 37°C in large breeds. A drop in body temperature is not necessary, it can be missed, and it is often the case that owners do not know how to measure body temperature properly, so it is essential to educate them on the correct way to take the temperature of a bitch (Johnson, 2009).

Late-term radiography is the best modality for accurate assessment of litter size and size of the fetuses. Radiography may also reveal an emphysematous fetus or fetal skeletal or skull

collapse, consistent with fetal death (Smith, 2007). Ultrasonographic measurements of inner chorionic cavity and the biparietal diameter offer additional value when assessing prepartum fetuses (Lopate, 2008; Beccaglia et al., 2016). Based on the size of the fetus, the size of its head, the appearance of particular structures on certain organs, we can estimate the time of parturition in bitches of small and medium-sized breeds quite accurately (Beccaglia and Luvoni, 2012), similar evaluation in large and giant breeds is more difficult (Münnich and Küchenmeister, 2008; Groppetti et al., 2015).

6.2.3 The effect of timing and type of parturition on survival

The duration of parturition has a demonstrable effect on the probability of survival of the offspring. Pup mortality is lower (5.8%) if stage II lasts 1-4.5 hours and higher if stage II lasts between 5-24 hours (13.7%), but the probability of pups dying is greater if stage II lasts longer than 24 hours. In this case, the prognosis is also worse for the female. The prognosis is better if the female is healthy and the fetal HR is normal (>180 beats/minute). The puppies are less likely to survive if the fetal heart rate is below 160 beats/minute. In this case, an emergency cesarean section should be performed immediately and the pups removed from the uterus as soon as possible (Smith, 2007).

In one study, all puppies from elective CS survived, whereas newborn death rate ranged from 3% to 20% in litters born from both natural whelping or emergency CS (Cain and Davidson, 2023). However in another study, where natural parturition, emergency and elective CS were compared, the type of the parturition had no impact on puppies' survival or weight gain (Plavec et al., 2022).

In emergency CS mortality was associated with puppy in the pelvic canal, duration of anesthesia > 80 minutes and dam's age of more than eight years. If the time from induction to start of surgery was longer than 30 minutes, this might have been associated with increased mortality (Schmidt et al., 2021).

6.3 Performing the procedure

Preoperatively, hematocrit, total protein, serum calcium and glucose are checked and fluid therapy is administered. Before anaesthesia is induced, the animal is preoxygenated and the surgical field is clipped and prepared (Pretzer, 2008; Traas, 2008).

After induction of anaesthesia, the final surgical preparation is made and laparotomy is performed. The incision should be long enough (from the halfway between the xiphoid and umbilicus to the pubic bone), otherwise it will be difficult to further expose the uterine horns. When multiple fetuses are present, the linea alba is often very thin and in contact with the uterine wall, so care must be taken not to inadvertently incise the uterine wall when performing the abdominal incision. Before the uterine incision is made, the surgical field is protected with moistened abdominal compresses to prevent uterine content spillage into the abdominal cavity. The uterine incision is usually made ventrally on the body of the uterus, the fetuses are removed (eviscerated) through this single incision, starting with the most caudal fetus. They are then usually removed alternately from the left and right horn. When the fetuses appear in the hysterotomy wound, they are pulled out with their placenta by slow traction, freed from the amniotic sac, and the

umbilical vessels are clamped and disconnected approximately 2-6 cm from the abdominal wall. The neonate is immediately handed over to an assistant to start supportive care. If the placenta is still firmly attached to the uterus, the surgeon releases the puppy or kitten from the amniotic sac, clamps and cuts the umbilical cord and hands over the puppy or kitten. The surgeon continues with the gentle traction to retained placenta to remove it before closing the hysterotomy site. If removal is impossible without excessive hemorrhage and trauma, the placentas are left to pass naturally (Traas, 2008; Fransson, 2018).

After completion, the uterus is sutured in one or two layers (appositional inner layer and inverting outer layer) of continuous suture with an absorbable monofilament suture material size 3/0 or 4/0 and the submucosa included in the suture line. The abdominal cavity is lavaged with up to 200 ml/kg saline solution at body temperature, aspirated and the surgical handgloves and instruments are replaced. The abdomen is closed routinely and skin closure is best performed with buried intradermal suture (Fransson, 2018).

If the owners decide to spay the bitch at the same time, this can be done after removal of the puppies as in a CS (but without closing the hysterotomy) and then completing the ovariohysterectomy (OVH) in a standard way. The other possibility is a so-called "en bloc" resection. In the latter case, the ovaries and uterus are removed together with the fetuses, which must be removed from the uterus within 60 seconds of the uterine blood vessels being severed. This shortens the duration of anaesthesia and reduces possible contamination of the abdominal cavity. The abdominal cavity is routinely closed (Traas, 2008; Fransson, 2018).

An older study reported that en block ovariohysterectomy (a technique that involves ovariohysterectomy before hysterotomy and removal of the neonates) resulted in similar neonatal survival when compared to the previous studies of medical and surgical management of dystocia in dogs and cats. The neonates were extracted in 30-60 seconds (mean 40) after clamping the uterus. Further, none of the owners reported the dams having lack of mil kor being poor mothers (Robbins and Mullen, 1994).

A newer study compared mortality, intra- and postoperative complications and decreased mothering ability in bitches undergoing CS and CS with OVH (96% of OVHs were performed after the hysterotomy and retrieval of all puppies) and there were no differences in anesthesia duration, intraoperative complications, postoperative complications, mothering ability, puppy survival to weaning, or other variables compared between groups. However, CS+OVH bitches had longer surgery times, longer time from delivery to nursing and were more frequently perceived as painful postoperatively. The average time from induction to delivery of the last puppy was 23.0 ± 10.0 minutes (Guest et al., 2023).

6.4 Immediate postoperative care of the neonates

All the necessary materials must be prepared in advance. Neonates that move and vocalise when they are born usually do not need much help. But when they are stressed, it is the quick and appropriate help that can change the outcome. Ideally, we should have 1 assistant for every 1 to 2 puppies/kittens at most. We need to have clean towels ready to wipe and rub the puppies to stimulate their respiratory system. We also need to have heating pads, oxygen and appropriate aspirators ready to clean the airways. Medication that may be needed during resuscitation should be within reach. When opioids were used in a dam during CS, it is essential to have naloxone ready (Greer, 2015).

If the puppy (or kitten) is not breathing independently after a minute of rubbing, more intensive help is needed. How intensive we need to be can be determined by the Apgar score, which is used to determine the vitality of puppies (Lee, 2003). The Apgar score was developed by the physician and anaesthetist Virginia Apgar to assess the health of newborn babies and, as a result, to quickly identify those newborns who need extra help after birth (Apgar, 1953). Although the assessment was named after its inventor, it is now an acronym, A for appearance, P for pulse, G for grimace, A for activity and R for respiration (Apgar and James, 1962).

6.5 Conclusion

For most owners CS is a very emotional event. Therefore, it is important for us to take a realistic and knowledgeable (as well as empathetic) approach. If we plan to perform an elective CS, we should do it at the optimal time. If we are dealing with dystocia, we use ultrasound and other diagnostic toopls to assess the viability of the fetuses and if the fetuses are stressed, a CS should be performed immediately. Prolongation is harmful to both the puppies or kittens and the mother. After CS, we assess the viability of the neonate and provide them with the necessary support.

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7 NEONATAL DEVELOPMENT IN PUPPIES AND KITTENS

Margret L. Casal, Maja Zakošek Pipan

In puppies and kittens, the first two to three weeks of life (neonatal period) are characterized by complete dependence on the mother because of incomplete neurological functions such as audio and visual abilities and proper spinal reflexes. During this time, a vast number of developmental processes occur that result in physiological differences when compared to adult dogs and cats. Here we highlight the most important differences during the first two to three weeks of life based on published scientific studies.

7.1 Body Temperature

7.1.1 Temperature Regulation

Right at birth, neonates have their mother's body temperature, but they are born with only brown fat and no white insulating fat. Neonates cannot regulate their body temperature at birth and will have a lower body temperature than a normal adult. Therefore, they are dependent on their mother's heat or an outside source to keep them warm. Food intake will increase metabolism, which will contribute to the ability to generate heat. Lastly, shivering reflexes are not present at birth and don't develop until after two weeks of life. Normal adult body temperature ranges should be present by 6 weeks of age (Figure 1).

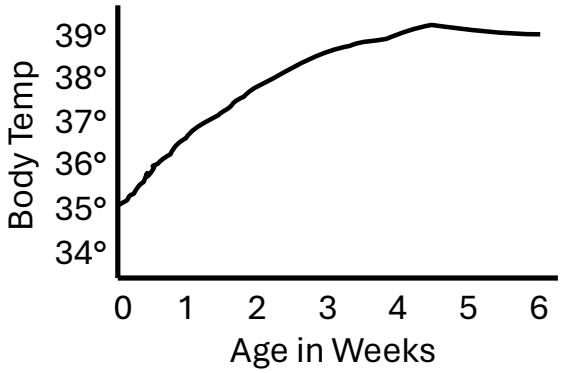

Figure 1: Increase in body temperature in neonatal puppies and kittens. Author: Margaret L. Casal

7.2 Integument, Bones and Muscles

7.2.1 Skin

At birth the skin is thin and somewhat fragile, resulting in an incomplete barrier function. At birth the hair coat should be complete except for some fine hair on the abdominal skin. By one week of age, the hair will have covered the abdominal skin. Within a few hours after birth, the umbilicus will have dried and fallen off by three days of life. The abdominal wall should be intact and there should be no bulging indicating a hernia or an infection. The eyes are closed at birth and should open by about ten days of age at the lid suture line. In some cat breeds, the eyes may open earlier but if they open shortly after birth, there may not be enough tear production to provide proper lubrication. Eyes of shorthaired breeds tend to open before 10 days and those of longhaired breeds after ten days. The ear canals are also closed at birth and open by two weeks of age.

7.2.2 Muscles

From birth until early adulthood the color of the muscle changes from pale tan red to a much deeper red, which is mainly due to the physiologic anemia that is present in neonates and then normalizes as the neonate ages. Parallel to the development of fetal muscle fibers to adult fibers, mobility develops. By 14 days of life, puppies and kittens should be able to crawl and within very few days thereafter (days 15 – 20) they will begin walking.

7.2.3 Skeleton

During the first two weeks of life, calcium concentrations in bone decrease and do not increase towards adult levels after three weeks of life irrespective of milk intake, the main source of calcium during the neonatal period. The epiphyses of the long bones increase dramatically during the first weeks of life and appear to do so as a percentage of the body weight. While both the size of bone and epiphyses will increase during this time, the epiphyses will grow at an accelerated rate when compared to bone itself. During pediatric development, osteoclasts and osteoblasts break down and build bone at an equilibrium that promotes bone growth. Calcium is resorbed during this process and reincorporated within the bone, which is made possible by bone alkaline phosphatase. These developmental processes explain the mild increases in serum alkaline phosphatase, calcium, and phosphorus seen in pediatric patients. Some centers of ossification are already present at birth such as in the diaphyses of the long bones, while many others won't be present until after 4-8 weeks of age. In general, closure of the growth plates begins in the front and gradually moves towards the hind limbs during postnatal development.

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7.3 Immune System

7.3.1 Thymus

At birth the thymus is in the anterior mediastinum where it takes up a large portion of the cranioventral thorax. A healthy thymus will be whitish grey in color and can be seen on radiographs. Between one week before and two weeks after birth the thymus weighs between 0.35 – 0.45% of the kitten's body weight (Casal ML, unpublished data). By 23 days it will have reached its highest thymus to body weight ratio. At birth, the thymus is not fully functional and will continue to develop for at least two to three more months. As the neonate grows, the thymus will regress until it is replaced by mostly fat tissue in young adulthood. The structure of the lymph nodes is normal at birth, but few lymphocytes are present. However, the lymphocytes increase in number during the first months of life. At birth the lymph nodes should be palpable. In general, though, facial nodes will be easier to palpate in neonates because of their immediate contact with pathogens and thus increased reactivity.

7.3.2 Cellular Immunity

IgM plasma cells have been demonstrated in bone marrow of 1-week-old kittens and puppies. However, IgG plasma cells were not present until adulthood in feline bone marrow. Secretory B cells have been observed in kitten and puppy spleens as early as 42 days of fetal life, but adult numbers of cells are not present in the spleen until well after the neonatal stage. The presence of IgM has been reported at birth in serum and bile suggesting that kittens and puppies are able to produce IgM on their own. However, increases in IgA are much more dramatic than in serum during early life suggesting that the secretory system develops much more rapidly in kittens and somewhat slower in puppies. Interestingly, many mucosal tissues show differential expression of immunoglobulin secreting plasma cells. Studies in dogs have shown inadequate neutrophil (phagocytic) responses because of incomplete development of the complement system. This may be true for the kitten as well. Therefore, puppies and kittens are born with an overall immature immune system and rely on the transfer of colostrum for protection against pathogens early in life.

7.3.3 Immunoglobulins

Virtually no antibodies are transferred in utero to canine and feline fetuses, and they are born immunologically immature. Therefore, neonates are dependent on colostral antibodies (passive transfer of immunity) for postnatal protection against infectious diseases. The colostral antibodies are taken up into the circulation from the intestinal lumen without being metabolized both by non-selective transport mechanisms and specific receptors. Changes in intestinal pH, motility and receptor expression within the first 24-36 hours of life do not allow for further uptake of immunoglobulins. In kittens and puppies, several studies have demonstrated that the majority of uptake of IgG and IgA occurs within the first 16-24 hours of life. Thereafter, the colostrally derived immunoglobulin serum concentrations decrease exponentially with a nadir around 8 days for IgA and 20-25 days for IgG in both kittens (Figure 2) and puppies. If there is no uptake, the risk for infectious diseases is greatest for neonates during the first four weeks of life before they begin to consistently make their own antibodies. While there is IgM in colostrum, it cannot be absorbed through the intestines because of the large molecular size. However, IgM can be present in precolostral kittens and puppies, and it has been shown to be produced shortly before birth in canine fetuses infected with distemper.

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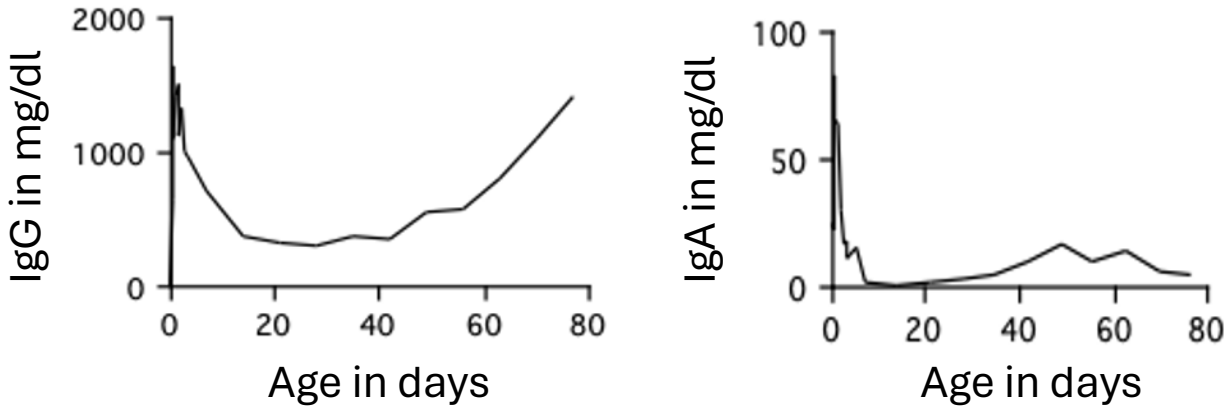

Figure 2. Mean serum IgG and IgA concentrations in kittens during the first 11 weeks of life. Author: Margaret L. Casal

7.4 Heart and Hematopoietic System

7.4.1 Heart and Heart Rates

Before birth, fetal pO2 is only 20 mm Hg. During birth, the placenta separates from the uterus cutting the maternal oxygen supply off. This results in hypoxia and induces gasping respiratory reflexes. The pO2 now rises to 50 – 60 mm Hg (Figure 3). By increasing the oxygen tension, the pulmonary vessels begin to dilate and the ductus arteriosus begins to narrow completely closing by 1-2 days. The increase in left sided pressure results in closure of the foramen ovale between both atria. The pO2 rises even more by day

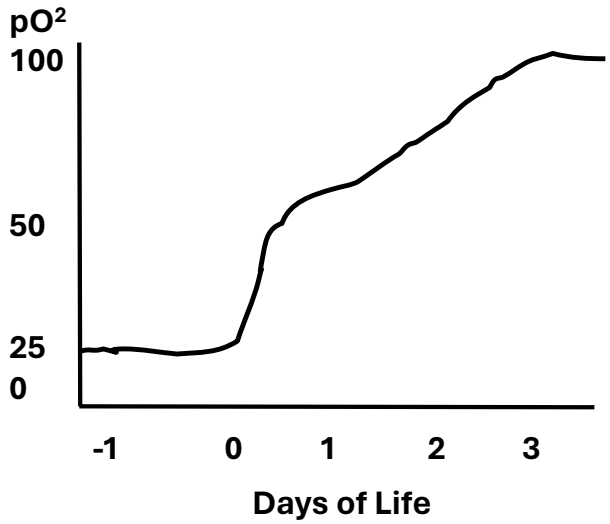

Figure 3: Increasing pO^2 in neonatal puppies. -1 days of life represents the fetal age.

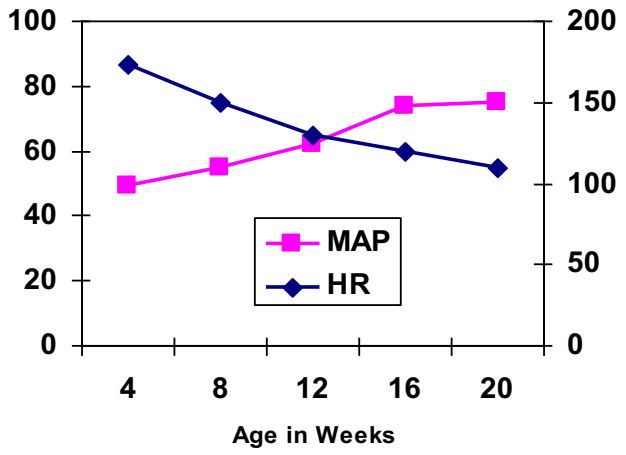

Figure 4: Heart rates (HR) and mean arterial pressures (MAP) in growing puppies.

2, correcting acidosis that develops in the neonate. At birth autonomic innervation of the heart and vasculature is incomplete, the myocardial cells are still dividing, and myocardial contractility is decreased. Thus, the ability to react to circulatory distress caused by acid-base shifts, blood loss and hyperthermia is decreased. Neonates may respond to hypoxia with bradycardia or no change in heart rate. The bradycardia is thought to reduce oxygen demand and thus be a protective mechanism. However, by 4 days of age, puppies and kittens have normal blood baroreceptor functions, and their heart rates will respond to hypoxia as in adults. At birth, the right and left ventricles have approximately the same mass changing to an ultimate adult ratio of 1:2 to 1:3 throughout puberty and change in the cardiac axis and shape. During this time, the canine heart changes from being ellipsoid at birth to more globoid in adulthood. At one month of age, puppies and kittens still have lower blood pressures, stroke volumes, and resistance in the peripheral vasculature than adults, but they have higher heart rates, cardiac outputs and central venous pressure. Overall heart rates right at birth range between 160 and 200 beats per minutes (bpm) in puppies and higher in kittens at 200 – 220 bpm. Heart rates may rise a bit during the first week of life (200-240 bpm in puppies and 220-275 bpm in kittens and then begin to decrease slowly as the mean arterial pressure rises.

7.4.2 Mean Arterial Pressures

Arterial blood pressure in kittens 17-21 days of age is much lower than adult cats (48.2+/-3.3 to 50.1+/-6.1 versus 95.9+/-2.5 to 114.9+/-14.1mmHg). In puppies, similar findings were noted: at 3-4 weeks of age, mean arterial pressures were around 50 mmHg versus 75-90 mmHg in our dogs (Casal, unpublished data; Figure 4).

7.4.3 Hematopoiesis

At birth the hematocrit of the newborn kitten or puppy is similar to that of the adult, because although the number of red blood cells is smaller, the fetal cells are larger. During the following weeks, the hematocrit is lower than in adults (physiologic anemia of newborns) due to decreased red blood cell production, the shortened lifespan of neonatal red blood cells, and hemodilution by an expanding blood volume. The neonates are born with fetal hemoglobin that switches over to adult hemoglobin, which results in a drop of red blood cells and hematocrit during the first weeks of life. This in turn results in a reticulocytosis. Nucleated red blood cells are also present during the first weeks of life. Hematopoiesis, particularly of megakaryocytes, is evident histologically in the liver and spleen for several weeks after birth (Casal, unpublished data; Figure 5).

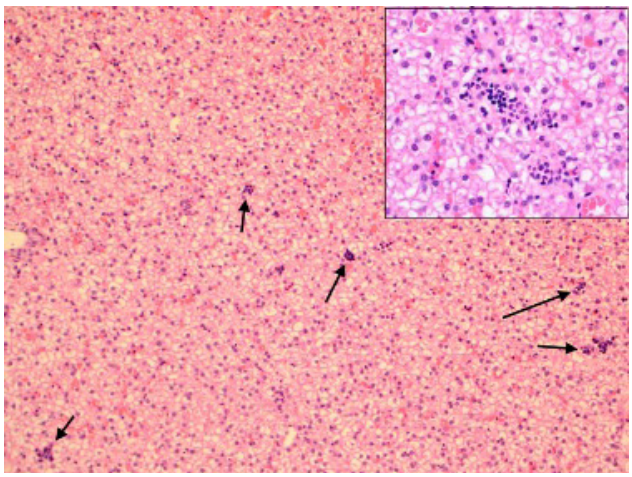

Figure 5: Microscopic image of a liver from a 2-week-old kitten. The arrows and the inset show extramedullary hematopoiesis. Author: Margaret L. Casal

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7.5 Pulmonary System

7.5.1 Lungs

Newborn kittens and puppies have an immature pulmonary system with a compliant chest wall similar to preterm humans. As such, their responses to hypoxia differ greatly from those in older kittens. Kittens and puppies less than 10 days of age have a biphasic response to hypoxia. Initially, there is a brief increase in ventilation followed by a short apnea and then a decrease in ventilation that is primarily due to a decrease in tidal volume. Interestingly, in kittens of all ages, electromyographic responses measured in the diaphragm were increased in response to hypoxia but respiratory rate increased only mildly in kittens less than ten days of age while the increase was more dramatic in older kittens. These findings may explain the irregular breathing patterns seen in newborn kittens and puppies and indicate that the responses are not centrally regulated. Unlike dogs, cats have vagal non-adrenergic noncholinergic inhibitory innervation. Vagal cholinergic innervation is completely functional at birth and vasovagal reflexes are present. Therefore, kittens and puppies should not be gripped tightly by the chest as they may become apneic and lose consciousness.

7.5.2 Effects of ambient temperature on respiration

A drop in ambient temperature results in increase in tidal volume, increase in CO_2 production, and increase in minute ventilation in neonates. Overall, this results in an increase in the metabolic rate. There is no effect on arterial CO_2 pressure. However, if neonates become hypoxic while at a lower than optimal ambient temperatures, tidal volume decreases, minute ventilation decreases, respiratory rate increases, gaseous metabolism decreases (O_2 consumption and CO_2 production), and the arterial partial pressure of CO_2 decreases. While hypoxia significantly reduced the metabolic rate in neonatal kittens at an ambient temperature of 30°C, the rate was reduced by twice that at 20°C ambient temperature.

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7.6 Kidney and Liver

7.6.1 Kidney

Nephrogenesis not complete at birth in dogs and cats until at least 3 weeks of life. Nephron development is centrifugal, begins in the juxtmedullary region and moves out towards cortex. Once umbilical cord is severed, renal blood flow increases dramatically. Renal blood flow development then depends on cardiac

output and fraction that perfuses kidneys which depends on renal vascular resistance. Renal blood flow triples from one day to one month of age. Autoregulation of renal blood flow in response to acute changes in systemic arterial pressure is not as efficient as it is in adults. Creatinine clearance is dependent on glomerular filtration alone and is directly proportional to lean muscle mass. Neonatal puppies and kittens lack lean muscle and, thus, creatinine concentrations are below normal adult levels. Fractional excretion of electrolytes varies greatly but dependent on rate of filtration, reabsorption and secretion. Renal plasma flow changes little because kidney grows at the same rate, but glomerular filtration rate increases markedly between 18 hours and 40 days. Tubular functions are incomplete at birth resulting in increased amino aciduria and glucosuria during the first 2-3 weeks of life. Neonates are unable to dilute distal nephron fluid, and this limits the ability to generate hypotonic urine and accumulate solute in the medulla. This limits the degree of concentration of urine. Between 2- and 77-days urine-to-plasma osmolality ratio increases 37-fold and leads to increases in urea retention, more efficient sodium retention and the decent of Henle's loops into the medulla. Up to 3 weeks of life, neonates are only able to excrete 10% of isotonic salt solution versus adults at 50%. All this leads to higher fluid requirements in neonatal puppies and kittens. Anatomically, the cortex is thinner in neonates than in adults and fetal glomeruli are still present (Figure 6).

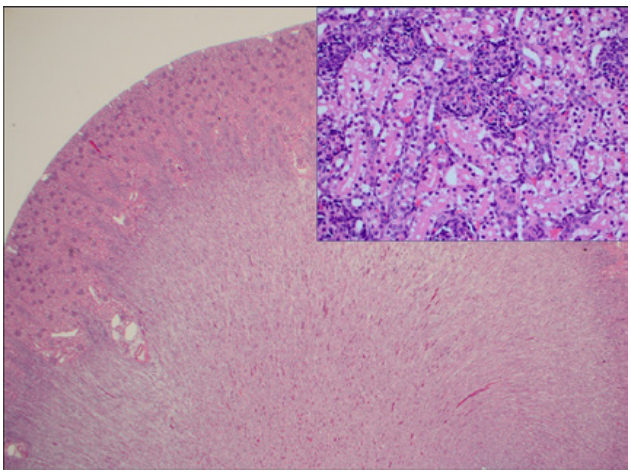

Figure 6: Microscopic image of a kidney from a two-week old kitten. Inset shows fetal glomeruli. Author: Margaret L. Casal

7.6.2 Liver

The development of hepatic functions has not been studied in detail in kittens and limited data are available. Studies in many other animal species have shown that hepatic enzyme function and metabolic capabilities are reduced well below adult levels for the first few weeks of life. Some enzymes are not functional in these species until almost six months of age. The same may be true for the cat. Studies in neonatal puppies showed that in vitro activities of glucose-6-phosphatase (G6P), UDP-glucuronyl transferase (GT) and P-450 were incomplete at birth and continue to develop over the next 4-8 weeks of life. This study also demonstrated that the water content of the neonatal liver was significantly higher and protein content lower at birth when compared to adults. Glycogen stores are limited in both neonatal puppies and kittens. Gluconeogenesis and their ability to mobilize glucose is also limited at birth, explaining the high susceptibility to hypoglycemia.

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7.7 Endocrine and Reproductive System

7.7.1 Cortisol and ACTH

Stress responses are incomplete early during development because high levels of adrenal hormones (mainly corticosteroids) would inhibit development of the nervous system as was shown in an early study in which neonatal kittens were administered corticosteroids. However, puppies and kittens can respond somewhat to stressors. Normally, corticotropin-releasing hormone is produced in the hypothalamus, acts on the pituitary to produce ACTH, which in turn acts on the adrenals to secrete cortisol. From the

time of birth, this is a well-regulated system with positive and negative feedback. However, there are differences that are note-worthy. In a study done in dogs, lower serum concentrations of cortisol were demonstrated in 1 – 6-week-old puppies (0.57±0.04 µg/dl) than in the 6 –12-week (1.04±0.06 µg/dl) and the 3 – 6-month (0.92±0.05 µg/dl) age group. Interestingly, breed related differences were noted in serum cortisol concentrations in adult dogs but the study did not include breed differences in puppies. While juvenile-onset hyperadrenocorticism due to adrenal hyperplasia or pituitary abnormalities may occur, it is most commonly caused by inappropriate use of steroids. Even topical steroids can be absorbed through the skin or through the conjunctiva. An ACTH stimulation test reveals a subnormal response, while exaggerated responses are noted in puppies with hyperplastic adrenals.

7.7.2 Thyroid

Serum thyroid hormones differ between puppies and kittens and their adult counterparts and differ significantly with time during the first 12 weeks of life. Therefore, it is critical to know the exact age of puppies or kittens and not to use the standard reference range for adult normal dogs or cats, respectively. In **puppies**, mean T_4 concentrations can increase to twice the adult normal upper limit during the first 4 weeks of life and then decrease gradually into the adult normal range by 7 weeks of life. T_3 concentrations are than adult normal values at birth from which point on they increase to the lower limit of the adult normal values between the 3rd and 4th week of life. In **kittens**, mean total T_4 concentrations are in the upper adult normal range and higher for the first 12 weeks of life. Thereafter, they are in the adult normal range. However, mean T_3 concentrations at birth are about a third of adult normal concentrations slowly increasing into the normal range by 4 to 5 weeks of age. There was no difference between males and females in either puppies or kittens.

7.7.3 Sex hormones and genitals

At birth, the sex of kittens can be determined by the anogenital distance, which is shorter in females (7.6±1 mm) than in males (12.9±1.5 mm)4. The genital opening in the male kitten is pinpoint, while the opening in the female is droplet shaped. Male kittens are born with descended testicles which are able to move freely in and out of the scrotum until five to seven months of age. Interestingly, serum AMH levels are beyond the assay's upper limit of detection in male kittens but in the low normal range for neonatal female kittens. Low levels of serum estradiol can be detected in male and female puppies during the first weeks of life, but it appears to be acquired through ingestion of their mothers' milk. In testes from male puppies 8-weeks-of-age, FSH and AMH expressions were quite high and decreased over time to maturity. There is no information on neonatal expression to date.

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7.8 Nervous System including Eyes and Ears

7.8.1 Eyes and ears

Kittens and puppies begin to open their eyes at about 10 days of age, although some breeds of cats (e. g. Abyssinians) may open their eyes as early as five days of age or be born with them open; the external ear canals open at around two weeks of age.

7.8.2 Brain and reflexes

The blood brain barrier is open in puppies and kittens during the first two weeks of life, which has implications for some drugs. The brain is softer than that of the adult because of the increased water content; neuronal development, migration, and myelination continue after birth. Sulci are incomplete at birth in puppies, and a full set should be present by two weeks of age. At birth, most neurological reflexes are not present yet. However, the righting, suckling, and rooting reflex are present for the first two weeks of life in normal puppies and kittens. For the righting reflex, the neonate is placed in dorsal recumbency and when let go, will flip over on its abdomen. The suckling reflex is tested by offering a finger or bottle nipple, whereby the normal puppy or kitten will readily suckle on the offering. The rooting reflex is tested by cupping a hand in front of the neonate's face. The neonate should press its muzzle into the hand as if searching for the mammary gland to suckle.

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7.9 Alimentary system and birth weight

7.9.1 Birthweight

Typical birthweights in kittens are 82-160g (mean 117 +/- 20 SD) and weight gain is about 10.4 +/- 1g per day. As a rule of thumb, kittens are born with about 100 grams, and they should gain about 100 grams a week. Thus, age can be estimated in an abandoned kitten by subtracting 100 grams from the current weight and dividing the result by 100, which results in an approximation of the kitten's age in weeks. Birthweight and estimation of weight at any given age is much more difficult in puppies, as the birth size variations are breed dependent. Birthweights range between as little as 120 grams or as much as 1.4 kilograms. However, despite these differences, as a rule of thumb, birthweights should be doubled by 10 days of age. Also, on average, puppies should gain about 5-10% over the weight the day before. For example, if a puppy is born at 200 grams, it should weigh between 210 and 220 grams the next day, although it is not uncommon for puppies and kittens to not gain weight the first day after birth.

7.9.2 Alimentary system

The neonate is born with a sterile gastrointestinal system, which will develop its own flora to aide in digestion during the first few days of life. Also, the gastrointestinal peristalsis is weaker (slower), there is lower intestinal blood flow, and the gastric is pH higher right at birth to aide in the uptake of colostrum. After the first 24-36 hours of life, the gastric pH decreases in order to digest milk. The fundus of the stomach is designed for storage in neonates, while the antrum for trituration of solid foods. Antrum responses not developed in one week old kittens. Fecal microbiomes were examined in neonatal puppies and compared to older puppies. The results showed that the bacterial species increased by 35% from 2 days of age to 3 weeks of age. Noninvasive markers of GI disease were measured in 2-week-old kittens with somewhat surprising results. Serum pancreatic lipase immunoreactivity and trypsin-like immunoreactivity generally remained within normal adult reference intervals. Serum cobalamin levels were quite variable at two weeks of age but mostly within normal ranges. However, they fell below normal in many kittens thereafter.

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8 NEONATAL PHARMACOLOGY

Margret L. Casal, Maja Zakošek Pipan

When treating diseases in neonates, age and maturation stage of the organs must be taken into consideration. Because kittens less than two weeks of age are equivalent in their development to prematurely born children and serve as a model for their treatment, several studies that have examined drugs that might be useful in increasing the human "premie's" chances of survival. Most studies examined the effects of a variety of drugs on the performance of the heart and lungs and the development of the brain. For all of the rest of the drugs, we rely on what is known in children and dogs and make an educated guess taking the physiological difference of the neonate and pediatric patient into account. Absorption, distribution, metabolism, and excretion of drugs in neonates differ not only from the adult dog and cat but vary from week to week during the first weeks of life. Some guidelines are given below to be able to decide if the drug in question should even be given, if there is a reasonable and safer alternative, and how dosing interval and total amount given should be adjusted, if needed.

8.1 Absorption

The rate of absorption depends on the age of the neonate, type of disease, route of administration, and type of drug given.

8.1.1 Oral

For oral drugs, age is particularly important as the neonate will have a higher gastric pH, gastro-intestinal peristalsis is slower, and intestinal blood flow is lower than an older kitten or puppy. During the first 12 -18 hours of life there is a potential for absorption of macromolecules along with colostral antibodies, which may lead to toxic plasma level of medications in this size range. The higher gastric pH leads to increased absorption of acid-labile medications because they are not broken down in the stomach. In addition, changes in ionization lead to increased and decreased absorption of weak acids and weak bases, respectively. Slow peristalsis and decreased intestinal blood flow in the young kitten and puppy result in increased uptake of some medications because of the delayed transit time. All of this leads to a decreased first pass effect during the first 24 hours of life. There is a decreased rate of absorption for lipid soluble drugs due to decreased bile production during the first few weeks of life. Hypothermia will also slow down gut, leading to reduction in uptake and possibly metabolism of a given drug. Absorption of lipophilic drugs given to nursing neonates or along with milk replacer may also be impaired. Other factors influencing oral absorption are vomiting, diarrhea, spitting the medicine out, and other concurrent disease, such as infectious diseases affecting the intestinal mucosa. In addition, the intestinal microflora is not fully developed until one month of age. Therefore, the use of antibiotics in young neonates disturbs the developing flora, which often results in diarrhea. Hypertonic solutions (>460 mOsm/kg) should be avoided as they have been shown to cause necrotizing enterocolitis. In general, uptake of medications from the gastrointestinal tract can be quite unpredictable during the neonatal age and alternative routes should be taken into consideration if possible. After the neonatal period, oral administration is generally the easiest. Finally, medications given orally may lodge in the esophagus if they are not given with a bit of water (or food if the medication allows). Regurgitation, dysphagia, choking, and gagging may result. Some medications may even lead to esophagitis such as doxycycline and clindamycin in kittens only, which may result in strictures that require treatment later in life.

8.1.2 Intramuscular

In the neonatal kitten and puppy, if possible, drugs should not be given via the intramuscular route because decreased muscle mass, decreased blood flow to the muscles, and inefficient muscle contractions make absorption and thus plasma concentrations unpredictable. Vasomotor reflexes are not fully

developed at this age resulting in exaggerated vasoconstriction contributing to the poor uptake from muscle tissue. The decreased muscle mass also makes IM injections very painful.

8.1.3 Subcutaneous

SC injections are often performed because it is the simplest route of parenteral administration. However, as with muscle tissue, absorption can be unpredictable. The lack of subcutaneous fat in the neonatal puppies and kittens leads to increased absorption rates, while hypothermia or cold ambient temperatures can result in decreased uptake.

8.1.4 Intravenous and intraosseous

The IV and IO routes are preferred in neonates, especially when accurate dosing is required, or the medication has a narrow margin of the safety. In a neonatal kitten, the jugular vein is suitable for placement of a ¾ inch 26 G IV catheter. The IO route using an 18 - 22 G needle (depending on the size of the neonate) is an alternative if a jugular catheter is not an option (Figure 1). The advantage is that no special needles or catheters are required. However, care must be taken when placing the needle. A second attempt of sticking a needle into the same bone will result in the fluid being administered to leak out of the first puncture made in the bone and subcutaneous fluid will accumulate. In addition, with bones that small severe damage to the bones and joints could occur.

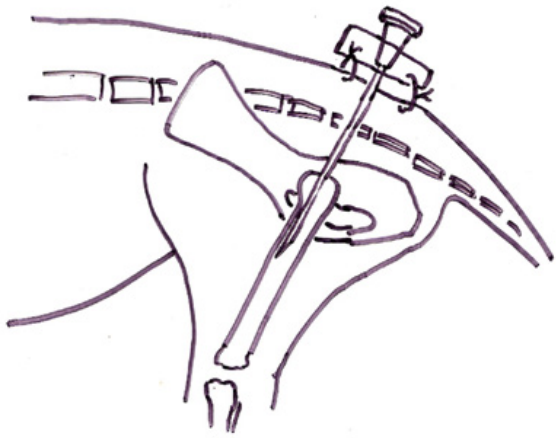

Figure 1: Intraosseous catheter. A 22G needle is placed through the intertrochanteric fossa into the medulla of the femur. The needle is secured at the hub with two tape "wings" that are held in place by stay sutures. MLC

8.1.5 Inhalants

In neonates, minute ventilation is much higher than in adults which results in increased absorption of inhalants, which will lead to a more profound effect of gas anesthetics.

8.1.6 Alternate routes

Other routes of administration include intraperitoneal (IP), rectal, endotracheal and topical. If IV or IO access is not available, drugs may be given IP. Careful placement of the needle is recommended to avoid damage to the internal organs. Drugs that need to be metabolized in the liver to be effective should not be given rectally, as this route bypasses the liver. Drugs that are given endotracheally are generally diluted lipid soluble substances and can include epinephrine, atropine, naloxone, and lidocaine. For the application of topical drugs, the correct environment must be provided. If the ambient temperature is too cold to heat up the patient, the peripheral blood flow will shut down, and the drug will not be taken up. In general, hypertonic solutions (>460 mOsm/kg) may cause intracranial hemorrhage and should therefore be avoided.

8.2 Distribution

The volume of distribution is an important concept in pediatric medicine and is calculated by dividing the total amount of drug in the body by the drug plasma concentration. By definition, it is the volume of fluid it would take to contain the total amount of drug, uniformly distributed, to achieve an observed blood concentration. It is not a real volume but aides in determining how to adjust doses of drugs for neonatal and pediatric patients. For example, if a water-soluble drug is given to an adult animal, a specific plasma concentration is expected from pharmacokinetic studies that had been performed previously.

8.2.1 Pregnancy

Physiologic changes during pregnancy lead to pharmacokinetic differences in the dam or queen and in the fetus. Increases in cardiac output, renal blood flow, glomerular filtration rate, renal and hepatic clearance, plasma protein binding, and increased volume of distribution are adaptive mechanisms during pregnancy and are related to the increased body water content in the dam or queen. While there is a "placental" barrier just like a blood-brain-barrier, it does not prevent drugs from getting through to the fetus. Drugs that are considered embryotoxic (<20 days canine fetus; <18 days feline fetus) lead to abortion in

general. Teratogenic drugs lead to congenital malformations, which are typically acquired after the embryonic phase. Examples of drugs that results in adverse effects in the fetus are tetracyclines (disruption of tooth and bone development), chloramphenicol (bone marrow suppression and fetal death), and griseofulvin (teratogenic in cats; Figure 2).

Figure 2: Neonatal kittens whose mother received griseofulvin during pregnancy. Courtesy: Dr. ME Haskins, UPENN

8.2.2 Nursing neonate

The distribution of drugs into the milk depends on lipid solubility, unbound fraction, and the extent of ionization in plasma versus milk. Milk has a lower pH than plasma. Therefore, weak bases tend to concentrate in the milk while weak bases will remain in the plasma and lipid soluble drugs will also accumulate in the milk. Macrolides are examples of drugs that tend to accumulate in the milk. On the other hand, penicillins and NSAIDs tend to remain in the plasma as they are poorly lipid soluble, weak bases. Lastly, some drugs are chelated by calcium and are therefore ineffective (tetracyclines and fluoroquinolones).

8.2.3 Neonate

We know that neonatal patients have up to 30% more body water than adults. This means that the volume of distribution for the water-soluble drug is much larger, despite the fact that the patient is smaller. If one would like to achieve the same plasma concentrations, more drug would have to be administered to compensate for the larger volume of distribution. Vice versa, if a lipid soluble drug is administered, the dose may have to be decreased because of the smaller body fat content and thus the lower volume of distribution for fat soluble drugs. Drugs can also be bound to plasma proteins, and this also needs to be taken into account because of the physiologically lower albumin and plasma protein concentrations in neonates. Although albumin concentrations are not much lower in pediatric neonates when compared to adults, they may have lower binding affinity leading to more free drug in circulation. Because of the incompletely developed blood brain barrier in neonates possibly up to 8 weeks of age, there is increased permeability to low lipid soluble drugs, such as some anesthetics, digoxin, and avermectins. In summary, high volume of distribution indicates high tissue drug concentrations and low plasma concentrations, and a low volume of distribution indicates low tissue concentrations and high plasma drug concentrations. Increased body water content, decreased fat stores, decreased plasma proteins, and incompletely developed blood brain barriers in neonates will shift the volume of distribution and thus the drug doses can be adjusted.

8.3 Metabolism and Elimination

Metabolism depends on a variety of mechanisms mainly in liver and kidney, but also in the intestinal wall, skin, and blood. Drugs are eliminated by transformation into mostly inactive metabolites for easier excretion through the kidneys. Processes include oxidation, reduction, hydrolysis, and conjugation. However, the immaturity of liver and kidney precludes rapid elimination of some drugs when compared to adult cats. In addition, some processes do not exist in cats, such as the inability to conjugate glucuronic acid resulting in sensitivity to phenols. Thus, the rate of elimination can be increased for certain drugs in pediatric neonates. The longer rate of elimination results in higher plasma and tissues levels for a longer period of time and drug dosing intervals may need to be increased to once daily rather than twice or twice daily rather than three times daily. Clearance is also directly related to the volume of distribution.

In neonates, it appears that the glomerular filtration rate matures by 3-6 weeks and the renal blood flow by 4-8 weeks. It also appears that microsomal drug metabolizing enzymes mature by 4-6 weeks of age. Complete details on liver and kidney metabolism are lacking in young neonates. However, it is safe to assume that hepatic metabolism is underdeveloped including enterohepatic circulation, which leads to prolonged half-lives and increased potential for toxicity. Drugs famous for their potentially toxic effects in young animals include barbiturates, antiepileptics, non-steroidal anti-inflammatory drugs,

sulfonamides, and choramphenicol. Antibiotics that are affected by hepatic metabolism and have been investigated in neonates include chloramphenicol, sulfonamides, tetracyclines, trimethoprim, and metronidazole. Drugs that are known to be affected by renal immaturity include ketamine, aminoglycosides, cephalosporins, penicillin, tetracyclines, sulfonamides, and trimethoprim. Interestingly, the immaturity of the proximal renal tubules does not allow for accumulation of aminoglycosides during the first 2-3 weeks of life, making them resistant to their nephrotoxic effects. However, to be safe, aminoglycosides should only be given once daily but at double the dose (increased volume of distribution!) in animals this young. In summary, drugs that are metabolized by liver and kidney should be avoided in neonates less than 4 weeks of age and the drug with the least toxic effects should be chosen for treatment.

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9 PYOMETRA IN BITCHES AND QUEENS

Stefano Romagnoli

9.1 Introduction

In small animals pyometra is a diestrual disease typical of adult intact females whose development is strongly influenced by sequential progestational stimulations (normal diestrus or treatment with progestins) of the uterus. Females giving birth regularly throughout their reproductive life almost never develop pyometra, while those who do so rarely or never in their lives have a greater chance of developing this condition. During a phase of progestational stimulation the canine and feline endometrium proliferates and starts secreting endometrial fluids (the so called "uterine milk") while the cervix remains closed and myometrial contractility is inhibited (Table 1) .

Table 1: Effects of estrogens and progesterone upon different uterine structures. These effects are observed during endogenous secretion as well as after exogenous administration. PMNs = polymorphonuclear cells

Structure	Estrogens	Progesterone
Endometrium	Growth, vascularity, edema of the whole endometrium	Proliferation and secretion of uterine milk by endometrial glands
Cervix	Relaxation and dilatation	Closure
Miometrium	Stimulates contractility	Inhibits contractility
Uterine Lumen	Stimulates PMNs to migrate into the lumen	Inhibits PMNs to migrate into the lumen

9.2 Physiopathogenesis of pyometra

Most bitches experience 2 estrous cycles/year, with development of corpora lutea and progesterone secretion for about 2 months. During the follicular phase estrogen cause outgrowth and branching of endometrial glands. The subsequent progesterone stimulation on the endometrium causes active secretion of endometrial glands with formation of cystic structures. Such endometrial production is very important for feeding the embryos, and if the female is not pregnant endometrial outgrowth starts regressing at the end the luteal phase, leaving the endometrium free to regenerate and be ready for the next chance for a pregnancy. If the female is rarely if ever mated some of these cystic structures may eventually persist, thereby making large sections of the endometrium unsuitable for the establishment of pregnancy. Fluid accumulates within the endometrial glands - which then dilate becoming fairly large (up to 0.3-2.0 cm diameter) – as well as it may leak into the uterine lumen. The endometrial pattern that develops is referred to as cystic endometrial hyperplasia (CEH) which is a prerequisite for the development of pyometra due to the fact that uterine milk in itself constitutes an inflammatory stimulus as well as an excellent culture media for bacteria. CEH is a physiological phenomenon whose regression starts during the second half of diestrus. However, with time and number of "open" (non-pregnant) cycles, CEH may not entirely disappear from some sections of the endometrium, thus increasing chances of causing endometrial inflammation. For unknown reasons gestation has a protective action on the canine endometrium, causing areas of the endometrium where placental attachment occurs not to develop any cystic endometrial hyperplastic lesion (although pyometra can occur in one horn with pregnancy in the opposite horn). As the disease progresses one or more of the following signs become manifest in the majority of females: purulent vulvar discharge (if the cervix is open), leukocytosis, abdominal enlargement, anorexia, polyuria and polydipsia. While diagnosis of overt cases poses no particular clinical problems,

recognizing a subclinical CEH may be a challenge. On ultrasound, CEH appears as very small hypoechoic areas of a few mm diameter within sections of the endometrium characterized by a normal texture.

Concurrent clinical problems - Faecal/perineal bacteria (*E. coli*, *Streptococcus* spp., *Staphylococcus* spp., *Klebsiella*, *Proteus* and *Pseudomonas* are the most common ones) often concur to the development of uterine lesions and clinical signs (especially if they cannot be cleared from the uterus prior to onset of the luteal phase), but are not necessary for the clinical manifestation of the disease. This has been clearly demonstrated by studies in which the experimental injection of bacteria in the uterus of ovariectomized bitches did not cause pyometra. High levels of progesterone (P4) concentration are necessary for pyometra to develop. Therefore, removing P4 from the general circulation is imperative if one wants to treat this condition effectively. When present, bacteria take advantage of uterine fluid (an excellent nutrient) and reproduce quickly causing the accumulation of pus and a well-known cascade of side effects. *E. coli* can produce an endotoxin which, upon bacterial death (i.e. following an antibiotic treatment), is released into the uterine lumen and absorbed. The resulting endotoxemia may cause a severe shock reaction and death of the bitch depending on the amount of endotoxin released and on the physical condition of the dog (antibiotics have no effect on concentrations of endotoxins). Clinical signs of endotoxemia include disorientation, hypothermia and shock. Renal lesions are frequent in bitches with pyometra (especially in older bitches), being due to either the disease itself (because of pre-renal azotemia due to dehydration or glomerular/tubular disease due to the bacterial infection or endotoxemia) or because they were already present when pyometra developed. Bone marrow, liver and spleen disease can also be either already present or caused by the disease itself in bitches with pyometra. Bitches with a closed cervix pyometra and with liver or kidney insufficiency are not considered good candidates for a medical treatment with aglepristone. On the other hand, in a bitch with a pus-distended uterus but with normal kidney, liver and cardio-pulomonary function the surgical procedure may be faster and simpler if the uterus has been emptied with aglepristone prior to surgery.

Pyometra may cause infertility in its initial stages (or s a sequel in females who have recovered) due to failure of embryo to attached to a damaged endometrium.

9.3 Treatment of pyometra

Being influenced by a progestational stimulus and characterized by accumulation of pus within the uterus, canine and feline pyometra has historically been approached from a clinical standpoint with drugs such as prostaglandin F2 α which provides the double action of luteolysis and uterine contractility. Other drugs which have proven useful for this condition include antiprolactinics. Recently, the progesterone antagonist aglepristone associated with prostaglandin E has become the treatment of choice.

Aglepristone (a P4-antagonist marketed for induction of abortion in mismated bitches) is currently the treatment of choice for canine/feline pyometra. It is supposed to be given twice at 24-hr distance and then repeated after one week (calculated form the first injection, e.g. day 0, 1 and 6) and then repeated again depending on need at weekly intervals. A protocol featuring injections on day 0, 2, 5 and 8 seems to be more effective (then the day 0-1-6 protocol) and not associated with relapses, although more research is needed on this aspect. The aglepristone dosage in bitches is 10 mg/kg (the same as for the induction of abortion). Side effects are none or very mild such as itching at the injection site. When treating pyometra one needs to be concerned about emptying the pus-filled uterus as well as causing luteolysis. When the cervix is open the uterus can be easily emptied stimulating miometrial contractility. Of all the drugs causing contraction of the uterine musculature (prostaglandins, oxytocin, ergot derivatives) prostaglandins (PGF and PGE) are the most indicated for the clinical treatment of pyometra because of their more physiological action on the diestrous uterus. Prostaglandin-based drugs which have been tested in bitches and for which safe dosages have been developed include natural PGF2α, the PGF2α synthetic analogues cloprostenol and alfaprostol, and most recently the PGE misoprostol (see table n° 2). Much less is known about the dosage or PGF2a in queens, who seem to be less prone to side effects than bitches (see over), and who may be treated with doses of up to 200 mcg/kg (although probably lower dosages are equally effective).

PGF2α offers the advantage of a double (miocontractile and luteolytic) action, but may cause some side effects in bitches depending on dosage. For instance, higher dosages of natural PGF2α (150-250 mcg/kg) are characterized in the bitch by a well-known cascade of side effects, which are rarely observed when using lower (<80 mcg/kg) doses. Side effects of prostaglandins should not be overemphasized as, when using i.e. doses of 50-100 mcg/kg of natural PGF2α ,they are not observed in all bitches, tend to subdue during the course of the treatment and are significantly less common when using dosages of natural PGF2α <50 mcg/kg. However, side effects of PGF2α compounds tend to be more evident in

bitches with pyometra, perhaps because of the deteriorated physical conditions which characterize the uterine disease. Efficacy of very low doses of natural PGF2α (50 mcg/kg) has been reported for bitches with pyometra (Lange et al., 1997). Such a treatment protocol is effective provided that prostaglandins are administered 3 times daily for as long as a vulvar discharge is present.

A synthetic analogue of prostaglandin E, misoprostol, has been recently used to help in evacuating uterine content in bitches with pyometra. Misoprostol is marketed as a human compound under different trade name in different European countries (i.e. Cytodec™ (France/Italy/Spain), Misodex 200™ (Italy), or Menpros® (Spain), and is available in 200 mcg tablets. In the bitch it is administered at 10 mcg/kg BID orally (1/2 tablets/10 kg). It has little if any side effects mostly only on the first day of treatment (vomiting = 25% of cases; diarrhoea = 30% of cases). PGE has been tested in dogs with pyometra and appears to be effective for causing evacuation of uterine content, although it does not have any luteolytic properties. Oxytocin and ergot derivatives induce very strong, short-lasting contraction of the uterine wall which are not thought to be adequate for emptying a pus-filled uterus. However, no specific studies have ever been done on efficacy of oxytocin or ergot derivatives in bitches with pyometra. Misoprostol pills can be dissolved in saline and administered intravaginally to help in opening the cervix.

Table 2 – Dosages of the most commonly used prostaglandin compounds in bitches to induce luteolysis and cause uterine contractility (PGF2α) or just uterine contractility (PGE). Prostaglandins should never be used to treat a closed-cervix pyometra because of the risk of uterine rupture or of pushing uterine pus retrogradely into the uterine tubes. When treating a bitch with any PGF2α product, start with half the normal dosage and gradually achieve the full dose within the first 2-3 days of therapy.

Prostaglandin	Daily dose in the bitch	Administrations/day
PGF2α, Natural compounds	Bitch – 50-80 mcg/kg (0.05-0.1 mg/kg)	3-2, SC
PGF2α, Cloprostenol	Bitch - 1 mcg/kg (0.001 mg/kg)	1, SC
PGF2α, Alphaprostol	Bitch - 20 mcg/kg (0.02 mg/kg)	2, SC
PGE, Misoprostol	Bitch - 10 mcg/kg (0.01 mg/kg)	2, per os

Aglepristone causes miometrial contractility but not directly, only due to the removal of the P4-induced blockade – and in fact miometrial contractility starts a few days after treatment onset – while prostaglandins act more rapidly. Therefore, aglepristone work well in conjunction with prostaglandins, and this association should be used whenever the uterus is filled with large quantities of pus (Table n. 3).

Table 3 – Example of a treatment protocol for a bitch with open-cervix pyometra treated for 15 days. Aglepristone and prostaglandin (PGF2α or PGE) are alternated in this case, but the two drugs can be given also on the same days (i.e. prostaglandins given every day). Antibiotics (Ab) should be chosen based on culture and sensitivity tests and administered on every day. Treatment length depends on the time needed for the female to recover and should not be decided in advance

	Days of treatment														
Aglepristone	1	2						8							15
PGF2α/PGE			3	4	5	6	7	8	9	10	11	12	13	14	
Antibiotics	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15

Using antibiotics - Antibiotic treatment should be specific as soon as culture results are available (a broad spectrum antibiotic such as ampicillin at 22 mg/kg 3 times/daily can be started in the meantime) and it should also continue for as long as a purulent vulvar discharge is present (which may persist for a few days after uterine diameter has become normal again). At the following proestrus a cranial vaginal culture should be taken and the bitch treated with a specific antibiotic until ovulation, and then bred to a proven fertile male at the proper time in order to ensure conception. When reproduction is not deemed necessary any longer the bitch should be spayed, as the recurrence rate of pyometra in older dogs may be higher than 50%.

Lenght of treatment –In order for a pus-filled uterus to be emptied prostaglandin administration should be continued as long as a vulvar discharge is present. Although large amounts of pus in the uterus may require treatments of several weeks duration, even when the uterus is not greatly enlarged caution should be taken not to stop the treatment too soon. The decision on when to stop the treatment should be based on a) evaluation of uterine dimensions before, during and after therapy (in order to confirm that uterine diameter has gone back to normal) as well as b) disappearance of fluid within the uterine lumen detectable on ultrasound; in normal conditions the uterine lumen is not detectable, but it becomes distinguishable when liquid accumulates within the uterus.

Risk of uterine rupture - The administration of miocontractants may be dangerous if the uterus is fully dilated with pus and/or the uterine wall is thin and atrophied or the cervix is only partially dilated, as this may cause either a) the pus to be forced backward into the uterine tubes and then into the abdomen, or b) the uterine wall to rupture. Uterine rupture is a very rare event, and can be caused by any drug stimulating uterine contractility. The risk of uterine rupture can be subjectively assessed looking at the thickness of the uterine wall with ultrasound using 7.5-10.0 MHz probes.

Treatment of closed-cervix pyometra

The availability of antiprogestin-based drugs has completely changed the clinical approach to a problem whose only solution for the decades has been only ovariohysterectomy. The administration of aglepristone during diestrus in the bitch will cause opening of the cervical os with consequent emptying of the uterine content. Following treatment with a dose of 10 mg/kg aglepristone administered twice at 24-hr interval (and then at weekly intervals if necessary) in bitches with closed pyometra, opening of the cervix occurs after 2-3 days in all treated animals.

Peculiarities of pyometra in queens

As in bitches, also in queens pyometra is the most common uterine disease which may start as a limiting factor for fertility but may easily turn into a life-threatening condition if it goes unnoticed. Pyometra is typical of intact, middle age or older queens but is occasionally encountered in young females and has been reported in neutered animals as well, both in queens showing signs of heat after spaying as well as in queens who never show signs of heat (de Faria and Nosworthy, 2008). Ovariectomized females receiving a prolonged treatment with a medium to high dosage of progestational drugs may develop pyometra (Agudelo, 2005). Physical examination of queens with pyometra usually reveals vaginal discharge in a mildly depressed, dehydrated cat with a normal or increased rectal temperature and palpably enlarged uterus; Signs such as polyuria/polydipsia, fever, dehydration, vomiting and weight loss are fairly unusual in the queen (5-30% of cases), and asymptomatic pyometra (as an incidental finding during ovariohysterectomy) is occasionally observed in felines. Vaginal discharge may be inapparent due to the queen licking on herself. Cats with closed-cervix pyometra show no vaginal discharge but generally have significant uterine distention causing abdominal enlargement and not unusually severe illness. Serum progesterone levels may be increased, although they often do not correlate with clinical disease. Ultrasonography generally confirms uterine enlargement showing often a thin uterine wall and presence of fluid of variable degree of echogenicity. Hematology and serum biochemistry generally reveal a leukocytosis with a left shift (but there are cases with normal leukocyte counts). Blood workup should never be forgotten as checking renal and liver function is mandatory in adult to elderly patients. Size of endometrial cysts in the queen may vary from 0.5 mm to 2-3 cm (Stabenfeldt and Pedersen, 1991).

Treatment of open cervix pyometra does not differ in queens from what is normally done in bitches and includes specific antibiotic therapy, administration of uterine contractant drugs such as prostaglandin F2α (PGF) until uterine size has returned to normal and the use of aglepristone when serum progesterone is > 1.0 ng/ml. Natural PGF at a dose of 200 mcg/kg IM twice a day can induce luteolysis (from mid-pregnancy on) and will cause miometrial contractions (at any stage) in the queen. Although the queen is much less sensitive to the action of PGF than the bitch, side effects (panting, salivation, defecation, grooming, vomiting and nausea) become evident after 10 minutes and may last from 45 minutes up to 3 hours depending on dosage (Nachreiner and Marple, 1974; Verstegen et al., 1993; Baldwin, 1995). It is advisable to start with half the dose and gradually increase dosage over a period of 1.5 to 3 days, monitoring speed and degree of uterine emptying.

Although little if any scientific information is available on efficacy of PGF doses similar to what is currently being used in bitches (50-80 mcg/kg), such low doses are likely to be effective also in the queen. When using natural PGF, therapy is successful if injections are given at least twice a day. In our feline patients we have used natural PGF at the dosage of 80-100 mcg/kg twice daily, starting with half the dose for the first day (or the first 2 administrations). Synthetic analogs of PGF, such as cloprostenol or alphaprostol have been effectively used in queens, with cloprostenol being used at a dose of 1.0 mcg/kg, administered three times at 48-hour intervals, while alphaprostol can be administered twice daily at the dose of 20 mcg/kg also in the queen.

Due to presence and degree of side effects and because of the small amount of information on the use of PGF in the queen, the treatment of a sick cat with PGF should be considered very cautiously and performed only with the owner's informed consent. Some authors question the use of PGF compounds in elderly (older than 8 years of age) queens with pyometra (Agudelo, 2005). A PGE dosage of ½ tablet/cat can be used in queens with pyometra.

Closed cervix pyometra is a therapeutic challenge also in the queen and should be considered an emergency. Aglepristone appears to be effective also in queens at 1.5 times the canine dose (15 mg/kg). The protocol for canine pyometra (aglepristone + prostaglandins+antibiotics) should be used also in the queen with pyometra. The use of PGF together with specific antibiotics based on cultural exam and sensitivity should be continued until vulvar discharge disappears and uterine size returns to normal. The average length of treatment with PGF for queens with open cervix pyometra is 2-5 days; however, clinicians should not be concerned about side effects if more than 5 days of treatment are necessary: queens easily adjust to PGF-related side effects, and treating a queen for 8-10 days should not be considered a problem. Prognosis for fertility after pyometra is good for young queens recovering promptly.

Diagnostic challenges – Diagnosing pyometra in the bitch or queen may be very easy, especially if uterine enlargement and leukocytosis (the only 2 clinical signs which are relevant to the diagnosis) can be appreciated along with the other classical signs of polyuria/polydipsia (in bitches), anorexia, depression, vulvar discharge (in case of open cervix pyometra) and leukocytosis. Females with a closed cervix pyometra with only a slight to moderate increase in uterine size are the most challenging cases, especially if leukocytosis is absent or if there is neutropenia (which may be due to endotoxemia). The range of WBC count reported for bitches with pyometra is 2500-196,800 cells/mm^3, with a left shift in 70-90% of cases. Average number of bands (toxic PMNs) is about 500 cells/mm^3, with degree of toxic change correlated with the severity of disease. Other abnormalities (anemia, azotemia, hypergammaglobulinemia, hypoalbuminemia, metabolic acidosis, decreased urine specific gravity, protenuria, bilirubinuria) may or may not be present in bitches with pyometra and when present may or may not be related to the uterine disease. Although not always relevant to the diagnostic process, haematology and biochemistry tests should be carried out to allow for a proper clinical management of the disease, thus avoiding pyometra patients to die i.e. for a renal complication once the uterine problem has been cured. Pregnancy should always be ruled out, as it may coexist with pyometra. Less common differential diagnoses include diabetes mellitus, hyperadrenocorticism, renal disease and diabetes insipidus.

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10 PYOMETRA – SURGICAL MANAGEMENT – INTERACTIVE

Tanja Plavec

Pyometra is a common illness in adult intact female dogs. It develops in up to 25% of intact bitches by the age of 10 years and is significantly less common in cats and other small animal species. The concept of cystic endometrial hyperplasia/pyometra states that hormonal changes during the luteal phase lead to cystic endometrial hyperplasia (CEH), which sets the ground for the establishment of infection with ascending opportunistic bacteria (most often *E. coli*) (Hagman,2022; Fransson, 2018).

Pyometra is a polysystemic disease, characterized by an acute or chronic suppurative bacterial infection of the uterus with accumulation of inflammatory exudate in the uterine lumen. A wide range of clinical signs are associated with the disease and the owners are advised to seek immediate veterinary care when pyometra is suspected. The diagnosis is generally straightforward but can be challenging when there is no vaginal discharge. While medical management may be possible in young or otherwise healthy animals or those with higher anestetic risks, it is contraindicated in patients with systemic illness, fever, hypothermia, organ dysfunction or peritonitis and sepsis. In these animals surgical ovariohysterectomy (OHE) is the treatment of choice (Hagman,2022; Fransson, 2018).

10.1 Surgical treatment

Ovariohysterectomy is the safest and most efficient treatment of pyometra, especially when patient's status deteriorates and in closed pyometra cases. It removes the source of infection and prevents recurrence (Fransson, 2018). The patient needs stabilization with adequate intravenous fluid therapy to correct hypotension, dehydration, shock, electrolyte, acid-base and coagulation disturbances as well as improve tissue perfusion and function of the organs (Hagman, 2022).

Intravenous broad-spectrum antibiotics, effective against most common pathogen *E. coli*, are indicated perioperatively in moderately to severely ill patients, sepsis or patients with serious complications (Hagman, 2022; Fransson, 2018). Antibiotic therapy should be continued postoperatively in systemically ill patients, those with urinary tract infection, increased complication risk and those with intraoperative contamination (Fransson, 2018). However, antibiotics do not need to be included in perioperative treatment in mildly depressed patients or those without concurrent diseases or complications. A combination of antibiotics is recommended for life-threatening peritonitis, severe sepsis or septic shock. Ideally, initial treatment is followed by culture and sensitivity testing, after which antimicrobial treatment is tailored to a narrower spectrum (Hagman, 2022).

Removal of the infection is a key and surgery should not be delayed, because endotoxemia and sepsis may develop. An OHE is performed with a few modifications to the standard technique. Uterus may be large, friable and prone to rupture; hence it should be isolated with moistened laparotomy sponges before gentle manipulation and transection. The suspensory ligament is often stretched, vessels in the broad ligament may be enlarged and require ligation (with resorbable suture material) and the uterine stump should not be oversewn to avoid leaving foreign material in an infected site. In generalized peritonitis the abdominal cavity needs to be lavaged copiously and later handled as septic peritonitis. Samples for bacterial culture are taken before abdominal closure if needed and abdomen is routinelly closed (Hagman, 2022; Fransson, 2018). Intensive postoperative monitoring is essential, but uncomplicated cases are discharged after 1-2 days of postsurgical hospitalization (Hagman, 2022).

Blood work abnormalities, encountered preoperatively, tend to improve rapidly after OHE. White cell count will increase within 48 hours after surgery, but band neutrophils will decrease (Fransson, 2018). Seven days after OHE the WBC counts will return to normal levels, comparable to clinically healthy dogs (Bartoskova et al., 2007).

Prognosis of survival is good and mortality rates are low (between 0-5%) (Fransson, 2018), however if systemic illnes or severe complications such as uterine rupture, peritonitis or septic shock are encountered, the mortality can be considerably higher (Hagman, 2022).

10.2 Differentiation between mucometra/hydrometra and pyometra

This differentiating is important for better therapeutical planning. However, it can be challenging, because the fluid in the uterus is present in both disease entities and clinical manifestation can be similar. Precise ultrasonographic examination demonstrating the difference in echogenicity of the intrauterine fluid and hemodynamic parameters appear as useful markers to differentiate uterine pathologic conditions but is not diagnostic (Bigliardi et al., 2004; Batista et al., 2016). More pronounced inflammatory response and more than three clinical signs of the disease are indicative od pyometra rather than mucometra/hydrometra (Hagman,2022; Franssonet al. 2004).

10.3 Stump pyometra

A stump pyometra is a disease entity when a pyometra develops in residual uterine tissue in incompletely spayed bitches and queens, most often as a part of the ovarian remnant syndrome (Hagman, 2022; Demirel and Acar, 2012; Ehrhardt et al., 2023). It has also been described without ovarian remnants, solely because of the treatment of spayed bitch with tamoxifen, which can cause pyometra in intact bitches as well (Ehrhardt et al., 2023). The clinical presentation is similar, except for a history of a previous spay. Ultrasonography usually shows areas of local fluid accumulation at the tissue stump, but the ovarian remnants may be difficult to find. Treatment consists of stabilisation (antibiotics if indicated) and surgical resection of both ovary and uterus (Hagman, 2022; Demirel and Acar, 2012).

10.4 Prevention of pyometra

Elective OHE is the best prevention of uterine disorders including CEH/pyometra complex. However, also correctly performed ovariectomy (as a predominant spay method in Europe) is preventative, because ovariectomized bitches lack hormonal production in the ovaries. There is also no fluid production in the uterus of spayed bitches and hence the uterus is less prone to ascending infection (Hagman, 2022; Urfer et al, 2019).

10.5 Conclusion

Pyometra is a potentially life-threatening illnes of middle-aged to older female pets. Hormones and bacteria are involved in the disease development. The diagnosis is usually straightforward, but may be challenging in less pronounced cases or in cases without vaginal discharge. The diagnosis is based on history, physical examination, laboratory tests and ultrasonography. The safest and most effective treatment is surgical OHE. Peritonitis, sepsis, endotoxemia and systemic inflammatory response syndrome are relatively common complications and demand appropriate management.

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11 POSTPARTUM CONDITIONS IN SMALL ANIMALS

Stefano Romagnoli

The puerperal period is a very delicate time for the dam who is undertaking important tasks such as uterine involution and lactation. Uterine, mammary as well as metabolic disorders can occur during the first 3-5 weeks postpartum (PP) which may place a considerable risk on the dam's as well as the neonate's life. Lactation is particularly demanding on the dam's organism. Partial, short-lasting anorexia may be observed already during pregnancy, and sometimes also in the early PP. Diarrhea, as well as hair loss can also be observed in the early PP. The daily measurement of PP rectal temperature (T) is a safety procedure which allows for early detection of PP complications whenever a T > 39°C is observed. In small animals lochia are brick red color, odorless and gradually decreasing over the first week PP, sometimes extending into the second or early third week. Mammary glands are typically symmetrically engorged, firm, with no dectable heat or reddening or intraparenchymal mass. Normal dams eat with appetite while lactating and caring for their pups.

11.1 Haemorrhage

Profuse vulvar hemorrhage is an uncommon complication in PP bitches and queens. It may be due to uterine or cervico-vaginal trauma, placental necrosis or sub-involution, or a coagulation disorder. The blood loss occurring with subinvolution of placental sites (see over) is typically mild and scant, while the remaining conditions can be characterized by a profuse and incontrollable blood loss. Small quantities of fresh blood are normally passed shortly after parturition. Such a discharge changes color rapidly due to the release of uteroverdin from the marginal hematoma, and decreases rapidly. The continuous passing of relevant amounts of blood is abnormal, and is frequently accompanied by unease, depression and pale mucous membranes. Vaginal (digital or endoscopic) inspection should be performed to rule out vaginal trauma. Traumas in the vagina can be dealt with by applying pressure or a vaginal tampon or clamping a bleeding vase. When bleeding comes from the uterus oxytocin (0.25-1.0 IU/dam) or ergometrine (0.2-0.5 mg/kg) should be administered to try and speed up uterine involution. If neither drug works and profuse bleeding continues a laparotomy should be performed immediately following a preliminary assessment of clotting function. Ovariohysterectomy is often necessary in such cases.

11.2 Agalactia

Agalactia is a rather obscure condition in bitches and queen, while it has been extensively studied in women. Milk production in breastfeeding mothers can be increased using a variety of drugs called galactogogues. These include antiemetics, antipsychotic and hormones.

11.2.1 Antiemetics

Metoclopramide is a Central Nervous System (CNS) dopamine D2 receptor antagonist used as an oral antiemetic drug. Its antagonizing action on the main prolaktin (PRL) inhibitor dopamine causes a powerful, albeit indirect stimulus to PRL release with reported high efficacy rates especially when metoclopramide is associated with oxytocin nasal spray. Maternal side effects in women include tiredness, headache, anxiety, nervousness and intestinal disorders and extrapyramidal signs (anxiety, agitation, movement disorders, dystonic reactions, ataxia) when used at higher doses (2-5 mg/kg). Metoclopramide is normally used in dogs as an antiemetic drug. It has been used also to stimulate canine PRL secretion, although scientific data with pre- and post-treatment PRL concentrations are available only for male dogs, in which a significant increase in serum PRL concentration is reported following treatment with 0.2 mg/kg 3 times daily. Effective use of metoclopramide in bitches with agalactia is anecdotal, with protocols varying from

low (0.1-0.5 mg/kg SC or PO, twice or three times per day) to high dose regimens (1-5 mg/kg beginning PO or SC, every 6-8 hrs). Extrapyramidal signs may occur in canines, and are a concern in nursing bitches, therefore higher dosages should best be avoided.

Domperidone is a peripheral dopamine receptor antagonist developed as an antiemetic agent and used for the treatment of nausea and vomiting. In women, domperidone significantly increases PRL secretion thereby enhancing breast milk production, and is therefore used (off-label) as a galactogogue in most Western countries. The maximum approved treatment protocol of domperidone in lactating women is 20 mg given 4 times daily; most authors advice using doses of 10 mg orally TID for 1-2 weeks. However, the minimum effective dose and the minimum duration of therapy have not been identified yet. It causes a significant increase in serum PRL concentrations and milk production in treated vs control mothers. Unlike metoclopramide, Domperidone is less permeable to the blood-brain barrier and is transferred in moderate quantities to maternal milk (milk-to-plasma ratio of 0.2-1.1), due to its high molecular weight and its 90% binding to plasma proteins. No side effects are reported in infants of mothers taking domperidone, while side effects in mothers include oral mucosal dryness, skin eruption, itch, headache and gastrointestinal disorders; extrapyramidal effects have been observed (dystonia) but are rare. Anecdotal use of domperidone in bitches and queens is known among small animal clinicians by "word of mouth" as effective at doses of 1.5-2.0 mg/kg in queens, and 2.2 mg/kg in bitches, per os for 1-3 weeks.

11.2.2 Antipsychotics

Chlorpromazine is an antagonist of D2 dopaminergic hypothalamic receptors, commonly used for the treatment of human psychosis including schizophrenia and depression. It has a wide action on different CNS receptors producing also anticholinergic, antihistaminic, as wells as weak antiadrenergic effects. In small animals, chlorpromazine is used as a second choice antiemetic drug when metoclopramide does not work and blood pressure is normal. Suggested antiemetic dosage is 0.2-0.5 mg/kg every 6-8 hrs. Only anecdotal information on the use of chlorpromazine in cases of agalactia is available for small animals (Johnston, Root-Kustritz and Olson, 2001). Some authors advice the use of acepromazine at 0.125-0.5 or 0.5-2.0 mg/kg, SC 2-3 times/daily. No data on clinical efficacy in bitches or queens with agalactia as well as milk:plasma ratio of transfer is available for antipsychotic drugs.

Sulpiride is a substituted benzamide used as an antipsychotic drug for the treatment of human psychosis including schizophrenia and depression. It is a strong antagonist of serotoninergic receptors as well as of muscarinic, alpha-adrenergic and histaminic receptors. Significant increases in milk production are reported only for primiparous mothers, not multiparous. Milk of treated mothers shows presence of the drug, although the milk:plasma ratio of transfer is lower than with metoclopramide or chlorpromazine. No information on the use of sulpiride in dogs or cats is available.

Oxytocin causes contraction of the myoepithelial cells that surround the alveoli as well as contraction of milk ducts, causing milk ejection. Oxytocin is widely used in small animals, mostly as a treatment for non-obstructive dystocia due to uterine inertia. However, no dose-response studies have ever been performed in bitches or queens. Its use in case of agalactia is anecdotally reported as effective following repeated dosing with the nasal spray or with IV or IM administrations of 2-5 IU IM prior to each suckling. Oxytocin will help to empty the mammary gland of previously produced milk, but it is unlikely to help increasing milk production by the mammary epithelium.

11.3 Retained placentas

Placental retention is quite uncommon in bitches and queens. It is fundamental to collect a careful history as to whether or not a sequential count of placentas and neonates was done during parturition as a) many owners/breeders make mistakes when counting placentas during delivery, b) placentas are sometimes expelled not after each fetus but grouped after a number of fetal expulsions, and c) the dam is sometimes quick to eat them. Reasons for placental retention in bitches and queens are still obscure. When this happens a muco-purulent vulvar discharge is often present, and both bitches or queens may show no signs or may occasionally become depressed, septicemic and with fever. Although the condition is frequently characterized by mild or absent clinical signs, the condition of the dam may worsen rapidly if not treated. Diagnosis requires performing a uterine ultrasonography as palpation can be misleading. When performed early, treatment can be done with oxytocin which continues to have a uterotonic effect for the first few days PP. From the 3rd-4th day PP onwards a prostaglandin treatment is indicated for as long as necessary until uterine ultrasonography looks normal and the dam has recovered completely. Broad spectrum antibiotics should be administered throughout the prostaglandin treatment, using antibiotics which are non-harmful for nursing neonates.

Metritis – This is an acute infection of the PP uterus which is often an emergency condition. The dam is typically depressed, with high fever, a purulent vulvar discharge, anorexia, decreased lactation and poor mothering behavior. Contamination from the lower reproductive tract is more commonly the reason for metritis (although hematogenous spread can occur) and *E. coli* is the most commonly isolated microorganism. Haematology and biochemistry indicate a systemic inflammatory condition with septicaemia and endotoxemia, while ultrasonography shows presence of fluid in the uterus and increased thickness of the uterine wall which is often completely inflamed. Retained palcentas and/or fetuses may be a complicating factor. Bacteriology of the vulvar discharge should be performed using a guarded swab to be submitted for aerobic/anaerobic culture and sensitivity testing. Antibiotic+uterotonic treatment should be continued until necessary. Antibiotic treatment can be given by IV infusion (along with electrolyte administration) in the early stages of the condition if necessary. Hand-rearing of the neonates should be carefully considered, particularly in chronic cases in which mastitis often develops as a consequence. Chronic metritis can cause long-lasting infertility.

11.4 Sub-involution of placental sites (SIPS)

Fetal trophoblastic cells normally are dislodged from placental sites in the endometrium within 2 weeks after parturition. Occasionally bitches may experience a prolonged bleeding following a normal parturition. Such bleeding is due to the persistence of trophoblastic cells in the upper connective tissue of the lamina propria of the uterus. Reasons for this to occur are unknown. These trophoblastic (or maternal decidual) cells reproduce very rapidly and do not allow adequate uterine healing. This results in a scant, drop-by-drop vulvar blood loss which is typically not associated with depression, anemia, anorexia or any other clinical sign. Affected bitches often are normal otherwise, except for this minor vulvar blood dripping which may continue beyond the first month and often may last for a few months, or occasionally may only stop at the beginning of the following proestrus. On manual transabdominal palpation uterine swellings of approx. 3 cm in diameter can be felt along the uterine horn/s. Polinucleated, heavily vacuolated giant cells can be observed on the vaginal smear of bitches with SIPS. Haematology, endoscopy, coagulation tests and uterine ultrasound are also indicated for a complete assessment of the case. However, diagnosis can only be suspected based on clinical signs (mild prolonged PP vulvar blood loss in the presence of normal health conditions); confirmation requires histopathology following ovariohysterectomy. As the condition is very mild and not life- or fertility-threatening, neither diagnostic confirmation nor treatment are normally necessary. Spaying should not be considered unless the bloody vulvar discharge becomes copious. The effect of prostaglandins or oxytocin for the treatment of bitches affected by SIPS (or for its prevention by early PP administration) is questionable and has never been rigorously investigated (also because this is a rare condition).

11.5 Eclampsia

Puerperal tetany or eclampsia is an emergency condition which is life-threatening in small animals, particularly common in small size bitches during the first month PP, although it has been reported also during the last 4 weeks of pregnancy. It is caused by a continuous depletion of extracellular Ca++ due to lactation, and it may be complicated by inadequate perinatal nutrition, calcium supplementation in pregnancy (which should NOT be done) and a strong, prolonged suckling by vigorous neonates. Also, metabolic alkalosis due to prolonged hyperpnea during stage 2 parturition may result in high protein binding of serum calcium which can promote or exacerbate hypocalcemia. The dam may initially show clinical signs due to subtle hypocalcemia (Ca 7.0-8.5 mg/dL = nervousness, panting progressing to behavioral changes, failure to care for the litter) which may or may not be followed by salivation and facial pruritus; signs of more evident hypocalcemia (Ca < 7.0 mg/dL) include stiff gait, limb pain and ataxia, following which the dam may become unable to stand with her hind limbs or all 4 legs rigidly extended. At this point she is also typically tachycardic and hyperthermic and seizures appear which, if not controlled by treatment will be followed by cerebral edema and death. Therapeutic intervention needs to be immediate with slow intravenous infusion of 10% calcium gluconate diluted 1:5 to 1:10 in saline given to effect. Mannitol can be used when seizures are present to control cerebral inflammation and swelling, while corticosteroids are contraindicted as they stimulate calciuria and decrease intestinal calcium absorption. As soon as seizures stop the IV infusion can be withheld and the dam treated with a similar dosage of calcium gluconate given SC diluted 1:1 with saline, repeated every 8 hrs until necessary. Once the bitch is stable, she may be sent home on an oral calcium diet (10-30 mg/kg every 8 hrs for a few days, or as long as she continues lactating) and vit. D. Lactation should be decreased (e.g. letting neonates suckle only once daily) or early weaning should be done if neonates are old enough.

11.6 Uterine prolapse

Prolapse of one or both uterine horns is rare in small animals but is reported both in bitches and queens. It follows intensive contractility of the myometrium coupled with abdominal contractions and tenesmus which, because of the dilated cervix, end up in forcing the uterus outside of the vulva. Uterine intussusception is probably an early feature of the process with the uterus ending up and with the endometrium visible on the outside while the uterine wall and serosal lining are internal. Placental retention may be a complicating factor A prolapsed uterus must be differentiated by a prolapsed bladder, and it should be kept in mind that a prolapsed uterine horn may contain intestinal loops inside. Once exposed to the environment, the mucosa of the prolapsed uterus quickly becomes dry and undergoes necrosis, and the broad ligament may rupture (risk of hemorrhage of uterine artery and hypovolemic shock) thus making uterine prolapse a true emergency. Treatment depends on a) the owner's interest for future fertility of the dam, b) degree of necrosis and c) content of the prolapsed uterus. Treatment options include amputation of prolapsed uterus or its replacement into the abdomen; both options should be better performed following laparotomy and replacement of the prolapsed uterus into the abdominal cavity by pulling the reproductive tract by its ligaments.

Amputation is a high risk surgery (the suspensory ligament may be hypertrophic due to tension and if not properly closed it may bleed profusely) and therefore is indicated in case of shock as an emergency procedure. Manual reduction requires accurate cleaning with sterile solution warmed up at 30°C, reduction of edema (if present) with 50% glucose, inverting and pushing back the prolapsed horn/s either by pushing it with a sterile, lubricated 10 cc glass tube or by lavaging with saline under moderate pressure. Prognosis depends on how quick veterinary intervention is: if immediately reduced with little or no contamination the dam may carry a pregnancy to term again. However, the risk of peritonitis is high. When just one uterine horn is prolapsed the presence of fetuses in the other horn should be checked.

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12 ARTIFICIAL INSEMINATION IN FEMALE CATS

Stefano Romagnoli

12.1 Semen collection and evaluation in tomcats

The cat is increasingly being used as a model for reproductive studies in endangered species, especially with regard to semen collection and freezing of wild felids, but also for basic research on in vitro maturation, in vitro fertilization, intra-cytoplasmic sperm injection and cloning. A great deal of new information on feline male reproduction has become available over the last decade, particularly thanks to research being conducted at the University of Bologna, Italy and the University of Uppsala, Sweden. The increasing level of knowledge in cat medicine as well as the increasing interest for the cats as a pet has prompted cat breeders to become more interested in assisted reproduction techniques in their animals. Efforts to adapt reproductive techniques currently used in dogs on a routine basis to the cat have so far been thwarted by the difficulty in collecting semen from tomcats. Work by Zambelli et al (2008, 2010) has shed new light on this aspect. Although a development of frozen semen banks similar to what has happened in the canine over the last 25 years is unlikely to occur in the near future for cats, artificial insemination can become a new tool for veterinarians doing feline reproduction work.

Semen collection - Feline semen can be collected using an artificial vagina during mating, using an electroejaculating device, performing a postcoital vaginal lavage in the queen, collecting urine by cystocentesis following ejaculation or by using a feline urethral catheter under sedation. A feline artificial vagina (AV) can be made using a 2-ml rubber pipette bulb cut off and the cut end attached to a small (4 cm long) test tube. The AV and the test tube are put into a 60-ml polyethylene bottle filled with water at 52°C thus providing an internal environment with at least 44 to 46°C. The use of an artificial vagina for semen collection requires a 2 to 3-week training period in docile tomcats, availability of an estrous teaser queens and the use of a protection for the operator's arm which the tomcat tends to bite during mounting and ejaculation. Average semen volume in the tomcat is 0.05 to 0.5 ml when collected with electroejaculation, 0.01 to 0.1 when collected with an AV (Dooley et al., 1991). Electroejaculation of tomcats has been described with an electric stimulator providing up to 180 2- to 8-V stimuli through a teflon probe which is inserted 9 cm into the rectum (Platz and Seager, 1978). Although considered a safe and reliable method, electroejaculation is not a practical procedure to be applied in a clinical setting.

Feline spermatozoa can be recovered from the vagina of a recently mated queen through a vaginal lavage using 1.0 ml of saline or by performing a vaginal smear. Lavaging the vagina may allow collection of semen samples on which sperms can be counted and morphologically evaluated, while only morphological evaluation can be performed on a vaginal cytology sample. A retrograde flow of spermatozoa at ejaculation is reported to occur in 15-90% of cases (Dooley et al., 1983; 1991). Therefore, semen presence and morphology can be assessed through collection of a cystocentesis urine sample right after breeding. The collection of urine by cystocentesis or urethral catheterization can be performed to simply assess whether testicles are capable of producing spermatozoa;

Urethral catheterization following sedation with medetomidine has been recently described by Zambelli et al (2008). This is a relatively easy technique which requires a 3 Fr or 4 Fr, 11-cm feline urinary catheter which must be cut 1.0 cm from the tip using a scalpel blade to remove the lateral openings thus obtaining a shorter, open-ended catheter. Following sedation with a single SC dose of 100 mcg/kg medetomidine, the urinary catheter is inserted for 9 cm into the urethra and then withdrawn: a minute quantity of highly concentrated semen is normally found in the more distal segment of the catheter. Such a quantity of semen is more than enough for a complete semen evaluation and can be used also for insemination by diluting it with a Tris-based diluent (see Table 2).

Semen Evaluation in cats is done similarly to the dog, although the amount of semen available for evaluation is much less, therefore the number of parameters to be assessed is lower. If semen needs to be used for artificial insemination (AI) 5 mcL are used for counting as follows:

- 5 mcL of semen in 295 mcL of water (or 5% formalin, if semen counting needs to be delayed), giving a final volume of 300 mcl with a dilution ratio of 1:60.
- Place 5 mcl of this solution in the haemocytometer and make the count.
- Multiply the final number by 10 (a factor which takes into account the volume of fluid used).
- Multiply by 60 (the dilution factor).
- Multiply by 1000 (from mcl to ml) to give the final concentration of sperm/ml.

A 5 mcl sample is used to assess motility, then after motility assessment the same drop is smeared to evaluate morphology, sediment, presence of white blood cells in the sperm sediment. We do not normally measure seminal plasma pH and alkaline phosphatase in cats. Criteria for assessing morphology, number of white blood cells as well as for judging results of bacterial culture are the same as for the dog.

12.2 Estrus and ovulation induction in queens

Ovulation must be induced in artificially inseminated queens as the insemination procedure will not achieve a stimulation of vaginal receptors sufficient to cause the release of LH from the pituitary. Ovulation induction is better performed in queens during a natural estrous period rather than during a pharmacologically induced estrus as most estrus induction regimens for cats will cause excessive ovarian stimulation. Induction of estrus in queens in anestrus can be easily accomplished by exposing queens to an increased photoperiod. The use of gonadotropins such as equine chorionic gonadotropin (eCG) followed by human chorionic gonadotropin (hCG) has been described as effective although associated with some excessive ovarian stimulation leading to formation of cystic ovaries. A more recent approach to estrus induction in queens which may be effective without causing excessive ovarian stimulation is the use of a 4.7 mg deslorelin implant, which causes the onset of estrus 5+2 days later in the majority of queens treated in anestrus or interestrus. Having a low (< 1.0 ng/ml) serum progesterone (P4) concentration is an essential prerequisite for any successful estrus/ovulation induction program for queens: the response rate is very low or absent in queens treated when serum progesterone is > 1.0 or 1.5 ng/mL. Once estrus is displayed, ovulation must be induced at the peak of estrus (which generally occurs on day 2 or 3) by IM administration of 100 IU hCG. If estrus induction is performed using deslorelin, the implant must be removed once heat is fully displayed and the ovulation induced

12.3 Artificial insemination of queens

The artificial insemination procedure should be ideally performed twice, 24 and 48 hours following hCG administration, respectively. Semen may be introduced in the vagina or directly into the uterus through the cervix. Intravaginal insemination can be accomplished using a 20 G, 9 cm long needle with a bulb connected to a 1.0 mL syringe; semen is deposited in the anterior vagina. The queen must be under general anesthesia during the procedure and then maintained in dorsal recumbency with her hindquarters elevated for 20 minutes post-AI to avoid excessive backflow of semen. Fresh or frozen semen may be used for the intravaginal deposition, although the intravaginal use of frozen semen is associated with a low success rate.

Intrauterine deposition of cat semen can be achieved surgically through a classical laparotomy approach or through laparoscopy. The surgical approach is not commonly used due to its invasiveness; an advantage of this approach is the possibility to confirm that ovulation took place and choose the uterine horn with the greatest number of ovarian follicles or ovulations for infusion of semen. Cervical catheterization has been achieved using a 2 mm glass speculum through which to insert a a 3 Fr tomcat catheter and gently push it through the cervix, although this method is unsuitable for queens whose cervix is narrower than 2 mm. The same 3 Fr catheter modified by the addition of a rounded tip needle at the end may be inserted into the vagina and guided through the cervix with the help of digital rectal manipulation, a procedure which requires some degree of practice and experience by the operator. Recently, the use of endoscopic transcervical catheterization in queens has been described using a human, semi-rigid sialoendoscope connected to a modified 3 Fr tomcat catheter with a 100 mm stainless steel, rounded tip

needle. This technique appears to be successful although more research is necessary to explore its clinical potentials; equipment cost is also a limiting factor.

Serum progesterone should be assayed 6 days after hCG administration to confirm that ovulation took place. However, the appropriateness of such blood sampling should be assessed as the entire AI protocol is somewhat stressing for the queen as the finding of a low serum P4 value does not allow to introduce corrective measures to achieve conception at this estrus cycle.

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13 ARTIFICIAL INSEMINATION IN DOGS: TECHNIQUES, BENEFITS, AND CONSIDERATIONS

Margret L. Casal, Maja Zakošek Pipan

Artificial insemination (AI) in dogs has become a valuable tool in modern veterinary practice and canine breeding. This technique involves the manual collection and deposition of semen into the reproductive tract of a female dog. AI offers numerous benefits, including genetic diversity, preservation of valuable genetic material, and the ability to breed dogs that are geographically separated. This paper delves into the various techniques of AI in dogs, its benefits, potential challenges, and considerations for successful implementation.

13.1 Techniques of Artificial Insemination

13.1.1 Vaginal Insemination

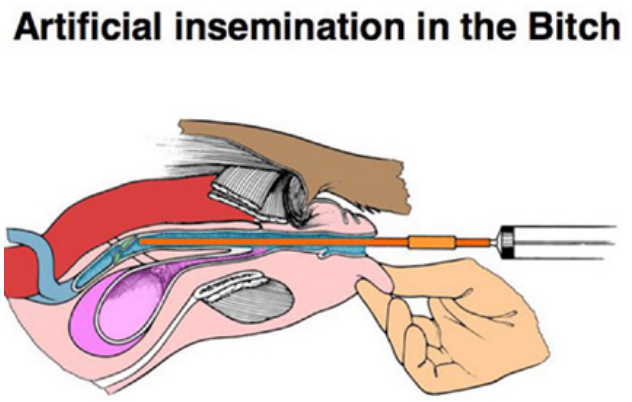

Figure 1: Vaginal insemination. Credit: University of Wisconsin, Madison

Vaginal insemination is the most straightforward and least invasive AI method. The process involves depositing semen directly into the vagina using a catheter attached to a syringe (Figure 1). This technique mimics natural mating but may have lower success rates compared to other methods if the semen quality is suboptimal or if the timing of insemination is not perfectly aligned with the female's ovulation. **Vaginal Insemination with fresh semen**: When breeders call and ask for AI, this is typically what they mean, although it is always best to question them as to the reason and their goals to make sure that the bitch is adequately timed and the procedure is reasonable. This is used when the timing is right, but the bitch will not accept the male, the owners are cautious about disease spread, or the male is not able to mount the bitch for health reasons (lameness, age, etc.). The semen is collected manually, evaluated (see semen evaluation), then loaded into a NON-rubber syringe and kept warm. The bitch is traditionally placed with her hindquarters elevated and an insemination pipette is passed into the cranial vagina to the level of the cervix (similar to how vaginal cytology is performed). The syringe is attached to the pipette and semen is deposited. A small amount of air follows to ensure that all of the semen is emptied from the pipette. Pregnancy rates and litter sizes with this technique mirror natural mating performed with similar ovulation timing. **Vaginal Insemination with chilled semen**. This is used with the stud dog is far from the bitch and travel of the bitch to the stud dog would be difficult and expensive. Typically, two collections are sent about two days apart with special semen extender designed for this purpose. Semen is generally inseminated directly out of the box as soon as it arrives at the destination. The semen should be evaluated by the collecting veterinarian and identification as well as a report should accompany the semen. A small drop of semen should be evaluated briefly by examining motility when warmed to 37 degrees C prior to inseminating the bitch. It is important to evaluate a warmed sample as chilling the semen depresses its motility. The insemination is generally done via vaginal insemination as

described above. However, alternative insemination techniques or selection of a different stud dog may be suggested if the semen is not of good quality.

13.1.2 Transcervical Insemination (TCI)

Transcervical insemination involves depositing semen directly into the uterus, bypassing the cervix. This technique can be performed using a rigid endoscope or a flexible endoscope (Figure 2). Before endoscopes were available, Norwegian or Scandinavian catheters were used, mainly in Europe (Figure 3). These required a high degree of skill, a relaxed bitch, and the ability to abdominally palpate the uterus and cervix. TCI offers higher success rates than vaginal insemination because it places the semen closer to the site of fertilization, thereby increasing the likelihood of conception. Specifically, TCI is generally performed with a long (28-35cm) rigid cystoscope with a 35-degree viewing angle is used to pass a 5-8 French catheter through the cervix. Due to the anatomy of the canine cervix, the use of a scope is extremely helpful. Fresh, chilled, or frozen semen may be used with this technique, however, the volume of the inseminate must be low (<2mLs) due to the size of the uterus. This is ideal for subfertile males to compensate for the lower normal sperm numbers. This is well tolerated by most bitches in estrus and can be performed awake with no sedation. It does require some practice on the part of the inseminator to become proficient in this technique.

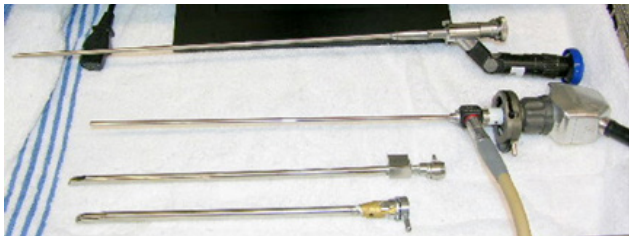

Figure 2: Rigid scopes for TCI. These are hooked up to a light source as well as a camera that can be attached to a monitor. Author: Margaret L. Casal

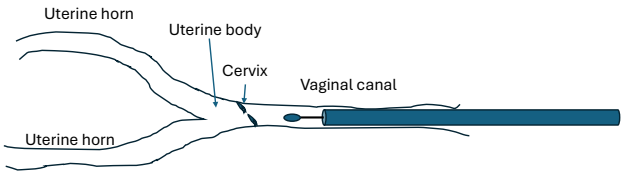

Figure 3. Norwegian catheter. Note the bulbed tip for easier access t:rough the cervix. Author: Margaret L. Casal

13.1.3 Surgical Insemination

Surgical insemination is a more invasive technique where semen is deposited directly into the uterus via a small incision in the abdominal wall. This method is typically reserved for cases where other AI techniques have failed or when the semen quality is poor. Like TCI, fresh, chilled, or frozen semen may be used with this technique, however, the volume of the inseminate must be low (<2mLs) due to the size of the uterus. This is ideal for subfertile males to compensate for the lower normal sperm numbers. The advantage is that no specialized equipment is needed, and this can be performed by any veterinarian that is proficient at a spay surgery. Disadvantages include only one insemination per cycle due to the invasiveness of the procedure, the requirement for general anesthesia, and the risk for surgical complications. The technique requires general anesthesia and aseptic preparation of the abdomen. A short midline incision (~2 cm) is made and both cranial uterus horns exteriorized. A 22G short (1 cm) IV catheter is placed into one horn directed cranially (Figure 4). The stylet is removed, and half of the semen is injected. The catheter can now be moved while pinching the uterus around the insertion site of the catheter for a few seconds to prevent semen leaking out into the abdomen and bleeding. Thereafter, the process is repeated on the other uterus horn. It is absolutely imperative that semen does not leak into the abdominal cavity, as this may lead to peritonitis. Lastly, there are countries in which this method is not allowed.

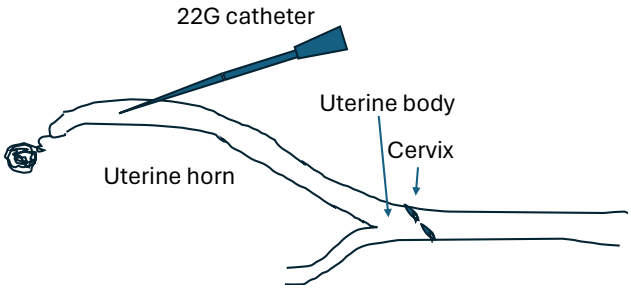

Figure 4: Diagram showing surgical insemination. The catheter will be attached to a non-rubbered syringe containing the semen. Author: Margaret L. Casal

13.1.4 Intratubal Insemination

Intratubal insemination involves placing semen directly into the fallopian tubes. This method is not commonly used in dogs due to its complexity and the need for specialized equipment and expertise. However, it may be considered in specific cases where other methods are unsuccessful.

13.2 Benefits of Artificial Insemination

13.2.1 Genetic Diversity

AI allows breeders to access and use genetic material from dogs located anywhere in the world. This ability to introduce new genetic lines helps prevent inbreeding and promotes genetic diversity, which can improve the overall health and vigor of the breed.

13.2.2 Preservation of Genetic Material

AI facilitates the preservation of semen from valuable or genetically superior dogs, which can be used long after the donor dog is no longer available for natural breeding. This is particularly important for endangered breeds or those with limited gene pools.

13.2.3 Overcoming Physical and Behavioral Barriers

AI is beneficial for breeding dogs that have physical or behavioral issues that prevent natural mating. For example, dogs with anatomical abnormalities, size mismatches, or aggression can still reproduce through AI.

13.2.4 Disease Control

AI reduces the risk of transmitting sexually transmitted infections (STIs) between breeding pairs. By using AI, breeders can ensure that the semen is free from pathogens, thereby protecting the health of both the female and her potential offspring.

13.3 Challenges and Considerations

13.3.1 Timing and Ovulation

Detection Accurate timing of AI is critical for success. Understanding the female dog's estrous cycle and precisely detecting ovulation are essential to ensure that the semen is introduced at the optimal time for fertilization. Techniques such as vaginal cytology, hormone assays, and ultrasonography are commonly used to determine the best time for insemination but typically, serum progesterone levels and vaginal cytology in concert with each other are ideal for accurate timing. Briefly, the bitch ovulates primary oocytes that must undergo a maturation that involves two meiotic divisions before they can be fertilized. This process takes about 2 days, and the mature oocytes can then survive for 2-3 days. Also, the sperm must undergo capacitation in the female's reproductive tract before it is capable of fertilization, which requires about 7 hours. Best conception rate occurs when the bitch is bred from 4 days before to 3 days after ovulation; best litter size is achieved when the bitch is bred 2 and 4 days after ovulation.

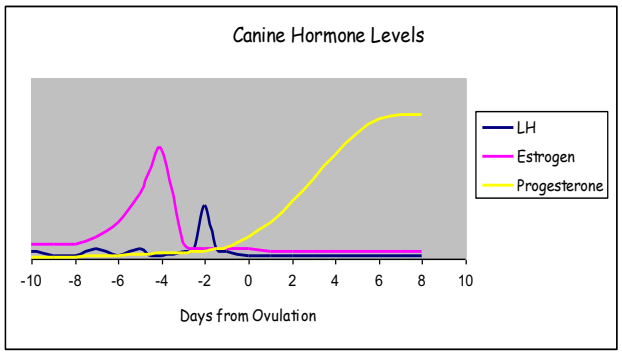

Figure 5. Diagram showing relative serum hormone levels during estrus. Author: Margaret L. Casal

13.3.2 Semen Quality and Handling

The quality of semen used in AI significantly impacts the success rate. Factors such as sperm count, motility, and morphology must be evaluated. Additionally, proper handling and storage of semen are crucial

to maintain its viability. Fresh, chilled, or frozen semen can be used, with each type requiring specific protocols for collection, storage, and thawing.

13.3.3 Ethical and Welfare Considerations

While AI offers numerous benefits, it is essential to consider the ethical implications and welfare of the animals involved. The procedures should be performed by trained professionals to minimize stress and discomfort. Furthermore, the decision to use AI should prioritize the health and well-being of both the female dog and her potential offspring.

13.3.4 Regulatory and Breeding Standards

Breeders must adhere to regulatory guidelines and breeding standards set by kennel clubs and veterinary organizations. These standards ensure that AI is used responsibly and ethically, promoting the health and sustainability of the breed.

13.4 Conclusion

Artificial insemination in dogs is a powerful tool that offers significant advantages in canine breeding. It allows for greater genetic diversity, preservation of valuable genetics, and the ability to overcome various breeding challenges. However, successful AI requires careful consideration of timing, semen quality, and ethical standards. By adhering to best practices and regulatory guidelines, AI can enhance breeding programs and contribute to the health and longevity of dog breeds.

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14 GATHERING AND EXAMINING FRESH SEMEN, ASSESSING SEMEN QUALITY AND CHILLING TECHNIQUES

Nataša Šterbenc

Interest in dog and cat breeding has increased considerably in recent decades. There is a great demand for assisted reproduction in dogs and cats, for the efficient breeding of companion animals and to ensure the diversity of genetic resources. This applies in particular to valuable stud dogs such as assistance dogs, stud dogs of rare dog breeds, genetically important dogs and dogs belonging to endangered dog species. Domestic cats can serve as a model for the reproduction of exotic and endangered cat species. Assisted reproduction in cats is mostly used for endangered and wild species, genetically valuable male cats as well as for domestic cats.

14.1 Gathering Fresh Semen from male dog

For the stud dog, the preliminary examinations are of great importance and include the breeding history, medical history and any medication or supplements administered in at least the last 6 months, as well as information on the environment and genetic or family factors. Semen samples from male dogs may be collected for the purposes of artificial insemination, cryopreservation, evaluation of semen quality and fertility assessment. The most commonly used method of semen collection in dogs is digital manipulation. Semen can also be obtained from the epididymis after castration, by electroejaculation or, less frequently by vaginal lavage after natural mating. For collecting the semen in male dog, teaser bitch in heat need to be present. In some cases, the most experienced dogs will ejaculate with simple hand stimulation, whereas in-experienced or younger dogs may require a teaser bitch in heat. The presence of a bitch in proestrus or estrus can increase the quality of the semen, especially the sperm concentration. Sometimes a bitch in heat may not be present, in which case vaginal swabs from healthy, *Brucella canis*-negative bitches in estrus can be preserved within gauze sponges from earlier examinations in a regular freezer (-20 °C) for future use, or canine pheromones can be used. If the stud dog is not familiar with a teaser bitch we will let them some time to get-to-know each other. Young inexperienced or usually nervous dog can sniff and play with the bitch before collection. All equipment should be at hand and warmed to body temperature and the dog should be walked before collection to allow opportunity to urinate. While the dog is sniffing the bitch's perineal area, the semen collector kneels or crouches on the left side of the dog (vice versa for left-handed people). He massages the foreskin over the penis with his right hand and moves the prepuce up and down the shaft of the penis. The semen collector's left hand holds the canine semen collection bag with top folded and places it on the tip of the foreskin. When the dog's penis has reached an erection of about half its size, the foreskin with the semen collection bag is pushed behind the *bulbus glandis*. If the *bulbus glandis* has enlarged to such an extent that the foreskin behind it can no longer be moved, remove the dog from the bitch and allow the erection to subside before trying again. Complete erection and ejaculation while the bulb is inside the prepuce can be painful and will result in incomplete semen release. A circular, firm pressure should be applied with the left hand to simulate copulatory closure or binding. The ejaculate is composed of three fractions: first (pre-sperm), second (sperm-rich) and third (prostatic fluid). The first fraction of semen is produced by the prostate and the urethral glands as a method to clear the urethra and distal prepuce of urine, cellular debris, dead sperm, and WBC. The first fraction is clear in quantity of 2 to 8 ml or more. The second milky-white semen-rich fraction in quantity of 0.5 to 2 mL (depending on the size of the dog) originates from the epididymis, is ejaculating during the fastest pelvic thrust and then descend and lift a hind leg as if trying to step over the bitch and reach the bond. The collector can feel a rhythmic pulsation of the penile urethra. Continue to apply pressure until crystal clear prostatic fluid is seen in the semen collection bag.

At this point, the penis is rotated approximately 180 degrees and directed caudally, continuing to firmly squeeze the *bulbus glandis* until the third prostatic semen fraction is ejaculated. The third fraction is the largest in volume (20 – 80 ml) and derives from the prostate only. Its purpose is to flush the urethra of all sperm that have been ejaculated, to provide a medium for sperm to swim in and to fill the vagina with fluid during a natural breeding to facilitate the sperm reaching their destination at the cervix.

After semen collection, the bitch is removed from the room while the dog is released. The dog will continue to ejaculate the rest of the prostatic fluid, which may take between 5 and 15 minutes. After the dog has finished ejaculating, the dog should be monitored until the penis is back within the prepuce. Some dogs prefer standing still, whereas others benefit from slow walking. Before the dog leaves the clinic, the vet must ensure that there is no *paraphymosis* issue, i.e. the inability to retract the penis into the prepuce due to constriction of the preputial orifice. *Paraphymosis* has been diagnosed in male dogs after semen collection.

Semen collection by electroejaculation is ethically questionable and is very rarely used in dogs.

14.2 Gathering Fresh Semen from tom cat

Semen samples from male cats can be obtained using various techniques, such as artificial vagina, electroejaculation, urethral catheterisation after sedation with medetomidine and collection of epididymal sperm. Collection with the artificial vagina usually requires a trained tomcat. The artificial vagina is made from a small rubber bulb for a Pasteur pipette and a small test tube which is warmed at 37°C. The tom is allowed to mount a female cat in heat and grabs her by the neck to restrain her. The artificial vagina is placed over the male's glans penis while he searches for the opening of the vulva with his thrusts. However, not all tomcats allow this procedure because of the behaviour and temperament of this species. Therefore, this method is largely restricted to research centres with large breeding colonies of females and trained males, while it is difficult to use with males that are only occasionally presented in daily practise. Electroejaculation is another technique for sperm collection in which the male cat has to be anesthetized during the procedure by using medetomidine (80-100 µg/kg IM) and ketamine (5 mg/kg IM) and a rectal probe with 3 electrodes is lubricated and inserted 6 to 8 cm deep into the rectum with electrodes directed ventrally. A series of electrical stimuli is then applied intermittently and are of low frequency. Different stimulation protocols for electroejaculation in cats have been described, but many use that of Howard et al. (1990) which consists of a total of 80 electrical stimuli of 2 – 5 volts applied in three consecutive series (30, 30 and 20 stimuli), plus 2 – 3 minutes of rest. To obtain semen, the cat's penis is extruded by applying light pressure to the base of the penis and the ejaculate is collected in a pre-warmed test tube placed over the glans penis. With this method, sperm can be obtained without prior training or the presence of a female in heat and is considered the method of choice for semen collection in non-domestic felids. However, it is ethically questionable and is therefore prohibited in several countries. A method that does not require training and is not ethically questionable is urethral catheterization. The method requires sedation of the cat with medetomidine (100-150 µg/kg), which stimulates the α2-adrenergic receptors and enables the release of a small amount of highly concentrated semen from the epididymal cavity into the urethra. The semen is collected using a urinary catheter (Buster® Cat Catheter, 1.0 mm x 13.0 cm), the tip of which is cut off to obtain a shorter catheter with an open end, which is inserted approx. 9 cm into the urethra, taking care to ensure that it does not reach the bladder. The catheter is then removed from the urethra and the semen sample is collected. Immediately after collection, the sperm sample is placed in a pre-warmed Eppendorf tube with a diluent. Currently, this method is probably the most practical and the least invasive procedure for collecting sperm from male cats in daily practise. Semen can also be obtained from the epididymis after castration, especially in genetically valuable males that have died unexpectedly.

14.3 Examining Fresh Semen and Assessing Semen Quality

The aim of semen evaluation is to predict the fertilizing capacity of a semen sample. Only males with optimal fertility produce semen with a high number of progressively motile, viable and morphologically normal spermatozoa. There are four basic components of semen evaluation: determination of ejaculate volume, percentage of progressively motile sperm, number of spermatozoa per ejaculate and percentage of morphologically normal spermatozoa.

The semen must be analysed immediately after collection. The semen should be protected from abrupt changes in temperature, excessive movement, water, germicides and cleaning agents. To avoid damaging the semen, it should be stored between body temperature (37 °C) and room temperature (20 °C). Equipment that comes into contact with the semen should be warmed to body temperature (37 °C).

All glassware (slides, coverslips, pipettes) should be warmed up before use. A warm microscope stage is ideal. Routine methods for evaluating semen quality are evaluation of colour, volume, motility (% of total and progressively motile spermatozoa), concentration and morphology. The volume should be determined using a syringe without a rubber stopper, as the latex of the stopper can have a spermicidal effect. The total sperm per ejaculate is dependent on a variety of factors. Very young and very old dogs produce less sperm. Sperm concentration is measured using counting chambers (haemocytometer) or a spectrophotometer, and motility is analysed using a phase contrast microscope. The concentration of spermatozoa in the sample is determined and multiplied by the total volume of the sample to determine the total number of sperm in the ejaculate. The subjective standard motility assessment method is performed using a phase contrast microscope. 10 µl of the sperm sample is placed on a pre-warmed (37 °C) slide and covered with a cover glass. At least 200 spermatozoa are observed in a sperm monolayer at 100-200x magnification. The overall motility and viability of the spermatozoa are analysed after incubating the samples in a water bath at 37 °C for 10 minutes. The objective measurement of sperm motility, kinematics and concentration is performed with a computer-assisted semen analyser (CASA) in a Makler counting chamber® warmed to 37 °C. Five automatically selected fields are analysed per sample. Motility is analysed as total and progressive motility. Total motility is the number of sperm that move, while progressive motility is the number of sperm that move in a straight line across the microscope's field of view. Sperm viability is analysed using the Viadent® assay to determine viable spermatozoa, according to the manufacturer's instructions. For viability staining, 30 µl of the prepared stain is incubated (Hoechst 33258) in a water bath (37 °C) for 5 minutes, 30 µl of semen sample is added and mix gently. The stained sample is incubated for an additional 5 minutes before analysis. During the incubation time, the stain penetrates the non-viable spermatozoa heads. By comparing the cells visible under standard and fluorescent illumination, the analyser can easily distinguish the live cells from the dead cells.

The hypoosmotic swelling (HOST) test evaluates the functional integrity of the sperm's plasma membrane and also serves as a useful indicator of its fertility potential. The hypoosmotic swelling test presumes that only cells with intact membranes (live cells) will swell in hypotonic solutions. The procedure is described by Jeyendran et al., (1992) an aliquot of 50µL of semen is diluted in 500 µL of hypoosmotic solution and incubated at 37°C for 30 min. A total of 200 spermatozoa are counted. Percentage of spermatozoa population with swollen and/or coiled tail is scored under a phase contrast microscope (400x magnifications).

In order to evaluate morphology, the semen must be stained and examined under very high magnification. The morphology of spermatozoa is determined after staining with numerous staining agents such as Wright-Giemsa, Diff-Quik, Papanicolaou, eosin-nigrosin, etc. The eosin-nigrosin stain is not only used for morphology, but is also considered a vital stain. If applied correctly to the sperm, it can also be used to test viability. Spermatozoa that are alive when the stain is mixed will not allow any dye to diffuse across their membranes, so they will appear white under the microscope; sperm that are dead when the stain is mixed will allow a pink dye to diffuse through the cell membranes, so they will appear pink under the microscope. The semen samples are analysed after incubation in a water bath at 37 °C for 10 minutes. Slides with fixed and stained semen samples are examined under a light microscope at 1000x magnification, whereby at least 100 spermatozoa are examined in each sample. The morphological changes are presented as a proportion of the total morphologically abnormal spermatozoa. The total morphologically abnormal spermatozoa are further divided into head abnormalities, acrosome abnormalities, neck/midpiece abnormalities, tail abnormalities and the presence of proximal, medial and distal cytoplasmic droplets. The morphological changes are classified according to anatomical location (head, neck, midpiece, tail), origin of the defect: primary (defect originates in the testis during spermatogenesis) and secondary (defect originates in the epididymis during maturation) and perceived negative impact on fertility: minor (structural defects but not with reduced fertility: compensable) and major (defects with proven infertility: non-compensable). For more detailed analyses, flow cytometry and some common semen tests are used to determine various characteristics and functions of the sperm (viability, acrosome, membrane and DNA integrity, etc.). All young dogs should have high semen quality with 85–90% motile, ≥70% progressively motile and >70% morphologically normal spermatozoa. Total ejaculate volume and concentration varies depending on the amount of prostatic fluid collected. Normal canine ejaculate is the colour of skim milk. The total number of spermatozoa should be 200–300 x 10^6, but this will vary with size of dog and ejaculation frequency The volume of the ejaculate of domestic cats is quite small and can best be measured with a variable micropipette. The volume of semen obtained by artificial vagina averages 0.034–0.04 ml (range 0.01–0.12 ml), by electroejaculation averages 0.076–0.22 ml (range 0.019–0.74 ml). The semen concentration obtained by artificial vagina averages 1730 x 10^6/ml (range 96 – 5101 x 10^6/ml), that by electroejaculation averages 168–361 x 10^6/ml. The total number of spermatozoa in the cat's ejaculate obtained with the artificial vagina is on average 57–61 x 10^6, with electroejaculation

on average 12–30 x 10^6. The quality of cat semen with highly variable motility averages 56–84%, and the large individual variation of morphologically normal spermatozoa averages 38.2% to over 90%.

14.4 Chilling Techniques and Freezing technique

Lowering the temperature by chilling or freezing the spermatozoa reduces the metabolic rate and thus increases the lifespan of the spermatozoa. As spermatozoa are relatively metabolically inert, the extracellular environment plays a crucial role in their survival. Sperm that are stored either chilled (4°C) or frozen (-196°C) should therefore be diluted in species-specific extenders. Semen extenders provide energy, stabilise pH and osmolarity, protect the spermatozoa membrane from injury during cooling and may contain antibiotics to prevent bacterial growth during transport or storage. Semen extenders should have general properties such as osmotic pressure, nutrient medium, absorption of metabolic residues and protection of spermatozoa from the harmful effects of cold. There are currently many different extenders on the market. In general, an extender should protect the spermatozoa from cold shock during cooling (by adding 10-20% egg yolk or skimmed milk), provide energy substrates (sugars such as fructose or glucose) and maintain the spermatozoa at a constant pH and osmolarity by adding buffers. To prevent the growth of bacteria, semen extenders should also contain some antibiotics (usually penicillin or streptomycin). If the semen is frozen, cryoprotectants such as glycerol should be added. These extenders can be prepared by veterinarians themselves or can be purchased from various commercial companies. Since egg yolk is a biohazardous compound, studies are being conducted in which egg yolk is replaced with egg yolk-derived phospholipids or vegetable lecithin to avoid the use of animal-derived substances. Extenders based on Tris-citric acid-egg yolk fructose (also known as Uppsala extenders) are probably the most commonly described and used extenders for the preservation of canine semen (1). Most commercially available extenders recommend a dilution ratio of at least one part semen to four parts of extender for maximal motility and fertility during cooled shipment or storage.

While cryopreservation of canine semen is a complicated and time-consuming process, the chilling of canine semen can be performed by veterinarians in their practises, provided they have acquired the basic knowledge of semen chilling and dilution. Immediately after collection and quality assessment, the semen is centrifuged at 3000 x g for 5 minutes and diluted 1:4 with a suitable extender and gradually chilled to 4 °C. The semen is then stored in a cool, dark place. Cooling the sample during storage reduces the metabolic rate, resulting in a longer sperm life. The quality and longevity of the semen after storage can be affected by the temperature to which the semen is cooled. Semen transported for insemination purposes should be chilled to 4 to 5 °C, as found in various studies over the last decade. Canine semen stored at 4 °C has a significantly longer lifespan than semen stored at 22 °C (2). Semen parameters are also preserved longer. Although various researchers have found spermatozoa viability to be around four to five days, semen can maintain good quality for at least 24 hours under ideal packaging and shipping conditions. Chilled semen can be transported short distances at 4°C in a thermos flask, a polystyrene box or a Minitübe neopore box.

If semen is going to be frozen, a cryoprotectant is required. The most common cryoprotectant is glycerol. When freezing canine semen, the optimal concentration of glycerol, the most commonly used cryoprotectant, varies between 2 - 4%. Before being exposed to low freezing temperatures, canine semen should equilibrate at a certain temperature and adjust for a period of time to allow the spermatozoa to develop a higher resistance to the effects of freezing. Equilibration should take place at 4 °C, and for canine semen 1-2 hours is usually ideal. Canine semen can be frozen in 0.25 ml and 0.5 ml straws. For identification purposes, the following information should be clearly labelled on the straw: breed, name, registration number or microchip number, date and place of freezing. For commercial purposes, controlled dynamic freezing, i.e. a variable nitrogen vapour flow, is recommended. However, these programmable freezers are expensive to purchase and operate, especially as dogs are usually only frozen 10 to 20 straws per run. Therefore, the method in which the straws are placed on a rack 4 cm above the surface of the liquid nitrogen in a styrofoam box for 10 minutes in liquid nitrogen vapour is usually used. The semen is stored in liquid nitrogen (-196°C) for an indefinite period of time.

Cat semen can be prepared for the chilling process. The semen sample is extended with an extender and subsequently cooled and stored at +5°C. This procedure allows short-term storage: overnight, for 24 hours or sometimes even up to 5–7 days.

The protocol for cryopreservation of cat semen was originally developed for canine semen. After collection of a very small amount of semen, it must be treated by dilution with a specific extender and/or concentration by centrifugation before cryopreservation. The speed and duration of centrifugation varies,

but 300 x g for 6–10 minutes has been commonly used. For feline semen, it is suggested that 4–5% glycerol is optimal for cryopreservation of feline semen.

Chilled and frozen semen has many advantages, such as obtaining multiple doses from one ejaculate, transporting semen to different locations, and with the addition of an appropriate extender, chilled semen can be stored at 5°C for 3 to 4 days, whereas frozen semen has no shelf life under suitable storage conditions.

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