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**CALCIUM CHLORIDE IN ALCOHOL NEUTER INJECTION;  
EVALUATION OF INTRATESTICULAR VS  
INTRAEPIDIDIMAL INJECTION  
IN STRAY OR OWNED DOGS**

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# ITALY - PUGLIA

## Italian

**law**(281/1991)

no kill policy

- Stray dogs are captured, neutered, dog shelters.

## Puglia

**law**(12/1995)

trap, neuter, and release  
(alternative to shelter).



# POPULATION

4 million  
Humans

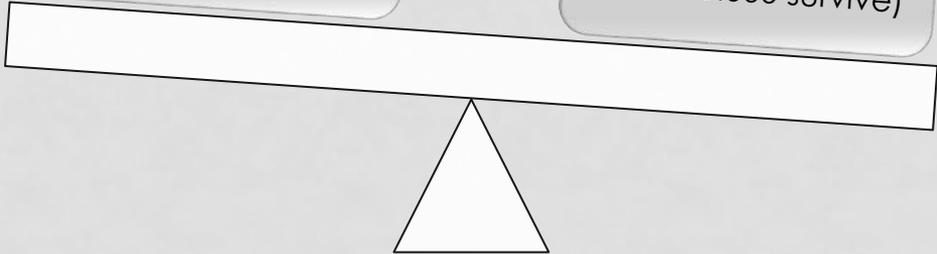
700.000  
Dogs

470.000 with owner  
(many free of  
roaming)

40.000 in 120 shelters  
(330 dogs each)

150.000 stray dogs  
(50.000 puppies/  
year: 15.000 survive)

Humans



NEWBORN/YEAR:

31.000  
Babies

vs

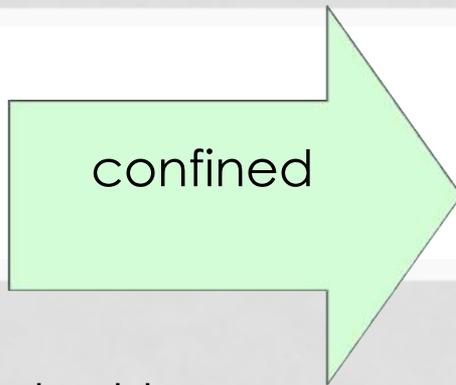
50.000  
Puppies

Every baby

2 puppies



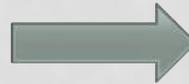
As long as the birth rates for dogs are so high, there will never be enough homes for all of them.



~~STRAY~~



It is important to **manage the dog population as a whole**, rather than just consider “strays” alone.



**46% of roaming dogs are actually owned dogs** allowed to roam and mate freely.



**Controlling reproduction of companion animals** to achieve effective population management is essential.

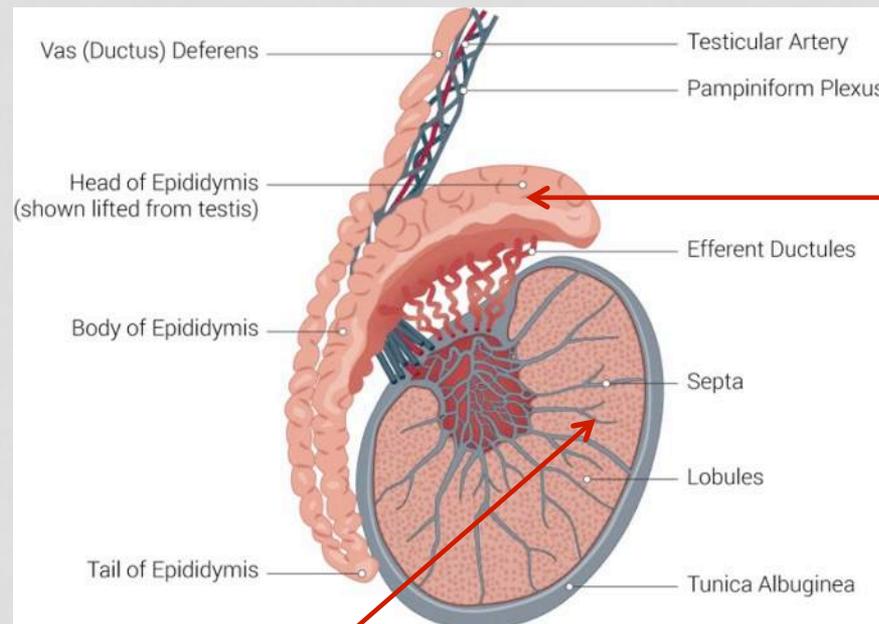


**Nonsurgical methods of sterilization** could be better accepted by reluctant owner and yield positive impacts on canine overpopulation.

# OUR RESEARCH



- INJECTION OF CALCIUM CHLORIDE INTRATESTICULAR VS INTRAEPIDIDIDIMAL
- BEST IN STRAY OR OWNED DOGS



Intraepididymal (head)

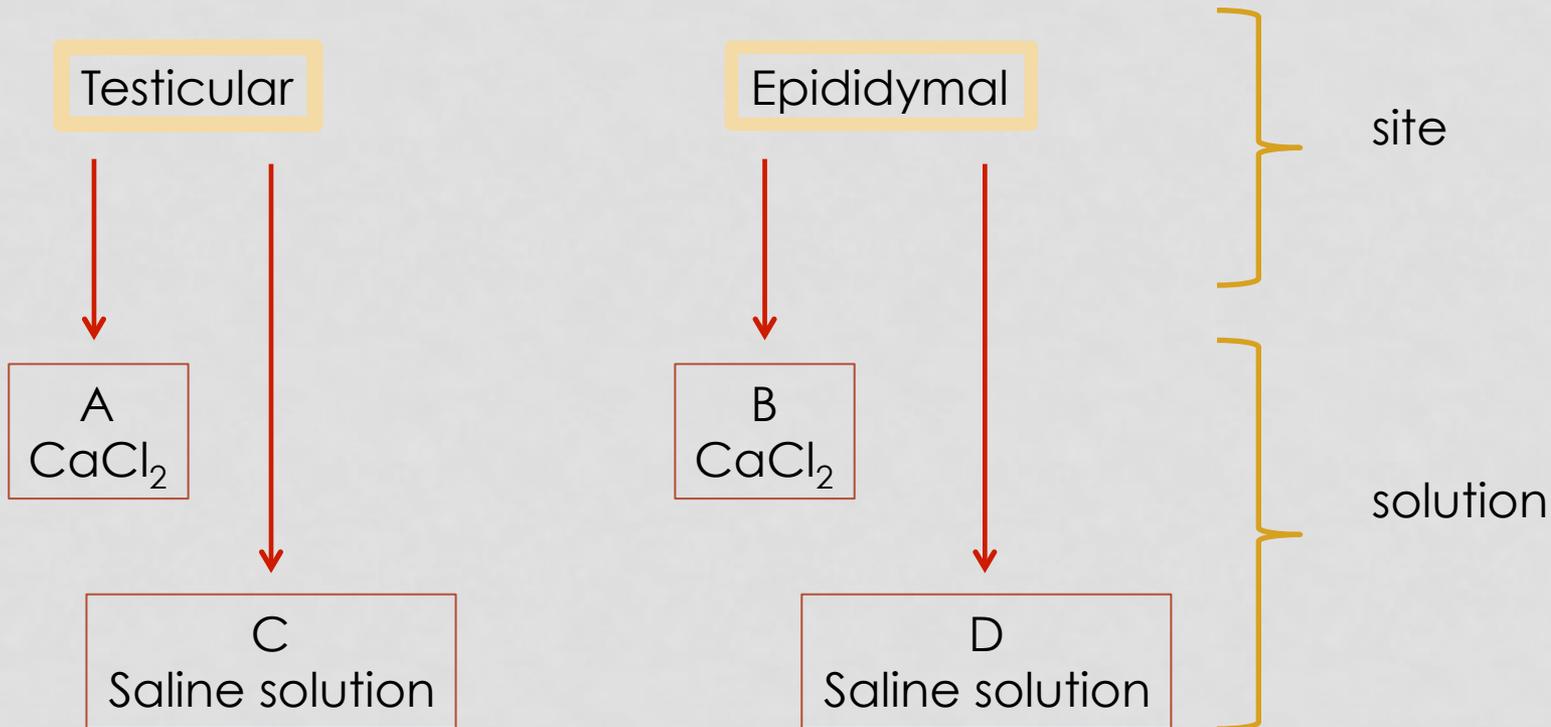
Intratesticular

# MATERIALS AND METHODS

**148 dogs**

4 groups (n=37)

crossbreed male  
18 - 26 months of age  
6 - 26 kg bw  
lightly sedated

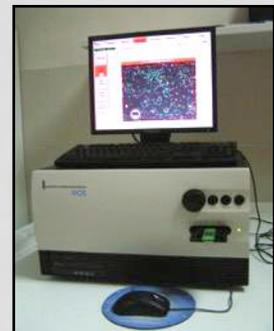


# MATERIALS AND METHODS

## Experimental protocol in brief

|                                 | $T_0$           | $T_3$          | $T_5$          | $T_6$          | $T_9$          |
|---------------------------------|-----------------|----------------|----------------|----------------|----------------|
|                                 | <i>Baseline</i> | <i>3 month</i> | <i>5 month</i> | <i>6 month</i> | <i>9 month</i> |
| <i>Injection</i>                | <u>X</u>        |                |                |                |                |
| <i>Testosterone</i>             | <u>X</u>        | <u>X</u>       |                | <u>X</u>       | <u>X</u>       |
| <i>Sperm analysis</i>           | <u>X</u>        | <u>X</u>       |                | <u>X</u>       | <u>X</u>       |
| <i>CEUS</i>                     | <u>X</u>        |                | <u>X</u>       |                |                |
| <i>Castration and histology</i> |                 |                |                |                | <u>X</u>       |

**9 months study**



# *FORMULA*

- 20 g of calcium chloride dihydrate powder brought to a final volume of 100 mL of 95% ethanol
- mixed, and sterilized in Falcon tubes.



# DOSE

## TESTICULAR

Group A - C

## EPIDIDYMAL

Group B - D

| Testicular Width | Dose per testicle |
|------------------|-------------------|
| 10-14 mm         | 0.25 ml           |
| 15-18 mm         | 0.5 ml            |
| 19-22 mm         | 0.8 ml            |
| 23 and above     | 1 ml              |

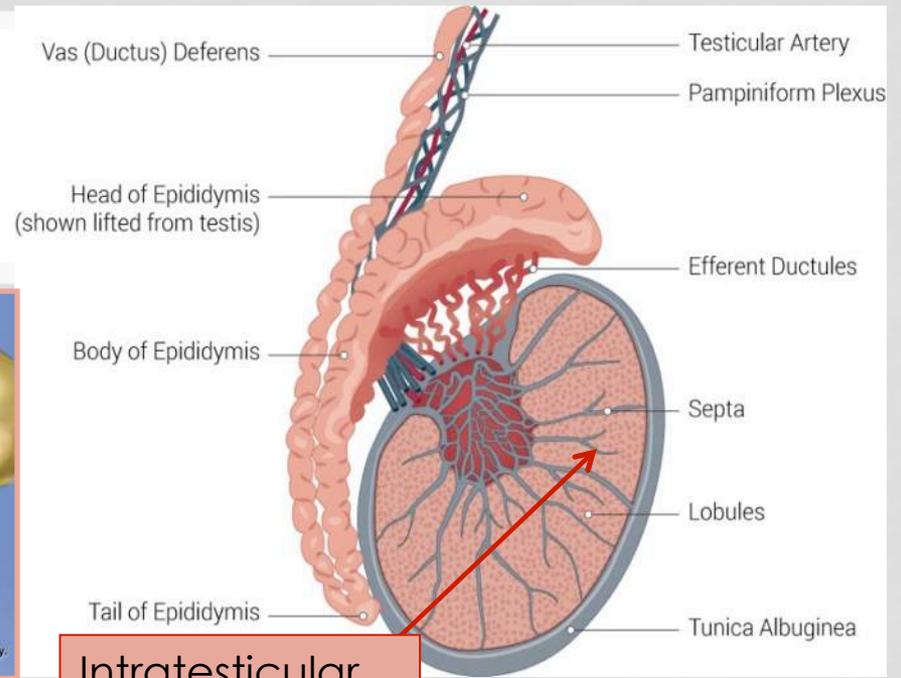
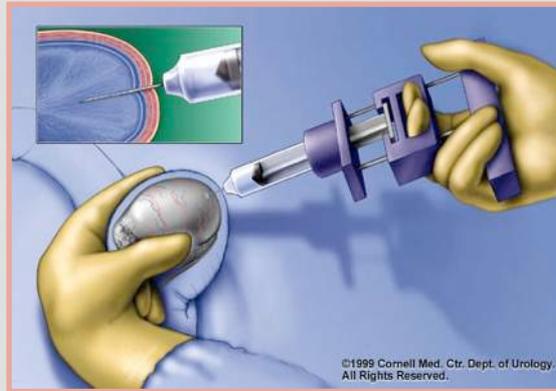
| Testicular Width | Dose per testicle |
|------------------|-------------------|
| 10-14 mm         | 0.06 ml           |
| 15-18 mm         | 0.12 ml           |
| 19-22 mm         | 0.2 ml            |
| 23 and above     | 0.25 ml           |

25% of the testicular dose

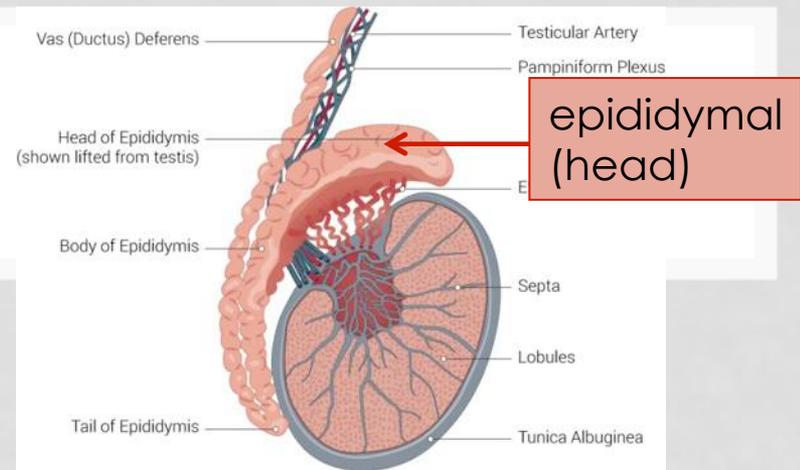
(epididymal volume is around 25% of the testicle)



# INTRATESTICULAR INJECTION



# EPIDIDYMAL INJECTION



- Ultrasound-guided
- Two operators

The epididymal head is ultrasonically located and injected percutaneously

# RESULTS



## **Routine clinical observation**

- All animals in the study tolerated the injections of  $\text{CaCl}_2$ .
- No adverse side effects
- At 3 months, the testes became atrophied in dogs in group A

|         | T0 vs T9   |             |
|---------|--|-------------|
|         | Baseline   | 9 months    |
| Group A | (*) <u>24 hours following injection</u> and continuing for the first 3-4 days a <b>slight increase in firmness</b> of testes on palpation<br>The increased firmness was slightly more than in other groups | Azoospermic |
| Group B | (*) the increase in firmness was focused on the epididymis.  | Azoospermic |
| Group C | (*)  | nd          |
| Group D | (*) the increase in firmness was focused on the epididymis.  | nd          |
|         |  |             |



- Semen evaluation (Computerized)

# RESULTS

| Injection   | Sperm concentration<br>(n. x 10 <sup>6</sup> /mL) | Total sperm<br>motility<br>(%) | Progressive sperm<br>motility<br>(%) | Serum testosterone<br>concentration<br>(ng/dL) |
|---|---|--------------------------------|--------------------------------------|--|
| CaCl <sub>2</sub> intratesticular<br>(group A)    | 167.4 vs 0*<br><hr/>                              | 95.1 vs 0*<br><hr/>            | 80.3 vs 0*<br><hr/>                  | 530.0 vs 174.4*<br><hr/>                       |
| CaCl <sub>2</sub><br>intraepididymal<br>(group B) | 384.4 vs 0*<br><hr/>                              | 94.0 vs 0*<br><hr/>            | 90.9 vs 0*<br><hr/>                  | 545.9 vs 557.8                                 |
| Saline control<br>intratesticular<br>(group C)    | 398.8 vs 403.8                                    | 93.5 vs 90.7                   | 89.4 vs 86.9                         | 551.2 vs 555.5                                 |
| Saline control<br>intraepididymal<br>(group D)    | 452.2 vs 470.0                                    | 93.0 vs 91.0                   | 88.7 vs 87.2                         | 575.6 vs 561.1                                 |

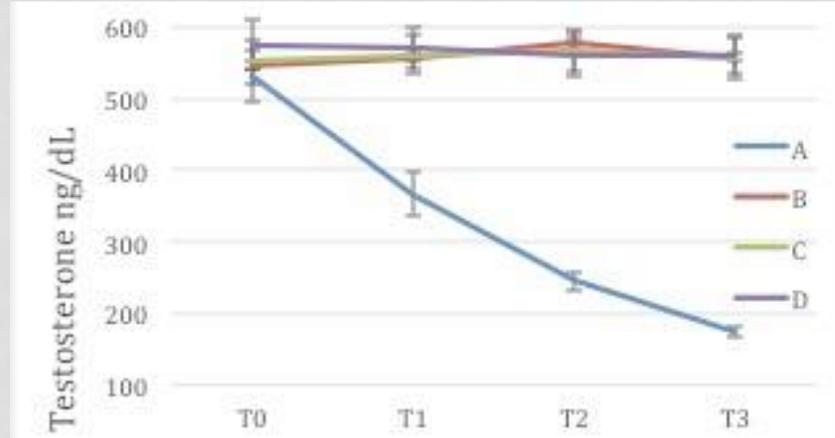
Table. Effects of intratesticular or intraepididymal injection of calcium chloride or saline (controls) on reproductive parameters. Data expressed in mean values within each group. T<sub>0</sub> vs T<sub>9</sub>. \*statistically relevant.

# RESULTS

## Testosterone/sexual behavior

INTRATESTICULAR (A): Testosterone drop.

A decrease in sexual behavior (i.e., loss of libido, mounting and dominance behavior) and aggression.



EPIDIDYMAL (B): no drop in the testosterone serum levels and no changes in sexual behavior (the same in control groups C and D).



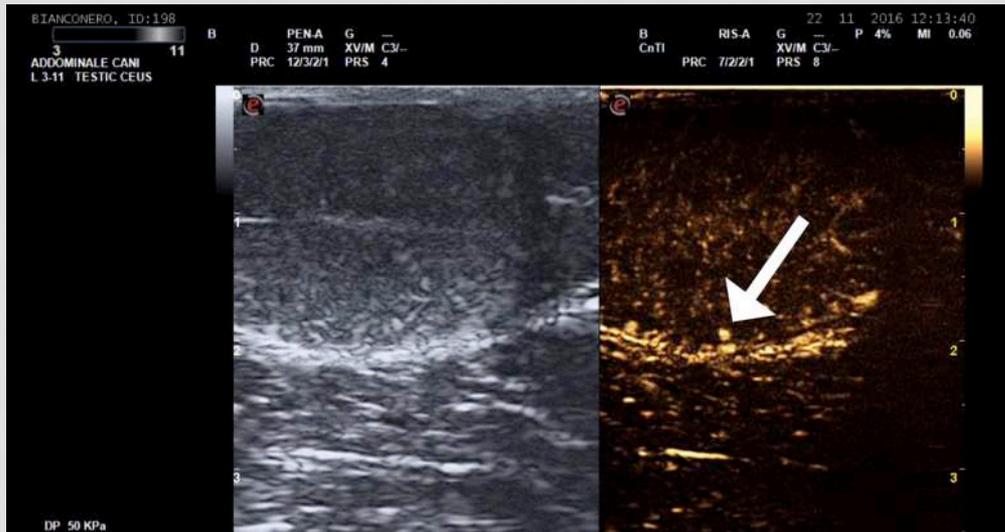
# CEUS

**Contrast Enhanced US** is ultrasound with contrast medium injected into the bloodstream, consisting of microbubbles

- It allows the echo-amplification of the structures under examination, in specific times for each organ
- Microbubbles reach the testicular parenchyma in about 15 seconds
- CEUS has been used to characterize testicular abnormalities and damage after  $\text{CaCl}_2$  injection.

# TESTICULAR INJ. AT CEUS

Baseline

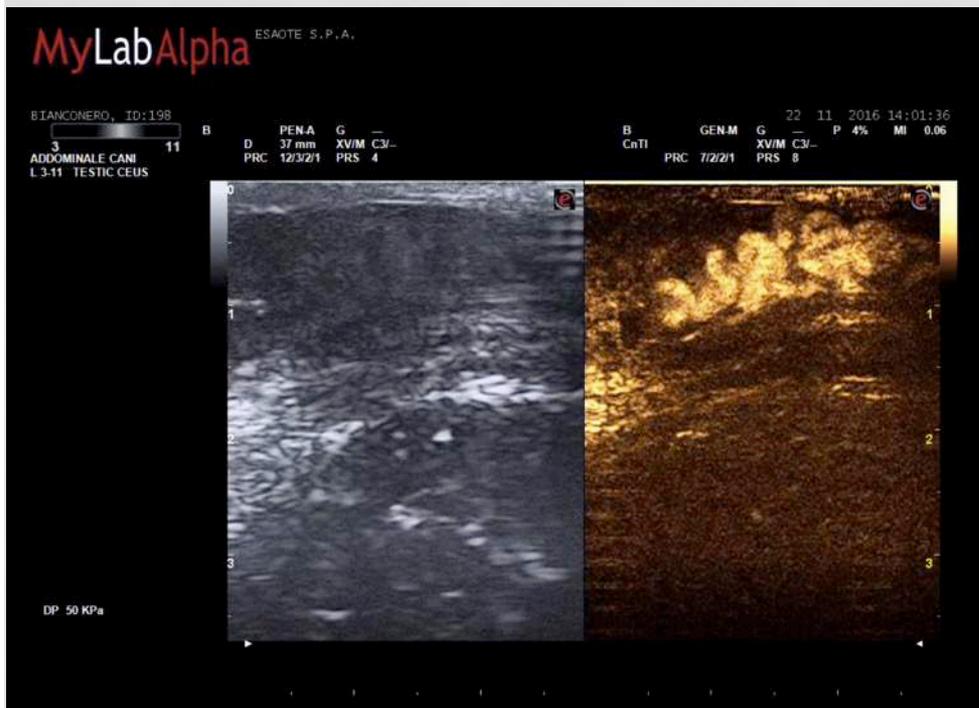


5 months later



# EPIDIDYMAL INJ. AT CEUS

Baseline



5 months later



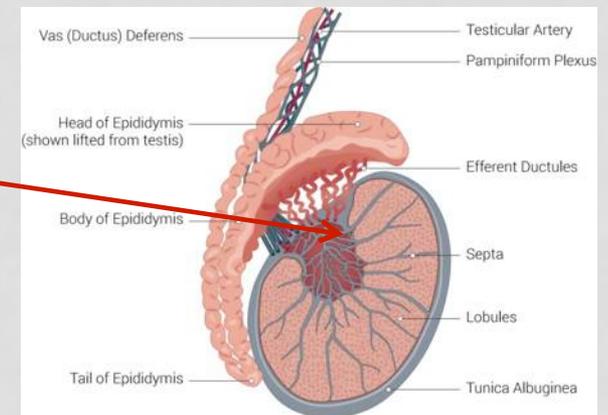
# HISTOLOGY

## EPIDIDYMAL

- no sperm cells
- Necrosis
- Damage in the area between the testis and epididymis

## TESTICULAR

- no sperm cells
- parenchymal degeneration



**PERMANENT DAMAGE**

# CONCLUSION

## **Injection in the epididymis**

- very challenging due to the small anatomical dimension and flexibility of structures. Better echographically guided injection.
- Sterility for at least 9 months (histological damage was permanent)
- no drop in the serum testosterone
- as time consuming as orchiectomy
- optimal for use in owned dogs where anatomical integrity and testosterone maintenance can induce reluctant owners to sterilize their animals.

## **Intratesticular injection**

- easily performed
- Sterility for at least 9 months (histological damage permanent) long-term efficacy
- reduced sexual behavior
- fulfilled the principal requirements for application to a population of stray canines

**The use of both methods together in stray and owned dogs may help fight the global problem of dog overpopulation.**

# ACKNOWLEDGMENTS



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Prof. Giulio Aiudi



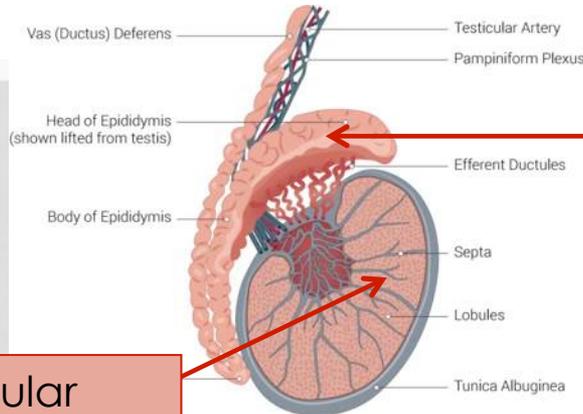
Joyce Briggs  
Valerie Benka

THANK YOU FOR YOUR ATTENTION





# WHY THE EPIDIDYMAL HEAD?



Intraepididymal  
(head)

Intratesticular

Sperm entering the caput epididymis are incomplete - they lack the ability to move forward (motility) and to fertilize an egg. It stores the sperm for 2–3 months while they mature. In the lower portion of the epididymis - the tail - the sperm are stored until they are transported to the ejaculatory duct during ejaculation

- Intratesticular injection results in decreased spermatogenesis
- intraepididymal (tail) injection **blocks sperm transport to deference tubules** but does not alter spermatogenesis.

Our goal was to inject the sclerosing/necrotizing agent right in the place where sperm are still immature. With the impairment of the epididymal head, **sperm will never become motile**; in the occurrence of regained ejaculation capacity, sperm would be unable to reach oocytes, leaving the dog infertile.