**Universidade de Évora - Escola de Ciências e Tecnologia**

Mestrado Integrado em Medicina Veterinária

Dissertação

# Endoscope-assisted prefemoral ovariectomy and ovariosalpingectomy in chelonians: a preventive and curative procedure

João André Gabão Martins

Orientador(es) | Maria Dias
Maria Teresa Oliveira
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A dissertação foi objeto de apreciação e discussão pública pelo seguinte júri nomeado pelo Diretor da Escola de Ciências e Tecnologia:

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Évora 2024

*To my beloved Filipa,*

*for teaching me even the sky is not the limit*

## Acknowledgments

Reaching this milestone has gotten me reminiscing about what a fun and challenging journey this was and how none of it would have been possible without an amazing support system.

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# Abstract

Female chelonians are frequently affected by a broad spectrum of reproductive diseases, with surgical correction being the preferred treatment for most disorders. This dissertation includes a literature review of the diagnostic and therapeutic approach to the most observed reproductive disorders in chelonians and a retrospective study of 36 cases of chelonians submitted to endoscope-assisted prefemoral ovariectomy and ovariosalpingectomy. The study aimed to characterize the affected population, the diagnostic and therapeutic approach, and the overall surgery, including complications, endoscopic findings, and outcomes associated to the latter. The surgery was successful in 94.4% of chelonians, given the two casualties reported. Complications were present in 86.1% of cases with 63.9% of patients exhibiting yolk coelomitis. During the first week of the postoperative period, 85.7% of females were found to have recovered uneventfully. The endoscope-assisted surgery appears to be a feasible and versatile option for treatment and prevention of reproductive disorders in chelonians.



**Keywords:** Chelonians; endoscopy; ovariectomy; ovariosalpingectomy; reproductive disease.

# Resumo


## Ovariectomia e ovariosalpingectomia pelo acesso pré-femoral com auxílio endoscópico em quelónios: um procedimento preventivo e curativo

As tartarugas fêmea são comummente acometidas por diversas doenças reprodutivas cujo tratamento de eleição passa frequentemente pela cirurgia. Esta dissertação engloba uma revisão bibliográfica da abordagem clínica às afeções reprodutivas mais prevalentes em quelónios fêmea e um estudo retrospetivo de 36 quelónios submetidos a ovariectomia ou ovariosalpingectomia pelo acesso pré-femoral com auxílio endoscópico. Este estudo pretendeu caracterizar a população afetada, a abordagem diagnóstica e terapêutica bem como o procedimento cirúrgico, evidenciando as complicações, os achados endoscópicos e os desfechos deste último. A cirurgia teve sucesso em 94,4% das tartarugas tendo sido registadas duas mortes. Foram observadas complicações em 86,1% dos casos, dos quais 63,9% exibiram celomite por gema de ovo. Uma semana após a operação, 85,7% das tartarugas tinham recuperado sem intercorrências. A esterilização realizada pelo acesso pré-femoral com auxílio endoscópico é uma opção prática e versátil para o tratamento e prevenção de doenças reprodutivas em quelónios.



**Palavras-chave:** Quelónios; endoscopia; ovariectomia; ovariosalpingectomia; doença reprodutiva.

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## List of Abbreviations

**AST** – Aspartate transaminase

**Ca –** Calcium

**$CO_2$** – Carbon dioxide

**IM** – Intramuscularly

**IV** – Intravenously

**LED –** Light-emitting diode

**MBD** – Metabolic bone disease

**NSAIDs** – Nonsteroidal anti-inflammatory drugs

**PCV** – Packed cell volume

**P** – Phosphorus

**POFS** – Preovulatory follicular stasis

**UA** – Uric acid

**TWBC** – Total white blood cells

**WBC** – White blood cells

# Preface

The present dissertation was written after the conclusion of a curricular internship at Exòtics Veterinària clinic in Barcelona, under the mentorship of Doctor of Veterinary Medicine Xavier Valls, specialist in exotic animal medicine. From the 1st of November until the 31st of March, the author participated in the clinic's routine activities, performing and assisting several clinical and surgical procedures in exotic animal species, with a special focus on haematology, radiography, ultrasonography, and endoscopy.

Given the author's ever so growing interest in herpetology, it was particularly beneficial to follow and learn from Doctor Xavier Valls' consults, and diagnostic and therapeutic approaches to numerous species of reptiles, as well as some amphibians. Specialized in endoscopy of exotic animals, the mentor provided the author the opportunity to closely observe and even practice common endoscopic techniques in avian and reptilian species.

In this context, it was made possible the elaboration of a study involving chelonians admitted for endoscope-assisted prefemoral ovariectomy and ovariosalpingectomy and retrieval of the according data.

# I. Literature Review

## 1. Female Chelonian Reproductive System

### 1.1. Anatomy

The female chelonian has two ovaries in the caudal half of the coelomic cavity, cranial to the kidneys, suspended dorsally from the mesovarium (Kuchling, 1999; McArthur et al., 2004; Chitty & Raftery, 2013a). They are made of blood vessels, nerves, connective tissue, epithelial cells, and germinal beds involved in a flexible tunic (Jacobson et al., 2021). The juvenile ovaries have a slender and elongated shape, occasionally appearing as lobular. The primordial follicles consist of a single squamous epithelial layer, a nucleus and ooplasm, and can be identified as pale granules on the surface of the ovary (Kuchling, 1999). Reproductively active mature ovaries present a hierarchy of follicles at different stages of development and vary in size depending on the stage of follicular activity (Chitty & Raftery, 2013a; Jacobson et al., 2021). At this stage, the follicles can either be previtellogenic (without yolk) or vitellogenic (contain yolk) (Stahl & DeNardo, 2019). Ovaries in preovulatory phase can occupy a significant area within the coelomic cavity and are comprised of numerous yellow follicles surrounded by a stretched ovarian wall (Kuchling, 1999).

The oviducts, supported by the mesosalpinx, follow ventrally and caudally to the ovaries (Kuchling, 1999; Innis & Boyer, 2002; Jacobson et al., 2021). In immature chelonians, the oviducts are straight and thin tubes, becoming wider and longer in mature chelonians (Kuchling, 1999). The oviduct contains mucous cells with cilia as well as nonciliated cells, and it also includes glands located beneath the epithelial lining responsible for the secretion of albumen and calcareous substances, which contribute to the formation of the shell and shell membranes (Stahl & DeNardo, 2019). Histologically, it can be divided into five segments: cranially to the ovaries, the thin and flattened *infundibulum* or *ostium*; leading caudally to the *pars albuminifera*; followed by the isthmus or aglandular portion; then the thicker walled shell gland or uterus; leading to the short vagina that opens into the urodeum of the cloaca (Kuchling, 1999; Girling, 2002; Chitty & Raftery, 2013a).

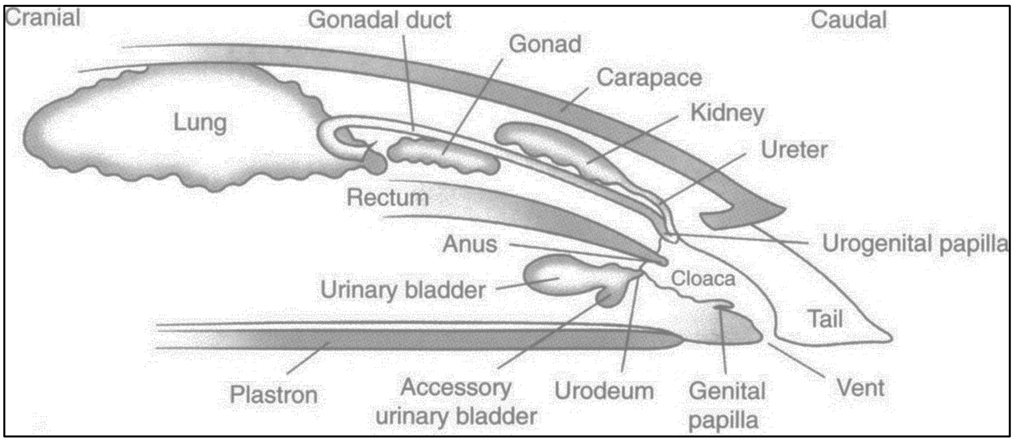



Fig. 1. Diagram of the anatomy of the chelonian cloaca and associated body systems (adapted from Hernandez-Divers, 2004).

## 1.2. Ovarian Cycle and Physiology

Identical to mammals, ovarian activity tends to be regulated by seasonal changes that influence the hypothalamic-pituitary-gonadotropin endocrine system (Vitt & Caldwell, 2014).

In temperate climates, chelonians are usually reproductively active during the warmest months of the year, where the length of day is longest (Perry & Mitchell, 2017). The ovulation and fertilization of female individuals occur during the spring season, followed by nesting in late spring and summer. New folliculogenesis starts in late summer and fall for next year's cycle (McArthur et al., 2004; Perry & Mitchell, 2017).

Changes in day length and temperature are minimal in tropical climates, causing reptiles in these regions to breed continuously throughout the year. Nonetheless, some species reproductive cycles are linked to variations in precipitation, being more active during rainfall or, otherwise, during dry season (McArthur et al., 2004; Blanvillain et al., 2011).

In the wild, sexual maturity is generally reached much slower than in captivity, leading to believe it is greatly influenced by rate of growth and size of the chelonian. For example, wild leopard tortoises, as old as 15 years, have been observed to not have yet reached sexual maturity, whereas captive individuals of the same species can often reproduce from four to six years. (Innis & Boyer, 2002; McArthur et al., 2004; Boyer & Charles J., 2019).

Folliculogenesis initiates with the secretion of a gonadotropin by the pituitary. Chelonian gonadotropins seem analogous and identical in function to follicle stimulating hormone and luteinizing hormone of mammals (Jacobson et al., 2021). The gonadotropin will stimulate the previtellogenic follicles to secrete oestradiol. The latter will, in turn, stimulate the liver to produce vitellogenin from the body's fat stores, which is then transported by the blood, and stored in the maturing oocytes. This process is called vitellogenesis (Vitt & Caldwell, 2014; Perry & Mitchell, 2017; Boyer & Charles J., 2019). During this period, the liver increases in size and exhibits a yellow hue, which is attributed to a physiological increase in intrahepatic fat and should not be confused with pathological hepatic lipidosis (Stahl & DeNardo, 2019). Increased concentrations of total protein, albumin, globulin, calcium (Ca), phosphorus (P), triglycerides, and cholesterol may be observed at this time (McArthur et al., 2004; Portas, 2017b).

The mechanism behind ovulation is yet to be fully comprehended, although surges in luteinizing hormone, progesterone, and testosterone have been reported during ovulation in numerous chelonian species (Jacobson et al., 2021). Most species can ovulate without copulating, and some only in the presence of a male counterpart (McArthur et al., 2004 Sykes, 2010; Boyer & Charles J., 2019). After ovulation, proliferating granulosa cells form the corpora lutea, that will liberate progesterone throughout the rest of the cycle (Jacobson et al., 2021).

Once ovulation occurs, the ova enter the oviducts where they can be fertilized in the infundibulum (Stahl & DeNardo, 2019). Female chelonians can reserve sperm for months to years, so copulation normally takes place before ovulation (Perry & Mitchell, 2017; Boyer & Charles J., 2019). The developing ova is then enveloped in membranes and albumin layers deposited by the albumin gland, followed by shell membranes and the eggshell when reaching the shell gland (McArthur et al., 2004; Sykes, 2010; Boyer & Charles J., 2019). Most species can retain the eggs for one to two months before oviposition. Gravid females may exhibit increased basking behaviour and partial or complete anorexia (Portas, 2017b). The formed egg then passes through the vagina, into the urodeum, and is finally expelled from the cloaca. At oviposition, female chelonians commonly excavate a nest, lay their eggs and cover them (Sykes, 2010). Progesterone plays a crucial role in facilitating the development of eggs. Following oviposition, a decline in concentrations of oestrogen, progesterone, and testosterone has been observed in many chelonian species (Jacobson et al., 2021).

All chelonians are oviparous. The eggs can either be soft or, more frequently, hard-shelled. Depending on the species, they can produce one or more clutches of eggs per breeding season, each clutch composed of single or multiple eggs (McArthur et al., 2004; Boyer & Charles J., 2019). However, the quantity of eggs deposited by a particular species may not be consistent (McArthur et al., 2004).

After nesting season, the corpora lutea regresses and the mature follicles that did not ovulate may undergo atresia, where the yolk is reabsorbed by infiltrative histiocytes and leucocytes. Ultimately, both the corpora lutea and regressing follicles convert into corpora albicans (Perry & Mitchell, 2017; Boyer & Charles J., 2019).

## 2. Diseases of the Female Chelonian Reproductive Tract

### 2.1. Preovulatory Disease/Preovulatory Follicular Stasis

Preovulatory follicular stasis (POFS), or follicular stasis, is a disease commonly reported in captive held reptiles that occurs when females fail to ovulate and to reabsorb matured follicles, likely repeating this process and accumulating follicles over successive cycles (Rivera, 2008; Portas, 2017a; Stahl, 2019a).

Multiple factors are implicated in the normal chelonian reproductive cycle. Inadequate nutrition and environmental conditions have often been associated with the development of this syndrome (Perry & Mitchell, 2017; Portas, 2017a; Roberts & Warner, 2020).

The static follicles may become necrotic and friable, and produce inspissated yolk, eventually rupturing and leading to chronic coelomitis (Rivera, 2008; Perry & Mitchell, 2017). Granulomatous and heterophilic infectious oophoritis is often seen secondary to chronic POFS, as yolk contained by the vitellogenic follicles is a great culture medium for circulating bacteria to colonize. The ovaries will frequently present adherences between follicles and to surrounding structures (Knotek, et al., 2017a; Johnson, 2019; Stahl & DeNardo, 2019).

Anorexia and lethargy are consistently the most common signs demonstrated by chelonians (Perry & Mitchell, 2017; Johnson, 2019). Coelomic distension, restlessness, pacing, and behavioural changes may also be observed (Stahl & DeNardo, 2019).

### 2.2. Periovulatory Disease

During ovulation, follicles may not proceed as expected through the oviduct due to obstructions, adhesions, retrograde oviduct peristalsis, trauma, or others, releasing ova into the coelomic cavity. These ectopic follicles will, over time, generate inspissated yolk, dystrophic mineralization, and inflammation (Mans & Sladky, 2012; Roberts & Warner, 2020).

### 2.3. Postovulatory Disease/Dystocia

Dystocia, egg-binding, or postovulatory egg stasis is defined as failure to complete parturition (or oviposition) within the acceptable time for a given species, and can essentially be classified as obstructive or nonobstructive (McArthur, 2004; Perry & Mitchell, 2017; Stahl, 2019a; Hellebuyck & Vilanova, 2022).

In captive reptiles, nonobstructive dystocia can arise from various underlying causes (Fig. 2), often happening concurrently, making its diagnosis more challenging (Innis & Boyer, 2002; Johnson, 2019; Stahl & DeNardo, 2019). It's commonly associated with poor physical condition of the chelonian and/or hypocalcaemia (McArthur, 2004; Hellebuyck & Vilanova, 2022). Inadequate nutrition, temperature, humidity, and substrate are all common predisposing factors to nonobstructive dystocia (Perry & Mitchell, 2017; Stahl & DeNardo, 2019; Hellebuyck & Vilanova, 2022). If the female is unable to dig a nest as deep as the length of its shell due to the harsh or shallow nature of the substrate, oviposition is likely to fail (Innis & Boyer, 2002).

Obstructive dystocia can be caused by several mechanical factors (Fig. 3), whether they be maternal or foetal in their origin, that prevent the regular progression of the eggs through the oviduct and cloaca (Roberts & Warner, 2020; Hellebuyck & Vilanova, 2022). This condition may develop due to compression of the oviduct from space-occupying processes in the coelomic cavity, oviductal torsion, strictures or adhesions, fractures or deformities of the pelvis, physical immaturity, prolapses of the reproductive tract, bladder or cloaca, inflammatory diseases, abnormal egg size, shape or mineralization, fractured eggs, and previously retained eggs or remnants of these (Rivera, 2008; Perry & Mitchell, 2017; Johnson, 2019; Stahl & DeNardo, 2019).

It may be difficult to assess whether the female is overdue or in need of immediate intervention, however, it is advised to intervene once the patient appears to be consistently straining without successfully being able to lay its eggs (McArthur, 2004). Even though dystocia is seldom a life threatening condition in chelonians, chronically retained eggs may produce secondary infectious salpingitis, coelomitis, septicaemia, urinary/colonic obstruction, and rupture of the oviduct leading to liberation of calcified eggs into the coelomic cavity (Sykes, 2010).

The dystocic female may exhibit lethargy, anorexia, repetitive straining, restlessness, excessive digging behaviour, prolapse of the oviduct, bladder or cloaca and hindlimb paresis (McArthur, 2004; Norton, 2005; Sykes, 2010; Perry & Mitchell, 2017; Portas, 2017a).

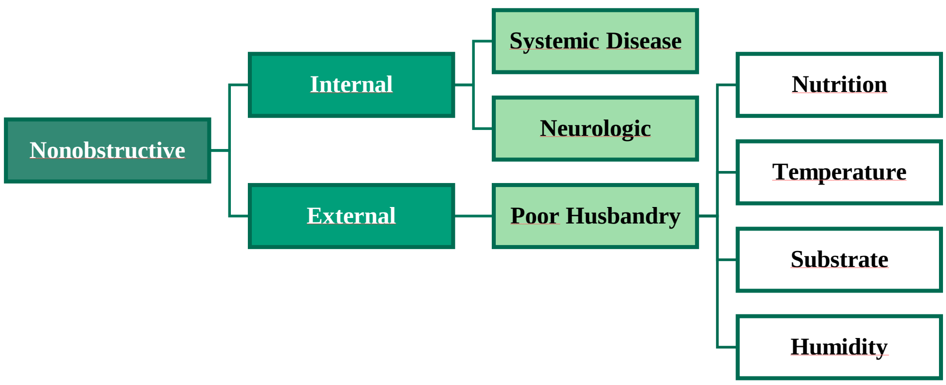



Fig. 2. Causes of nonobstructive dystocia.

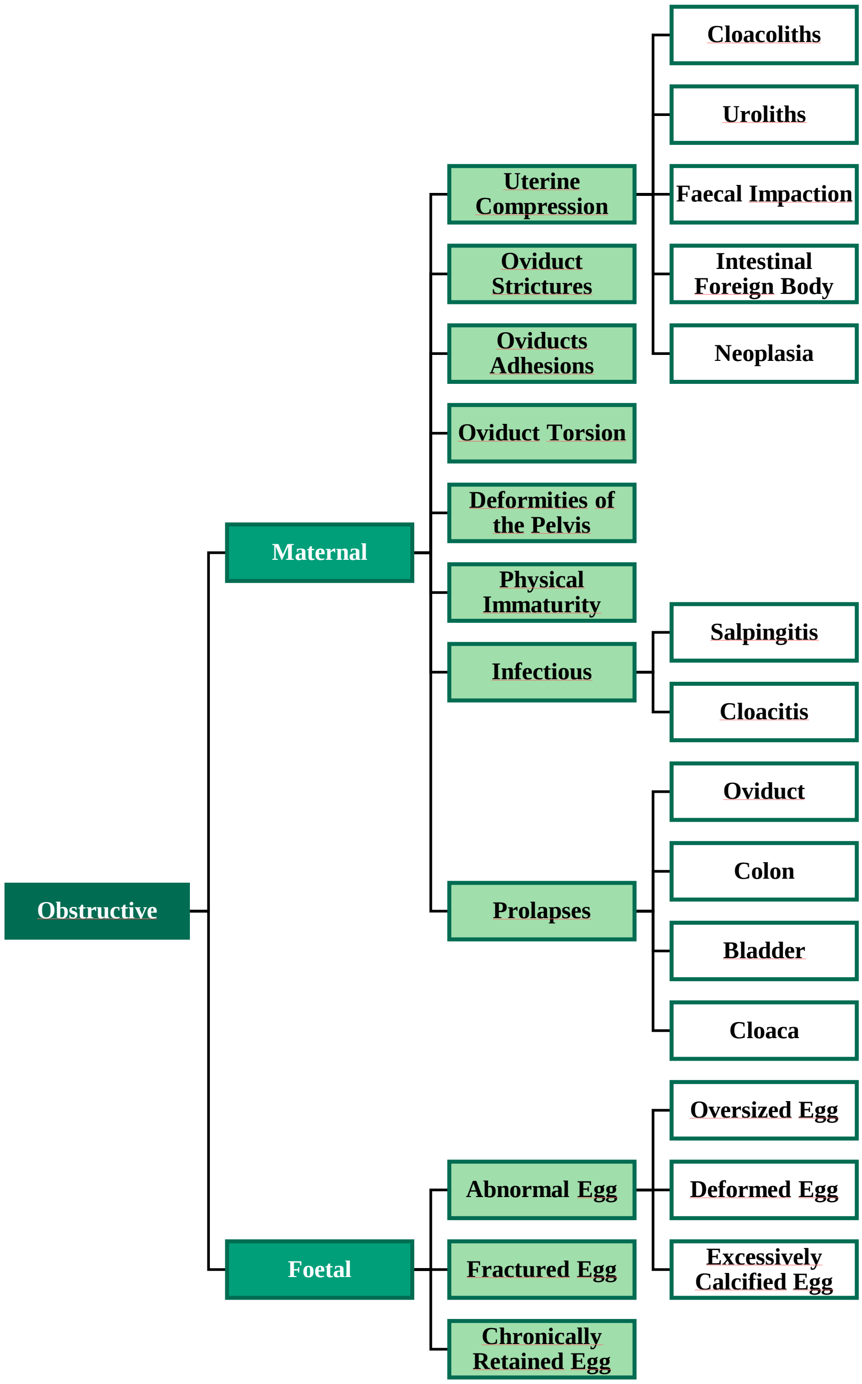



Fig. 3. Causes of obstructive dystocia in chelonians.

## 2.4. Ectopic eggs

Ectopic eggs are an infrequent but possible complication of other reproductive disorders, appearing free in the coelomic cavity or inside the bladder (Mans & Foster, 2014; Stahl & DeNardo, 2019).

The release of eggs into the coelom can be due to reverse oviductal peristalsis or secondary to oviductal rupture, often by wrongful administration of oxytocin in an obstructive dystocia (Sykes, 2010; Mans & Sladky, 2012; Johnson, 2019).

Ectopic eggs in the urinary bladder have also been historically associated with the oxytocin therapy in chelonians (Mans & Foster, 2014). After the egg follows its natural course into the cloaca, its propelled backwards through the urethra into the bladder (Innis & Boyer, 2002; Perry & Mitchell, 2017).

The presence of ectopic eggs in the coelom and bladder usually produces an inflammatory reaction which may lead to infection, culminating in sepsis. Furthermore, it can hinder the expulsion of other eggs remaining in the reproductive tract (Stahl & DeNardo, 2019).

### 2.5. Yolk Coelomitis

Egg yolk coelomitis is a common cause of morbidity and mortality across all female reptiles, and appears as an acute or chronic disease (Stahl, 2019b; Roberts & Warner, 2020).

Yolk coelomitis occurs when the liberation of yolk material into the coelom exceeds the coelomic capacity to reabsorb it (Stacy et al., 2008; Cornax et al., 2013). This condition occurs secondary to, or in combination with POFS, oophoritis, salpingitis, dystocia, oviductal rupture, trauma, rough palpation, and other disorders (Innis & Boyer, 2002; Perry & Mitchell, 2017; Roberts & Warner, 2020).

Yolk and other embryonic tissues are irritants to the coelomic cavity and can provoke a remarkable inflammatory reaction (McArthur, 2004; Portas, 2017a). Additionally, the yolk material promotes bacterial growth resulting in vulgar secondary infections (Portas, 2017a; Roberts & Warner, 2020).

In chronic cases of egg yolk coelomitis, a foul-smelling, dense, yellowish exudate may be noted adhered to the serosal surfaces (Stahl, 2019b; Roberts & Warner, 2020).

As with other reproductive disorders, clinical signs are typically nonspecific and include a decrease in urine/faecal output, lethargy, anorexia, diarrhoea, and weight loss (Innis & Boyer, 2002; Sykes, 2010; Perry & Mitchell, 2017).

### 2.6. Prolapse of the Cloaca and Oviduct

Prolapses have been depicted as common disorders in reptiles, nevertheless, Hedley and Eatwell (2014) demonstrated a prevalence of only 2.4% in chelonian species.

Chelonians might present with oviductal and/or cloacal prolapse linked to conditions that incite straining or increase intracoelomic pressure, such as dystocia, parasitism, constipation, and cystic or cloacal calculi (McArthur, 2004; Norton, 2005; Hedley & Eatwell, 2014; Portas, 2017a). Other reproductive and metabolic disorders can also predispose to oviductal prolapse, such as POFS, salpingitis, hypocalcaemia, neoplasia, among others (Stahl & DeNardo, 2019).

The condition of the oviduct can vary depending on the duration of the prolapse, with possibilities including oedema, trauma, or necrosis (Portas, 2017a). It is important, and often difficult, to swiftly identify the protruding structure and assess its viability (De Voe, 2002 cited in Roberts & Warner, 2020; Norton, 2005; Hedley & Eatwell, 2014).

### 2.7. Neoplasia

The prevalence of neoplasms in chelonians is low, with studies recording percentages of 1.2% (Sykes & Trupkiewicz, 2006) and 2.7% (Garner et al., 2004). Although none of the neoplasms observed in these studies originated from the reproductive tract, there are individual case reports of ovarian carcinomas, dysgerminomas and teratomas, and oviductal leiomyomas in different species of the Testudine order (Frye, 1994; Newman et al., 2003; Christman et al., 2017; LaDouceur, 2020; Simard, 2021; Hellebuyck & Vilanova, 2022).

# 3. Diagnostic Approach to the Female Chelonian with Reproductive Disease

When a chelonian is brought in due to a reproductive concern, it is crucial for the clinician to not focus solely on that aspect as it may overshadow the aetiology of the issue, resulting in flawed management of the disorder (Perry & Mitchell, 2017).

## 3.1. Clinical History and Physical Examination

The way historical information is conveyed and displayed can exhibit significant differences. In any case, an extended review of the reptile's husbandry will allow a good understanding of its housing conditions (lighting, temperature, humidity, cage size, ventilation, substrate, nutrition, and interactions with same species individuals or others) and whether these enable/encourage species-specific behaviours (Boyer & Boyer, 2019; Rossi, 2019; Skurski et al., 2019).

It's often difficult to differentiate the chelonian in reproductive distress from regular reproductive cyclicity (McArthur, 2004; Portas, 2017a). Owners have described their pets as becoming restless, pacing and climbing across the enclosure, probably searching for potential partners or suitable nesting locations (Innis & Boyer, 2002). Other common testimonies state a decrease or absence of the reptile's appetite, coelomic distension, and lethargy (Portas, 2017a; Johnson, 2019). Repetitive straining with unsuccessful oviposition is an indicative sign of dystocia (Stahl & DeNardo, 2019).

During the physical exam, clinical signs are typically nonspecific or nonexistent (Portas, 2017a). With severe or chronic illnesses such as yolk coelomitis, ectopic eggs, or prolapses (Fig. 4), the chelonian becomes more likely to appear in an apathetic or obtunded state (Stahl & DeNardo, 2019).

In chelonians, palpation can be done through the inguinal or prefemoral fossa, while holding the patient vertically and slightly tilting it to the side being palpated. Even though the shell is a limiting factor in coelomic palpation, preovulatory follicles and postovulatory eggs may still be perceived, although with some difficulty to distinguish between (Perry & Mitchell, 2017; Portas, 2017a; Stahl & DeNardo, 2019). These must also be differentiated from faecoliths and cystic calculi (Portas, 2017a).

Fig. 4. Cloacal prolapse in a red-eared slider (*Trachemys scripta elegans*) (Courtesy of Dr. Xavier Valls).

### 3.2. Haematology and Biochemistry

A complete blood work may be necessary to search for any underlying conditions or systemic illness, although uncomplicated cases may reveal parameters within the normal range (McArthur, 2004; Perry & Mitchell, 2017; Portas, 2017a).

With POFS, biochemical analysis may reveal hypercalcemia, hyperproteinaemia, hyperalbuminaemia, hyperphosphataemia, and elevated creatinine kinase and alkaline phosphatase, which can occur physiologically in the preovulatory phase. Hypocalcaemia is a common finding in reptiles with dystocia instigated by nutritional deficiencies (Rivera, 2008; Sykes, 2010; Portas, 2017a).

The female may exhibit haemoconcentration due to anorexia and dehydration, or anaemia in more chronic cases. It often presents with a stress leucogram, or leucocytosis/leucopenia with a left shift when inflammation or infection are present. Heterophils may demonstrate toxic changes (Fig. 5) (McArthur, 2004; Portas, 2017a; Stacy & Harr, 2021). Leucopenia and heteropenia may be observed in chronic cases as well (Rivera, 2008).

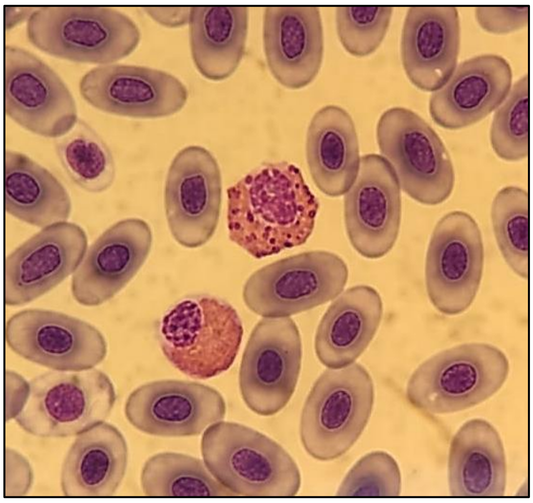


Fig. 5. Toxic heterophil next to a nontoxic heterophil in the blood film of a red-eared slider (*Trachemys scripta elegans*). Note the dark purple granules in the cytoplasm of the toxic cell.

### 3.3. Radiography

Radiography of the reproductive tract normally involves whole body dorsal and lateral projections (Gumpenberger, 2017). It is a crucial diagnostic exam to help distinguish between follicular stasis and dystocia (Holmes & Divers, 2019).

Due to the chelonian shell, coelom detail is lost in radiographs, thus soft tissue structures like follicles can rarely be identified unless the enlarged preovulatory follicles produce a mass effect in the mid coelom, dorsally compressing the lungs (Gumpenberger, 2017; Knotek, et al., 2017b). When POFS or other ovary or salpinx disorders are suspected, ultrasound and computed tomography may serve as a second diagnostic tool (Holmes & Divers, 2019).

The mineralized chelonian eggs are promptly identified as thinly radiopaque oval or oblong structures in the caudal half of the coelomic cavity (Fig. 6) (Holmes & Divers, 2019). It's essential to critically assess the number of eggs, their integrity, size, and distribution in the coelom, as well as any shell abnormalities that may be contributing to a dystocic parturition (Norton, 2005; Perry & Mitchell, 2017; Stahl & DeNardo, 2019). Fractured eggshells, oversized eggs compared to pelvic dimensions, and ectopic eggs are common radiographic signs of dystocia (Gumpenberger, 2017).

Smooth and thickened eggshells might indicate prolonged egg retention within the distal portion of the oviduct, while eggs inside the bladder may reveal an increase in mineralization and coarse shell surface due to mineral and uric acid (UA) deposition. Although radiographs may readily identify mineralized eggs, it might not be easy to infer whether they are located inside de oviduct or free in the coelomic cavity (Perry & Mitchell, 2017; Holmes & Divers, 2019).

Radiography may also identify concomitant or facilitating morbidities, including constipation, calculi, coelomic masses, and metabolic bone diseases (MBD) such as nutritional secondary hyperparathyroidism and renal secondary hyperparathyroidism (Norton, 2005; Stahl & DeNardo, 2019).


Fig. 6. Dorsoventral radiograph of a female river cooter (*Pseudemys concinna*) carrying eleven eggs. The eggs appear similar in size and shape, differing in the degree of mineralization of the eggshell (Courtesy of Exòtics Veterinària).

### 3.4. Ultrasonography

Ultrasonography is the preferred noninvasive imaging technique for assessing reproductive function, staging, and detecting diseases in juvenile and adult individuals (Hochleithner & Sharma, 2019).

Evaluation of the female chelonian reproductive tract may be done using the prefemoral acoustic window (Fig.  7), which allows visualization of the gonads, oviduct, intestine, kidneys, and bladder (Meireles et al., 2016; Knotek, et al., 2017).

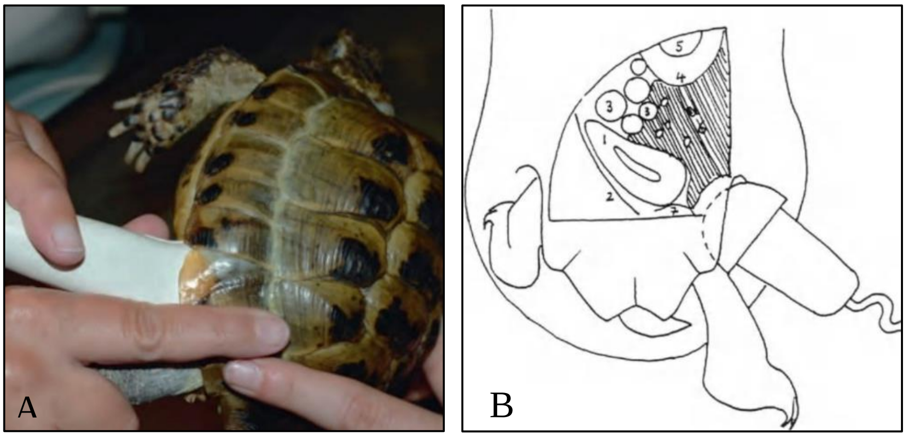



Fig.  7. (A) Prefemoral window access in chelonians (adapted from Hochleithner & Sharma, 2019). (B) Illustration of the ultrasonographic view through the prefemoral window of a female chelonian. 1 - kidney, 2 - carapace, 3 - ovarian follicles, 4 - egg with calcified shell, 5 - yolk, 6 - urinary bladder containing hypoechoic urine and hyperechoic urate crystals, 7 - leg musculature (adapted from Wilkinson et al., 2004).

In juveniles, it may be challenging to identify the small and underdeveloped gonads that are frequently concealed by the intestines. At this stage, follicles may be visualized as round and anechoic or hypoechoic structures. With time, the follicles' size and echogenicity tends to increase proportionately (Meireles et al., 2016; Hochleithner & Sharma, 2019).

Mature follicles (Fig.  8.A) appear as large, homogenous, and spherical structures, hyperechoic relative to the oviduct (Meireles et al., 2016; Hochleithner & Sharma, 2019). These can be observed filling the caudal coelom in preovulatory stage (Portas, 2017a). After ovulation (or failure to ovulate), during reabsorption or atresia of the clustered vitellogenic follicles (Fig.  8.B), these diminish in size, acquire a more heterogeneous echogenicity, and progressively lose their round shape and well defined contour (Gumpenberger, 2017; Hochleithner & Sharma, 2019).

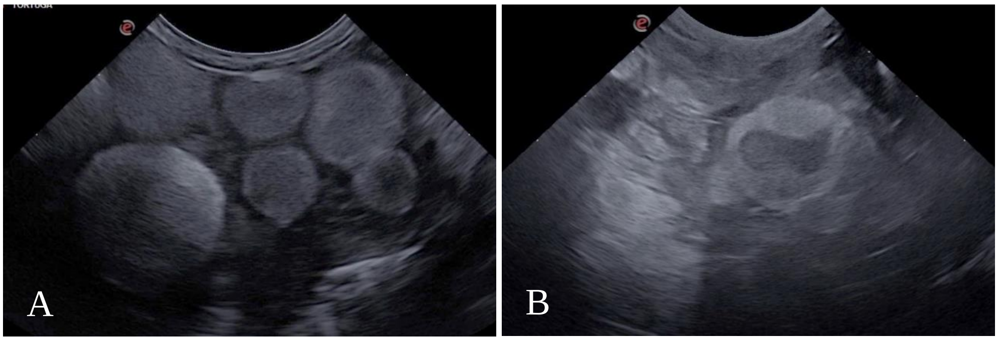



Fig. 8. Chelonian ultrasound images (prefemoral window) of mature vitellogenic follicles (A) and of a degenerated follicle (B) (Courtesy of Exòtics Veterinària).

Follicular stasis is best identified with ultrasonography. Sonographically, the nonovulated follicles will maintain their large diameter instead of decreasing in size (Perry & Mitchell, 2017; Stahl & DeNardo, 2019). Anechoic fluid is frequently observed surrounding the coalescing follicles, and may contain free-floating debris that indicate an inflammatory response, suggesting the need for surgical resolution (Gumpenberger, 2017; Hochleithner & Sharma, 2019).

The ovulated ova become more elliptical, and acquire a smooth and hyperechoic outer shell (Fig. 9), more calcified in chelonians than other reptiles, capable of producing an acoustic shadow (Meireles et al., 2016; Gumpenberger, 2017). Sonographically, the outer albumin layer appears anechoic while the inner portion (the yolk) appears as hyperechoic (Meireles et al., 2016; Hochleithner & Sharma, 2019).


Fig. 9. Ultrasound image of a mineralized egg of a golden thread turtle (*Mauremys sinensis*). 1 - yolk, 2 – albumin layer, 3 - outer eggshell (Courtesy of Exòtics Veterinària).

In the female chelonian with dystocia, irregularities may be observed in the eggshell surface suggesting the egg is not transiting correctly (Portas, 2017a; Hochleithner & Sharma, 2019). The echogenicity of the egg contents may become heterogenous, and fluid may be noted filling the distal oviduct and/or the coelomic cavity (Gumpenberger, 2017; Perry & Mitchell, 2017; Hochleithner & Sharma, 2019). When these signs are present, probability of successful egg laying becomes low, and surgical intervention is indicated (Hochleithner & Sharma, 2019; Stahl & DeNardo, 2019).

Ectopic eggs can also be identified via ultrasound. These may incite an inflammatory response, observed sonographically as free anechoic fluid in the coelomic cavity with floating echogenic debris (Gumpenberger, 2017; Portas, 2017a). Coelomic effusions may be aspirated, with the assistance of the ultrasound, to conclude on cytology if the flocculent content is inflammatory and/or infectious in nature. It may be difficult to distinguish eggs located free in the coelom from eggs located within the bladder (Stahl & DeNardo, 2019).

Identifying a coelomic neoplasia originating from the gonads can be challenging due to the inconsistent visual characteristics of the gonads. As a result, it may require excluding other normal coelomic structures to reach a diagnosis (Hochleithner & Sharma, 2019).

### 3.5. Computed Tomography

Computed tomography permits the evaluation of each individual follicle and egg, unlike radiography. It can diagnose and indicate the need for intervention in follicular stasis and dystocia based on the distinct horizontal levelling of the follicle and egg contents, respectively (Gumpenberger, 2017).

### 3.6. Endoscopy

Endoscopy has become a very important diagnostic tool throughout the years, especially in reptiles where medical history, physical examination, and imaging modalities have often offered limited and lacklustre diagnostic value (Schildger, 1999; Divers, 2019b).

Endoscopy allows exploration of the inner organs with less procedural risks, less anaesthesia time, and a faster recovery than the traditionally used transplastron coeliotomy (Proença & Divers, 2015). This minimally invasive technique also permits the realisation of therapeutic procedures and the collection of tissue samples for further histopathology and/or microbiology analysis, significantly improving antemortem diagnosis and treatment success (Schildger, 1999; Divers, 2019b; Stahl & DeNardo, 2019).

### 3.6.1. Equipment

Rigid endoscopy remains the most versatile telescope in reptile and other exotic pets' medicine, mainly due to its capacity to access areas of interest (Schildger, 1999; Proença & Divers, 2015; Divers, 2019a).

The 2.7 mm scope (Fig. 10) with 30 degrees field of view and 18 cm in length allows for examination of reptiles that weigh from 50 g up to 20 kg (Schildger, 1999; Divers, 2010). It can be handled with a 3.5 mm protection sheath or with a 4.8 mm sheath with working channels that permit infusion of gas or fluid, and introduction of 1.7 mm (5Fr) instruments (Fig. 10) (Divers, 2019a). The 1.9 mm endoscope may be an option for procedures with a narrow entry point, such as cystoscopy (Di Girolamo & Selleri, 2015).

The endovideo camera attached to the telescope is a crucial component of the system, not only does it improve the surgeon's success, it also facilitates recording of photos and videos from the procedures with the photodocumentation ability of the device (Schildger, 1999; Divers, 2019a).

The light used for visualization of internal structures is guided by a fibre-optic cable, and emitted by xenon, halogen or LED light source (Proença & Divers, 2015; Divers, 2019a).

Insufflation or irrigation with sterile saline can be used to mildly distend the working area to allow for better visibility and to enable surgical procedures. In coelioscopic procedures, introduction of filtered air or $CO_2$ gas can be regulated via an endoflator, though air can also be delivered with syringes or small air pumps (Schildger, 1999; Divers, 2019a).

Warm saline can be infused with a syringe or via dripping through a fluid line, mainly when examining a hollow viscus (Divers, 2019a).

The most important commonly used instruments are the biopsy forceps, scissors, retrieval forceps, and needle, although other instruments such as the radiosurgical needle electrode, the wire basket retrieval device, and the polypectomy snare can be irreplaceable in distinct circumstances (Divers, 2019a).

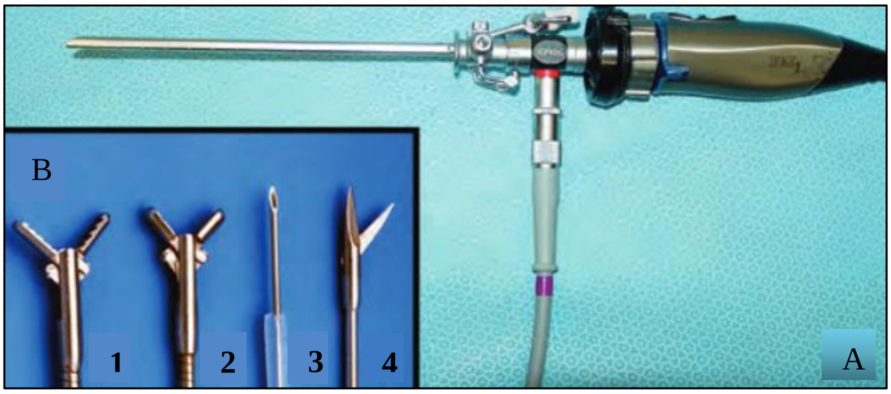



Fig. 10. A 2.7 mm rigid endoscope system. (A) A 2.7 mm telescope set within a 4.8 mm operating sheath, connected to a light cable and an endovideo camera. (B) Most relevant 1.7 mm instruments that can be used through the operating channel, including: 1 - retrieval forceps, 2 - biopsy forceps, 3 - remote injection/aspiration needle, 4 - single-action scissors (adapted from Divers, 2019a).

### 3.6.2. Patient Preparation

Performing standard assessments for preanaesthetic evaluation and surgical preparation is necessary. As a general rule, the reptile patient should be fasted for 24 to 72 hours, or skip a feeding cycle (Proença & Divers, 2015; Divers, 2019b; Hellebuyck & Vilanova, 2022). The chelonian should be stimulated to urinate and defecate before the procedure to avoid any unnecessary iatrogenic trauma. It can be done so by stimulating the cloaca with a digit or swab, or by shallow bathing (Proença & Divers, 2015; Hellebuyck & Vilanova, 2022).

The patient should be fully anaesthetized with diligent observation of cardiac and respiratory functions for this procedure, and analgesia with opioids, nonsteroidal anti-inflammatory drugs (NSAIDs), and/or local anaesthetics must be taken in consideration in order to effectively manage the associated pain (Schildger, 1999; Innis, 2010; Divers, 2019b). The reptile should be kept warm in its preferred optimal temperature range until, throughout, and after the procedure (Schildger, 1999).

### 3.6.3. Cloacoscopy

The chelonian cloaca is routinely evaluated with the animal in dorsal recumbency (Divers, 2019b). The endoscope is gently introduced in the cloaca and warm sterile saline is infused through the sheath system (Fig. 11). The fluid-filled and dilated cloaca provides exceptional means for appraisal of the urodeum, coprodeum, and proctodeum (Innis, 2010; Divers, 2019b). It allows assessment of the cloacal mucosa, urethra, bladder, urogenital papillae, distal oviducts, and distal colon (Fig. 12) (Divers, 2019b).

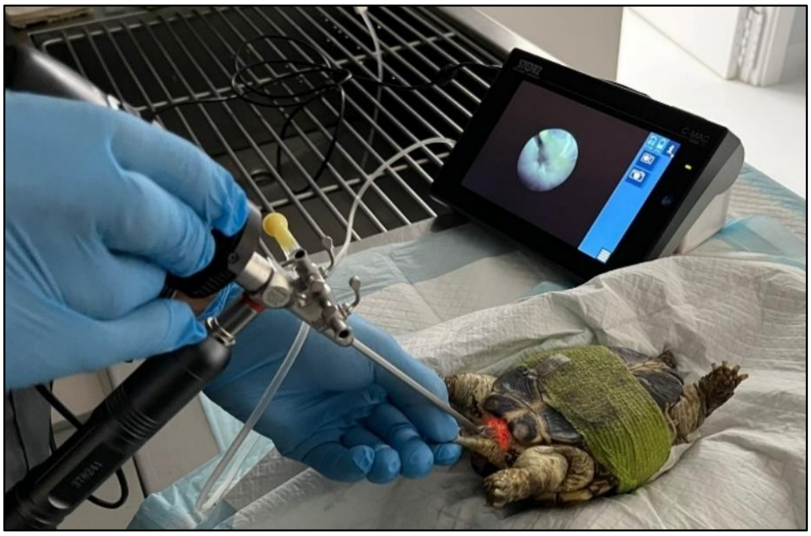


Fig. 11. Cloacoscopy on a Russian tortoise (*Testudo horsfieldii*). Note the fluid line and the portable endoscopy device connected to the telescope (Courtesy of Dr. Xavier Valls).

Cloacoscopy has been employed to identify and remove eggs from the distal oviducts and bladder, and to identify and correctly reposition prolapsed structures (Divers, 2019b).

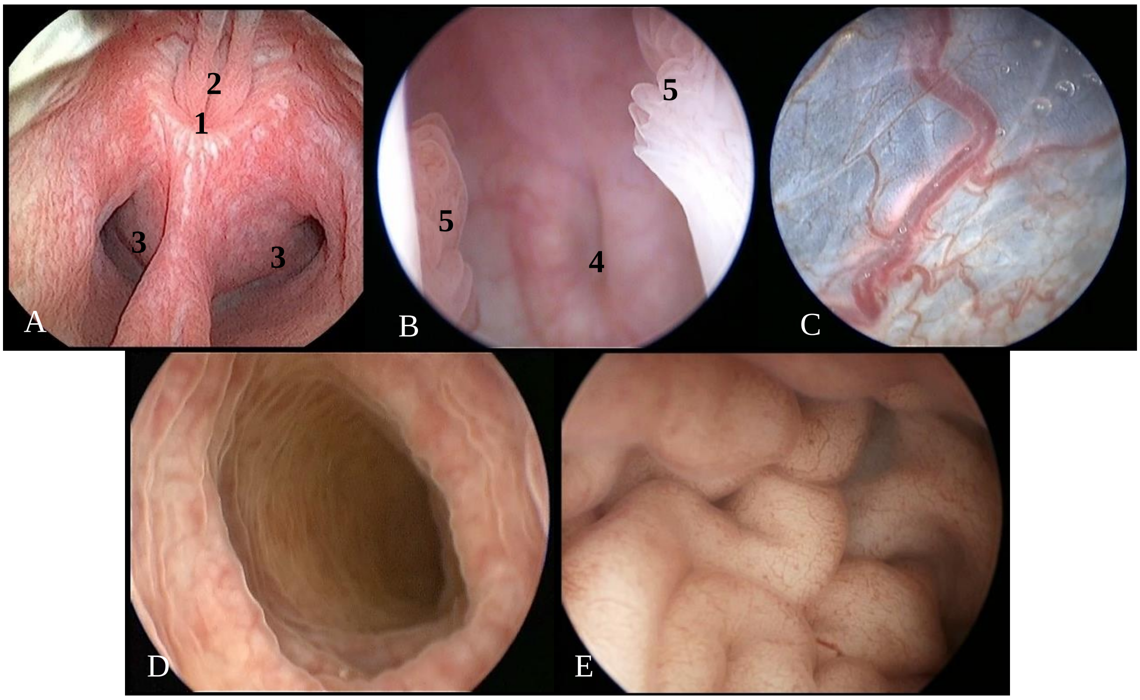



Fig. 12. Chelonian cloacoscopy. (A) Inside the cloaca, the endoscope can used to inspect the: 1 - colon, 2 - bladder and oviducts, 3 - accessory bladders. (B) Urodeum. 4 - urethral opening, 5 - genital papilla. (C) Cystoscopy. Note the thin, see-through, highly vascularized bladder wall. (D) Colonoscopy. Note the transversely corrugated structure of the colon. (E) Salpingoscopy. Note the convoluted appearance of the oviduct. (Courtesy of Dr. Xavier Valls).

### 3.6.4. Coelioscopy

Coelioscopy through the prefemoral window has many advantages over transplastron osteotomy. Entry in the coelom through soft tissue coeliotomy and not by creation of a bone flap in the plastron offers a less traumatic, less painful, and faster approach. Additionally, the patient returns to normal function faster and the surgical incision heals in about six to eight weeks instead of the months to years seen with osteotomy (Innis, 2010; Divers, 2019b, 2019d).

Endoscopic examination of the chelonian requires general anaesthesia and lateral recumbency positioning (Schildger, 1999; Innis, 2010; Proença & Divers, 2015). After creation of the entry into the coelom through the prefemoral fossa, the endoscope may be introduced to start evaluating internal organs. All organs, except for the spleen, can be appreciated with great satisfaction (Hernandez-Divers, 2004).

Endoscopic examination often reveals a range of pathologies, sometimes with minimal observable radiographic or ultrasonographic alterations (Divers, 2019b). Changes in the anatomical shape, surface, location, or colouration of the organs may suggest the presence of pathology and warrant biopsy for further diagnosis. Diagnostic coelioscopy often detects secondary complications of reproductive disorders (Fig. 13), such as oophoritis, salpingitis, oviductal torsion, oviductal rupture, ectopic eggs/follicles in the coelom, free fluid, and adhesions in the coelom (Proença & Divers, 2015; Perry & Mitchell, 2017). The latter two may make the procedure more difficult by impairing endoscope movement and visualization (Stahl & DeNardo, 2019).

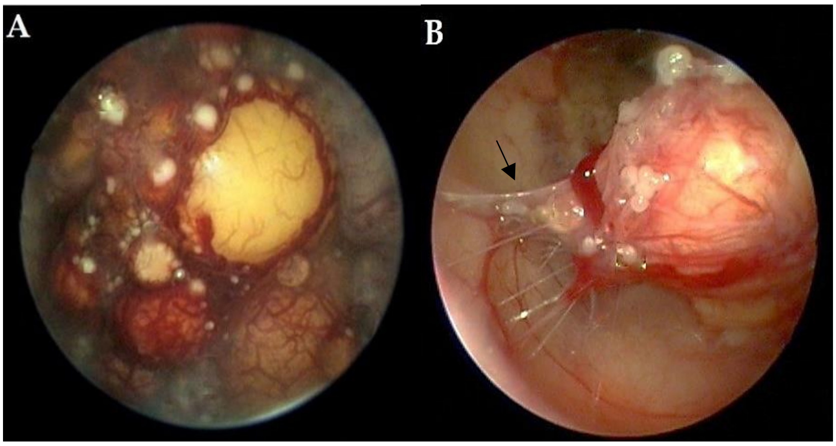



Fig. 13. Common snapping turtle (*Chelydra serpentina*) with oophoritis (A) and follicles adhered (arrow) to the coelomic membrane (B) (adapted from Hellebuyck & Vilanova, 2022).

Biopsies can be sent for cytological, histopathological, and microbiological diagnosis, and are considered to be more valuable than those taken with the guidance of ultrasound (Rawlings et al., 2003). Minor bleeding after biopsy is expected, but neglectable (Divers, 2019b). Tissue samples are commonly taken from the liver that may present focal or diffuse abnormalities (Fig. 14) (Divers, 2019b). The ovaries may also be biopsied when oophoritis, neoplasia, or infertility are suspected (Stahl, 2019b).

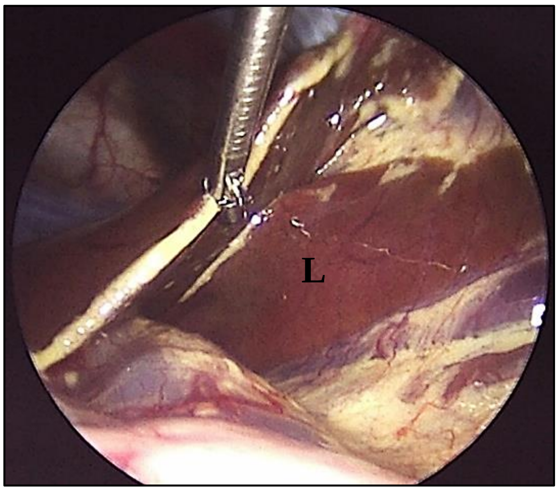



Fig. 14. Liver lobe (L) edge biopsy performed on a chelonian during coelioscopy using the 1.7 mm biopsy forceps. Note the yellowish-white fibrinous exudate covering the coelomic surface, and the rounding and white discolouration of the liver's edges (Courtesy of Dr. Xavier Valls).

## 4. Therapeutic Approach to the Female Chelonian with Reproductive Disease

The therapeutic approach varies considerably depending on the disorder at hand, the species involved, the status of the patient and affected organs, and the owner's objective, whether the chelonian has an intended use for breeding or not.

### 4.1. Medical Management

For most reproductive conditions, medical management should only be resorted to if the reptile is not in need of immediate stabilization, if it is a mild case, or if the reptile's reproductive success is to be preserved. Otherwise, chronic cases, cases of advanced infection, unstable patients at presentation, or patients kept as pets and, therefore, not intended for breeding, all indicate for surgical management (Perry & Mitchell, 2017; Portas, 2017a; Johnson, 2019; Stahl & DeNardo, 2019).

Medical management of POFS is commonly unsuccessful, however, if the chelonian appears healthy, it may be sent home where a nest box and calcium gluconate supplementation should be provided for two to three weeks, after which the female should be reevaluated to establish if ovulation or resorption has happened (Portas, 2017a; Stahl & DeNardo, 2019). Females intended for breeding can also be paired with a male and be given an appropriate nesting site. After this period, unaltered or degenerative follicles confirmed on ultrasound or computed tomography deem the patient eligible for surgical management (Stahl & DeNardo, 2019).

Mild cases of oophoritis and salpingitis may be treated early with NSAIDs and appropriate antimicrobials based on culture and sensitivity testing (Stahl & DeNardo, 2019). Chronic cases or complicated infections warrant ovariectomy or ovariosalpingectomy, depending on the organs involved (Proença & Divers, 2015; Portas, 2017a; Stahl & DeNardo, 2019).

Differing from other reptiles, dystocia in chelonians is seldom an emergency, and can often be managed medically or by correcting environmental/husbandry factors (Perry & Mitchell, 2017; Stahl & DeNardo, 2019). A healthy female chelonian with suspected nonobstructive dystocia may be given an appropriate nesting box and oral calcium therapy for up to two weeks. Failure to lay the eggs, or observed deteriorating condition of the female within the time period, entails reevaluation and additional medical therapy (Portas, 2017a; Stahl & DeNardo, 2019). Before commencing oxytocin therapy, the patient should first be stabilized if it is not considered to be in a healthy condition. Correction of dehydration, electrolyte imbalances, and hypocalcaemia is of great importance for a successful medical management, as well as administration of antibiotics, analgesics or anti-inflammatories, if necessary (Norton, 2005; Portas, 2017a). The use of oxytocin or, if available, arginine vasotocin, should be reserved for confirmed cases of nonobstructive dystocia, otherwise it may lead to broken eggs, eggs pushed into the bladder, rupture of the oviduct, and considerable haemorrhage (Stahl & DeNardo, 2019). Chelonians respond better than other reptiles to oxytocin, so administration can be given slowly intravenously (IV), intramuscularly (IM), or subcutaneously at lower doses (1-4 IU/Kg) to induce oviposition (McArthur, 2004; Perry & Mitchell, 2017). Di Ianni et al. (2014) showed oxytocin administrated IV required less administrations and induced oviposition more rapidly than the intramuscular route. Although the eggs are frequently expelled without laying behaviour after administration, a nest box should still be provided

(Stahl & DeNardo, 2019). The dose may be repeated or increased every three to 12 hours, for the next 48 hours, even though chances of successful oviposition fall dramatically after the second or third administration without results (Norton, 2005; Portas, 2017a; Stahl & DeNardo, 2019). Other adjunct medications have been utilized such as beta-adrenergic blockers like propranolol and atenolol, and prostaglandins F2α and E, that are thought to potentiate oxytocin effect and aid in egg expulsion, though further studies are needed to verify their efficacy (Innis & Boyer, 2002; Sykes, 2010).

Some forms of obstructive dystocia can be resolved per cloaca. Eggs visible inside the cloaca or near the oviductal opening into the urodeum may be grasped and extracted with forceps or punctured with a large-gauge needle (14-18 G) to aspirate its contents and collapse the egg, facilitating its expulsion or removal (Norton, 2005; Stahl, 2013; Portas, 2017a). This procedure can be repeated for the remaining eggs. Nonetheless, the typically hard-shelled chelonian eggs tend to break during ovocentesis, making the process more laborious as shell fragments must be removed piece-meal (Innis, 2010; Perry & Mitchell, 2017). Cloacoscopy permits a more careful and effective approach (Stahl & DeNardo, 2019). Eggs adhered to the mucosa, oversized eggs, and eggs that are out of reach, require surgical resolution (Innis & Boyer, 2002; Portas, 2017a).

Cloacoscopy is a noninvasive technique that not only allows entry, visualization, and labour inside the cloaca and oviducts, it can also be utilized to manage ectopic eggs inside the bladder (Innis, 2010; Di Girolamo & Selleri, 2015). Where before cystotomy was the only means for solving this problem, now cystoscopy permits manipulation and removal of retained eggs with grasping forceps or other grasping instruments (Innis, 2010; Mans & Foster, 2014; Stahl & DeNardo, 2019). After removal of all egg contents and fragments, a complete lavage of the bladder should be performed (Knotek, 2013; Di Girolamo & Selleri, 2015). The patient should be under general anaesthesia for this procedure (Stahl & DeNardo, 2019).

Cloacoscopy may additionally be used to facilitate replacement of mild or partial oviductal prolapses with viable tissue. The prolapsed tissue must be kept clean and moist and must be handled carefully. Inversion and replacement can be done with insertion of a blunt instrument in the exteriorized opening of the organ, and gentle guidance back into the cloaca (Stahl & DeNardo, 2019). However, more frequently the prolapsed tissue is necrotic and desiccated at presentation, requiring surgical amputation, and further ovariosalpingectomy to prevent recurrence (Portas, 2017a; Stahl & DeNardo, 2019). In addition, it is essential to tackle the root cause. Affected chelonians should receive appropriate antibiotic therapy, analgesia, and symptomatic support as necessary (Portas, 2017a).

Reproductive tract neoplasia may be treated with chemotherapy, radiation, and/or surgery, based on histology and staging of the mass. Early diagnosis and benign neoplasms usually point to surgical removal, whereas malignant neoplasia and presence of metastases suggest the oncology therapy to be more focused on palliative care (Christman et al., 2017; Mayer & Moore, 2019).

## 4.2. Surgical Management

Surgical intervention is recommended when medical management fails; when the patient presents in unstable condition; presents disorders with advanced chronicity or infection; or when the reproductive future of the chelonian is neglectable and a permanent solution is preferred (Proença & Divers, 2015; Perry & Mitchell, 2017; Portas, 2017a; Stahl & DeNardo, 2019).

Most reptiles with underlying chronic diseases require some form of supportive care and stabilization before undergoing a surgical procedure and being submitted to anaesthesia. Fluid therapy, antimicrobial/antifungal therapy, and analgesia should all be considered. The patient should also be maintained in its preferred optimal temperature zone throughout all the perioperative period (Schumacher & Mans, 2014; Mans et al., 2019; Schnellbacher & Shepard, 2019; Sladky & Mans, 2019).

### 4.2.1. Surgical Coeliotomy

Entry to the coelom can be granted by surgical coeliotomy, where the majority of internal organs may be examined, biopsied, and submitted to surgical interventions. Selection of the preferred approach should take into consideration the species involved, the targeted organ or site, and the procedure's objective (Wüst & Divers, 2019).

#### 4.2.1.1. Prefemoral Coeliotomy

This soft-tissue approach has made most surgical procedures faster, easier, less traumatic, and with a faster and uneventful recovery in the postoperative period, comparative to the traditional transplastron osteotomy (Innis et al., 2007; Proença & Divers, 2015; Wüst & Divers, 2019).

The entry cranial to the hindlimb provides good access to the kidneys, bladder, intestines, reproductive tract, liver, and caudal portion of the lungs, being an important staple in reproductive tract surgery (Sykes, 2010; Knotek, 2013; Proença & Divers, 2015).

The anaesthetized chelonian is positioned in lateral recumbency and held in place by a vacuum bean bag or towels (Schildger, 1999; Proença & Divers, 2015; Wüst & Divers, 2019). The suspended hindlimb is retracted and taped caudally to the other limb (Fig. 15), shell, or table to expose the prefemoral fossa (Innis, 2010; Proença & Divers, 2015). The entry site and surrounding shell is then aseptically prepared and draped according to conventional methods (Divers, 2019b; Wüst & Divers, 2019).

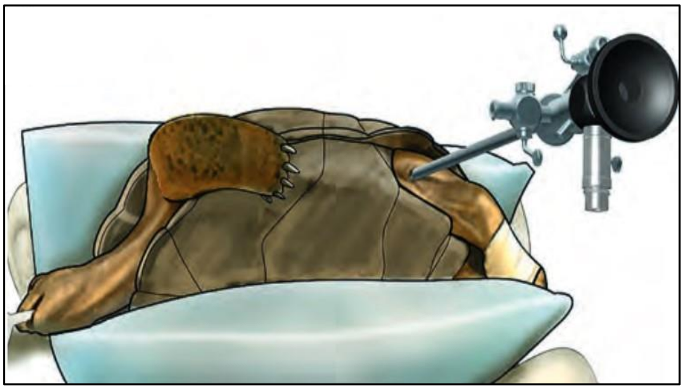


Fig. 15. Illustration of prefemoral approach in a chelonian submitted to coelioscopy (adapted from Divers, 2019b).

A skin incision is created in the centre of the prefemoral fossa, from cranial to caudal direction, extending from the skin near the plastrocarapacial junction to the skin cranial to the limb musculature (Innis, 2010; Wüst & Divers, 2019). The size of the incision varies depending on the objective of the procedure, whether it involves sole examination, or surgical manipulation (Innis, 2010). Blunt dissection of the subcutaneous connective tissue and fat, cranial to the sartorius and ventral to the iliacus muscles, using scissors or haemostats, exposes the oblique and transverse abdominal muscles (Fig. 16). The coelomic aponeurosis and peritoneal serosa can then be perforated using haemostats or a spring-loaded needle directed cranially and parallel to the table (Schildger, 1999; Innis, 2010; Divers, 2019b; Wüst & Divers, 2019). A retractor ring and elastic stays can be placed to improve exposure of the coelom (Proença & Divers, 2015).

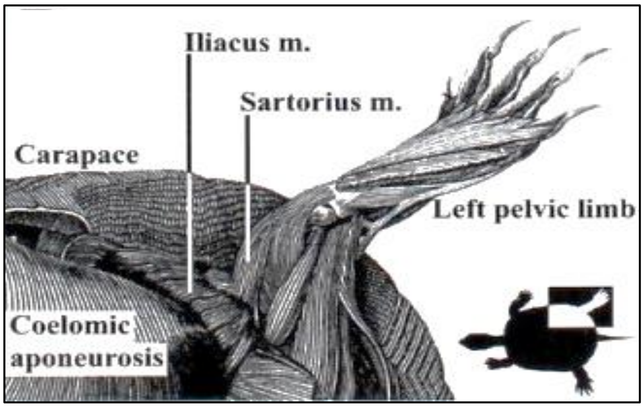



Fig. 16. Illustration of the prefemoral fossa (ventral view) and the anatomical relationship between the sartorius and iliacus muscles, and the coelomic aponeurosis (adapted from Hernandez-Divers, 2004).

Generally, in aquatic species, a single incision may be sufficient for bilateral reproductive surgery, while a large bladder and obstructive ligaments can be found to be hindrances in tortoise surgery, often resulting in a bilateral approach (Innis et al., 2013; Paries et al., 2014; Proença et al., 2014b).

After completing the exam, the coelom should be thoroughly lavaged (Schildger, 1999; Wüst & Divers, 2019). Closure of the peritoneum and aponeurosis can be done with absorbable sutures in a simple interrupted or continuous pattern, while the skin suture should be nonabsorbable or delayed absorbable, in a simple continuous pattern (Innis, 2010; Wüst & Divers, 2019). Aquatic turtles may access water 24h after the procedure (Innis, 2010). Sutures can be removed six to eight weeks after (Divers, 2019b).

The patient should be monitored for cardiorespiratory function and temperature until the appearance of first movements and recovery from anaesthesia (Schildger, 1999; Divers, 2019b). The most valuable indicators of overall improvement often involve the resumption of usual behaviours and weigh gain (Divers, 2019b).

#### 4.2.1.2. Transplastron Coeliotomy

This traditionally used approach involves incising the bone, meaning it causes more pain, trauma, and slower healing than the previously discussed prefemoral coeliotomy (Divers & Wüst, 2019). The prefemoral approach should be the chosen technique whenever possible, nevertheless, by providing a broader window, transplastron osteotomy is still indicated for the removal of large foreign objects, cystic calculi, and masses that otherwise would not fit through the prefemoral gap (Rodrigues et al., 2015; Divers & Wüst, 2019; Hellebuyck & Vilanova, 2022).

The patient is set in dorsal recumbency and held by towels or bean bags, so that the plastron can be aseptically prepared with the use of a brush and draped (Divers & Wüst, 2019). Before incising the plastron, it should be taken into consideration the regional anatomy beneath, location of the hinge, plastron thickness, and the degree of access needed (Divers & Wüst, 2019; Hellebuyck & Vilanova, 2022).

The incisions are done with the use of an oscillating sagittal saw or, less advisably, a rotating saw, at an angle of 45 degrees (Alworth et al., 2011; Divers & Wüst, 2019). The two lateral incisions are the first to be made, in full thickness, followed by the caudal and cranial cuts that do not require bevelling. Generally, the cranial cut is made last, and the caudal cut is incomplete. By leaving a few millimetres of bone thickness to create a hinge, it helps stabilize the loose fragment while maintaining blood supply, thus potentiating primary healing (Alworth et al., 2011; Hellebuyck & Vilanova, 2022). The created segment is then lifted using a periosteal elevator, and the inserted muscles are bluntly dissected close to the bone. The plastron flap is reflected caudally and kept moist with a sterile gauze drenched in saline (Alworth et al., 2011; Divers & Wüst, 2019). The coelomic cavity can be accessed with a midline incision of the coelomic membrane in most tortoises that possess two abdominal veins, or with a unilateral paramedian incision in aquatic chelonians that possess a singular abdominal vein (Divers & Wüst, 2019; Hellebuyck & Vilanova, 2022).

For closure, the coelomic membrane is sutured in a simple continuous or interrupted pattern using absorbable sutures, while the bony segment is drilled in each corner and fixed to the plastron with sutures. Antibiotic can be instilled in the incision line, topped by acrylic or epoxy resin to seal and protect the incisions (Alworth et al., 2011; Divers & Wüst, 2019).

The quality of the reduction of the bony segment and repair of the plastron generally dictates whether the healing occurs by first or second intention. Frequently, the bone flap is seen to work only as protective sequestrum for new bone developing dorsally (Alworth et al., 2011; Divers & Wüst, 2019). Healing typically takes 12 to 18 weeks, however, the epoxy or acrylic cover should only be removed after six to 12 months, as complications may occur, extending the expected healing period. Radiography is rarely beneficial when evaluating the progress of healing due to the tendency for radiolucent lines to persist even after the plastron is healed (Divers & Wüst, 2019).

### 4.2.2. Surgical Procedures

Sterilization by ovariectomy or ovariosalpingectomy is the preferred treatment for the majority of reproductive diseases, though hemi-ovariectomy or hemi-ovariosalpingectomy can be performed for breeding chelonians suffering from unilateral disease (Norton, 2005; Innis, 2010; Proença & Divers, 2015; Stahl & DeNardo, 2019; Antunes et al., 2020).

#### 4.2.2.1. Ovariectomy

Sterilization provides a permanent solution for unwanted breeding, it is indicated for the treatment/prevention of reproductive diseases such as follicular stasis, oophoritis, salpingitis, dystocia, ectopic follicles/eggs, yolk coelomitis, and oviductal/cloacal prolapses (Proença & Divers, 2015; Perry & Mitchell, 2017; Stahl & DeNardo, 2019)

The minimally invasive endoscope-assisted prefemoral ovariectomy is often the preferred method, however, ovariectomy can also be performed through the prefemoral approach without the use of an endoscope. Transplastron coeliotomy should be reserved for conditions whose treatment may be hindered by the limiting nature of the prefemoral fossa (Innis & Boyer, 2002; Innis, 2010; Proença & Divers, 2015; Di Girolamo & Mans, 2016). Complete endosurgical ovariectomy has been performed in many species, but it is best employed in immature chelonians, as a preventative measure, due to their small and underdeveloped ovaries (Divers, 2019c; Stahl, 2019b).

After entering the coelom, the ovaries are identified dorsally and caudally. The ovarian interfollicular tissue is grasped and gently handled with atraumatic grasping forceps towards the prefemoral incision, exteriorizing the follicles, the mesovarium, and the ovarian vessels (Stahl, 2019b). The mesovaria vasculature is ligated with vascular clips or absorbable monofilament suture and transected distally to remove the ovary. Whenever possible, the contralateral ovary should be removed in the same fashion through the same prefemoral coeliotomy, otherwise, a second incision might be needed. Before routine closure, the vascular pedicles should be inspected for bleeding and the coelom lavaged (Proença & Divers, 2015; Stahl, 2019b).

#### 4.2.2.2. Salpingectomy

Salpingectomy is commonly indicated in chelonians for the treatment of oviductal diseases such as postovulatory egg stasis or obstructive dystocia, and oviductal prolapse. The ipsilateral ovary must always be removed when performing salpingectomy (Knotek & Wilkinson, 2017; Stahl, 2019b).

The prefemoral coeliotomy approach is often the preferred choice for this procedure, especially when there is few or no eggs involved (Stahl, 2019b).

Entering the coelom, the oviducts are found adjacent to the ovaries and are readily identified when filled with eggs. The oviduct is carefully exteriorized through the incision starting at the fimbria and ending on its junction with the urodeum. The fimbria and the vessels in the mesosalpinx are then clamped, ligated with absorbable sutures or vascular clips, and transected with radiosurgery or laser (Stahl, 2019b). The distal oviduct is also clamped and ligated using a circumferential transfixing ligature and/or vascular clips, finally being transected (Proença & Divers, 2015; Knotek & Wilkinson, 2017). This process may then be replicated in the contralateral oviduct, unless specified otherwise. As oviductal disease is frequently associated with ectopic eggs and coelomitis, a lavage with sterile saline before closure is advisable (Stahl, 2019b).

#### 4.2.2.3. Salpingotomy

Salpingotomy is often the choice for reptiles with retained eggs, whose reproductive function is to be maintained, and that previously failed less invasive treatments (Stahl & DeNardo, 2019). However, as medical management for nonobstructive dystocia is commonly successful in chelonians, this procedure is not performed frequently (Innis & Boyer, 2002). The preferred approach for salpingotomy is the same as for salpingectomy (Stahl, 2019b).

After exteriorizing the oviducts as described earlier, they are inspected for their condition, and number and location of static eggs. The incision should be made longitudinally in the antimesenteric border of the oviduct, positioned to enable the extraction of the maximum number of eggs through it (Di Girolamo & Mans, 2016; Stahl, 2019b). The incision can be extended as necessary; however, secondary complications of dystocia commonly involve adhesions of the eggs to the oviductal mucosa, meaning multiple incisions should be made to remove the eggs that otherwise cannot be moved through a single incision. The pelvic inlet should be inspected to verify extraction of all eggs. Closure of the salpinge can be performed with a fine monofilament absorbable suture in a continuous or inverting pattern (Alworth et al., 2011; Stahl, 2019b).

#### 4.2.2.4. Cystotomy

Cystotomy is indicated for removal of calculi or ectopic eggs (whether they are the cause or result of dystocia), and for sampling the bladder wall for microbiology culture in cases of complicated infectious cystitis (McArthur, 2004; Knotek & Wilkinson, 2017; Divers, 2019e).

The bladder may be accessed through prefemoral coeliotomy or transplastron coeliotomy depending on the relation between the size of the prefemoral gap and the proportions of the unit to be removed. This decision can be objectively made by comparing radiographic and prefemoral measurements (Divers, 2019e).

Care must be taken entering the coelom, as iatrogenic rupture of the large and frail bladder is a common complication (Proença & Divers, 2015). The bladder is generally easily identified and pulled towards the incision. To minimize bladder movement and urine contamination, the coelom should be packed with moistened gauze, and the bladder should be fixed with stay sutures secured by haemostats. The cystotomy incision is performed in a region of the ventral or lateral bladder wall that is less vascular, while cautiously aspirating the bladder fluid. Marsupialization of the bladder incision to the prefemoral skin may help avoid further contamination (McArthur, 2004; Divers, 2019e). An angled spoon can be used to remove small eggs or calculi. Larger eggs are better removed after collapsing, and large calculus after drilling with wooden screws to break into smaller pieces or simply to help with extraction (Divers, 2019e). Once the removal is completed, inspection and copious flushing of the bladder with saline should ensue (Knotek & Wilkinson, 2017). Closure of the bladder incision is made with fine absorbable monofilament suture primarily in a simple continuous pattern, proceeded by an inverting layer oversewing the first (McArthur, 2004; Divers, 2019e).

#### 4.2.2.5. Ectopic Eggs/Follicles and Yolk Coelomitis

Yolk coelomitis and ectopic follicles/eggs frequently arise from other reproductive disorders, and can be very debilitating, requiring surgical management to be effectively treated (Portas, 2017a; Stahl & DeNardo, 2019).

It is important not to overlook this pathology during reproductive tract surgery. The coelomic cavity and reproductive tract should be thoroughly evaluated, and any ectopic follicle/egg or other irritant source debrided and removed (Perry & Mitchell, 2017; Stahl & DeNardo, 2019). Ovariectomy or ovariosalpingectomy are advised to avoid recurrence (McArthur, 2004; Mans & Sladky, 2012; Portas, 2017a). Before closure of the coelom, further diagnostic samples can be taken from diseased organs for histopathology and/or microbiology, and a thorough lavage of the coelom with warm sterile saline should be made to eliminate any residual or disseminated yolk material (Stahl, 2003; Perry & Mitchell, 2017; Portas, 2017a).

### 4.2.3. Endoscope-Assisted Ovariectomy and Ovariosalpingectomy

Endoscope-assisted procedures not only have the advantages related to the prefemoral approach, but also provide great coelomic visualization and the option to perform the procedure intracorporeally (Proença & Divers, 2015). Furthermore, it allows retrieval of diagnostic valuable biopsies where further diagnostic sampling is warranted.

The endoscope is used to help locate, grasp, and then exteriorize the desired structure through a small incision, so that procedure can be completed outside the coelom. The endoscope has been used to assist in many different surgical procedures, of most importance to the reproductive disorders, ovariectomy, ovariosalpingectomy, salpingotomy, and cystotomy (Divers, 2019c). Any ectopic eggs or follicles free in the coelom may also be readily identified during coelioscopy and removed.

The patient is positioned in lateral or dorsal recumbency, and the coelom is accessed unilaterally through the prefemoral fossa. The telescope is introduced inside the coelomic cavity in order to identify the ipsilateral ovary and guide its exteriorization with the endoscopic atraumatic grasping forceps (Fig. 17.A). The ovary is grasped at its interfollicular connective tissue and gently pulled towards the prefemoral incision with caution not to rupture any follicles (Fig. 17.B). When the grasped portion is being held near or out the incision, the endoscope may be set aside, and the remainder of the ovary carefully brought out. Routine ovariectomy is performed, and the same process is repeated for the contralateral ovary (Fig. 17.C). The oviducts should not be removed except when diseased, in which case, identification, grasping, and exteriorization for routine salpingectomy can be achieved aided by endoscopy. Frequently, bilateral ovariectomy is performed through the same incision, nonetheless, some terrestrial chelonians may require a bilateral approach due to obstruction caused by their large intestine and bladder, and their small prefemoral gap (Innis, 2010; Divers, 2019c). Lastly, the endoscope may be used to inspect the ligation sites to assess for any haemorrhage or leftover ovarian tissue (Innis, 2010).

Complications associated with this procedure include organ perforation when creating the entry to the coelom, haemorrhage, ectopic or ruptured follicle/egg, follicle/egg unable to pass through the prefemoral incision due to size, among others. Infections are rarely seen after the surgery (Proença & Divers, 2015).

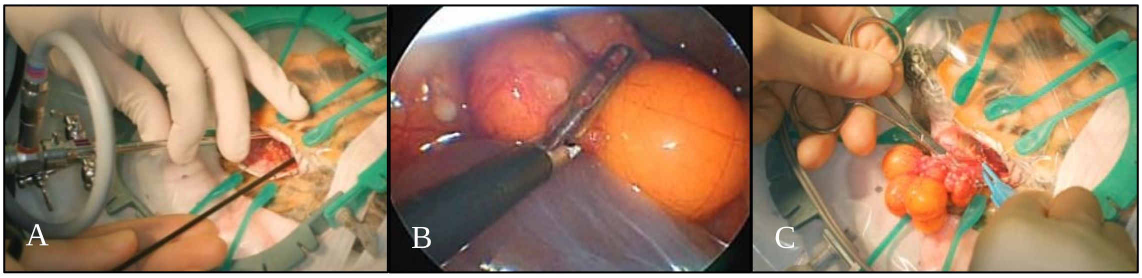



Fig. 17. Endoscope-assisted prefemoral ovariectomy in a female chelonian. The telescope is used to identify the ovary (A), assure its correct grasp at the interfollicular connective tissue using grasping forceps (B), and guide its exteriorization where routine ovariectomy can be carried out (C) (adapted from Innis et al., 2007).

### 4.2.4. Prognosis and Prevention

The prognosis for diseases of the reproductive tract can vary depending on the chronicity and extent of the disease, as well as with the state of the patient at presentation. Early detection and intervention generally imply a more favourable outcome compared to chronic disorders that may present degenerative changes, and likely require surgical resolution. Generally, in the chelonian patient, most reproductive disorders have a good prognosis for survival of the individual, but a poor one for its reproductive success (Stahl & DeNardo, 2019).

For nonbreeding chelonians, reproductive disorders may be effectively prevented with elective ovariectomy or ovariosalpingectomy. However, in a general sense, good husbandry practices with an appropriate environment and nutrition for the species, careful breeding practices, and routine veterinary evaluations may be helpful in lowering the incidence of reproductive disease, especially in chelonians intended for breeding (Portas, 2017a; Stahl & DeNardo, 2019).

# II. Endoscope-Assisted Prefemoral Ovariectomy and Ovariosalpingectomy in Chelonians: a Preventive and Curative Procedure

## 1. Introduction

Chelonians, right from the onset of their reproductive maturity, exhibit a susceptibility to a wide array of reproductive diseases (Innis & Boyer, 2002). Many of these ailments arise as cascading consequences, with some being directly linked to others. Moreover, certain reproductive diseases stem from the challenges posed by captivity, including inadequate housing conditions and the absence of essential social and environmental cues (Rivera, 2008; Chitty & Raftery, 2013c; Stahl & DeNardo, 2019).

Female chelonians are commonly affected by reproductive diseases such as POFS, dystocia, ectopic eggs, oophoritis, salpingitis, yolk coelomitis, and cloacal and oviductal prolapses (Innis et al., 2007; Rivera, 2008). These disorders, although rare emergencies, exert a deleterious impact on the chelonian's health over time, often resulting in the development of chronic conditions that tend to resurface in sync with the reproductive cycle (Perry & Mitchell, 2017; Stahl & DeNardo, 2019).

To ensure a correct diagnosis and application of a fitting treatment plan, it is vital to pursue a thorough medical history, with a detailed physical exam, followed by haematological analysis and diagnostic imaging (Innis & Boyer, 2002). Endoscopy too, may be employed as a great diagnostic tool (Hernandez-Divers et al., 2005).

Although medical therapy has relative success managing egg retention in chelonians, other conditions chiefly require surgical intervention (Innis & Boyer, 2002; Hellebuyck & Vilanova, 2022). Henceforth, sterilization emerges as the preferred treatment for the majority of reproductive disorders, particularly in nonbreeding reptiles (Stahl & DeNardo, 2019).

Traditionally, gonadectomy was favoured solely as a curative practice, given that it required accessing reproductive organs through an invasive and traumatic osteotomy (Proença & Divers, 2015). The development of both soft-tissue approaches and endoscopic techniques has resulted in a more streamlined alternative, with a shorter surgery time, less trauma, and a swifter recovery (Innis, 2010; Proença et al., 2014a; Divers, 2019c). In light of this, the endoscope-assisted prefemoral approach should always be the preferred technique unless contraindicated (Di Girolamo & Mans, 2016; Hellebuyck & Vilanova, 2022). Recommended by the expanding workforce of endoscopy and herpetology specialists, this surgery has gained popularity in recent years as an elective procedure due to its appeal for owners of nonbreeding chelonians who would rather not only avoid the deterioration of their pet's health, but also the financial hassle that comes with it, year after year (Innis, 2010; Proença & Divers, 2015). Endoscope-assisted prefemoral ovariectomy and ovariosalpingectomy has been described as a solution for several reproductive diseases in many different species of turtles and tortoises (Innis et al., 2007; Mans & Sladky, 2012; Proença et al., 2014a; Antunes et al., 2020; Hellebuyck & Vilanova, 2022).

## 2. Objectives

This retrospective study compiles data from 36 cases of chelonians admitted for endoscope-assisted prefemoral ovariectomy and ovariosalpingectomy. The study aims to characterize the affected population, to elucidate the diagnostic and therapeutic approach to female chelonians suffering from reproductive disease, and to report the complications, endoscopic findings, and outcomes of the surgical procedure.

## 3. Materials and Methods

Chelonians eligible for this study were patients admitted for endoscope-assisted prefemoral ovariectomy or ovariosalpingectomy during the course of one year, from June 2022 to June 2023. The procedure was performed by Dr. Xavier Valls, generally in Exòtics Veterinària clinic in Barcelona, Spain. Data was retrieved, with permission, from the medical reports of the clinic and from Dr. Xavier Valls' endoscopy visual media archives.

The variables reviewed for every patient of this study included the age and species of the chelonians, clinical history, and findings registered during physical examination on initial presentation.

The medical records of the ensuing diagnostic tests (all performed in-house) were also included, such as hemogram, biochemical analysis, radiography, and ultrasonography. However, not all patients underwent every aforementioned test, as every case followed its individual path towards a diagnosis. Consequently, the number of animals for each diagnostic test varied.

Cases where medical therapy was attempted prior to surgical management were analysed for the efficacy of the treatment.

Video and photo recordings taken during surgery with the endovideo camera were reviewed for findings and complications encountered amidst the procedure. Records of the follow-up consults and phone calls after sterilization were also included in this study.

### 3.1. Characterization of the Population

The chelonians involved in this study belonged to a variety of aquatic and land species, among them *Trachemys* spp., *Mauremys* spp., *Pseudemys* spp. *Graptemys* spp., *Testudo* spp., *Chelonoidis* spp., and *Kinixys* spp., totalling at 14 species operated. The age of the patients was considered for the making of the study.

### 3.2. Clinical History and Examination

All 36 chelonians underwent a prompt clinical examination after a thorough review of their medical history. The study exhibits the reason for presentation of the patients along with the clinical findings obtained during the physical examination. With the purpose of facilitating interpretation of the results: lethargy, dehydration, cloacal prolapse, and oedema of the prefemoral area were classified as nonspecific signs; skin abrasions, erythema, and rhamphotheca overgrowth were categorized as dermatological signs; blepharitis and ocular discharge were grouped as ocular signs; respiratory signs included nasal discharge and dyspnoea; palpation of eggs through the prefemoral fossa was labelled as a reproductive sign; and fractures of the shell as a traumatologic sign.

### 3.3. Hemogram and Biochemical Analysis

Blood samples were generally taken from the jugular vein, as this site is less likely to contaminate the sample with lymph (Stacy & Harr, 2021; Campbell & Grant, 2022a). Occasionally, whenever restraint of the head was made challenging or impossible by the patient, blood would be taken from the dorsal coccygeal vein. The site was aseptically prepared with 70% ethanol. Blood was withdrawn using a 23- or 25-gauge needle and a 1 mL syringe, both previously heparinized. Blood smears were prepared soon thereafter and stained using Diff-Quik stains. The remaining blood was put in a microhaematocrit tube and/or collection tube and centrifuged.

Some of the data regarding the haematocrits was lost and unable to be retrieved. Even so, the haematocrit of 24 chelonians was reviewed.

Blood samples of 23 females were submitted to biochemical analysis, where the following parameters were quantified: albumin, total proteins, aspartate transaminase (AST), UA, total bile acids, sodium, chloride, potassium, calcium, and P. These assays enabled the assessment of the number of chelonians with changes in their biochemical parameters, as well as which parameters were most frequently altered.

This study includes the leucograms of the 32 patients operated in Exòtics Veterinària, as access to the remaining four chelonians was not possible. Total white blood cell (TWBC) counts were estimated by counting all leucocytes in 10 different monolayer fields at 40x magnification under microscopy, averaging the numbers obtained, and multiplying it by 2000, giving the results in white blood cells (WBC) per microliter (Campbell & Grant, 2022b). The WBC differentials were performed under immersion oil by classifying 100 leucocytes into heterophils, lymphocytes, eosinophils, basophils, or monocytes. The obtained percentage of each cell type multiplied by the TWBC estimate provided the absolute number of that leucocyte (Campbell & Grant, 2022c).

Reference values for healthy chelonians were taken from “Reptiles” in Carpenter’s Exotic Animal Formulary (6th edition) by Carpenter & Harms (2023) and from “Hematology and Biochemistry Tables” in Mader's Reptile and Amphibian Medicine and Surgery (3rd edition) by Divers & Stahl (2019).

### 3.4. Diagnostic Imaging

Radiography was carried out in 30 chelonians. In general, whole body dorsoventral and lateral projections were performed, without the need for restraint of any kind. Radiography would permit differentiation between follicular stasis and dystocia by evidencing eggs with calcified shells, eggs with deformities, and possible ectopic eggs. Moreover, conditions such as fractures and MBD could be identified from the assessment of bone integrity and mineralization.

Sonography was performed in 35 chelonians, through the inguinal window, using a high frequency microconvex transducer, and, less frequently, a linear transducer. This imaging modality would additionally enable evaluation of the soft tissue structures of the caudal coelom. The ovaries were appreciated for the size of their follicles, echogenicity, and homogeneity, while eggs identified on ultrasound were assessed for shell mineralization, deformities, degenerative changes, and ectopic locations. Other conditions such as coelomitis, oviduct torsion, and masses could also be evaluated.

### 3.5. Medical Therapy

On imaging exams, the presence of eggs and the absence of an obvious physical impediment to egg laying was identified in 16 chelonians. In these cases, a presumptive diagnosis of nonobstructive dystocia was made, warranting oxytocin therapy. A first oxytocin (5 IU/kg) administration was given IM on the hindlimbs and, if complete clutch expulsion was not seen, a second injection would be given with the same dose 24 hours later. Radiographs were repeated to confirm therapy success. The treatment was considered effective in cases where all eggs were expelled, partially effective in cases where clutch expulsion was incomplete, and ineffective in cases where no eggs were laid.

### 3.6. Endoscope-assisted Prefemoral Ovariectomy and Ovariosalpingectomy

The coelioscopic component of the procedure enabled documentation of surgery findings and surgical complications, further permitting identification and characterization of coelomitis cases. The presence of coelomic fluid, adhesions, diphtheroid plaques, fibrinous exudate, and/or opaqueness or hyperaemia of serosal surfaces were considered compatible with a coelomitis diagnosis.

#### 3.6.1. Patient Preparation and Anaesthesia

Patients in unstable condition were first rehydrated and stabilized mainly with fluid therapy. Hospitalized chelonians were maintained in temperatures of 25°C or above and supplemented with UVB lighting. Prior to anaesthesia and surgery, a feeding cycle was skipped.

Temperature in the operating room and recovery room was equally kept at 25°C. Chelonians were sedated with midazolam (2 mg/kg) and methadone (1-1.5 mg/kg) IM 30 minutes before anaesthetic induction with alfaxalone (10-20 mg/kg) IV. After induction, the patients were intubated, positioned in oblique recumbency on the heated surgical table with the help of a vacuum bean bag, and connected to a mechanical small animal ventilator for intermittent positive-pressure ventilation (IPPV) (4 to 6 breaths per minute at 10 to 12 mmHg). Depth of anaesthesia was maintained and regulated with isoflurane at 1 to 2%, while the heart rate was monitored using a Doppler probe placed on the thoracic inlet. The suspended hindlimb was taped caudally to the shell or, as seen in Fig. 18, to the other leg, so that the now exposed prefemoral fossa, along with the surrounding shell, could be aseptically prepared with chlorhexidine gluconate 4% and draped. Lastly, lidocaine (4 mg/kg) was infiltrated in the incision site.


Fig. 18. Spanish pond turtle (*Mauremys leprosa*) prepared for surgery: 1 - the heart rate is being monitored with the Doppler probe placed on the right thoracic inlet; 2 - the patient is intubated and connected to a mechanical ventilator to maintain the anaesthetic plane with isoflurane supplied by IPPV; 3 - the hindlimbs are taped together to expose the entry point (Courtesy of Dr. Xavier Valls).

### 3.6.2. Surgical Procedure

The skin was incised with a scalpel blade from cranial to caudal in the centre of the inguinal fossa. Scissors were used to bluntly dissect the fat and connective tissues between the sartorius and iliacus muscles until exposure of the oblique and transverse abdominal muscles. Entry to coelom was achieved by perforation of these tendinous aponeurosis with a Veress needle.

The 30°, 18 cm x 2.7 mm rigid endoscope (connected to a LED light source) was introduced in the coelom for a quick survey of the internal organs and identification of the ipsilateral ovary. Whenever visualization of internal structures was considered suboptimal, the 4.8 mm working sheath would be attached to the telescope for gas infusion into the coelom, allowing improved visibility.

After recognition of the ovary, the skin and muscle incisions were extended using scissors, facilitating the introduction of atraumatic grasping forceps. These were used to grasp an avascular area of the ovarian interfollicular connective tissue (Fig. 19) and pull the ovary toward the incision under endoscopic guidance.

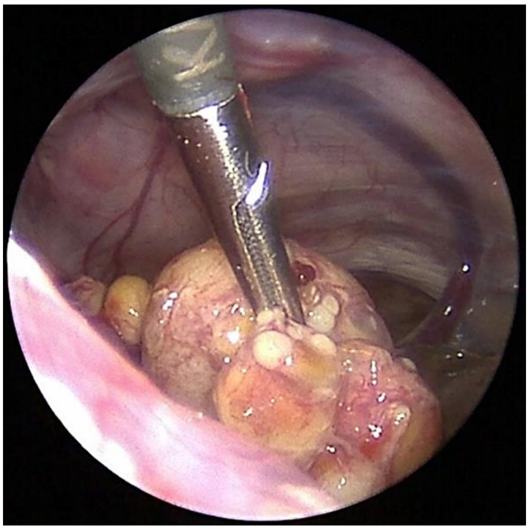


Fig. 19. Coelioscopic guidance for the grasping forceps to grip and traction the interfollicular connective tissue during ovariectomy (Courtesy of Dr. Xavier Valls).

With the ovary held near the incision, the endoscope was removed and set aside. The numerous follicles were then gently exteriorized, often requiring further extension of the incision. Following complete exteriorization, the ovarian vasculature was ligated with stainless-steel surgical ligation clips and the mesovarium was transected with the use of bipolar cautery forceps (Fig. 20). Coelioscopic examination of the ligation site was performed to verify a correct haemostasis and complete excision of all ovarian tissue. The second ovary was attempted to be removed through the same prefemoral incision in the same manner as the first.

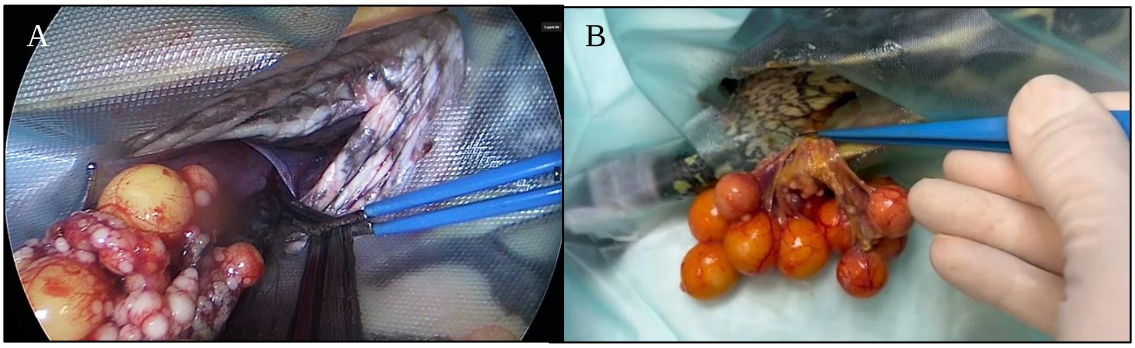



Fig. 20. Transection of the ovarian pedicle (A) and mesovarium (B) using bipolar radiosurgery after ligating the ovarian vasculature (Courtesy of Dr. Xavier Valls).

In the cases where bilateral ovariosalpingectomy was advised, succeeding ovariectomy, the ipsilateral oviduct was identified with coelioscopy and exteriorized from cranial to caudal with atraumatic grasping forceps or haemostats. The fimbria and vessels in the mesosalpinx were clamped with haemostats and ligated with polypropylene-polyethylene sutures and/or stainless-steel surgical ligation clips and transected with radiosurgery.

The distal oviduct was clamped with haemostats and ligated using a circumferential transfixing ligature and/or stainless-steel ligation clips, finally being transected with bipolar radiosurgery (Fig. 21.A and B). Ligation sites were inspected with coelioscopy to verify correct haemostasis (Fig. 21.C). Ovariosalpingectomy of the contralateral organs was attempted similarly through the same incision.

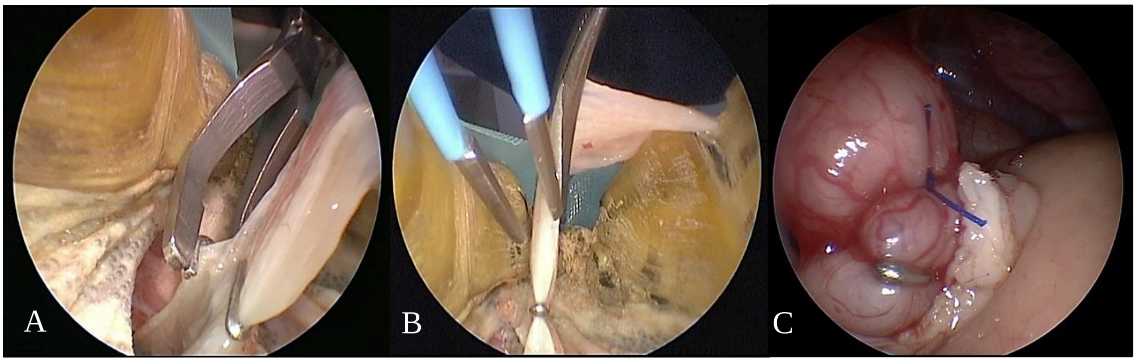



Fig. 21. (A) Ligation of the oviduct with stainless-steel ligation clips. (B) Transection of the oviduct using bipolar cautery forceps. (C) Verification of the ligation sites through coelioscopy to guarantee there is no bleeding (Courtesy of Dr. Xavier Valls).

When performing the surgical procedure through a singular prefemoral incision proved unfeasible, that incision would be closed, the recumbency altered to the other side, and the surgical procedure repeated.

Liver biopsies and ovary samples were sent for histopathologic evaluation whenever warranted and substantiated by owner compliance. Biopsies were taken from the edge of the liver lobe, under coelioscopy, using biopsy forceps.

Ectopic eggs inside the bladder had to be removed through cystotomy after unfruitful attempts of removal through cloacoscopy. The bladder would be identified and brought to the incision, later being stabilized with stay sutures secured by haemostats and by packing the coelom with moistened gauze. An incision would be made on a less vascularized portion of the lateral bladder wall while simultaneously aspirating the overflowing urine. Eggs were removed through a combination of gentle manipulation, collapsing, and the use of forceps and other surgical instruments.

Ectopic eggs and follicles inside the coelom were removed after careful debridement of adhesions between these and surrounding structures.

Before closure, a lavage of the coelom was executed with either warm saline or a 5 mL 1:100 diluted solution of amikacin (250 mg/mL).

Polypropylene-polyethylene sutures were used for the closure of every layer. The coelomic membrane was sutured in a simple continuous manner while the aponeuroses of the abdominal muscles were closed in an interlocking pattern. Lastly, the subcutaneous fat and skin were closed separately, also with a simple continuous suture.

### 3.6.3. Postoperative Care

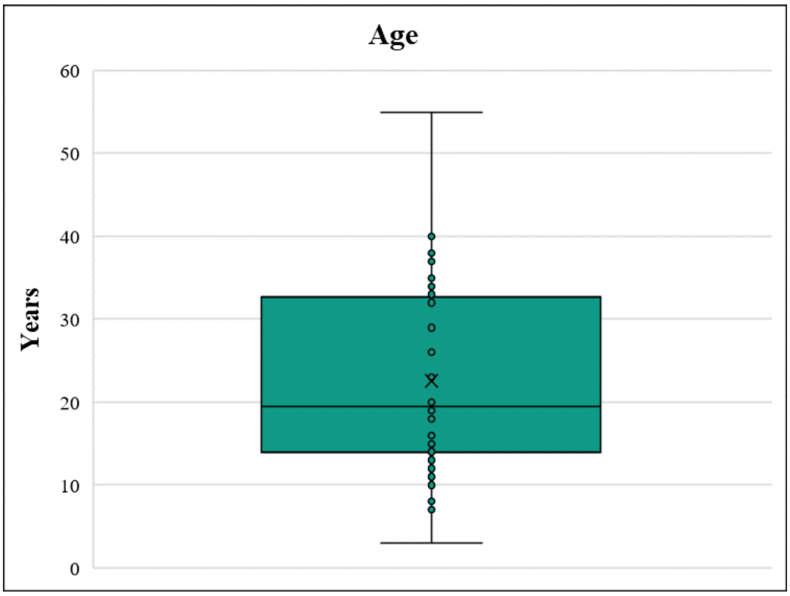
Following the surgical procedure, isoflurane administration was stopped, and the opioid and benzodiazepine anaesthetics were reverted using naloxone (0.04 mg/kg) and flumazenil (0.01 mg/kg) IM, respectively. At this point, patients were disconnected from the anaesthetic circuit and ventilation was performed manually with the use of an Ambu bag. The chelonians were monitored for their heart rate and kept on the heated surgical table in ventral recumbency until resumption of spontaneous breathing and restoration of pinching, withdrawal, and palpebral reflexes. Postoperatively, ceftazidime (20 mg/kg) was administered IM every 48 hours for 15 days and meloxicam (0.2 mg/kg) was administered IM every 24 hours for five to seven days.

Aquatic turtles were granted full access to water 24 to 48h after. Patients were dispatched in the immediate days following the procedure unless apathy persisted. A control call or, preferably, control visit was generally made seven, 14, 30, 60, and 90 days after surgery to infer on the patients' well-being and surgical wound healing, although the number and timing of the controls varied greatly between patients. Chelonians were considered fully recovered from surgery after returning to normal activity levels and regaining their appetite.

# 4. Results

## 4.1. Characterization of the Population

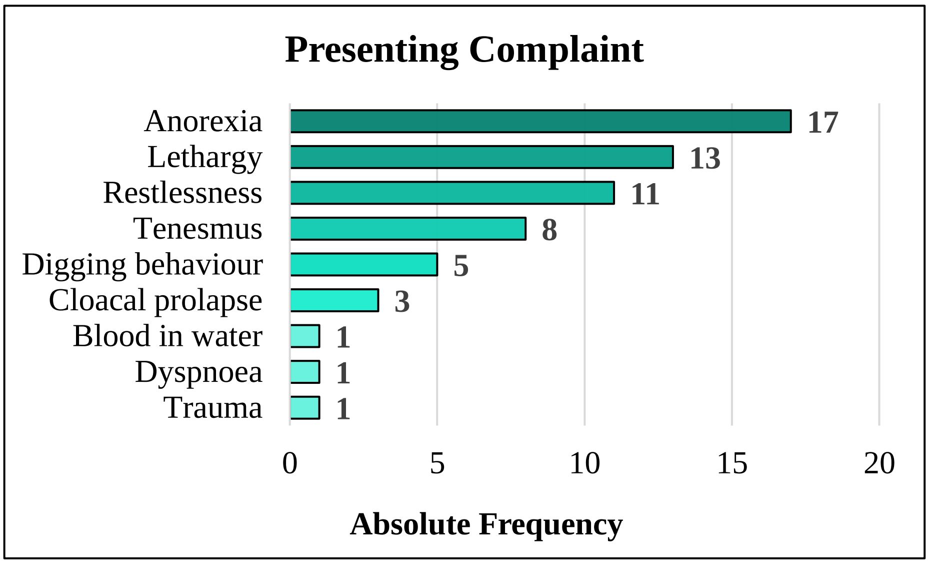
Thirty-six animals fulfilled the inclusion criteria and were admitted to the present study. Graphic 1 illustrates the age distribution of the patients admitted for sterilization. It shows the youngest admitted chelonian was three years old, while oldest was 55 years. Additionally, the mean age of the group at initial presentation was 19.5 years, with half of the sample being contained between the ages of 14 and 33 years.




Graphic 1. Boxplot of the age (in years) of chelonian operated.

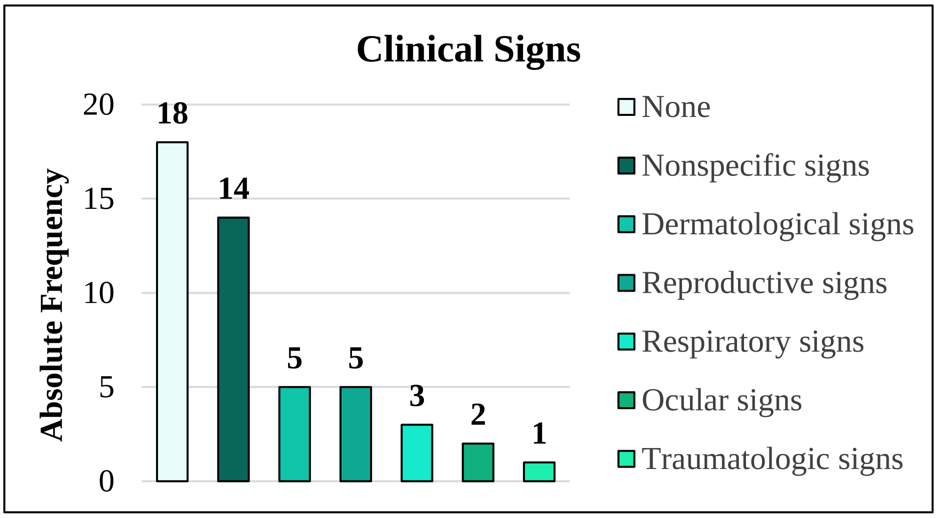
## 4.2. Clinical History and Examination

As evidenced by Graphic 2, the most common reasons for presentation given by the owners were hyporexia/anorexia (n=17/36; 47.2%), lethargy/apathy (n=13/36; 36.1%), restlessness (n=11/36; 30.5%), tenesmus (n=8/36; 22.2%), digging behaviour (n=4/36; 11.1%), and cloacal prolapse (n=3/36; 8.3%). Out of the 36 chelonians in this study, 20 had previously produced and laid eggs (55.6%), nine of which suffered from recurrent egg-binding (25.0%).




Graphic 2. Presenting complaints represented by absolute frequency.

Clinical signs observed on the first consult are demonstrated on Graphic 3. No remarks were reported in 18 of the 36 chelonians (50.0%). Clinical signs observed were frequently nonspecific (n=14; 38.9%), dermatologic (n=5; 13.9%), and reproductive (n=5; 13.9%).

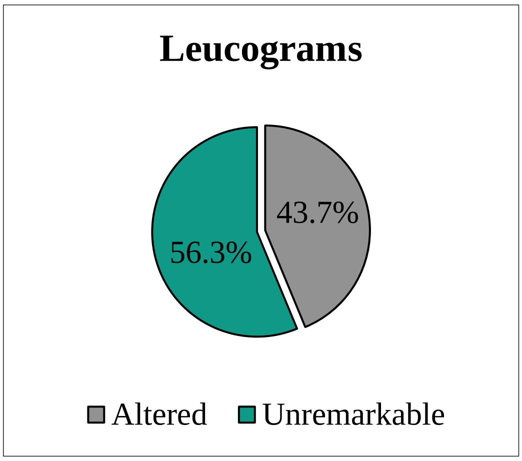



Graphic 3. Clinical signs recorded at initial presentation in absolute frequency.

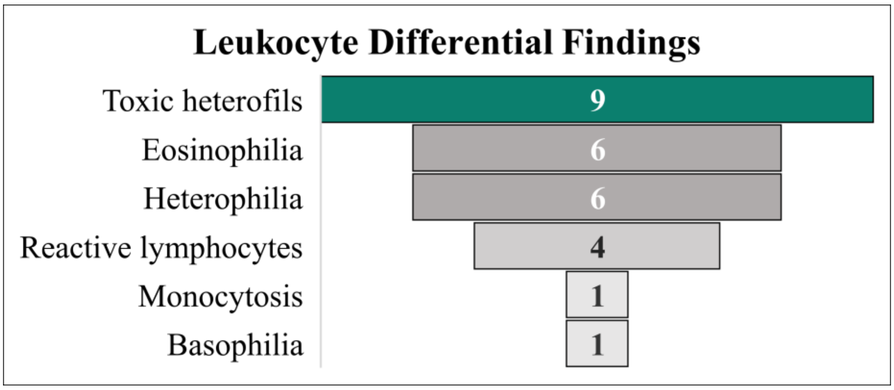
### 4.3. Hemogram and Biochemical Analysis

Out of the 24 haematocrits retrieved, eight (33.3%) were increased beyond the species' range and four (17%) had a decreased packed cell volume (PCV).

Analysis of the leucograms of 32 chelonians showed 18 had all values within their normal range (56.3%), while the remaining 14 had some alteration in one or more white blood cell lines (43.7%) (Graphic 4). TWBC counts revealed leucocytosis in 10 patients (31.3%). Graphic 5 demonstrates the findings registered during the manual leucocyte differential. The most frequent changes in the WBC lines were heterophilia (n=6) and eosinophilia (n=6), whereas basophilia and monocytosis were each seen only once. Nine chelonians exhibited some degree of toxicity of the heterophils, while four displayed reactive lymphocytes.




Graphic 5. Relative frequency of chelonians with altered and unremarkable leucograms.



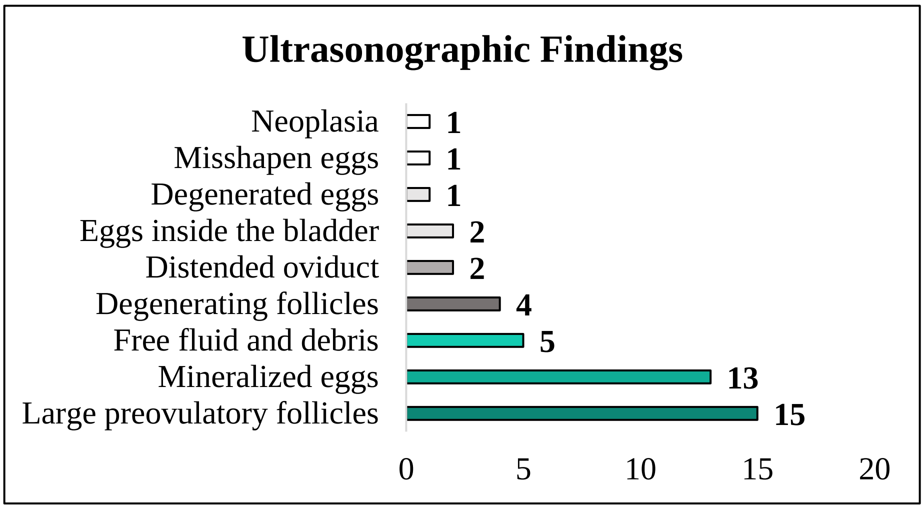

Graphic 4. Findings of manual leucocyte differential count in absolute frequency.

Regarding the biochemical assays, 18 of the 23 analyses showcased all parameters within the normal range for the species. Out of the remaining five, the most common increases reported were in UA (n=4), AST (n=3), and P (n=2).

### 4.4. Diagnostic Imaging

Out of the 30 radiographs taken, 17 evidenced calcified eggs (56.7%), two of which further showed abnormally formed eggs (7.7%) and another two suggested ectopic eggs inside the bladder (7.7%). One of the gravid chelonians also had a visible shell fracture.

On ultrasound, common observations included: numerous large preovulatory follicles filling up the caudal coelom (n=15/35; 42.9%), calcified eggs inside the oviduct (n=13/35; 37.1%), anechogenic fluid with floating echogenic debris (n=5/35; 14.3%), and deformed and heterogenous follicles (n=4/35; 11.4%). Less frequently, ultrasonography was able to identify distension of the salpinx filled with fluid (n=2/35; 5.7%), eggs inside the bladder (n=2/35; 5.7%), degenerated eggs (n=1/35; 2.9%), misshapen eggs (n=1/35; 2.9%), and a heterogenous and irregular mass in the anatomic region of the ovary (n=1/35; 2.9%). The ultrasonography findings are expressed in Graphic 6 and depicted in Fig. 22.

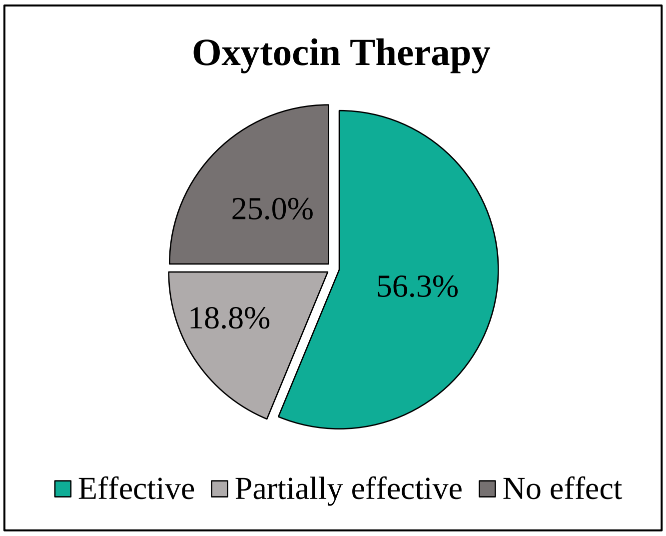



Graphic 6. Ultrasonographic findings expressed in absolute frequency.


Fig. 22. Examples of the ultrasonographic findings encountered. (A) Large preovulatory follicles. (B) Mineralized eggs. (C) Heterogenous and irregular mass (confirmed later to be an ovarian neoplasia) (Courtesy of Dr. Xavier Valls).

### 4.5. Medical Therapy

Medical management using oxytocin administrations proved effective in nine chelonians (56.3%), partially effective in three chelonians (18.7%), and ineffective in four (25.0%). These results are expressed in Graphic 7.




Graphic 7. Effectiveness of oxytocin therapy expressed in relative frequency.

### 4.6. Endoscope-assisted Prefemoral Ovariectomy and Ovariosalpingectomy

Out of all 36 chelonians sterilized, ovariectomy was conducted in 28 females, while ovariosalpingectomy was performed in eight. The procedure was elective for 12 patients.

#### 4.6.1. Complications

Complications (whether surgical or secondary to reproductive disease) were reported in 31 of the 36 chelonians operated (86.1%).

As a result of the hindering complications, two chelonians died (5.6%). One did not regain consciousness, passing away immediately after the procedure (Fig. 23), and the other during the late postoperative period. On another instance, the procedure had to be interrupted and rescheduled due to extended surgery time.

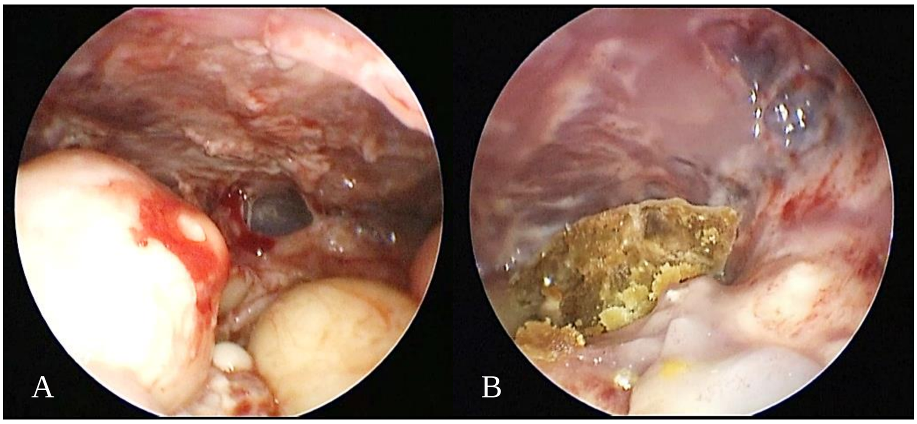



Fig. 23. Coelioscopy of the 38-year-old red-eared slider (*Trachemys scripta scripta*) that died immediately after surgery. (A) Appearance of the coelomic cavity after ovariectomy. Note the pinkish-white fibrinlike yolk material covering every serosal surface of the coelom and the haemorrhage created during ovariectomy. (B) Ectopic egg free in the coelom with marked degeneration. Note the opaqueness and petechiae of the coelomic wall serosa and the abundant thick, white exudate (Courtesy of Dr. Xavier Valls).

#### 4.6.1.1. Surgical Complications

The most commonly arising surgical complications (Table 1; Fig. 24) were follicles found in ectopic locations in the coelom (n=5; 13.9%), haemorrhage (n=5; 13.9%), rupture of follicles during ovary traction with consequent yolk leakage (n=4; 11.1%), and iatrogenic bladder perforation or tear (n=3; 8.3%).

Table 1. Surgical complications encountered during the endoscope-assisted prefemoral ovariectomies and ovariosalpingectomies, expressed in absolute and relative frequency.

| **Complications** | **n** | **%** |
|---|---|---|
| Ectopic follicles free in the coelom | 5 | 13.9 |
| Haemorrhage | 5 | 13.9 |
| Follicle rupture | 4 | 11.1 |
| Bladder rupture | 3 | 8.3 |

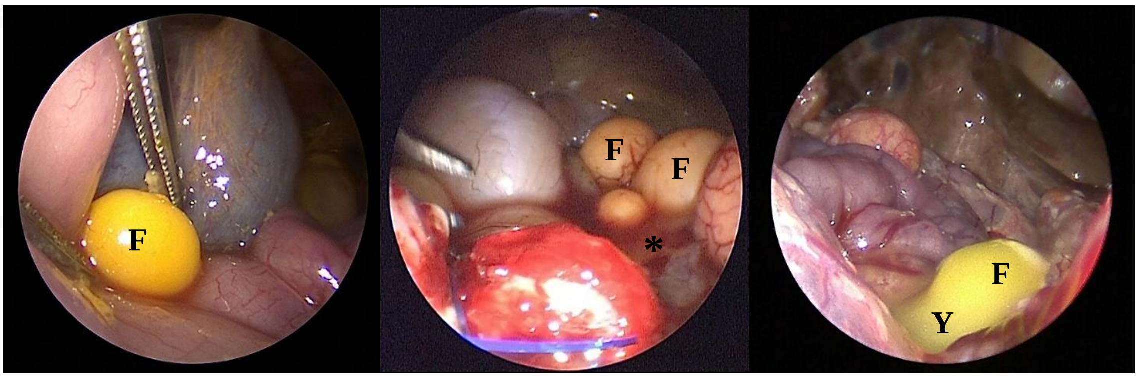



Fig. 24. Surgical complications observed during ovariectomies/ovariosalpingectomies of chelonians. (A) Ectopic follicle (F) free in the coelomic cavity. (B) Haemorrhage (*). (C) Follicle rupture with yolk (Y) leakage (Courtesy of Dr. Xavier Valls).

#### 4.6.1.2. Complications Secondary to Reproductive Disease

During the surgical procedures, it was frequent the registration of findings secondary to reproductive disease (Table 2; Fig. 25). Most of these had not been diagnosed in previous exams and were found to often interfere and considerably extend total surgery time. These complications included yolk coelomitis (n=23; 63.9%), follicles found in ectopic locations in the coelom (n=4; 11.1%), ectopic eggs inside the bladder requiring cystotomy (n=3; 8.3%), ectopic eggs free in the coelom (n=3; 8.3%), oviduct torsion (n=2; 5.6%), and cloacal prolapse (n=2; 5.6%).

Table 2. Complications secondary to reproductive disease encountered during the endoscope-assisted prefemoral ovariectomies and ovariosalpingectomies, expressed in absolute and relative frequency.

| Complications | n | % |
|---|---|---|
| Yolk coelomitis | 23 | 63.9 |
| Ectopic follicles free in the coelom | 4 | 11.1 |
| Ectopic eggs inside the bladder | 3 | 8.3 |
| Ectopic eggs free in the coelom | 3 | 8.3 |
| Oviduct torsion | 2 | 5.6 |
| Cloacal prolapse | 2 | 5.6 |

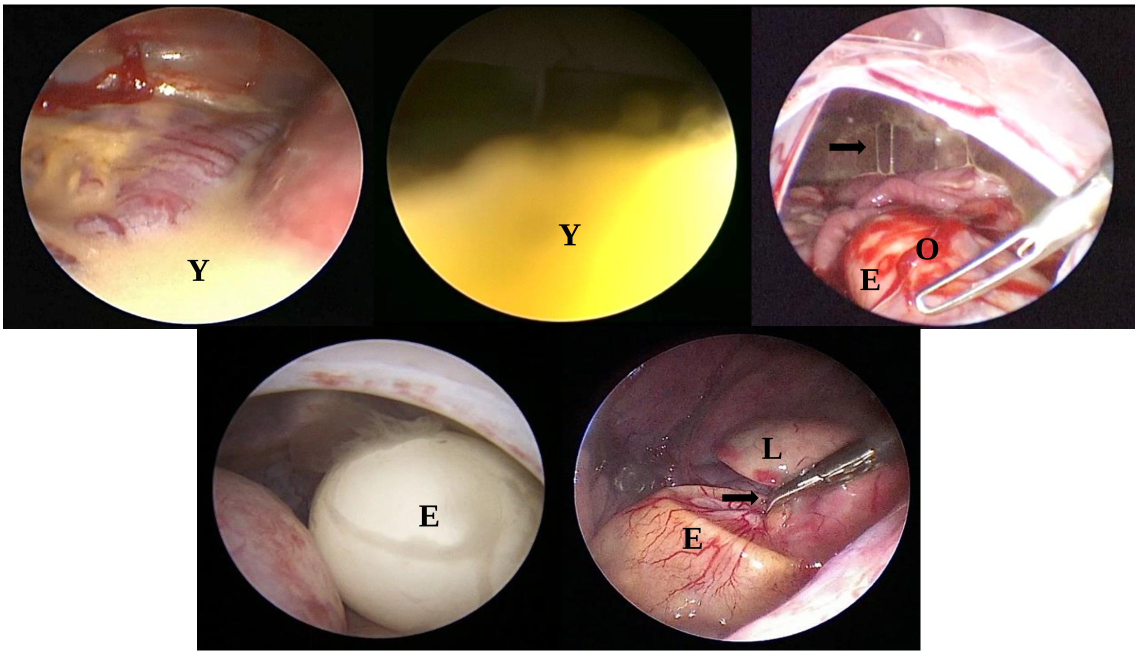


Fig. 25. Complications secondary to reproductive disease observed during ovariectomies and ovariosalpingectomies of chelonians: coelomitis with hyperaemia of the coelomic serosa, adhesions (arrow), and free yolk (Y) capable of obscuring the telescope lens; torsioned oviduct (O) with egg (E) inside; ectopic eggs inside the bladder; and ectopic eggs in the coelomic cavity adhered to livre (L) (Courtesy of Dr. Xavier Valls).

### 4.6.2. Yolk Coelomitis Characteristics

Visualization of internal organs during coelioscopy permitted identification of lesions compatible with coelomitis in 23 of the 36 chelonians (63.9%) operated. While presentation of the condition varied substantially between females, certain prominent characteristics remained consistent across numerous cases.

Coelomic fluid was observed from moderate quantities of free egg yolk to dense fibrinous exudates covering the entire coelom (Fig. 26).

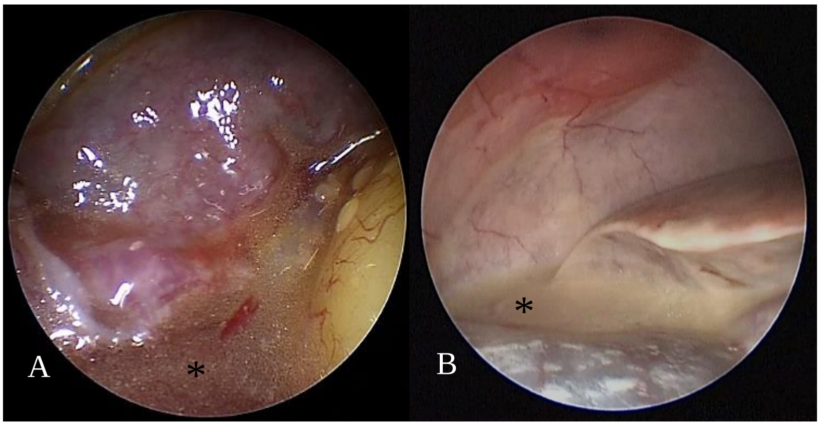



Fig. 26. Coelomic fluid (*) seen ranging from a blurry appearance with lipid droplets (A) to a thick brownish-white exudate (B) in coelomitis cases (Courtesy of Dr. Xavier Valls).

Neovascularization and adhesions (Fig. 27) were found primarily between the ovaries and surrounding structures. In more advanced cases, these were encountered dispersed throughout all coelomic serosal layers.

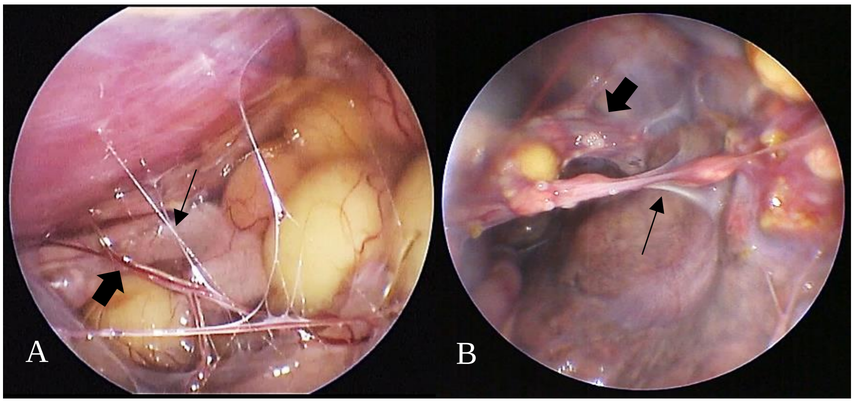



Fig. 27. Neovascularization (thick arrow) and fibrous adhesions (thin arrow) formed between the ovarian follicles and the coelomic wall (A) or liver (B) of chelonians with coelomitis (Courtesy of Dr. Xavier Valls).

Serosal surfaces of the coelomic walls and organs were often seen hyperaemic and opaque, acquiring a yellow, white, grey, or even brown colour (Fig. 28).

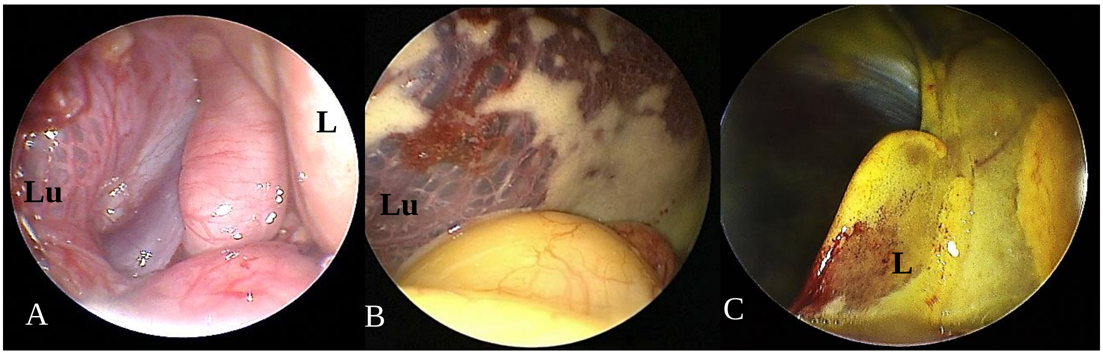



Fig. 28. Comparison between the serosal membranes of the healthy chelonian (A) lung (Lu) and liver (L), and the serosal membranes of chelonians with coelomitis (B and C). Note the pale pink colours of the organs being replaced with a dark red or white and yellow staining in the diseased reptiles (Courtesy of Dr. Xavier Valls).

### 4.6.3. Biopsies

Biopsies were retrieved from the liver (n=8) and ovaries (n=3) of eight patients and submitted for histopathologic evaluation.

Laboratory reports of the liver samples revealed all chelonians presented hepatic lipidosis (100%). Seven of the eight (87.5%) samples also displayed hepatocyte hyperplasia and hypertrophy containing vacuoles of phagocytized yolk, and mesothelial lesions provoked by the yolk material free in the coelomic cavity, ultimately diagnosing visceral egg yolk coelomitis. One of the liver biopsies additionally reported the presence of a metaplastic oviductal tissue thought to be an adenocarcinoma.

Histopathology of the ovary samples diagnosed fibrinous-lipogranulomatous oophoritis with follicular atresia and degeneration in two females, and one other case of an ovarian teratoma.

### 4.6.4. Postoperative Follow-up

On the first follow-up visit or call, it was confirmed that 30 of the 35 chelonians (85.7%) had recovered uneventfully from the surgery having resumed their regular behaviours and regained their appetite. Conversely, in the other five cases, chelonians remained apathetic and anorectic during the succeeding seven to 21 days. One chelonian died 20 days after the procedure, while the remaining four went on to recover following additional treatments.

On monthly control ultrasonographies, two of 13 chelonians (15.4%) displayed ovary remnants with developing follicles, while the female of the extirped ovarian neoplasia presented multiple small masses developing throughout the coelomic cavity.

## 5. Discussion

Most chelonian species are reproductively active for a vast period of their lives, having the potential to live for at least half a century or even longer (Vitt & Caldwell, 2014; Boyer & Charles J., 2019). The youngest and the oldest chelonians in this study were three and 55 years old, respectively. Additionally, Graphic 1 reveals that 25.0% of the operated females were 14 years or younger. This data conveys that sexual maturity (seen to be reached as soon as three years of age in this study) can be, in fact, much more precocious under captive conditions (Innis & Boyer, 2002). The wide range of ages in this study further corroborates that age may not be a key underlying risk factor in the onset of reproductive disorders, as stated by Hellebuyck & Vilanova (2022).

During the visits, owners reported their pet being mostly hyporectic/anorectic (n=17; 47.2%), lethargic/apathetic (n=13; 36.1%), and/or restless (n=11; 30.5%). This data is in agreement with the existing literature which frequently describes the expression of reproductive disease as nonspecific (Innis & Boyer, 2002; McArthur, 2004; Portas, 2017a; Stahl, 2019a). Although anorexia may not be considered a clinical sign as it can be physiological in gravid reptiles (Portas, 2017b). Other signs such as tenesmus (n=8; 22.2%), digging behaviour (n=4; 11.1%), and cloacal prolapse (n=3; 8.3%), although still vague, can be more telling of a reproductive process. Straining and nesting behaviour are generally indicative of dystocia and may eventually lead to oviductal or cloacal prolapse (Innis & Boyer, 2002; Portas, 2017a). Prolapses are also known to occur as a result of the increased intracoelomic pressure provoked by follicles, neoplasia, or other conditions (Hedley & Eatwell, 2014; Stahl & DeNardo, 2019).

Graphic 3 displays the symptomatology recorded on the first consult prior to the surgery. Half of the chelonians (n=18) were alert, responsive, and showed no clinical signs during the physical exam, while the other half displayed mostly nonspecific (n=14; 38.9%), dermatological (n=5; 13.9%), and reproductive (n=5; 13.9%) signs. In chelonians, reproductive disorders such as POFS, dystocia, and egg yolk coelomitis are often progressive and insidious conditions that may go unnoticed for long periods, or simply manifest as typical "ill turtle" clinical signs, with anorexia, lethargy, an coelomic distension (Innis & Boyer, 2002; Mans & Sladky, 2012; Johnson, 2019; Stahl & DeNardo, 2019). For this reason, clinical signs for most reproductive diseases are usually nonspecific or absent, which is precisely what was found on the present study. Eggs were able to be palpated through the prefemoral fossa in only five of the 17 gravid females (evidenced by radiography), proving these may not always be palpable, and not for all species (Innis & Boyer, 2002). The dermatological sings consisted primarily of skin abrasions, thought to be a consequence of the restless and distressed females accidentally scrapping on their enclosures, and erythema, generally of bacterial, fungal, or viral origin, secondary to abrasions or septicaemic states. Respiratory and ocular signs were likely unrelated to the reproductive issue, as they commonly occur in chelonians with bacterial or viral infections of the upper respiratory tract (Boyer, 2019). The traumatology case of this study was due to a fall that fractured the gravid female's shell, consequently leading to dystocia.

Reference intervals for haematological values can be challenging to determine for reptiles and other ectotherms, owing to the potential physiological adaptations that can occur in response to many intrinsic (e.g., age, sex, species) and extrinsic (e.g., season, temperature, diet) factors (Heatley & Russell, 2019b; Campbell & Grant, 2022a). Even so, a PCV, total solids concentration, and blood smear are recommended as the bare minimum to further obtain important clinicopathologic data (Heatley & Russell, 2019b).

Of the 24 haematocrits retrieved, half were altered beyond the species' range. Eight chelonians (33.3%) had an increased PCV suggesting the animal was dehydrated, as diseased turtles tend to reduce their food and water intake and true polycythaemia has not been reported in reptiles (Sykes & Klaphake, 2015; Perry & Mitchell, 2017; Stahl & DeNardo, 2019). The remaining four (16.7%) had the PCV below the normal range for their species, which could be due to an incorrect venipuncture technique and consequent lymphodilution, or anaemia, the development of which has frequently been reported in reptile patients with chronic reproductive diseases (Stacy et al., 2011; Sykes & Klaphake, 2015; Heatley & Russell, 2019b; Stahl & DeNardo, 2019).

More than half of the 32 leucograms analysed were unremarkable (n=18; 56.3%) in terms of both TWBC and differential counts. In reptiles, the diagnosis of an infectious process may be difficult to make off of a singular leucogram, furthermore, it is not uncommon to observe a normal leucogram in diseased reptiles (Sykes & Klaphake, 2015). Leucocytosis was present in 10 patients (31.3%), heterophilia and eosinophilia in six patients (18.8%), and monocytosis and basophilia in one patient each (3.1%). Leucocytosis could possibly reflect inflammation, an immune response, infection, and/or stress (Sykes & Klaphake, 2015; Heatley & Russell, 2019b). Increases in TWBC counts have been documented in cases of follicular stasis, dystocia, and egg yolk coelomitis (Stahl & DeNardo, 2019). The lower incidence of leucocytosis compared to the shifts in leucocyte percentages in the chelonians of this study, supports that the latter may occur more commonly in response to disease (Heatley & Russell, 2019b). Heterophilia has been linked to inflammatory responses, often prompted by infectious agents, tissue injury, necrosis, gravidity, stress, or neoplasia, all of which may be seen with most reproductive disorders (Stahl & DeNardo, 2019; Stacy & Harr, 2021). Eosinophilia has been associated to parasitism and nonspecific immune stimulation. In chelonians, eosinophils are proven to phagocytize immune complexes, which may suggest the presence of infection (Stacy et al., 2011).

Toxic heterophils were identified in the blood smears of nine chelonians (28.1%), while reactive lymphocytes were present in the blood of four (12.5%). Both morphologic changes are frequent nonspecific alterations in reptiles with inflammatory or infectious diseases, that can correlate the severity of the disease with the degree of toxicity of the leucocyte (Sykes & Klaphake, 2015; Stacy & Harr, 2021).

In the context of biochemical assays, 18 out of the 23 analyses (78.3%) demonstrated all parameters within the healthy range for the species. McArthur (2004) affirmed that uncomplicated cases of POFS could reveal biochemical parameters within normality, which might hold true for other reproductive diseases as well. However, the present study also revealed a great number of complications and chelonians suffering from reproductive disease. Lack of clinical chemistry data, accurate baseline data, and the fluctuating physiology of ectotherms may be in the genesis of this contrasting information (Heatley & Russell, 2019a).

The most frequently increased parameters were UA (n=4), AST (n=3), and P (n=2). Some authors recommend assessing at least the Ca, P and UA levels in chelonians with reproductive disease as these parameters could give a good indication of dehydration, hypocalcaemia, and renal insufficiency (Portas, 2017a). Renal dysfunction may be suspected in cases of dystocia and yolk coelomitis with elevated uric acid values and an inverse Ca-P ratio (Innis & Boyer, 2002; Rivera, 2008; Chitty & Raftery, 2013b; Portas, 2017a; Roberts & Warner, 2020). Nevertheless, UA lacks both sensitivity and specificity as an indicator for renal disease in reptiles, as its levels may rise in correlation with severe dehydration, recent consumption of a carnivorous meal, or reduced body temperature. Based on this, evaluation of renal function should require the quantification of all chelonian nitrogenous end-products, including blood urea nitrogen and ammonia, and repetition of the analysis once the patient is rehydrated (Heatley & Russell, 2019a; Wilkinson & Divers, 2020). The enzyme AST is not specific to a particular organ and its increase may simply signify tissue damage (Chitty & Raftery, 2013b; Heatley & Russell, 2019a).

Radiography of 30 chelonians evidenced 17 gravid females with mineralized eggs (56.7%). Out of these 17 chelonians, two exhibited abnormally shaped eggs, while another two displayed eggs in the centre of the caudal coelom (from dorsal projection), pointing to possible egg ectopia inside the bladder. Having more than half of the chelonians radiographed in this study with retained eggs highlights the importance of this imaging modality in identifying cases of dystocia and distinguishing these from cases of follicular stasis (Holmes & Divers, 2019; Stahl & DeNardo, 2019). The misshapen and misplaced eggs found in the study represent radiographic signs of egg-binding, although the latter must be confirmed on ultrasound or, ideally, with cystoscopy (Di Girolamo & Selleri, 2015; Gumpenberger, 2017; Fazio, 2021). Additionally, other causes such as MBD, pelvic or shell fractures, obstipation, and urinary calculi were able to be discarded, with only one patient showcasing a shell fracture (Welsh, 2011; Gumpenberger, 2017; Knotek, et al., 2017b).

Graphic 6 illustrates the ultrasound results of 35 chelonians. The most common observation found was numerous large preovulatory follicles filling up the caudal coelom in 42.9% of patients (n=15). This finding, although not completely diagnostic of follicular stasis, expresses the space-occupying effect the follicles in preovulatory stage can exert, displacing and obscuring other intracoelomic organs (Fazio, 2021). For a definitive diagnosis of POFS, serial ultrasound studies with measurements would need to be performed to assess correct follicular ovulation or resorption (Stahl & DeNardo, 2019).

In four instances (11.4%), follicles were found deformed and of heterogeneous echogenicity, which are ultrasonographic signs compatible with follicular degeneration and possible oophoritis (Gumpenberger, 2017). Hypoechoic to anechoic fluid with echogenic floating debris was observed between the follicles and coelomic organs of five chelonians (14.3%), hinting at an inflammatory process, presumably egg yolk coelomitis and/or oophoritis (Gumpenberger, 2017; Hochleithner & Sharma, 2019).

Comparable to the existing literature, ultrasonography discerned with less frequency other uncommon but possible crucial signs, namely the distension of the fluid-filled salpinx, suggesting salpingitis or oviductal torsion (n=2; 5.7%), ectopic eggs inside the bladder (n=2; 5.7%), heterogeneous (n=1; 2.9%) and aberrant eggs (n=1; 2.9%), and a heterogeneous and irregular mass in the anatomic region of the ovary (2.9%), later confirmed to be an ovarian neoplasia (Gumpenberger, 2017; Knotek, et al., 2017b; Hochleithner & Sharma, 2019).

The diagnostic imaging techniques utilized in this study proved that while radiography is more sensible for detecting (and establishing the number of) mineralized eggs (n=17) than ultrasonography (n=13), ultrasound is significantly more sensible for the evaluation of soft tissue structures of the reproductive tract (follicles and salpinx) and of other contents of the coelomic cavity (Gumpenberger, 2017).

Medical management with oxytocin therapy was attempted in 16 females presumably with nonobstructive dystocia. This treatment was effective in 56.3% of chelonians. The effectiveness of the treatment with oxytocin performed in this study seems to be much lower than other studies that reported overall success rates for induction of oviposition in chelonians higher than 90% (Tucker et al., 2007; Di Ianni et al., 2014). However, in

Tucker et al. (2007), a study involving 253 female chelonians, success is defined as the turtle retaining two or fewer eggs, which does not align with the definition of success established for the present study. Other authors registered a wider range of success, differing substantially between chelonian species (Feldman, 2007). In Di Ianni et al. (2014), oxytocin was administered IV at a lower dose (2 IU/Kg) and repeated in significantly shorter intervals (60 minutes after the first injection and 120 after the second), reporting a shorter oviposition time and requiring less administrations until complete clutch expulsion, when compared to the IM administered group. The intramuscular route of administration and the long interval between injections (24h) used in this study may both be contributing factors to the lower than expected effectiveness of the treatment. Oxytocin administered IV appears to be a promising alternative, although further studies with bigger sample sizes are lacking in order to establish more concrete evidence of its success, as well as to narrow the guidelines for medical management of nonobstructive dystocia in chelonians.

In the three cases (18.7%) where oxytocin therapy was partially effective, the remaining eggs were found inside the bladder, not previously noted on ultrasound. This corroborates the proposition that oxytocin administration is a predisposing factor for ectopic eggs inside the organ (Minter et al., 2010; Gumpenberger, 2017; Stahl & DeNardo, 2019).

Four females (25.0%) failed to lay their eggs after oxytocin therapy. Cases of chelonians that do not respond to oxytocin are usually due to an undiagnosed obstructive cause or, more frequently, an underlying medical condition (Innis & Boyer, 2002). The improper use of oxytocin (i.e., in the presence of obstructive dystocia or a dehydrated or unstable patient) may result in worsening of a metabolic disorder, oviduct rupture, egg fracture, ectopic eggs forced into the bladder or the coelomic cavity, or severe haemorrhage, jeopardizing the patient's life by creating an additional problem while failing to solve the initial one (Perry & Mitchell, 2017; Johnson, 2019; Stahl & DeNardo, 2019).

Regarding the surgical management, the endoscope-assisted prefemoral ovariectomy/ovariosalpingectomy was successful in 34 of the 36 female chelonians operated (94.4%), given the two casualties that resulted from the surgery (5.6%). Overall, it may be interpreted as a high success rate considering that complications were reported in 86.1% (n=31) of the surgical procedures. To the author's knowledge, no other studies exhibiting the frequency of complications found during this surgery exist.

Some surgical complications occurred naturally during the procedures, like ectopic follicles in the coelom (n=5; 13.9%) and follicle rupture (n=4; 11.1%), as follicles were seen to occasionally be torn from the interfollicular connective tissue during traction of the ovary and gathering the ectopic follicles was not always an easy task owing to their frail and slippery nature. Both follicle ectopia and rupture have similarly been accounted for by Proença et al. (2014a).

Other surgical complications arose more frequently as a consequence of the resolution of complications secondary to reproductive disease.

Haemorrhage was seen in 13.9% (n=5) of cases, generally during the debridement of adherences and handling of hyperaemic coelomic contents of patients with coelomitis. Less frequently, haemorrhage was caused by faulty ligature of the ovarian or oviductal vessels. The chelonian that did not regain consciousness, passed away immediately after the procedure presumably due to the extensive loss of blood experienced during ovariectomy. The turtle presented with a severe case of coelomitis with neovascularization and multiple deep adhesions between the inner organs and the entire coelomic cavity (Fig. 23). This complication has been reported in ovariectomies and ovariosalpingectomies of other chelonians, but mostly due to oviduct rupture (Mans & Sladky, 2012; Proença & Divers, 2015). The present study depicts more clearly the association between chronic yolk coelomitis and the increased risk of haemorrhage during the procedure.

Two of the three reported iatrogenic bladder tears surged while resolving both cases of oviduct torsion during debridement of the adherences between the inflamed, congested, and necrotic organ and the bladder, requiring suturing of the inadvertent ruptures. The death of the chelonian (35-year-old *Trachemys scripta elegans*) in the late postoperative period occurred presumably as a consequence of a leaking bladder suture or an unnoticed bladder tear, associated to the salpingectomy of its torsioned oviduct. Blood analysis revealed leucocytosis, hyperuricaemia, and hyperphosphataemia two days before death and, a postmortem coelioscopy found uric acid crystallizations scattered all throughout the coelom. These findings were both compatible with renal insufficiency and gout (Divers & Innis, 2019; Wilkinson & Divers, 2020).

The most commonly encountered complication secondary to reproductive disease in this study was coelomitis in 63.9% (n=23) of cases. This disorder has been documented in previous case reports of chelonian endoscope-assisted ovariectomy and ovariosalpingectomy (Mans & Sladky, 2012; Proença et al., 2014a; Hellebuyck & Vilanova, 2022). Coelomitis findings will be discussed in further detail below.

In four cases (11.1%), chelonians with coelomitis were observed to have ectopic follicles adhered to the coelomic wall, liver, or other organs, with no connection to the ovary.

Oviductal torsions (n=2; 5.6%) and ectopic eggs in the coelom (n=3; 8.3%) have been referenced in the literature as possible complications (Innis et al., 2007; Mans & Sladky, 2012; Proença & Divers, 2015; Stahl & DeNardo, 2019; Hellebuyck & Vilanova, 2022). Resolving these was made challenging mainly by the existing adhesions between the salpinx or eggs and the surrounding organs.

Ectopic eggs inside the bladder (n=3; 8.3%) and cloacal prolapses (n=2; 5.6%) were considered complications by the author of the present study, since the warranted cystoscopy/cystotomy and prolapse replacement were additional techniques performed during surgery to avoid anaesthetizing the patient twice. Furthermore, removal of eggs through cystoscopy was first attempted, which too contributed to an extended surgery time. This was the case for the chelonian that had its surgery interrupted and rescheduled.

Although this surgery has been successfully performed without the use of endoscopy before (Takami, 2017), Proença et al. (2014a) discourages performing sterilization of female chelonians with reproductive disease under restricted visualization (without endoscopy), has it could originate more complications, including the previously discussed.

As previously mentioned, during the surgical procedure, a coelomitis diagnosis was made in over half of chelonians through coelioscopy. In general, coelomitis not only made coelioscopy more challenging (Fig.  25), but additionally predisposed to other complications (Stahl & DeNardo, 2019).

Endoscopy has been praised as the best diagnostic modality for yolk coelomitis (Innis & Boyer, 2002). The high percentage of cases reported in this study although significant, could be expected, given that this condition occurs secondary to many other reproductive diseases (Perry & Mitchell, 2017; Portas, 2017a; Stahl & DeNardo, 2019; Roberts & Warner, 2020). Yolk coelomitis has also been referred to as egg-related coelomitis in avian medicine, considering that the irritant source may be more than just egg yolk (e.g., eggshell or albumin) (Greenacre, 2015). This disorder is characterized by the presence of hyperaemia, free fluid, altered serosal membranes, and adhesions on the coelomic cavity (Innis & Boyer, 2002; Stahl & DeNardo, 2019; Roberts & Warner, 2020), all of which were recorded in multiple patients of this study.

The coelomic fluid observed in coelomitis cases was moderate to abundant and consisted of serous fluid or yolk material with lipid droplets in acute cases, and a progressively thicker, whiter, and opaquer fluid in more chronic cases (Fig. 26). Similar recounts to the coelomic fluid found in the acute coelomitis cases of this study have been made for chelonians undergoing the same surgery (Mans & Sladky, 2012; Proença et al., 2014a), as well as for reptiles of the saurian suborder (Stacy et al., 2008). Yolk discovered inside the coelomic cavity is presumably due to the leakage and rupture of degenerative follicles associated with POFS (Stahl & DeNardo, 2019).

The coelomic fluid changes witnessed with chronicity can be explained by the avian and reptile primary defence mechanism of fibrin exudation. In order to prevent the dissemination of an inflammatory source, whether a pathogen or a sterile irritant, fibrin is deposited locally to contain it (Huchzermeyer & Cooper, 2000; Roberts & Warner, 2020). Failure to prevent the spread and/or occurrence of repeated aggressions likely resulted in the coelomic cavity covered in a fibrinlike yolk material and adherences seen in Fig. 23 and similar cases. In the chronic cases where the fluid was additionally malodorous, bacterial infection might have been present (Roberts & Warner, 2020).

Adhesions and neovascularization were often seen between multiple follicles and the coelomic wall or liver (Fig. 27), which usually occurs in reptiles with chronic oophoritis triggered by POFS and/or yolk coelomitis (Stahl & DeNardo, 2019; Roberts & Warner, 2020). Severe, chronic, and infectious cases of coelomitis frequently displayed these findings across the entire coelom involving every internal organ, similar to the reports of Stacy et al. (2008), Mans & Sladky (2012), and Couture et al. (2017).

In the coelomitis cases where the coelomic serosa was not covered by either abundant coelomic fluid or adhesions, hyperaemia and opaqueness could be noted. The serosal membrane was commonly observed to acquire a similar colour to that of the coelomic fluid present (Fig. 26.B and Fig. 28.B and C), probably on account of the attempt to remove the irritant by the phagocytic activated mesothelial cells composing the coelomic serosal layer (Cornax et al., 2013).

Histopathologic analysis of the liver diagnosed hepatic lipidosis in 100% (n=8/8) of biopsies and yolk coelomitis in 87.5% (n=7/8). The diagnosis of hepatic lipidosis could be a challenging one in chelonians, especially for females with reproductive disease, due to their physiologic prereproductive intrahepatic fat storage (Stahl & DeNardo, 2019). Nevertheless, some degree of inflammatory reaction was detected in all samples by the pathologist making the diagnosis clearer (Boyer & Scott, 2019). The overlapping diagnosis of hepatic lipidosis and yolk coelomitis in almost all samples may indicate that the latter, along with the associated chronic hyporexia and stress, may be the inciting factor for the lipid metabolism disruption.

Coelioscopic liver biopsies have been demonstrated to be satisfactory and diagnostic in turtles (Divers et al., 2010). However, their use might be best employed for bacterial culture rather than for diagnosing coelomitis through histopathology, given that the condition can be diagnosed through coelioscopy alone and is frequently accompanied by infection (Innis & Boyer, 2002; Perry & Mitchell, 2017; Stahl & DeNardo, 2019). Still, histopathology of one liver biopsy revealed ectopic metaplastic oviductal tissue, thought to be a metastasis of an oviduct adenocarcinoma, that otherwise would not have been identified. To the author's knowledge, this type of neoplasia has not yet been reported in chelonians, having mostly been documented in snakes (Pereira & Viner, 2008) and lizards (Kubiak et al., 2020). Further histopathologic analysis of the oviduct would need to be made to confirm the suspicion.

Out of the three ovary samples submitted for histopathological examination, two exhibited fibrinous-lipogranulomatous oophoritis accompanied by follicular atresia and degeneration. Follicular degenerative changes are typically the result of delayed atresia seen with preovulatory disease, which may develop into a granulomatous oophoritis over time (Stahl & DeNardo, 2019; Roberts & Warner, 2020). Considering this, along with the concomitant yolk coelomitis (diagnosis made through coelioscopy and histopathology of the liver) present in these two cases, it is possible that they were the result of chronic POFS. Although few definitive diagnoses of oophoritis were made, there is a strong possibility that the condition was underdiagnosed, given the previously mentioned high percentage of cases presenting adhesions between the ovaries and the surrounding structures. Both oophoritis and salpingitis seem to be underestimated, particularly in chelonians, due to the general challenges in effectively visualizing and sampling the reproductive tract posed by the shell (Hellebuyck & Vilanova, 2022).

The ovarian teratoma, suspected on ultrasound and confirmed through histopathology, belonged to a 19-year-old red-eared slider (*Trachemys scripta elegans*). Despite the low prevalence of neoplasms in chelonians, similar case reports of this type of ovarian neoplasia have been made for this species (Newman et al., 2003; Hidalgo-Vila et al., 2006). No distinction was made regarding the malignancy of the mass; however, it was described as highly invasive but unlikely to metastasize. This corresponded to a prevalence of 2.7% (confirmed) neoplasia in chelonians with reproductive disease in this study. A retrospective study reviewing biopsies and necropsies of 1067 chelonians, demonstrated the exact same prevalence of neoplasia occurrence, albeit with no reported reproductive tract neoplasia (Garner et al., 2004).

On the first follow-up visit or call after the surgical procedure, 85.7% (n=30/35) of patients had recovered their habitual appetite and returned to their normal activity levels. This result resembles the 81.8% of uneventful recoveries seen in Innis et al. (2007), a study describing the use of coelioscopic-assisted prefemoral oophorectomy in 11 chelonians, where two chelonians died in the postoperative period. Other smaller studies and case reports of this surgery in chelonians showed no complications or deaths in the postoperative period (Mans & Sladky, 2012; Proença et al., 2014a; Hellebuyck & Vilanova, 2022).

The five females (14.3%) that presented apathy and anorexia in the subsequent days following the procedure were submitted to fluid and nutritional therapy, some requiring oesophagostomy tube feeding. In one case, the ceftazidime used was changed for marbofloxacin (5 mg/Kg IM every 24 hours) following a control leucogram indicating an untreated infection (heterophilia with toxic heterophils). The chelonian left with a leaking bladder suture succeeding salpingectomy of a torsioned oviduct, started fluid therapy only 18 days after surgery, dying two days later, while the other four females went on to recover between the second and the fourth week of the postoperative period. This emphasizes the significance of promptly intervening and providing supportive care during the post-surgical recovery of chelonians that have not fully recuperated, particularly in more complex cases (Perry & Mitchell, 2017; Stahl & DeNardo, 2019).

Two months after the surgery, two chelonians displayed multiple maturing follicles on ultrasound, meaning remnants of the ovaries had been left behind. Incomplete procedures may result in regrowth of even the smallest remnants, causing folliculogenesis and egg laying to resume (Cardona et al., 2011). On more than one occasion, exteriorizing the complete ovary was a time consuming and arduous task, mainly due to the multiple adhesions between these and the coelomic contents. Additionally, the presumably inflamed organ would often tear during traction to the incision, requiring further localization and retrieval of the remaining segments. Girolamo et al. (2022) assessed the macroscopic presence of remnant ovary tissue in a study involving 16 female chelonians, where the turtles were euthanized for postmortem examination after being submitted to a prefemoral ovariectomy. The study reported a lack of grossly visible ovarian tissue in all 14 chelonians that had a complete surgery, however, none of these animals exhibited complications during the procedure, unlike the chelonians of the present study. In fact, the only reported chelonian with severe coelomitis and adhesions between the ovaries and liver in that study, was not able to have its surgery completed and, therefore, was not included in the postmortem inspection of ovary tissue remnants (Girolamo et al., 2022). In the present study, few chelonians (n=13) had a control ultrasound in the first four months following surgery, so the number of females in need of a revision surgery may be higher. Given this information, it is reasonable to advise a control ultrasonography two to four months after the surgery, particularly in patients that had more technically challenging surgeries.

The female of the extirped ovarian neoplasia presented four months later, on ultrasound, multiple heterogenous small irregular masses, distinct from follicles, developing on the serosal surfaces of the coelomic cavity, similar to the findings of Cardona et al. (2011). Given the low likelihood of metastasis of the teratoma observed in this study, the nodules are thought to have originated from direct implantation of neoplastic cells disseminated during removal of the mass. This finding is an indicator of malignancy in teratomas (Newman et al., 2003; Bel et al., 2016). Under these conditions, palliative treatment with supportive care and pain relief was agreed to be the most suitable option for the patient.

As mentioned earlier, while age does not seem to be key factor for the manifestation of reproductive disease, it does appear to be correlated with the number and severity of complications found. Taking into account the ages of the two deceased chelonians of this study (35 and 38 years old), it is possible to theorize that with each reproductive cycle the female goes through, the chances of encountering complications such as ectopic follicles/ eggs, chronic POFS/oophoritis, coelomitis, and haemorrhages increase. This further suggests that the prognosis for female chelonians undergoing surgical sterilization worsens with age. From this perspective, when dealing with older chelonians, the surgeons should prepare accordingly for the eventuality of having to perform a more challenging procedure. Catheterization prior to surgery and even intraoperative fluid therapy may be advised in these cases (Divers, 2019d).

## 6. Conclusions

The present study permitted a detailed overview of the clinical management of chelonians with reproductive disease ultimately submitted to endoscope-assisted ovariectomy or ovariosalpingectomy. The number of female chelonians and chelonian species involved in this study, alongside the diverse reproductive conditions described are unprecedented for a single study of this nature.

Captive held chelonians frequently suffer from a wide range of reproductive diseases, often chronic and recurrent, that may manifest from the moment the chelonians reach sexual maturity, to the rest of their reproductively active lives. Overtime, most of these disorders further lead to infections and coelomitis, capable of greatly impacting the reptiles' welfare.

It is paramount for the clinician to include both elements of haematology and imaging in the diagnostic approach, as results may prove to be inconclusive or nonspecific on their own. The therapeutic approach will commonly vary significantly based on the diagnosis made. Options might consist of correcting husbandry related issues, addressing the disease medically or, most advisably, addressing the disease surgically.

The endoscope-assisted prefemoral ovariectomy/ovariosalpingectomy is the preferred choice for most reproductive disorders, especially for nonbreeding chelonians. This minimally invasive and versatile technique offers exceptional visual aid for discerning gross anatomopathological lesions, verifying haemostasis, retrieving valuable diagnostic biopsies, and identifying ectopic follicles and eggs, as well as other complications. Postoperative recovery and wound healing are also unmatched when compared to traditional plastronotomy.

In this study, the surgical procedure demonstrated a high success rate given the considerable intraoperative complications encountered, particularly with over half of the patients displaying coelomitis. Nevertheless, the perceived success rate may not be as high when factoring in the females known to need revision surgery after the detection of ovary remnants on ultrasound, as well as those that might reveal this finding on future controls.

Performing this surgery in chelonians with chronic reproductive disorders regularly yields an increased anaesthetic and surgical risk owing to the initial presentation of these reptiles (e.g., apathetic, dehydrated, with multiple organ dysfunction) and to the complications that become more prevalent (e.g., coelomitis, haemorrhage, ectopic follicles/eggs). For this reason, the endoscope-assisted prefemoral sterilization should not only be recommended as a curative procedure, but also as preventive measure, notably when employed earlier in the life of the chelonian.

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