Department for Small Animals and Horses

Platform for Artificial Insemination and Embryo Transfer

University of Veterinary Medicine Vienna

# Could saline solution be a possible chemical castration method for low cost population management of stray male dogs?

Diploma Thesis

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Submitted by

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## 1. Introduction

In cities and in rural areas all over the world stray dogs cause problems and especially their overpopulation is difficult to control. Hughes and Macdonald (2013) estimates the dog population worldwide at 700 million in 2010, with 4.3 million in Europe and 72 million in North America (Hughes and Macdonald 2013). Futhermore, Hughes and Macdonald (2013) estimate that 75 % of world’s dog population is ownerless, resulting in apporximatley 525 million stray dogs worldwide.

Management of this population is important because stray dogs lead to various problems. On the one hand they form a reservoir for pathogens – not only parasitic diseases have zoonotic potential, also the rabies virus can be transmitted by dogs. On the other hand, stray dogs are often the cause of car accidents, noise pollution, scattering of garbage from rubbish bins and many other issues (Aurich 2018, Hassan and Fromsa 2017, Taylor et al. 2017).

To control these issues, stray dogs have been castrated to prevent their reproduction. Surgical castration of male dogs is still state of the art and is used by veterinarians all over the world. But well-structured programs and funding are the biggest challenges. Due to management, ethical and these financial reasons, however, chemical castration is getting more interesting as an alternative method for castration of dogs and other species (Aurich 2018).

Gonadotropin releasing hormone (GnrH) analoga/-agonists and -antagonists, immunovaccination (Improvac for pigs or Equity for horses) and intratesticular/intraepididymal injection (ITI) of contraceptive solutions/chemosterilants are some of the available alternatives on the market. Furthermore studies looked into sodium chloride as a potential intratesticular contraceptive as a very cheap and easy producible solution (Aurich 2018, Bertone et al. 1990, Canpolat et al. 2016, Emir et al. 2008, Emir et al. 2011, Kwak and Lee 2013, Leoci et al. 2014b, Neto et al. 2014, Oliveira et al. 2017).

The following chapters will give a brief overview of the most important anatomical and histological structures, surgical and chemical castration methods are described and the reasons for castration are discussed in more detail.

## 1.1. Anatomy, Histology and hormonal Regulation

### Anatomy

The male reproductive system consists of the testicles, the *epididymis*, the *ductus deferens*, the *funiculus spermaticus*, the *tunicae testis*, s*crotum*, the accessory genital glands, the penis and the prepuce (Gasse 2004). According to the scope of this thesis, only testicles, *epididymis*, *funiculus spermaticus*, *scrotum* and *tunicae testis* will be described in more detail.

The testicle is enveloped in several layers of different tissues within the *scrotum*. The *testes* produce spermatozoa and sex steroid hormones that are responsible for the typical male appearance and sexual behavior. Dependent on species and age of the individual male, *testes*, s*crotum* and *epididymis* vary in its size. In difference to wildlife animals, domestic animals seasonal changes in testicular size are moderate (Gasse 2004).

As shown in Figure 1 the head of the *epididimys* is attached to the *extremitas capitata* of the *testis* and the tail of the *epididymis* to the *extremitas caudata*. Whereas the *margo liber* is not attached to the *mesorchium* or the *epididymis*. The *margo epididymalis*, however, attaches to both, the *epididymis* and the *mesorchium*. The lateral and medial part of the *testis* is also named as the lateral and medial surface. In detail, the testes are enclosed by a fibrous capsule containing the superficial branches of the testicular artery and veins, the *tunica albuginea.* The *tunica albuginea* is not elastic and therefore keeps some pressure on the testicular parenchyma. Therefore, cutting into this tissue during surgical castration leads to a noticable protrution. Furthermore, the *tunica albuginea* is coverd with a serosa (Gasse 2004).

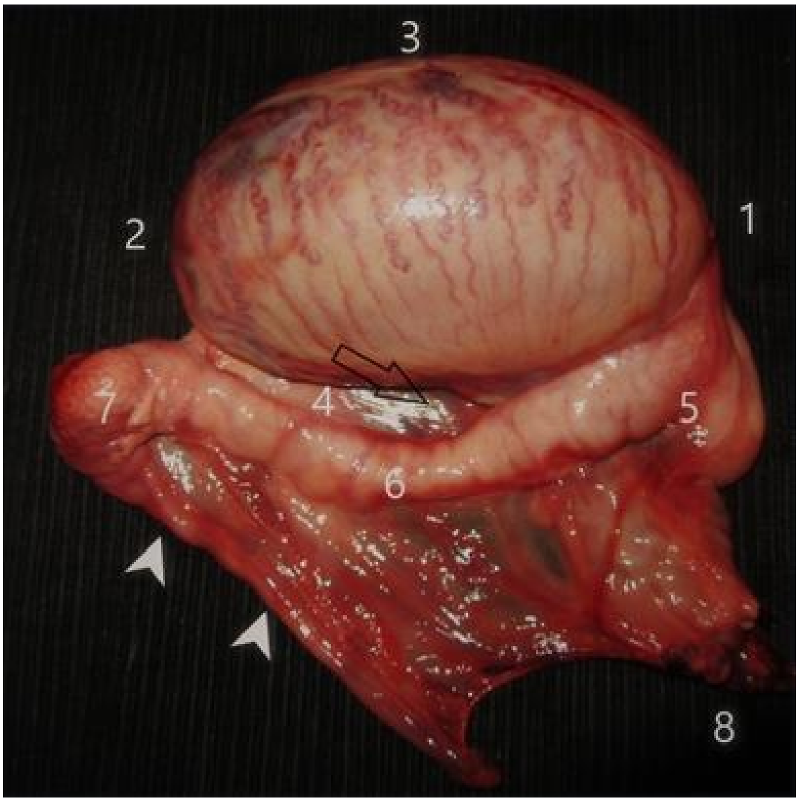


**Figure 1: Testis, epididymis and adjacent tissue from a stallion after inguinal castration**
1: *extremitas capitata*; 2: *extremitas caudata*; 3: *margo liber*; 4: *margo epididymalis*; 5: *caput epididymis*: 6: *corpus epididimys*; 7: *cauda epididymis*; 8: *funiculus spermaticus*. The *funiculus spermaticus* or spermatic cord contains the *arteria testicularis*; the *vena testicularis* forming the *plexus pampiniformis*, the *ductus deferens* with the *arteria* and *vena ductus deferentis*, lymph vessels and testicular nerves forming the *plexus testicularis*; black arrow: entrance to the *bursa testicularis*; white arrows: *ductus deferens*

Small connective tissue septa (containing blood vessels and nerves) divide the testes in several lobules, starting from the internal part of the *tunica albuginea* and ending in the *mediastinum testis* (see Figure 2). These lobules contain fine tubules, which form the *rete testis*. In the periphery, the tubules are tortuous, forming the *tubuli seminiferi contorti*. Approaching the *mediastinum*, they get more straight and bigger, resulting in the name *tubuli seminiferi recti*. From the *rete testis*, spermatozoa are carried in the *ductuli efferentes* which are connected with the tubular system of the *caput epididymis*. The connective tissue in the lobes of *the ductuli efferentes* is highly vascularized. More details on the structure of the seminiferous tubules, endocrine cells and other cell types in the connective tissue are given in the histology part (Gasse 2004)

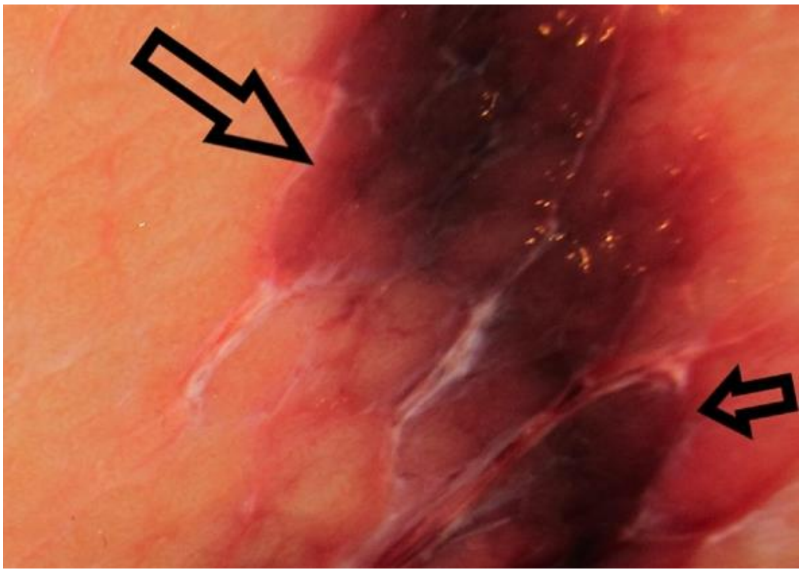

**Figure 2: Longitudinal section of a horse testicle**

Black arrows pointing at clearly defined lobular septa which separate each testicular lobe. In this case, bleeding occurred because of local anesthetic injection during castration.

The *tunicae testis* are important during surgical castration for orientation and because of the surgical technique applied. Starting from the outside, the first detectable structure is the *scrotum* or *cutis* containing sweat and sebaceous glands. This is followed by the *tunica dartos* and *tela subdartoica*. The *tunica dartos* is directly attached to the inner side of the *cutis* and cannot be separated (see Figure 3, Figure 4). It contains muscle cells, which in combination with the *musculus cremaster,* push by contraction the testes into the direction of the inguinal canal or sometimes even into the abdominal cavity. After relaxation of this muscle cells, the testes are released back to its typical position.

**Figure 3:** ***Regio scrotalis*** **of a 35 kg dog**

Incision along the *raphe scroti* with view to the *cutis*, *subcutis* and *tunica dartos.*

*The arrow is pointing to the subcutis.*

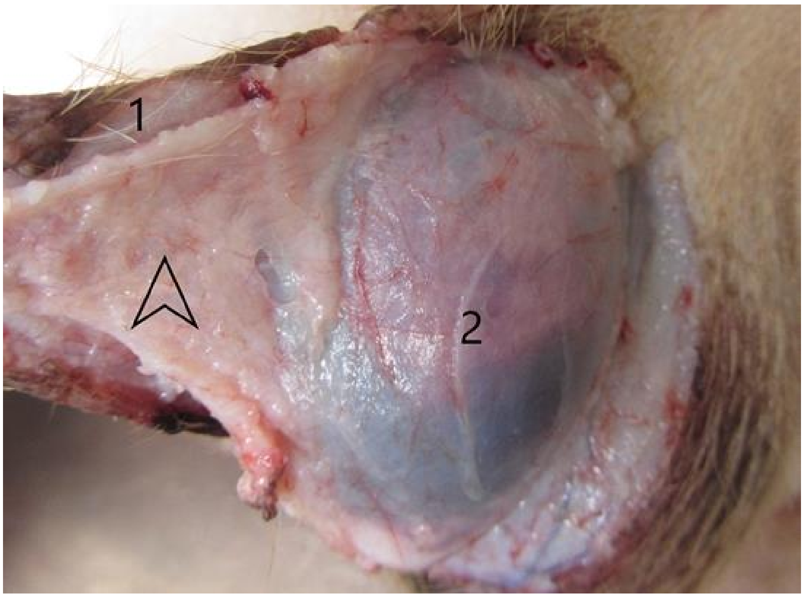


**Figure 4: Preparation of the** ***tela subdartoica*** **of a 35 kg dog**

1: *cutis*; *subcutis*; *tunica dartos*; 2: *fascia spermatica externa;* Black arrow head: *tela subdartoida*

The next layer is the *fascia spermatica externa* which derives from the *fascia trunci* followed by the *fascia spermatica interna* deriving from the *fascia transversalis*. In the proximal part of the *scrotum* and closer to the inguinal canal the *musculus cremaster* lies between the last two layers, it derives from the *musculus obliquus internus abdominis* which passes through the inguinal canal. The vaginal process is made of the *fascia spermatica interna* and *tunica vaginalis*. Seen as a glittering surface, the *lamina parietalis* of the *tunica vaginalis* is directly attached to the inner side of the *fascia spermatica interna*. The *lamina parietalis* origins from the *peritoneum* coming from the abdominal cavity (Figure 5, Figure 6).

The serosal layer attached to the testes side is the *lamina visceralis* of the *tunica vaginalis*. Directly below the final layer, the *tunica albuginea* is following and surrounds the parenchyma of the *testis* (Gasse 2004, Künzel 2011).

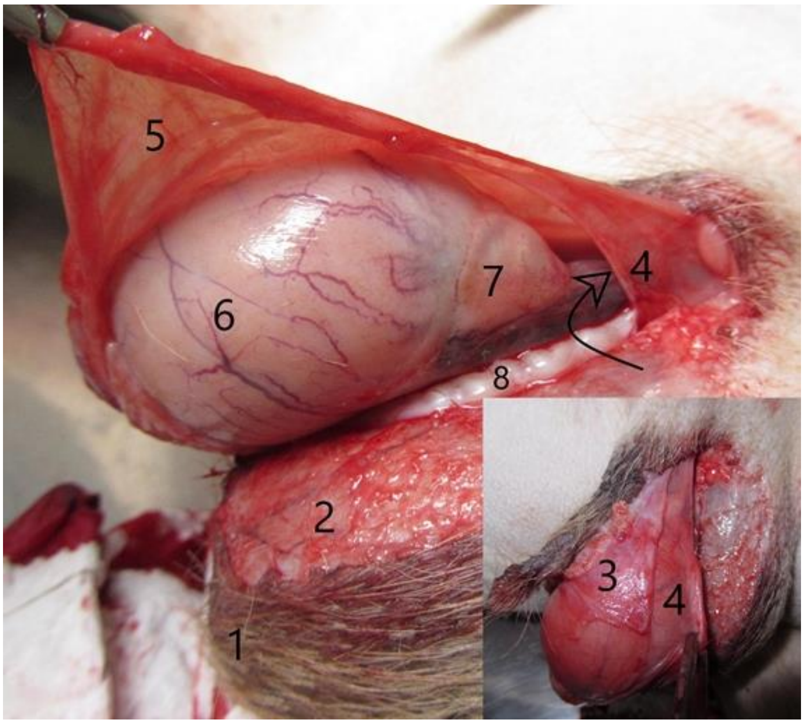


**Figure 5: Preparation of the *processus vaginalis* of a 35 kg dog**

1: *cutis*; 2: *tunica dartos*; 3: *fascia spermatica externa*; 4*: fascia spermatica interna*; 5: *lamina parietalis* of the *tunica vaginalis* attached to the *fascia spermatica interna*; 6: *lamina visceralis* of the *tunica vaginalis*; 7: *caput epididymidis* *8: ductus deferens;* Arrow: *cavum vaginale*

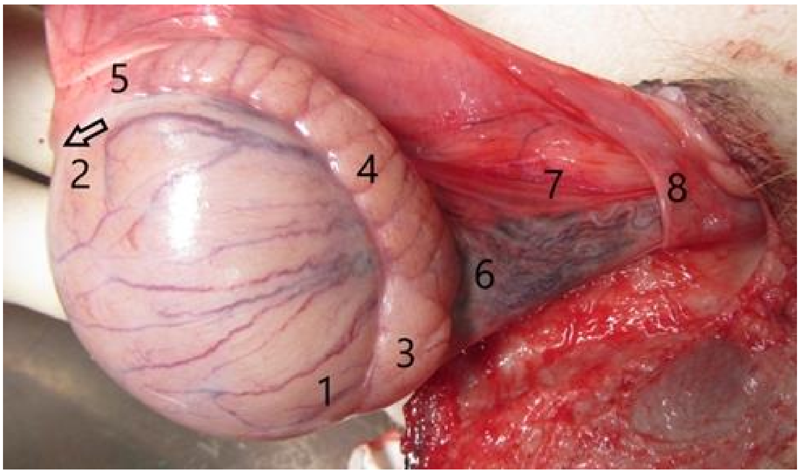


**Figure 6: Preparation of the *plexus pampiniformis* of a 35 kg dog**

1: *extremitas capitata*; 2: *extremitas caudata*; 3: *caput epididymis;* 4: *corpus epididymis*; 5: *cauda epididymis*; 6: *plexus pampiniformis*; 7: *ducuts deferens* 8: *funiculus spermaticus*; Arrow: showing the area where the *ligamentum caudae epididymis* attaches

The *epididymis* can be divided in head, corpus and cauda. It is located along the epididymal border of the *testes*. The head of the *epididymis* is formed by several *ductuli efferentes* coming from the testicle and merging into the beginning of the epididymal duct, which is passing through the *corpus epididymis* and the *cauda epididymis*. The *cauda epididymis* is attached via the *ligamentum proprium testis* to the *extremitas caudata*. After maturation in the *epididymis*, the spermatozoa are stored in the *cauda epididymis* until ejaculation. The *ductus deferens* origins directly from the epididymal tract, connecting the pelvic part of the *urethra* with the gonads. Connected to the *mesoductus deferens*, it runs medial to the *epididymis* in the spermatic cord together with the testicular arteries, veins and nerves. Passing the inguinal canal and going to the cranial part of the pelvis. The terminal part of the *ductus deferens* differ between species. In the dog, stallion and the ruminant, located at the bladder neck, the terminal part is enlarged and called *ampulla ductus deferens*. In the cat and the pig, the ampulla is absent. Every species, however, contains glands in the terminal part of the *ductus deferens* (Gasse 2004).

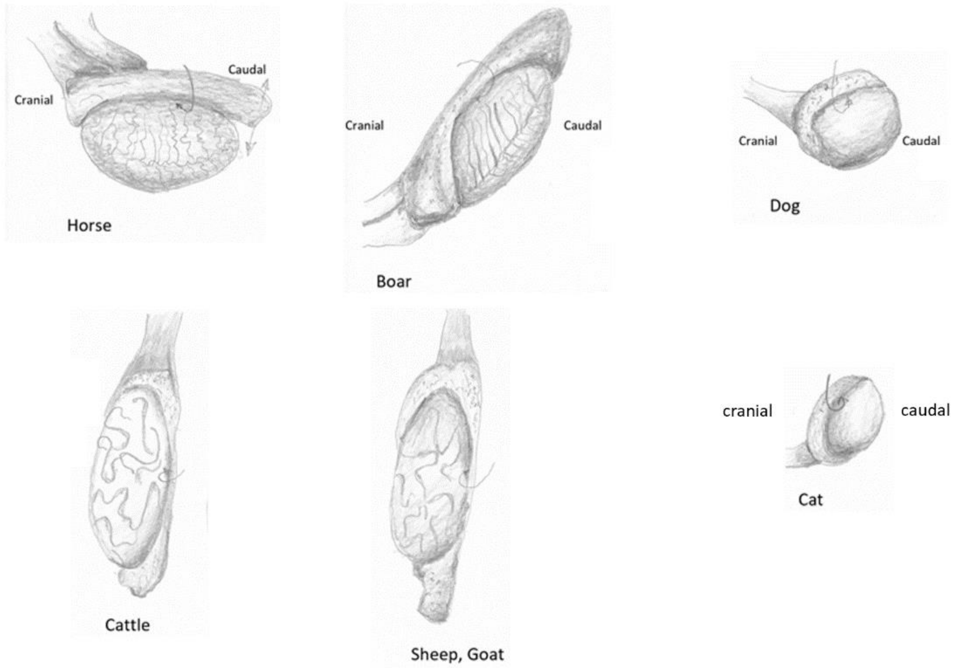


**Figure 7: Testicular position in the *scrotum***
The position of the testis in the *scrotum* varies among species as shown in the picture. Lateromedial view for cattle, sheep and goat. Ventrolateral view for horse, boar, dog and cat. The arrows point at the entrance to the testicular bursa (Gasse 2004).

Comparing with other species the position of the testicle within the scrotum differs in the dog. The *margo liber* points caudoventrally, the *caput epididymis* points ventrally and the *cauda epididymis* dorsally (see Figure 7) (Gasse 2004).

### Vascularisation

The *funiculus spermaticus* or spermatic cord contains the *arteria testicularis*, the *vena testicularis* forming the *plexus pampiniformis* (see Figure 6), the *ductus deferens* with the *arteria* and *vena ductus deferentis*, lymph vessels and testicular nerves.

In dogs the *arteria testicularis* origins directly from the aorta in the area of the first two lumbar vertebrae. In rare cases, the *arteria testicularis* derives from the *arteria renalis* instead. After arriving at the *testis*, it spreads in the *rami epididymalis* and the *rami ductus deferentis* (Waibl et al. 2005).

In the dog, cat, pig and cattle, the *arteria umbilicalis*, which later releases the *arteria ductus defferentis*, deriving from the external iliac artery. In the dog the *ramus ductus deferentis* is more prominent forming the *arteria ductus deferentis* The *scrotum* is vascularized by the perineal branch which derives from the external pudendal artery (Waibl et al. 2005).

The *vena testicularis* sinistra leads directly to the *vena cava caudalis*. In dogs the *vena testicularis sinistra* runs to the *vena renalis sinistra*. Passing the *canalis inguinalis* with the corresponding artery in the *plica vasculosa*, it leaves the same branches as the artery, but the veins also form the so called *plexus pampiniformis* (see Figure 6). This venous plexus is responsible for cooling the arterial blood, flowing from the warmer region of the abdominal cavity into the cooler region, the *testes* (Vollmerhaus and Roos 2005).

**Lymph nodes**

The *lymphonodii iliaci mediales* are tributary lymph nodes for the testis and the *lymphonodii lumbales aortici* for the *epididymis (Vollmerhaus and Roos 2005)*.

**Sensible innervation**

The origin of the sensible innervation of the testicles differ with regard to species.

In carnivor species the *nervus genitofemoralis*, origins from L3-L4 (dog) and L4 (cat), passing through the *musculus psoas major* near the femoral artery and splitting up into two parts afterwards. It passes the *canalis inguinalis* directly caudomedial of the deferent duct (Nickel et al. 2004).

After splitting again into a medial and lateral branch, it innerves the *ductus deferens*, the *tunicae testis* and the *preputium*, but not the *scrotum*. Heading to the testicles, it also releases small branches to the *musculus cremaster* and the *musculus obliquus internus abdominis* (Nickel et al. 2004). The *scrotum* is innervated by sensible *nervi scrotales dorsales* which derive from the *nervus perinealis superficialis* from the *nervus pudendus* (Nickel et al. 2004).

**Histology and endocrine activity of the testicles**

The *tunica vaginalis* consists of *mesothelium* (serosal characteristics) and connective tissue. The *tunica albuginea* is following the *tunicae vaginalis* directly laying on the testes forming a capsule around the *testes*. It consists in grand part of collagen fibers, some elastic fibers and myofibroplasts which can be contracted. From the testes border, *septula* are running to the *mediastinum testis* as connective tissue. *Lobuli testis* are formed and contain the different ductuli, but also important endocrine cells in the intertubular space. Every lobule contains one to four convoluted seminiferous tubules. There are also blood and lymph vessels, fibrocytes and free mononuclear cells. Only in the cat, intertunical endocrine cells are known (Samuelson 2007).

The forementioned endocrine cells in the intertubular space are also called Leydig cells and synthetize steroid hormones, mainly testosterone and estrogens. They are large polymorphous cells with a spherical nucleus forming cords or clusters and are often accompanied by a capillary. The most important organelle in the Leydig cell is the smooth endoplasmatic reticulum because it contains the important enzymes for steroid biosynthesis whereas the small Golgi complex in Leydig cells has no relevant function for hormone production (Samuelson 2007).

Each tubule is lined by the seminiferous epithelium, the germinal stratified epithelium that is the site of spermatogenesis. This *epithelium* forms a basal lamina that is attached to a thin wall of connective tissue, the *lamina propria*, consisting of collagen type one and four. The cells within the connective tissue are myofibroblastic, lying near to the germinal epithelium. The fibrocytes are located more peripherally. The seminiferous epithelium is composed of two cell types: sustentacular cells and spermatogenic cells (Samuelson 2007).

The sustentaculum cells or Sertoli cells can be divided in two parts, a basal compartment and an apical compartment. Starting from the basal compartment and moving to the apical compartment, germinal cells develop finally into spermatids which leave the *tubuli contorti* and mature in the epididymis (Samuelson 2007).

The origin of all the germinal cells is the spermatogonium:

- Reserve cells or dark type A spermatogonia: do not divide often, heterochromatic nuclei witch little cytoplasm (Samuelson 2007).

- Light type A spermatogonia: similar to dark type A cells but with pale or euchromatic nuclei, influenced by testosterone to divide mitotically again and again and producing this way also type B cells, not only light type A cells (Samuelson 2007).
- Light type B spermatogonia: similar to light type A cells but with a round nucleus, they are also influenced  to divide too, but in difference to the light typ A spermatogonia they develop to primary spermatocytes (Samuelson 2007).
- The primary spermatocytes pass to the apical (adluminal) compartment and form the so called *zonulae occludentes*, which are tight junctions between them and the sustentaculum cells. They are important for establish the blood-testes barrier. They are the biggest cells and show condensed chromosomes in the nucleus. This cell type goes in prophase I of the meiosis cycle which contains the leptotene, zygotene, pachytene and diakinesis (Samuelson 2007).

This leads to:

- Secondary spermatocyte: They possess the same one and a half number of chromosomes that the primary spermatocytes possess. They develop very quickly to spermatids passing a brief prophase II, metaphase II, anaphase II and telophase II finally containing a hapoloid complement of DNA (Samuelson 2007).
- Spermatides: Initially the nucleus is small and placed centrally in to the cytoplasma than the spermatogenesis begins. The Golgi phase, the cap phase, the acrosomal phase and the maturation phase change the spermatide (Samuelson 2007).
- After the so called spermiation, the cells have become spermatozoa with the already typical morphology and are released into the tubular lumen (Samuelson 2007).

The important tasks of Sertoli cells are:

- Formation of the blood-testis barrier with tight junctions
- Nutrition of spermatogonia
- Production of Inhibin
- Phagocytosis of the residua from spermatozoa which left their place at the sustentaculum cell
- Production of the Anti-Muellerian Hormone (Antiparamesonephric hormone)

- Control of the influence of the hormones, testosterone and follicle stimulation hormone (FSH)
- Androgen binding proteins like transferrin (Samuelson 2007).

The *tubuli seminiferi contorti* develop into the tubuli recti. There luminal epithelium is cuboidal with apical microvilli and cilia, containing also lymphocytes and macrophages (Samuelson 2007).

The *rete testis* does also have a cuboidal epithelium. From the *rete testis*, eight to 20 tubules merge into the *epididymis*, also known as the *ductuli efferentes*. The epithelium starts at the beginning as a cuboidal epithelium. Passing to the *ductus epididymidis*, it gets low columnar, being in part ciliated. The non-ciliated have microvilli. The microvilli and ciliated cells are important for the transport of spermatozoa towards the epididymal duct. The connective tissue in this part consists of elastic fibers and also of myofibroblasts with sympathetic and parasympathetic innervation (Samuelson 2007).

The ductus *epididymidis* can be divided into the head, body and tail. The head and the body have a simple high prismatic and double rowed epithelium with the tall principal cells and the short polygonal basal cells. The principle cells are highly prismatic with stereocilia. In beteween these cells, clear cells and apical cells can be seen. Lymphocytes and macrophages remove germination residues. The basal epithelium at the non-luminal part is attached directly to connective tissue and the connective tissue directly to smooth muscle cells, which is important for the transport of maturating spermatozoa from the head and the corpus to the *cauda epididymidis*. Coming to the ductus deferens the pseudostratified epithelium gets smaller and the smooth muscle thickens (Samuelson 2007).

The *ductus deferens* is the continuation of the *ductus epididymidis* and it consists of a *tunica mucosa*, *tunica muscularis* and *tunica serosa* or *adventitia* depending on the location.

- *Tunica mucosa:* consists of a double rowed columnar epithelium with short stereo ciliated principal cells and an underlying loose connective tissue (Samuelson 2007).
- *Tunica muscularis:* is formed by three smooth muscle layers which form the prominent *tunica muscularis* (Samuelson 2007).
- *Tunica serosa* or *adventitia*: serosal layer covering the *tunica muscularis (Samuelson 2007)*.

## 1.2. Surgical and chemical castration methods

### <u>Surgical castration</u>

Every surgical procedure should be prepared according to aseptic principles and a thorough cleaning and disinfection of the surgical field should be performed. Before any castration it should also be ensured that the patient is not a cryptorchid and that a general anesthesia would not endanger the patient's life. Generally, absorbable suture materials are used for subcutan structures. Absorbable or non-absorbable sutures can be used for the cutis. Absorbable suture material would be an advantage, especially for stray dogs, where you often cannot do a post op check and pull out the stitches. The vessels, nerves and the spermatic cord can be ligated with a surgeon’s knot. This can be used with a transfixation ligature too. For wound closure of the skin as mentioned before absorbable suture material is advantageous if stray dogs are castrated. Also electrosurgery could be used for vessel ligation (Hedlund 2007).

<u>Closed orchiectomy through the *scrotum*</u>

The *testes* are manually pressed into the *scrotum* and an incision, paramedian to the *raphe scroti* (median septum) is made. The incision should pass through the *cutis*, *tunica dartos*, *fascia spermatica externa* but not the *fascia spermatica interna*. Once the forementioned structures are opend, the testicle can be advanced by controlled pressure. The testicle itself, *epididymidis*, *ductus deferens, funiculus spermaticus* and the *ligamentum caude epididymidis* are identified. Then the *ligamentum caudae epididimydis* is carefully ruptured with the fingers and separated from the *tunicae*. The *ductus deferens* and the vascular cord can be ligated separately or together and an encircling ligature is added around both. Between the testicle and the ligature, with sufficient distance to the ligature, a haemostatic forceps is placed and the transection is made between clamp and ligature. The *musculus cremaster* and *tunica vaginalis* should be ligated too. No closure of the subcutis and cutis is necessary (Hedlund 2007).

<u>Closed prescrotal castration</u>

Closed presctoral castration differs from closed scrotal castration only in regard to the access to the testicles. The skin incision is not made directly on the scrotum, but in the pubic region, paramedian in the caudal abdomen. The testicle is pressed caranially as much as possible into the prescrotal area. The skin and subcutaneus incission is made along the median raphe over the displaced testicle. Again, the *fascia spermatica interna* is not opened. For the next step a gauze sponge is used to brush fat and fascia proximally. This way the testicle can be exteriorized maximally. The whole spermatic cord and tunics are ligated. For better ligation a trans-fixation ligature is recommended, ligating the *musculus cremaster* and again the *ductus deferens*, lymph and blood vessels and nerves in once. Finally, the subcutis is closed with a continuous suture and the cutis with an intracutaneous, continuous or single-button suture, depending on the surgeon's preference (Hedlund 2007).

<u>Open prescrotal orchiectomy</u>

The testicle is pressed cranially as much as possible into the prescrotal area. The skin and subcutaneus incission is made along the median raphe over the displaced testicle. In this technique the *fascia spermatica interna* is opened and thus also the attached *lamina parietalis* of the *tunica vaginalis*. The *ductus deferens* and the vascular cord could be ligated separately or together and an encircling ligature is added around both. Between the testicle and the ligature, with sufficient distance to the ligature, a haemostatic forceps could be placed and the transection is made between clamp and ligature. The *musculus cremaster* and *tunica vaginalis* should be ligated too. The closure of the cutis and subcutis follows the same principles as in the closed prescrotal orchiectomy (Hedlund 2007).

<u>Perineal castration</u>

The perineal castration follows the same principles as the open prescrotal castration, but the access to the testes is different. Between the anus and the *scrotum,* in the midline, a vertical incision is made through the c*utis* and *subcutis* opening the *perineum*. Once a testicle is protruded, the *tunicae testis* are opened to the f*ascia spermatica interna* and the same procedure is made as in the open prescrotal castration (Hedlund 2007).

<u>Vasectomy</u>

With this technique, a segment of the *ductus deferens,* before entering the inguinal canal, is removed. The skin incision is made at the cranial border of the scrotal region. First the spermatic cord between the scrotum and the inguinal canal is individuated. Then the *cutis*, *subcutis* and *vaginal tunic* is opened, isolating the *ductus deferens*. Double ligation and section of at least 0.5 cm of the duct is following. The same must be done on the other side and finally closed with a subcutan and cutan suture (Hedlund 2007).

## Non-surgical castration

Immunocastration and interruption of the Hypothalamus-Pituitary-Gonadal axis

In immunocastration animals are vaccinated with a vaccine which contains GnrH on a carrier protein. Provoking a systemic production of antibodies against endogenous hormones like GnrH or FSH. If enough antibody titers are present contraception can work (Aurich 2018). In a new trial study from February 2020 1 ml of 550 µg GnRXG/Q recombinant protein, 500 µg of low molecular weight chitosan as adjuvant and 1 ml NaCl 0.9 % as solvent were injected subcutaneously. The vaccinations were done at day 0 and 30 of the trial, resulting in specific immunity until day 150. This shows that infertility in the vaccinated males can be expected for approxamtely 150 days (Siel et al. 2020).

Giving a male individuum GnRH agonists or antagonists, will lead to a down-regulation of GnRH receptors and thus the FSH and LH production will be terminated and the germ cell production will be stopped (Aurich 2018). The negative feedback mechanism of the hypothalamus-pituitary-gonadal axis is responsible for this mechanism. In male dogs slow releasing subcutaneous implants containing 4.7 mg (efficient for 6 month) or 9.4 mg (efficient for 12 month) of GnRH analogue Deslorelinum (Suprelorin®, Virbac Schweiz AG Europastrasse 15, Postfach, 8152 Glattbrugg) resulted in infertility and positive effects against benign prostate hyperplasia in dogs (Aurich 2018).

Intratesticular injection (ITI) of chemosterilant solutions

The objective is to bring the chemical solution to the germinal cells, Leydig cells and sustentaculum cells (Sertoli cells) and finally induce apoptosis, necrosis or anyother failure and thus should stop hormone and germ cell production. The result is infertility. A lot of the chemical solutions for castration are very aggressive and lead to necrosis. If other structures than the testicular parenchyma are also infiltrated accidentally, it results in necrosis of them. Too much pressure on the testicle, too much pressure on the syringe during application or a leacking testicle because of a too big diameter of the needle or wrong application, can result in complications (Fagundes et al. 2014, Forzán et al. 2014).

For ITI, the dog should be fixed to avoid movement and when necessary also sedated. A new, small diameter needle should be used (28 g and 12 mm in dogs) on each testis. The needle should be positioned as close as possible to the ductuli efferentes and almost parallel to the sagittal line of the testicle. Pressure on the testes at the time of injection should be avoided by holding the skin below the testis rather than the testis themselves. The injection should be made slowly and before removing the needle, wait for 3 seconds (Cavalieri 2017, Oliveira et al. 2012).

This location is chosen because of the closeness to the *ductuli efferentes*. If after injection some intact and fertile areas of the testis are present, but the zone around the *ductuli efferentes* and thus the way from the sperm cells to the *epididymis* is closed, infertility is probably reached.

Contraceptive solutions used so far for ITI:

Solutions described in several scientific works and reviewed by Cavalieri (2017):

- Zink Gluconate (Fagundes et al. 2014, Oliveira et al. 2012, Woodward et al. 2017)
- Cadmium described in bitches by intragonadal injection (Chatterjee and Kar A. 1969, Pariazek 1957)
- Calcium Chloride (Jana et al. 2005, Jana and Samanta 2006, Kuladip and Prabhat Kumar 2007)
- Chlorhexidine (Scully et al. 2015)
- Bacillus Calmette Guerin (Naz and Talwar 1981)
- Zinc tannate (Migally and Fahim 1984)
- Lactic acid (Fordyce et al. 1989)
- Ethanol (Freeman and Coffey 1973)
- Silver nitrate (Freeman and Coffey 1973)
- Acetic acide (Freeman and Coffey 1973)
- Formaldehyde (Freeman and Coffey 1973)
- Sodium tetradecyl sulfate (Freeman and Coffey 1973)
- Sodium morrhuate (Freeman and Coffey 1973)
- Glycerol (Weinbauer et al. 1985, Wiebe and Barr 1984, Wiebe and Dinsdale 1991)

### 1.3. Reasons for castration of male dogs

Especially in urban areas stray dogs present a big problem. Roaming the streets in search of females for copulation, they create traffic accidents or traffic jam. Some male dogs show aggressive behavior against other owned dogs. If stray dogs find garbage bins which they can reach they spread the contents and therefore facilitate the spread of rodents. Hygiene in such areas is not maintained. Dogs also make noise and might limit tourism in these cities (Taylor et al. 2017). Most of these stray dogs are neither vaccinated nor regularly dewormed and thus they are potential reservoirs for diseases like rabies (Taylor et al. 2017). Being infected from parasites and eliminating them with their feces they do not only store them but also spread the parasites and their eggs. These are infectious for other animals, domestic dogs and humans. Roaming groups of hungry dogs show predator instincts like wolves and or may attack foals, cattle or sheep and goat herds, provoking economic damage to the farmers. This predator instinct is enforced when wolves (*canis lupus*) reproduce with domestic dogs (*canis lupus familiaris*) and often the result is a so called hybrid with high predator instinct and less fear from urban areas (Godinho et al. 2011, Verardi et al. 2006). A short summary of these problems is displayed in ***Table 1***.

**Table 1: Problems associated with stray dogs** (Hassan and Fromsa 2017).

| | |
|---|---|
| Public Health | • More than 100 zoonotic disease identified that can be transmitted from dog to human, e.g. rabies<br>• Dogs may be responsible for bit occurrences |
| Environmental contamination | • Deposition of excreta near or in areas inhibited by people<br>• Potential genetic contamination of wild Canide populations |
| Nuisance factors | • Noise: Barking, howling, aggressive interactions<br>• Odor/aesthetics: Territorial urin marking, fecal contamination and deposition of urin |
| Wildlife | • Hunting smaller wild mammals |
| Damage to property and livestock | • Result from accidents<br>• Hunting of livestock |
| Animal welfare of stray dogs | • Injury resulting from car accidents<br>• Injury from aggressive confrontation during competition for limited resources<br>• Malnutrition due to limited availability of sustainable food source<br>• Disease susceptibility<br>• Inhumane culling methods, stray control measures<br>• Persecution/deliberate abuse by members of the community |

## <u>Overpopulation and accociated problems</u>

In dogs, the most common reason for castration is overpopulation, especially in stray dogs. In regions where dogs can reproduce nearly uncontrolled because the owner does not look after them or a lot of stray dogs are present, castration is a very important tool for regulation of uncontrolled reproduction (compare ***Figure 8***).

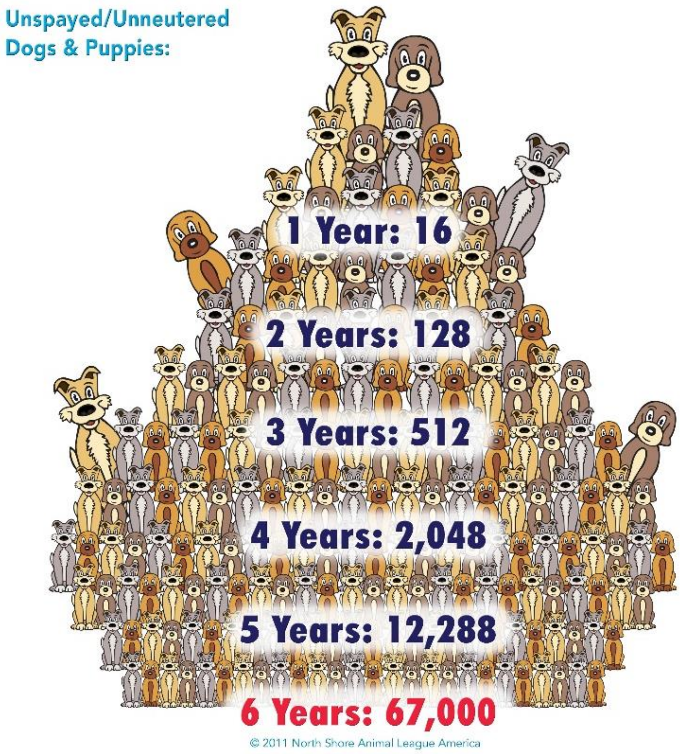


**Figure 8: Reproduction pyramide of an unspayed dog population**

This graphic clearly shows the problem of overpopulation of stray dogs. Note the rapid reproduction rate of an uncontrolled dog population. In only two years a dog population of 16 individuals increases to 128, in 5 years this population increases a hundredfold and in 6 years it is estimated to be about 67000 individuals (The Grey Area News 11.06.2020).

In the USA, in addition to surgical castration, euthanasia in shelters is a common means to regulate the overpopulation of ownerless dogs. *Fehler! Verweisquelle konnte nicht gefunden werden.* shows the high numbers of euthanatized dogs in the some of the states (Rowan and Kartal 2018, Taylor et al. 2017, WHO Expert Consultation on Rabies 2013).

**Table 2: Number of dogs euthanized in US states** (Rowan and Kartal 2018)

| State | Year | Dogs euthanized in shelters | Human population (2010) | Dogs/1000 people (2011) | Dogs euthanized per 1000 people | % of pet dogs in state euthanized in shelters |
|---|---|---|---|---|---|---|
| California | 2011 | 176907 | 37253956 | 177 | 4.69 | 2.65 % |
| Colorado | 2013 | 6968 | 5029196 | 264 | 1.36 | 0.52 % |
| Delaware | 2011 | 2012 | 897934 | 180 | 2.22 | 1.23 % |
| Maine | 2012 | 644 | 1328361 | 226 | 0.48 | 0.21 % |
| Maryland | 2011 | 10477 | 5773552 | 157 | 1.80 | 1.15 % |
| Michigan | 2013 | 22909 | 9.883.640 | 206 | 2.32 | 1.13 % |
| Nevada | 2011 | 14679 | 2700551 | 212 | 5.39 | 2.54 % |
| New Hampshire | 2012 | 346 | 1316470 | 161 | 0.26 | 0.16 % |
| New Jersey | 2011 | 6023 | 8791894 | 152 | 0.68 | 0.45 % |
| North Carolina | 2013 | 62269 | 9535483 | 261 | 6.45 | 2.47 % |
| Virginia | 2013 | 16519 | 8001024 | 210 | 2.04 | 0.97 % |
| Total for 11 States | | 296867 | 90512061 | | 3.28 | |
| Estimated USA Totals (from PetPoint & AVMA data) | 2010 | 1723039 | 309350000 | 225 | 5.57 | 2.48 % |

## Rabies and its costs

Another major problem of uncontrolled strayed dogs are human death due to rabies infections. Domestic dogs are responsible for over 99 % of human deaths due to rabies. Rapid population turnover (due to high death rates) of both owned and unowned dogs can present a significant challenge for the maintenance of high vaccination coverage.

Humans bitten by dogs count as the most important transmission route for rabies. In Tanzania, 23709 bites have been documented in six years (Cleaveland et al. 2002, Taylor et al. 2017, WHO Expert Consultation on Rabies 2013).

In Asia, the largest number of rabies deaths is present, *i.e.* 30000 human deaths per year. In Africa approximately 24000 rabies fatalities per year are recorded (Lembo et al. 2010). Often the high number of deaths in certain regions is justified because of the high costs of prophylaxis. The costs for post exposure prophylaxis are immense, estimated at around US $ 1.5 billion (WHO Expert Consultation on Rabies 2013).

"The Humane Society of the US estimates that each year $ 2.5 billion is spent by human organizations and $ 800 million to $ 1 billion by animal control organizations on managing the pet overpopulation problem" (Taylor et al. 2017).

**<u>Welfare</u>**

Stray dogs are often victims of traffic accidents or deliberate abuse by humans. Moreover teritorial fights, malnutrition and a higher susceptibility to diseases cause injuries or lead to the necessity of medical interventions (Hassan and Fromsa 2017).

Furthermore castration, surgical or chemical, is also used for owned dogs with behavior problems. Behavioral disorders often lead to dogs being ownerless and ending up in an animal shelter or the like and ultimately suffering themselves. Genetic predispositions to diseases, *e.g.* prostate cancer, testicular neoplasia and general tumor prophylaxis, are more often reasons for castration in domestic dogs (Aurich 2018).

### 1.4. Legal basis: Castration of animals

In the EU, there are currently no clear and rather unambiguous regulations regarding the castration of dogs. In most cases this is internally regulated by each state. In addition, it is unclear whether chemical castration by ITI is legal or not.

For example in Germany and Austria the castration of the dog means the surgical removal of its gonads. In male dogs, the testicles are removed, in bitches the ovaries and sometimes also the uterus. Both sexes lose their ability to reproduce through castration (Bundesministerium der Justiz und für Verbraucherschutz 20.06.20, Rechtsinformationssystem des Bundes 11.06.2020).

In Austria and Germany castration is therefore the removal of organs, this is generally forbidden for vertebrates, including dogs (Rechtsinformationssystem des Bundes 11.06.2020).

Exceptions are only made:

1) If veterinary indication is given

2) To prevent uncontrolled reproduction

3) For the further use and keeping of the animal

(Rechtsinformationssystem des Bundes 11.06.2020).

Therefore, it is legally allowed to control stray populations by castration in Austria and Germany, however, the laws do not mention ITI as a means to castrate animals. Thus chemical castration in general is not regulated by current laws in these two countries.

Since the laws can be defined differently from country to country, the individual law of a country must be taken into account when spaying dogs.

### 1.5. Objective

Considering the facts that overpopulation of stray dogs is a current problem and that despite attempts to castrate as many male and female dogs as possible the problem still exists; one is looking for new and above all cheap castration methods. One of these potentially very cheap and simple castration methods could be the ITI with saline solution. So the aim of this diploma thesis is to show if saline solution could be a possible chemical castration method for low cost population management of stray male dogs.

## 2. Materials and Methods

For research, only scientific papers with topics for chemical castration of male dogs with saline solution were searched. Papers mentioned in scientific journals, veterinary journals or other veterinary literature were selected.

In the following, the procedure for researching and selecting relevant literature for the main subject area – chemical castration with saline solution - is described.

For the research on chemical castration of male dogs with saline soluiton, the following databases were searched for scientific publications:

- PubMed

- Scopus

In order to enable a structured query and evaluation of the search results, the respective search queries were developed according to the PICO scheme (Richardson et al. 1995). The formulation of the questions is based on population, intervention, comparators and outcome of interest.

1. population: dogs, no restrictions regarding age or breed
2. intervention: chemical castration with sodium chloride
3. comparators: surgical castration and/or other intratesticular injected solutions
4. outcome of interest: chemical castration with saline solution techniques, success rates, adverse effects, costs

This structure resulted in a search query in Pubmed:

[1] *castration*

[2] *AND saline solution*

In the Scopus database, the query was designed as follows:

[1] TITLE-ABS-KEY (castration

[2] AND saline

[3] AND solution)

As in the course of the literature search only a small number of papers met the different selection criteria, only point 2 of the PICO schemes was applied in the search. In the further selection of relevant literature, a simple three-step procedure was followed. In the first step, the title of the respective publication was examined and, if necessary, the publication was excluded. For the remaining hits the summary was reviewed according to the defined PICO criteria and further publications were sorted out (stage 2). Only in the last and third stages were the remaining scientific articles subjected to a full text review. In these, the references for similar publications were searched too.

Furthermore scientific papers not published in either English, German or Italian were excluded.

## 3. Results

The literature research on chemical castration with saline solution on Pubmed and Scopus has produced the results presented in ***Table 3***.

**Table 3: Number of publications found in the Pubmed and Scopus databases**

| | |
|---|---|
| **Pubmed (total)** | 165 |
| **Scopus (total)** | 65 |
| **Total number of publications** | 230 |
| **Minus duplicates** | 185 |
| **Remaining after step 1** | 41 |
| **Remaining after level 2** | 14 |
| **Remaining after level 3** | 1 |

The main reasons for eliminating publications in regard to the PICO question were:

- Chemical castration with other substances than saline solution
- Other species than dogs
- Languages other than English, German or Italian

Resulting in only one publication matching all criterias:

*Canpolat I, Karabulut E, Eroksuz Y. 2016.* ***Chemical Castration of Adult and non-Adult Male Dogs with Sodium Chloride Solution.***

Objective: “The purpose of the study was to determine the efficacy of intratesticular injection of sodium chloride at 20 % concentration on chemosterilization outome of the young and adult male dogs.” (Canpolat et al. 2016)

Although this publication might be considerd as a randomized controlled trial and thus should be rated with a high quality of evidence, the overall quality of the study can only be rated medium to poor. Main reasons for this rating are:

- No controll group
- Only two groups with each 6 dogs (young vs. adult)
- No futher description of the population used

- Not reproducible regarding materials and methods (insufficient description of instruments, contradiction in dates removing testes)

Nonetheless, the findings regarding the outcome of interests are the following:

Chemical castration with saline solution techniques

Before injection the solution was prepared and 200 g sodium chloride was dissolved in one liter of pure water. This liquid was autoclaved and stored at 4 °C until use. For ITI skin was scrubed with iodine solution. Under general anesthesia with propofol (6 mg/kg) followed from 3 % sevoflurane, the 20 % solution was injected intratesticular until tension occurs. The author describes neither needle size, amount of applied volume nor complications.

The results were evaluated ultrasonographically looking at differences between height, width and length of the testicle and histologically if changes in the parenchyma were obtained. For histological analysis the dogs were castrated surgical.

Success rates

The testicular volume decreased significantly in non-adult dogs after 3 weeks. In non-adult males most of the testicular parenchyma became necrotic. Histopathologic indications show focal coagulation necrosis with severe degenerative changes in seminiphorus tubuli. Also sparrse Leydig cells were observed in tubular lumen.

The testicles from the adult male dogs show only well demarked necrotized regions.

Ultrasonographic examination one day after injection shows diffuse echotexture and increased echogenite. Hyperechogenic areas were observed. 3 non-adult dogs show clear testicle atrophy at day 30, but none of the adult male dog showed this.

No success rate was mentioned.

Adverse effects

Reaching the peak after 48 hours, testicular swelling was evident in every dog. One non-adult dog showed fistula and sloughing of the scrotal skin. Necrotized tissue was the reason.

Costs

No further information about costs of the procedure and no control group.

## 4. Discussion

There is, surprisingly, only one paper fulfilling all inclusion criteria of the research question. Being of rather moderate to poor quality, it is not possible to give an evidence based answer to the objective of this thesis. Extending the focus of this theses, however, leads to further and more reliable results regarding ITI techniques for castration of dogs.

### Comparison with other species and other ITI solutions

Testicular anatomy varies considerably among domestic animal species. In rams, bulls and boars, the Leydig cells take 1 %, 5 % or even 20 % to 30 % of testicular volume. In dogs and boars, the testicular parenchyma is completely separated by septa whereas other species only have inconspicuous connective tissue strands. In rodents and horses, the mediastinum testis is small and less centrally located than in ruminants, pigs, cats and dogs (Eurell and Frappier 2013). All these factors are assumed to influence the distribution and therefore the efficiency of the chemical castration substance within the parenchyma. Species with bigger pampiniform plexus, bigger veins at the testicular surface and complex vessel patterns like the stallion or ruminants can be more predisposed for scrotal ulcer than species with small vessels.

The diffusion of the injected solution seems to be the problem also when testes size increase. Maybe a higher volume of the respective solution or a higher number of injections is needed in these cases. ***Figure 9****Fehler! Verweisquelle konnte nicht gefunden werden.* depicts a testicle after surgical castration. Before incision intratesticular lidocaine was applied. Testicular bleeding through the incision canal is obvious. This shows that if a too big needle is used for ITI and needle is moved too much in the parenchyma bleeding is increased. The blood-ITI solution can flow through the incision canal backwards in direction to the testicular capsule and flow out through vaginal tunics. Scrotal swelling, fistula and scrotal ulcer result (Forzán et al. 2014) .

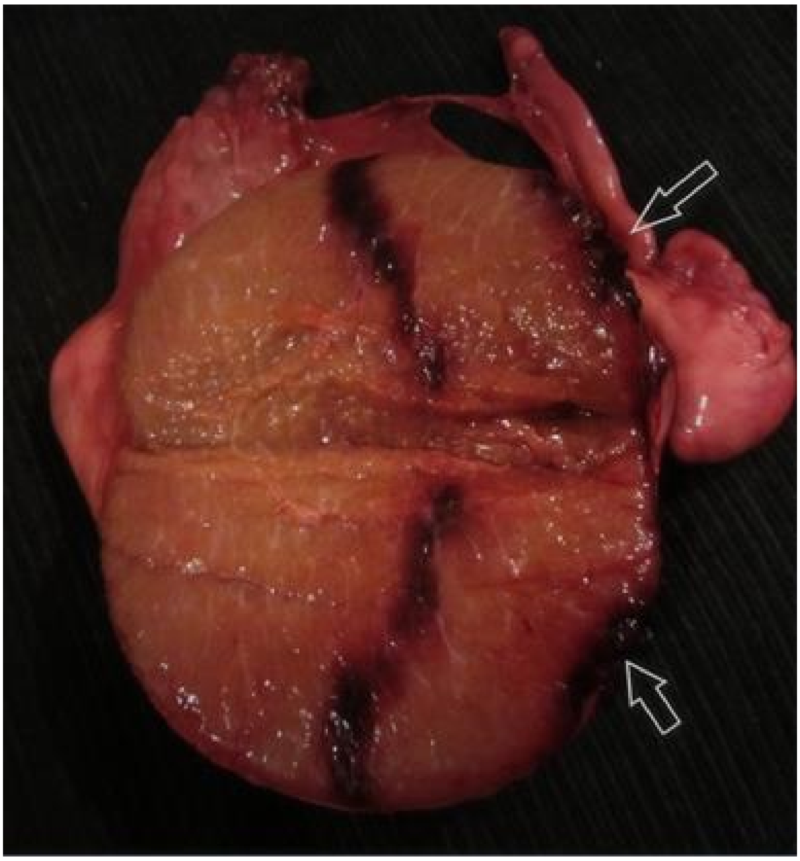

**Figure 9: Longitudinal section of a horse testicle after inguinal castration**
Bleeding occurred, do to intratesticular Lidocaine application before surgical castration. During ITI bleeding can happen too.

If one now compares solutions for chemical castration by ITI, Zinc Gluonates and CaCl clearly stand out in the research. In order to compare these with the few studies on chemical castration with NaCl, in detail Zinc Gluconate and CaCl:

Zinc Gluconate

Zinc Gluconate (Esterisol/Zeuterin, New York City, NY, USA; Neuterisol, Pet Healthcare International, Columbia, MO, USA). Zinc gluconate has received US Food and Drug Administration (FDA) approval as chemosterilant for use in male dogs in the USA and also some other countries. Some zink gluconate drugs also contain dimethylsulfoxide (DMSO) or are neutralized with arginine. Testoblock is also a zink gluconate based chemosterilant. Zink-gluconate derives from natural ingredients like zinc, glucose and arginine. This product is absorbed after 72 hours post injection and leads to fibrosis of the seminiferous tubules, rete testis and epididymis. This product is mostly used in the USA and costs around 30 $/ml. Depending on size of the dog and his testicles, 0.4 ml to 2.0 ml for each dog are used. Adding 5 to 15 $ for materials and light anesthesia. Some authors describe mild to moderate side reactions.

In a study from 2010, 249 dogs were neutered with zink gluconate and only in three of them, adverse reactions appeared (ACCD- Alliance for Contraception in Cats and Dogs 2015)**.**

A study applying Neuterisol was presented at the second International symposium on non-surgical contraceptive methods for pet population control in 2004, recommending dosages corresponding to testicular Injection dose in dependence of testicular width (compare ***Table 4***) (The Alliance for Contraception in Cats and Dogs 2004).

**Table 4: Recommended injection dose of Neuterisol in dependence of testicular width**

| Testicular Width (mm) | Dose Per Testis (ml) |
|---|---|
| 10-12 | 0.2 |
| 13-15 | 0.3 |
| 16-18 | 0.5 |
| 19-21 | 0.7 |
| 22-24 | 0.8 |
| 25-27 | 1.0 |

Pharmaceutic guidelines are defined and also a measuring template is provided. On this instrument, the ml needed for injection can be read (The Alliance for Contraception in Cats and Dogs 2004). Some studies mention adverse effects, mostly scrotal swelling or irritation after injection, caused from a mild inflammation (Levy et al. 2008, Soto et al. 2009).

In a large study including 270 dogs, 6 dogs showed vocalization during injection, one kicking, 17 scrotal pain (most in first two days), three scrotal irritation, two biting and licking, two scrotal swelling irritation and dermatitis, one scrotal ulceration, one subcutaneous infection, one dog dry scrotal skin, preputial swelling and scrotal score. Out of 270, 17 dogs showed neutrophilia, 12 vomiting (ten of the 12 dogs vomited within 1 minute and 4 hours after the injection), eleven anorexia, six lethargy, five diarrhea and two leukocytosis (The Alliance for Contraception in Cats and Dogs 2004).

Similar results were described in other studies. The treatment causes azoospermic, necrospermic or oligospermic ejaculates (Jana et al. 2005, Woodward et al. 2017). a libido rise reduction of testicular size and sperm motility (Soto et al. 2009), sperm vigor, ejaculate volume, sperm cell number decrease and abnormal sperm cell number increase (La Croix 2006, Soto et al. 2009, The Alliance for Contraception in Cats and Dogs 2004). Histological changes range from decrease of germ cell number, atrophy signs, fibrosis, loss of testicular architecture, focal presence of mononuclear inflammatory cells, neutrophilic infiltration in the testis and epididymis to calcified areas (Soto et al. 2009). By ultrasonographic examination, heterogeneous echotexture, mild evidence of mediastinal line, presence of hypoechogenic areas and parenchyma heterogeneity was detected. This was associated with reduced numbers or complete absence of spermatozoa in the epididymis (Soto et al. 2009).

<u>$CaCl_2$</u>

$CaCl_2$ was used in different concentrations and solvents, like saline solution (Leoci et al. 2014b), alcohol or lidocaine (Leoci et al. 2014a). One study used 5 mg/kg, 10 mg/kg, 15 mg/kg and 20 mg/kg bodyweight solved in lidocaine hydrochloride for injection (Kuladip and Prabhat Kumar 2007). Other authors used 10 %, 20 %, 30 % and 60 % $CaCl_2$ solution solved in saline solution. In a concentration of 30 % and 60 %, $CaCl_2$ solution was too aggressive and adverse reactions like scrotal swelling, ulcer and fistula and necrosis occurred. In 60 % of the 10 % group and 80 % of the 20 % group, azoospermia and in 20 % oligospermia was demonstrated. Sperm motility decreased in the 10 % group down to 10 %+/- 5.2 %, and in the 20 % to 5 % ± 2.1 %. Testicle width decreased in treated groups. Histology revealed similar results as treatment with zinc gluconate. In the 30 % and 60 % group also calcification and interstitial calcium deposits were found (Leoci et al. 2014b). In a follow up to the study where saline solution or local anesthetics like lidocaine as solvents induced too much discomfort and side reactions, alcohol was used as solvent for calcium chloride. Less dogs showed adverse reactions to the injection and treatment in general. Zero sperm counts, 0 % motility, fibrotic testicles, lower serum testosterone and raised sexual behavior over a year period was noticed (Leoci et al. 2014a).

Canpolat et al. (2016) reported similar results but it cannot confirmed that 20% saline solution has the same effect as zinc gluconate (Canpolat et al. 2016).

Sodium chloride for chemical castration in other species

Neto et al. (2014) used a 20 % hypertonic sodium chloride solution in one to five, 15 to 20 and 25 to 30 days old calves for contraception. The histological changes post ITI were very similar to injection of zinc gluconate or calcium chloride. Relevant changes like coagulative necrosis of the seminiferous tubules and Leydig cells but also dehydration of testicular parenchyma and fibrosis was described. A GnRH stimulation test after twelve month in treated groups only in the 25 to 30 days old calved resulted in an increase of testosterone leading to the conclusion that sodium chloride should be injected in the first 20 days of life to induce infertility (Neto et al. 2014). With regard to stress factors, injection of 20 % sodium chloride did not induce a significant stress response in comparison to orchidectomized controls when assessed by calf's eye corner temperature. Cortisol level increased 30 and 60 minutes in orchiectomized, in saline only after 30 minutes. Higher scrotal temperature 96 hours after injection and surgery was observed, indicating a slight inflammation after treatment in both methods. Elevating the percentage of the NaCl solution to 30 % improved the outcome in older calves (Oliveira et al. 2017).

**Side effects and complications associated with different castration methods**

A disadvantage of surgical castration is the risk of infections. The animal needs a relevant recovery time after being left into its habitual environment. If castration is done for population control, many dogs may be involved and place for recovery is thus limited which may increase the risk for infections. Surgical techniques that require a suture increase the risk for infections especially during warm seasons because of an increased occurrence of insects. They may not only spread bacteria but in some regions, may deposit eggs. This is usually prevented when chemical castration is used instead.

Scrotal hematoma (Adin 2011), peri-incisional bleeding, skin bruising, and swelling (Miller et al. 2018), partial wound opening, scrotal swelling, shaving wounds and postoperative hypothermia are common complications after surgical castration (Airikkala-Otter et al. 2018).

In ITI side effects frequently occur with dogs showing adverse reactions with similar complications as surgical castration. Infection can be a problem if scrotal fistulas develop or scrotal chewing occurs but seems to be less dangerous than in surgical wounds with site infection.

## Costs

Comparing the costs of surgical castration in Italy, Austria and Germany with the material costs of sterile saline or zinc gluconate, chemical castration is significantly cheaper. With injection of a volume of 1ml per testicle in a large testicle-sized dog, costs would approximate 60 US $ which is slightly below the costs for surgical castration in Austria, Italy and Germany (***Fehler! Verweisquelle konnte nicht gefunden werden.Table 5***). With saline solution, the costs would be much cheaper which is of great significance for population control.

**Table 5: Costs for castration in Austria, Germany and Italy**

| Country | Castration costs prescrotal/scrotal technique | Anesthesia | Total |
|---|---|---|---|
| Austria (2019) Veterinary Medicine University of Vienna | <10kg- 145 €<br>>30kg- 291 €<br>10-30 kg- 175 € | <10kg 50 €<br>>30kg 100 €<br>10-30 kg 54 € | 195 -391 € |
| Germany | 51.31 € | Intubated: 38.48 €<br>Injektion: 19.24 € | 70.55- 89.79 € |
| Italy | | | 80-150 € |

## Efficiency

One main advantage of surgical castration is, however, that the efficiency is close to 100 %, *i.e.* 100 % male dog will become infertile. The lower efficiency of chemical castration is associated with animal age, testicular size and also testicular anatomy (Aurich, 2018). In contrast, chemical castration has a lower efficiency and to the best of our knowledge there is no chemical castration method with an efficiency close to 100 %, leaving a considerable number of chemically castrated male dogs fertile. The results of chemical castration should not be underestimated. Pronounced effects of chemical castration on in semen characteristics like azoospermia, necrospermia, and oligospermia together with low semen motility and impaired libido have been demonstrated in several studies abnormality of spermatozoa increase (Jana et al. 2005, La Croix 2006, Soto et al. 2009, The Alliance for Contraception in Cats and Dogs

2004, Woodward et al. 2017). This clearly suggests that the treatment with the aim to provide population control and not infertility of one individual animal might provide an interesting and promising approach.

## Conclusion

After comparing the different methods, it becomes apparent that surgical castration is the most efficient approach for contraception. It is also the only acceptable method for castration of animals with cryptorchism or testicular anomalies. With regard to population control, chemical castration is, however, appealing because the side effects and costs of surgical castration are high and post-surgical control in stray dogs is usually not possible. Pain management and an adequate anesthesia are often missing. Some sterilant solutions have been reported to work very well and are associated with lower costs, less side effects and faster technique in comparison to surgical approaches. Some veterinarians did not even use sedation for chemical castration in dogs (Soto et al. 2009). Planning of castration days in castration projects is thus much easier. The chemo sterilant can be easily stored and transported in abounding amounts. If ultrasound-controlled injection is preferred a low-cost rectal ultrasound probe could be used. If sedation of animals is needed, waiting time is still much shorter than for surgical castration with a Ketamine or Isoflurane based anesthesia protocol. Such an owner-friendly management is not possible with surgical castration. If done appropriately, chemical castration might be an interesting alternative in such cases. Especially, application of hyperosmotic sodium chloride solution appears very interesting for chemical castration in non-adult male dogs. Chemical castration has thus considerable advantages in comparison to surgical castration in field castration campaigns or for population control in stray dogs. Under these circumstances, a certain percentage of male dogs that remain fertile may well be acceptable. Future studies will determine the most effective treatment protocol also well acceptable with regard to animal welfare.

## 5. Abstract

Stray dogs are a major concern for many countries in the world. Dogs as reservoir for zoonotic diseases (e.g. rabies), causing accidents, noise and pollution, furthermore ethic issues regarding malnutrition, injuries and last but not least large-scale euthanasia, to get the situation under control, are just a few challenges. To face these issues, affected countries and cities have to bear a substantial financial burden. Thus millions of dollars are invested in stray dog population management every year. Alongside vaccination projects, surgical castration of male and female individuals is performed on a regular basis. This method is still considered the gold standard for induction of infertility in male dogs. As surgical interventions, however, bear certain risks especially under field conditions, there is a need for new, cheaper, faster, less painfull and more efficient ways of castration. One promising potential method is the intratesticular injection of sterilizing solutions. As part of this thesis a comprehensive literature research on intratesticular injections of saline solution for this purpose was performed. A systematic search resulted in merely one published scientific paper on neutering male dogs with saline solution. In this study six juvenile, up to six month old, and six adult dogs where neutered with a 20 % intratesticular saline solution. Only the group of juvenile dogs showed desired effects like atrophy of testicles, necrotic parenchyma and sonographic hyperechogenicity. Due to a small number of cases and no control group in this study and a lack of studies in general, it is necessary to do further research. Especially application technique, efficient dosages and concentration should be of great interest of this promising alternative.

## 6. Zusammenfassung

Weltweit stellen streunende Hunde die Bevölkerung vor mehrere Probleme. Hunde als Krankheitsreservoir für Zoonosen (z.B. Tollwut), Ursache für Unfälle, Lärmbelästigung und Verunreinigungen, auch ethische Fragen hinsichtlich Abmagerung, Verletzung und nicht zuletzt gezielte Tötungsaktionen, um dem Problem Herr zu werden, sind hierbei nur einige Herausforderungen. Zur Bekämpfung müssen betroffene Länder und Ortschaften teilweise erhebliche finanzielle Belastungen erdulden. Jährlich werden Millionen an Dollar in das Populationsmanagement investiert. Neben Impfungen werden auch Kastrationen von männlichen und weiblichen Individuen sehr häufig durchgeführt. Das gebräuchlichste Verfahren zur Kastration ist zurzeit die chirurgische Kastration. Diese Methode gilt als sehr sicher, weil männliche Tiere mit großer Zuverlässigkeit unfruchtbar gemacht werden können. Da chirurgische Eingriffe insbesondere unter Feldbedinauch immer Nachteile mit sich bringen, wird nach neuen günstigeren, schnelleren, weniger schmerzhaften und effizienteren Arten zur Kastration gesucht. Eine dieser Methoden ist die intratestikuläre Injektion von sterilisierenden Lösungen. Im Rahmen der Diplomarbeit wurde eine umfassende Literaturrecherche zur intratestikulären Injektion von Kochsalzlösung gemacht. Nach systematischer Selektion ergab die Literaturrecherche nur eine publizierte wissenschaftliche Arbeit zur Kastration männlicher Hunde mit Kochsalzlösung. In dieser wurden juvenile, bis zu sechs Monate alte Rüden und adulte, über sechs Monate alte Rüden, mit 20 % Kochsalzlösung intratestikulär kastriert. Gewünschte Effekte zeigten sich nur in der juvenilen Gruppe, in welcher sich Hodenatrophie, Parenchymnekrose und Hyperechogenität im Ultraschall zeigten. Da die Fallzahl jedoch sehr klein ist (zwei Gruppen mit jeweils sechs männlichen Hunden), es keine Kontrollgruppe gibt und generell noch wenig über diese Kastrationsmethode geforscht wurde, ist es notwendig weitere Versuche durchzuführen, um die richtige Applikationstechnik, Menge und Konzentration zu ermitteln.

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## 9. Tables and figures



## 10. Abbreviations

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| FDA | Food and Drug Administration |
| FSH | Follicle stimulating hormone |
| GnrH | Gonadotropin releasing hormone |
| ITI | Intratesticular/intraepididymal injection |
| LH | Luteinizing hormone |
| PICO | Population, intervention, comparators, outcome of interest |