

# THERAPEUTIC ULTRASOUND AS A POTENTIAL MALE DOG CONTRACEPTIVE: COMPARISON OF TWO APPLICATION PROTOCOLS

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## INTRODUCTION

Ultrasound (US) was first reported as a male contraceptive in 1975 and has been applied to several species including dogs and humans<sup>1,2</sup>. US application is intended to create an environment not suitable for spermatogenesis. A more recent study recognized US to be a potential method to sterilize dogs non-invasively<sup>3,4</sup>. Standard treatment regimens have not been established, therefore, research needs to identify optimal conditions including the least number of applications necessary, the shortest time interval among applications, the best testis area to direct US treatment, optimal frequency and power, and so forth.

## THE STUDY - Used dogs as a model for humans to better approximate the contraceptive effects of US looking at two different treatment protocols.

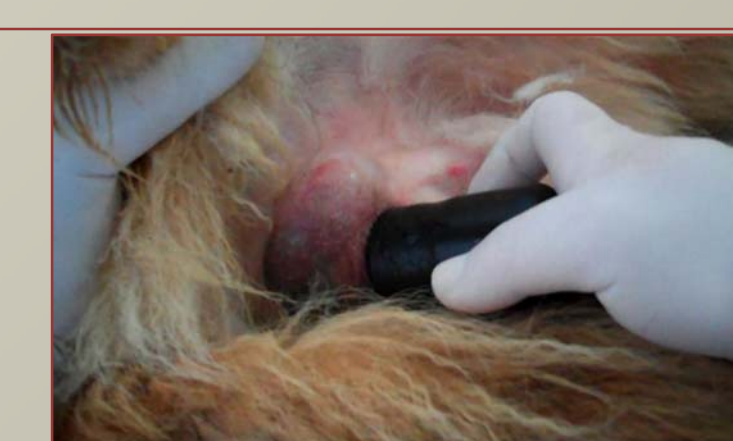
### MATERIALS AND METHODS

- ANIMALS** - 20 intact healthy male dogs of proven fertility
- TREATMENT** - All subjects were exposed to 1.5 watt/sq cm of US for 5 minutes per testicle on alternate days for one week (**Pictures 1-2**)
- Morphological and sperm evaluations were made before and 25 days after the end of treatments
- All dogs were castrated at day 40 and gonads were histologically examined
- Testicular volume data were statistically analyzed with "Wilcoxon matched pairs signed rank sum" test ( $p < 0.05$ ); semen evaluation was statistically analyzed with ANOVA test ( $p < 0.01$ )

- Group A (n=10)**: 1MHz all over the testicle
- Group B (n=10)**: 3MHz in the dorso-cranial area of the testis
- Length and testicular width were echographically measured to calculate the volume according to the formula for a prolate spheroid<sup>5</sup>:  $L \cdot W^2 \cdot 0.52$
- Semen collected was examined using an integrated visual optical system for semen analysis for sperm concentration and for percentage of total and progressively motile sperms (**Pictures 3-4**)



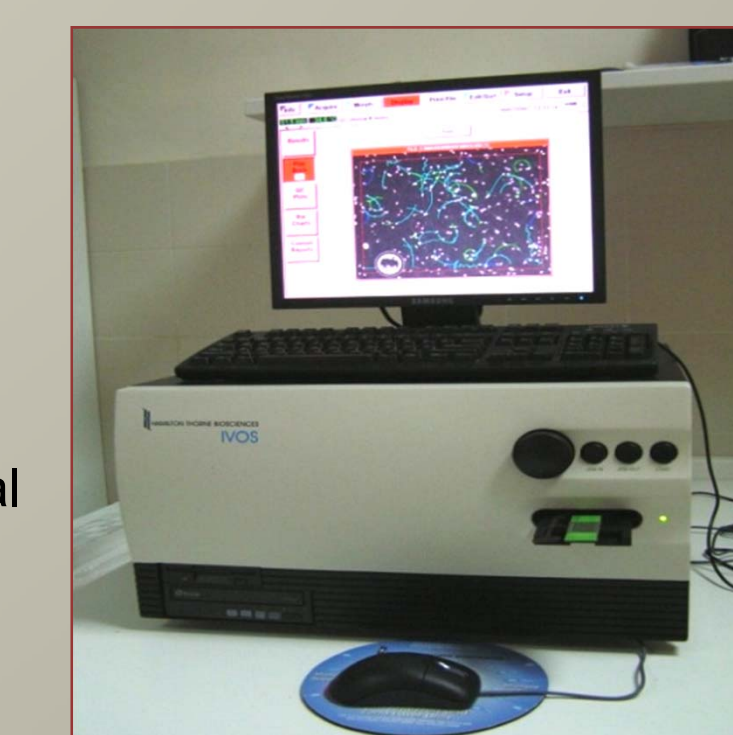
**Pic. 1** - Ultrasonic device: Vetri-son Clinic, Physiomed® Elektromedizin AG, Germany; 2.5 cm<sup>2</sup> transducer.



**Pic. 2** - Ultrasonic treatment



**Pic. 3** - Dog semen manual collection by latex cone.

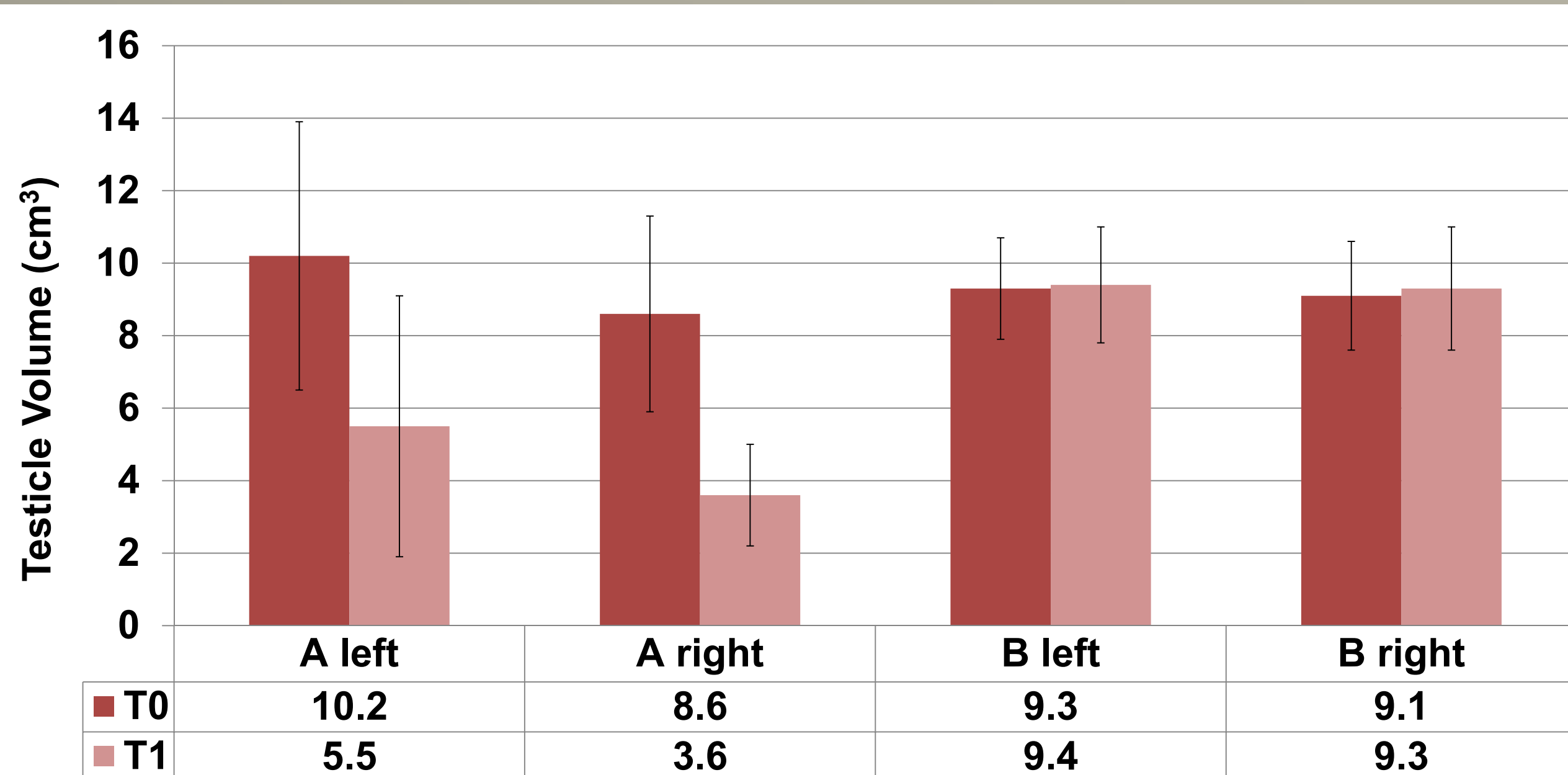


**Pic. 4** - Computer Assisted Semen Analysis (IVOS 12, Hamilton Thorn Biosciences, USA).

## RESULTS

- Group A exhibited marked tenderness to testicular palpation. No other remarkable local or systemic adverse effects were observed including pain or skin burns.

- Before the US treatment ( $T_0$ ), mean volume of ejaculates was  $12 \pm 4.5$  ml, sperm concentration was  $301.9 \pm 28.8 \times 10^6$ /ml with an average percentage of total and progressive motile sperms of  $87.2 \pm 5.5$  and  $58.4 \pm 7.7$ , respectively. After the US treatment ( $T_1$ ), a zero sperm count was noticed in A ( $p < 0.01$ ), and no variation in B group.



**Graph 1** - Testicular volume ( $T_0$  vs  $T_1$ ) of both testicles in both groups (mean values and standard deviation).

## SUMMARY AND CONCLUSION

After the US treatment, a zero sperm count was noticed in group A ( $p < 0.01$ ), and no variation in B group. These changes were confirmed by the histologic exam. Our results demonstrated that US at group A parameters (1MHz over the entire testes) leads to irreversible testis damage, while 3MHz in the dorso-cranial area of the testis is ineffective on dog fertility.

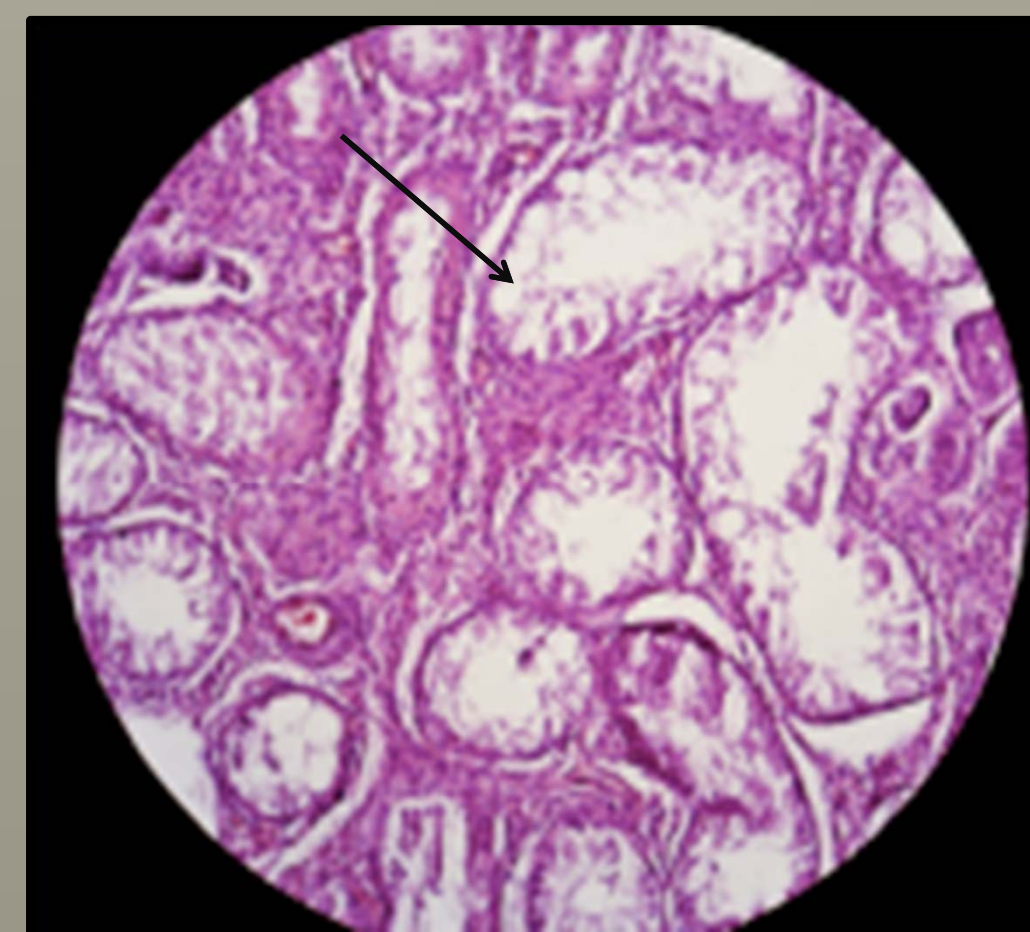
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Table 1	GROUP A	GROUP B
Palpation	Marked tenderness	Normal
Semen analysis	<b>AZOOSPERMIA</b>	Normospermia
Testicular Volume (Grap.1)	Left: $10.2 \pm 3.7$ vs $5.5 \pm 3.6$ /cm <sup>3</sup> * Right: $8.6 \pm 2.7$ vs $3.6 \pm 1.4$ /cm <sup>3</sup> *	Left: $9.3 \pm 1.4$ vs $9.4 \pm 1.6$ /cm <sup>3</sup> Right: $9.1 \pm 1.5$ vs $9.3 \pm 1.7$ /cm <sup>3</sup>
Histology	Interstitial fibrosis, widespread tubular atrophy and hyalinization of the basement membranes ( <b>Picture 5</b> )	No changes ( <b>Picture 6</b> )

**Table 1** - Summary of results. \* indicates values that are statistically different ( $p < 0.05$ )



**Pic. 5 (Left)** - Group A histology: interstitial fibrosis, widespread tubular atrophy and hyalinization of the basement membranes. Absence of sperm cells (arrow).



**Pic. 6 (Right)** - Group B histology: normal testicular tissue.