

Dr. RAFFAELLA LEOCI

DVM, PhD

University of Bari Aldo Moro (Italy)

Department of Veterinary Medicine

Section of Surgery and Obstetric

leocivet@yahoo.it

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

Raffaella Leoci, Giulio Aiudi, Giovanni Michele Lacalandra

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

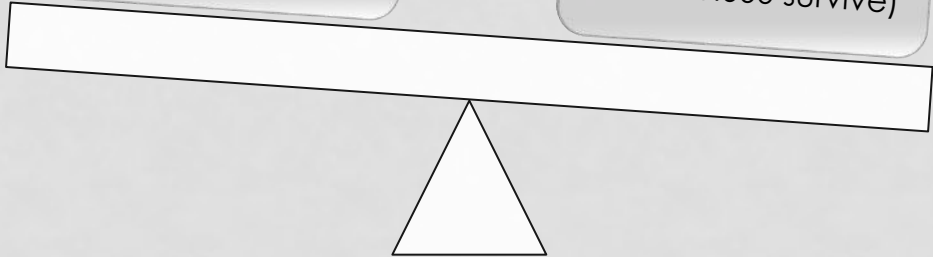
700.000
Dogs

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.



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.

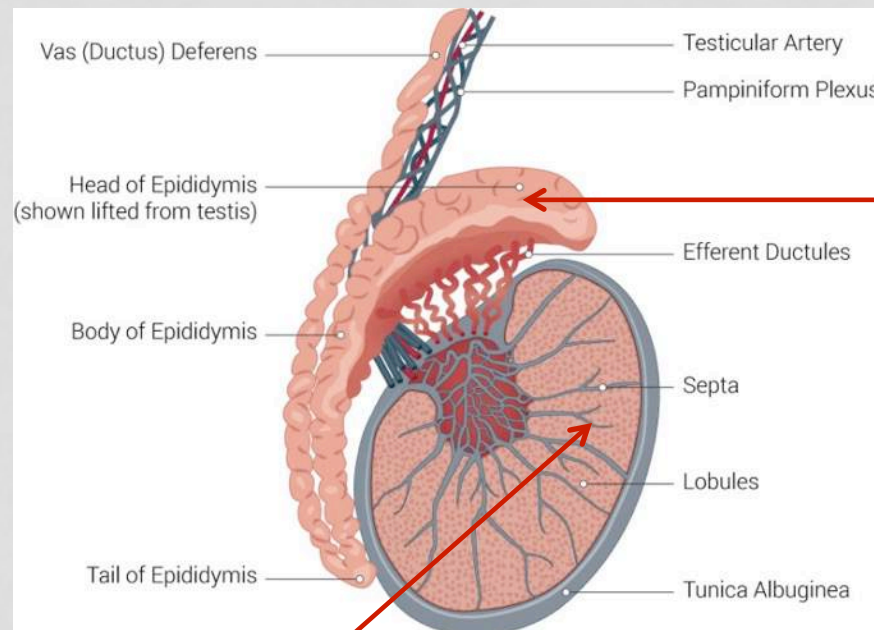
Controlling reproduction of companion animals to achieve effective population management is essential.

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)

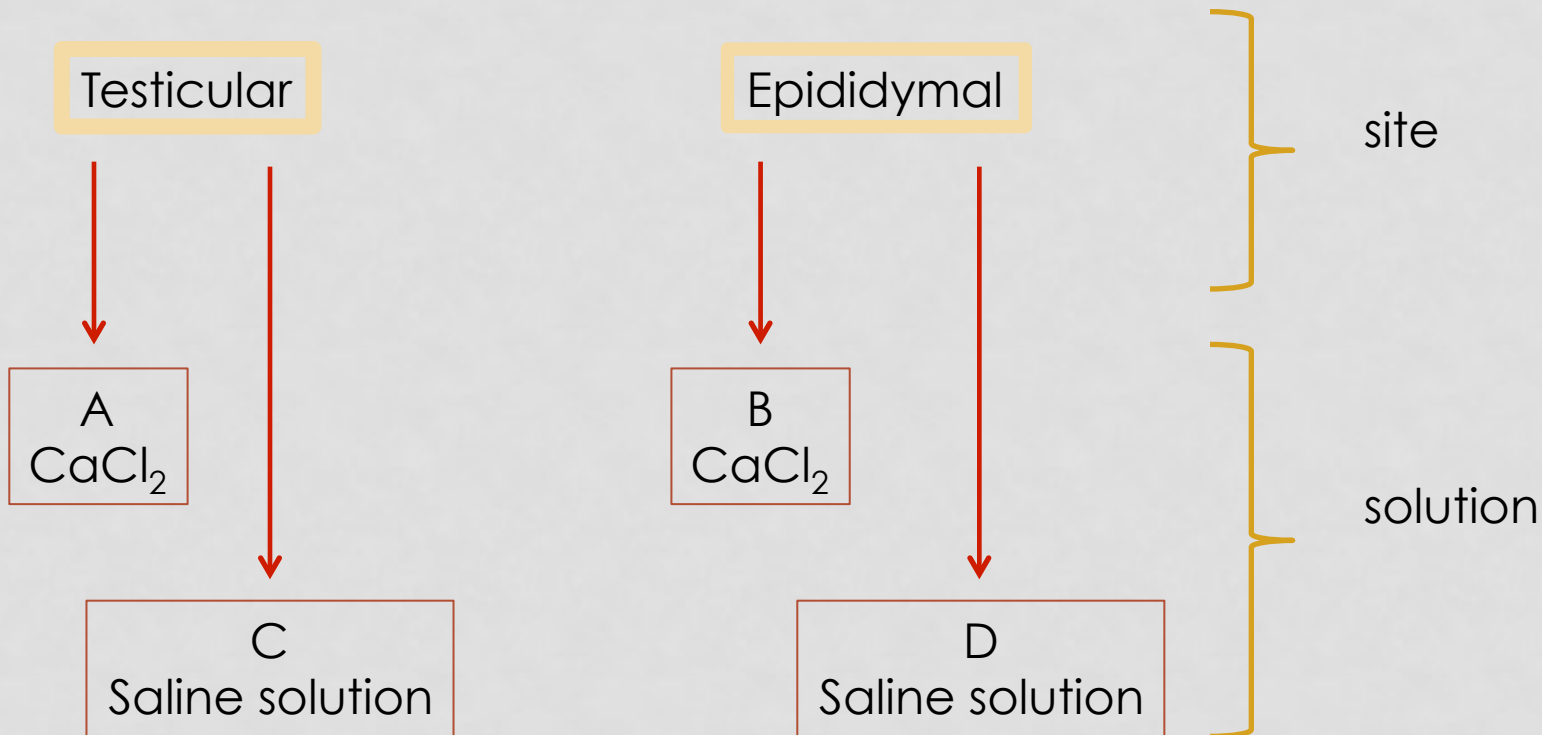
Intratesticular

MATERIALS AND METHODS

148 dogs

4 groups (n=37)

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

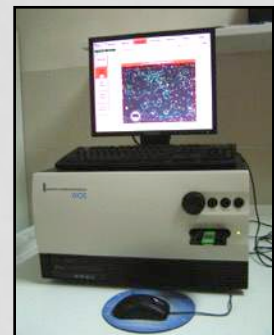


MATERIALS AND METHODS

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

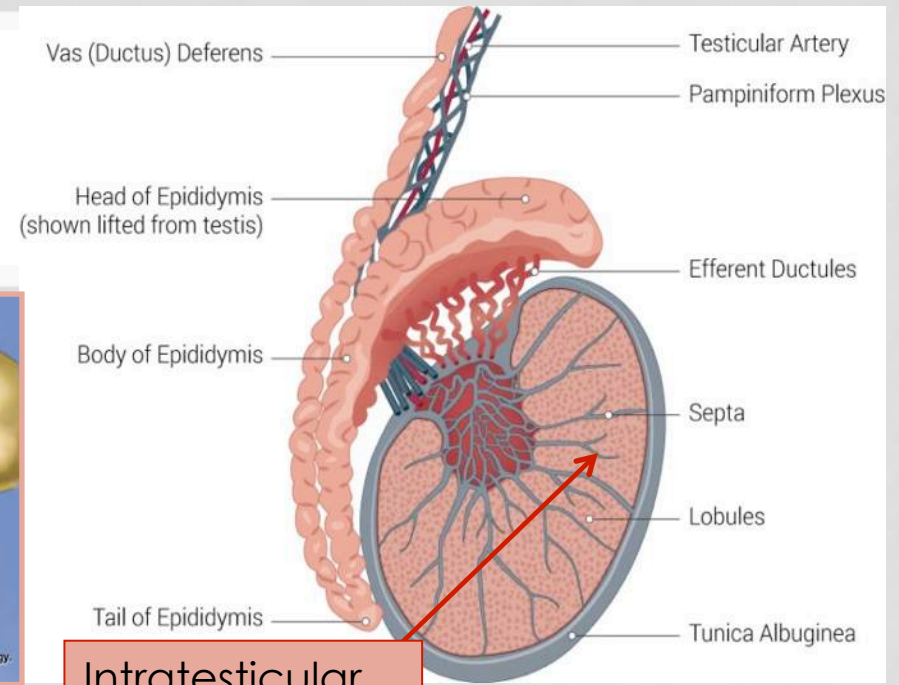
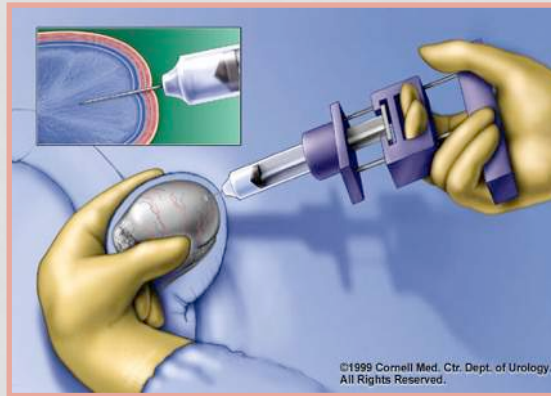
Testicular Width	Dose per testicle
10-14 mm	0.06 ml
15-18 mm	0.12 ml
19-22 mm	0.2 ml
23 and above	0.25 ml

25% of the testicular dose

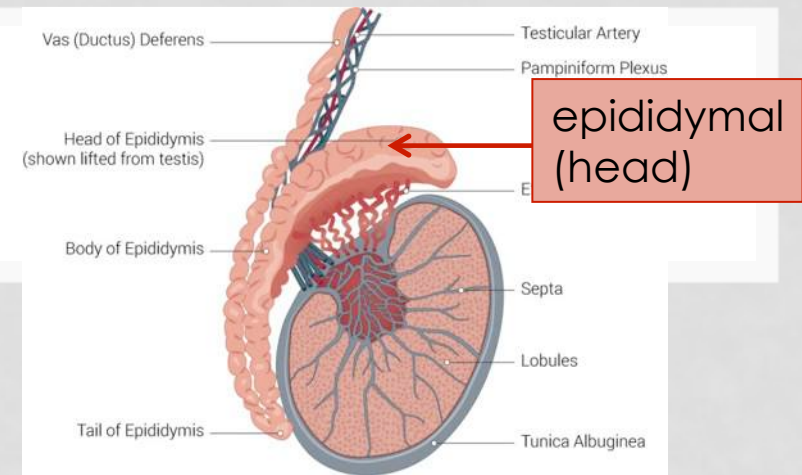
(epididymal volume is around 25% of the testicle)



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

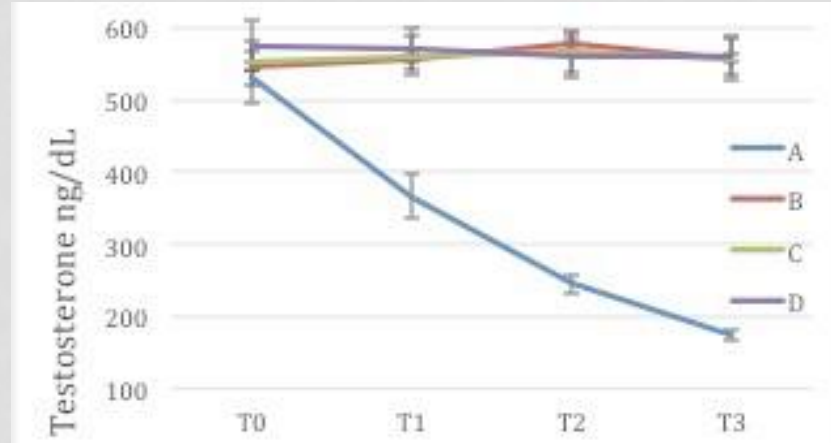
Table. Effects of intratesticular or intraepididymal injection of calcium chloride or saline (controls) on reproductive parameters. Data expressed in mean values within each group. T₀ vs T₉. *statistically relevant.

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).



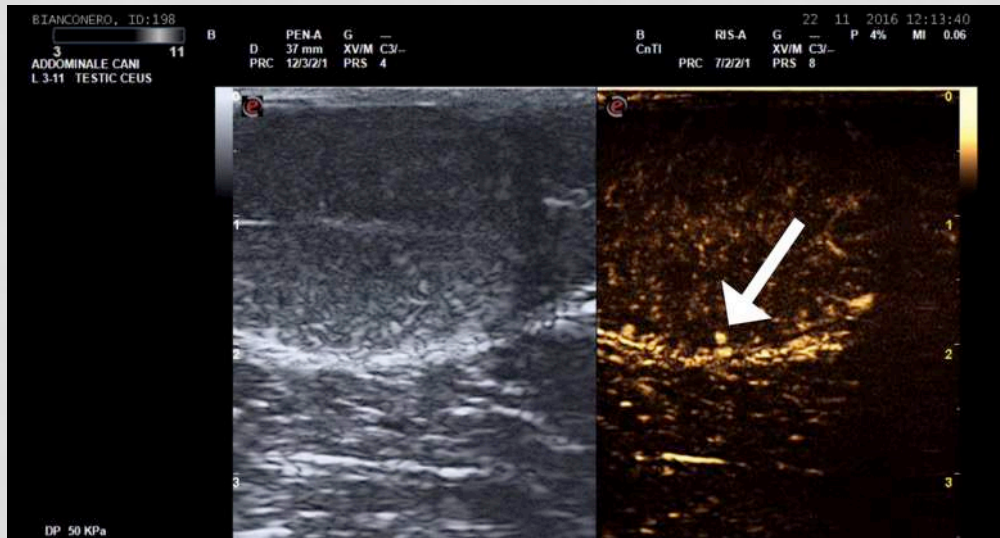
CEUS

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 CaCl_2 injection.

TESTICULAR INJ. AT CEUS

Baseline

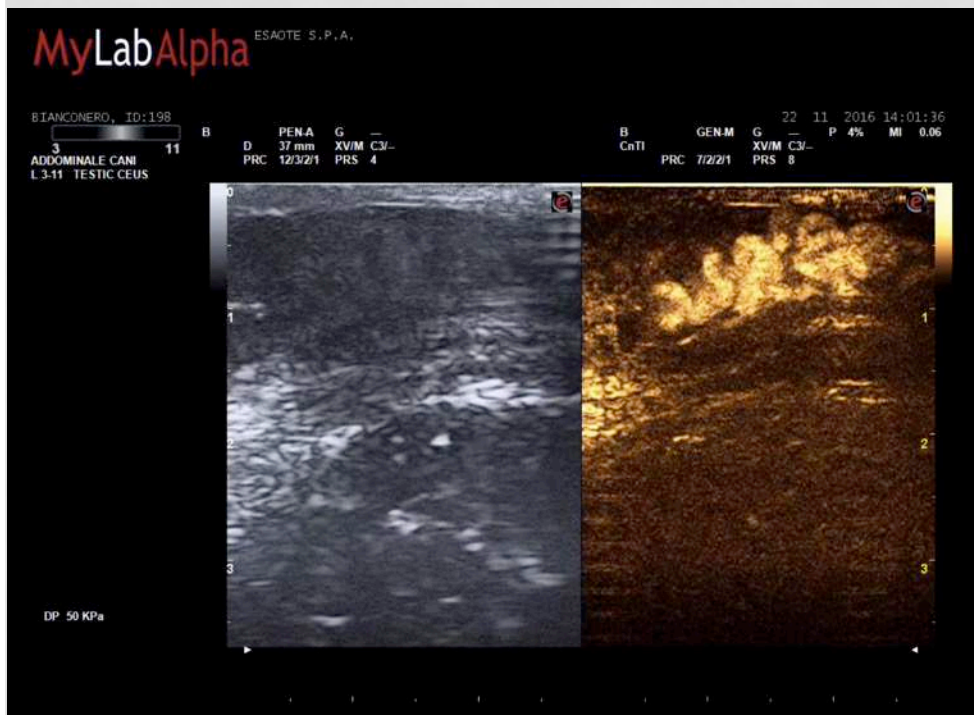


5 months later



EPIDIDYMAL INJ. AT CEUS

Baseline



5 months later



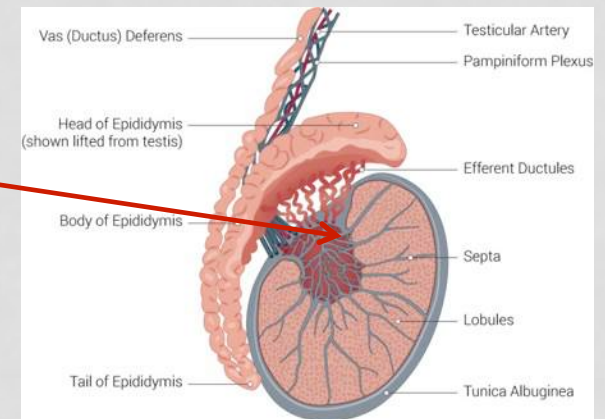
HISTOLOGY

EPIDIDYMAL

- no sperm cells
- Necrosys
- 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.

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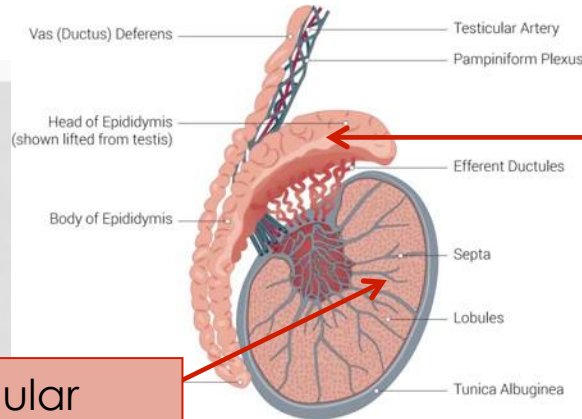
Joyce Briggs
Valerie Benka

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.