INTRODUCTION

Because of the loss of productivity and the personal and societal toll taken by surgery, surgical complications, and the cost of anesthesia, a great need continues to exist for nonsurgical methods of fertility control that are satisfactory, affordable, and consistently available to users. In particular, new methods for male contraception are needed because currently the only options for men are condoms (which have a high misuse rate and are subject to supply disruptions in low-income countries) and vasectomy (which is surgical and generally permanent). Both surgery and sterilization are psychological barriers for many men. We therefore seek to develop permanent, nonsurgical methods of contraception for males. Owing to their efficiency and cost-effectiveness, non-surgical, non-invasive methods are also sought for control of feral and street populations of animals such as dogs, cats, and monkeys.

Prof. M.S. Fahim at the University of Missouri demonstrated that brief applications of testicular ultrasound waves were effective at reducing or eliminating sperm in rats, cats, dogs, rabbits, monkeys, and man \(^{(1-5)}\). Prof. Fahim passed away in 1995 and this research was not further pursued. We wish to replicate Dr. Fahim’s work in monkeys to determine whether the effect can be confirmed in an independent laboratory with a method that could be applied in men.

METHODS

Animals: 4 adult rhesus (Macaca mulatta) males (6-15 yrs, BW 10-15 kg)
Testicle size measurements collected and volume calculated.

Ultrasound exposure: 2.5W/cm\(^2\) for 30m for all males, 3 times, every other day on a Monday, Wednesday, Friday schedule.

Two methods of Ultrasound Application:
1) Cup method (N=2): Animal’s scrotum positioned in the cup and sound waves emitted from the transducer at the base of the cup through a media solution of 3% NaCl before radiating the testes.
2) Direct method (N=2): Application of the probe directly to the scrotal surface.

Semen collection: Male monkeys are trained to chair restraint for electro-ejaculation, performed using defibrillation gel penile electrodes, with no anesthesia

Semen quality was evaluated a minimum of three times over two months prior to ultrasound exposure and weekly for 9 weeks following exposure.

- Evaluated for volume, sperm density, percentage of motile sperm, and total numbers of sperm according to the WHO '87 methods.
- Sperm morphology: washed sperm samples from each male were layered onto glass slides, dried, and stained using the method of Papanicolaou. Two hundred sperm per male per treatment were scored for various categories of abnormal forms according to Tollner et al. \(^{(7)}\).
- Computer assisted sperm analysis (CASA): Sperm were washed by centrifugation in modified BWW medium with 0.3% BSA (mBWW) and resuspended into mBWW for videomicrography as performed by Tollner et al. \(^{(8)}\).
- Motion characteristics of the recorded sperm were analyzed using the HTM Ceros, version 10.9d (Hamilton Thorne Biosciences, Inc.).
- At least 200 sperm per semen sample were analyzed for curvilinear velocity (VCL), straight-line velocity (VSL), and amplitude of lateral head displacement (ALH).

REFERENCE
1. TSC average across first five SEs following US treatment x 100.

The following trends were consistent across males following ultrasound treatment. Cup Method...
- Suppresses total sperm count for 7-8 weeks with a maximal average inhibition of sperm numbers of 93%.
- Results in transient reduction of % motility.
- Reduces both VCL and ALH, measures of sperm vigor.

Both Methods...
- Increase the number of sperm with tail defects
- Reduce sample-to-sample variability in the total number of normally shaped sperm.
- Result in a recovery in semen quality in weeks to months following ultrasound treatment.
- Produce no detectable undesirable side effects.

DISCUSSION

The cup method, a modification of methods used successfully in rats and dogs to suppress spermatogenesis, results in reduction of semen quality in adult rhesus monkeys. Unlike in other animal models, treatment did not induce azoospermia, yet the numbers of sperm that were vigorously motile and with normal morphology were exceedingly low. Within exposure method, ultrasound treatment appeared to be most effective for males with smaller testes suggesting that higher levels of exposure may be required to achieve contraception in individuals and animals with greater testicular mass.

CONCLUSION

We confirm in a non-human primate species with testicles that compare in size with those of men that ultrasound treatment results in reduced sperm numbers and quality. This preliminary study provides a proof of principle that testicular ultrasound exposure has the potential to be a viable approach for contraception in humans.

Acknowledgements:
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Table 1

<table>
<thead>
<tr>
<th>Animal</th>
<th>Ave. Testicle Volume (mm(^3))</th>
<th>% Inhibition* of TSC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cup</td>
<td>1</td>
<td>2.0 x 10^5</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>3.0 x 10^5</td>
</tr>
<tr>
<td>Direct</td>
<td>3</td>
<td>2.9 x 10^5</td>
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<tr>
<td></td>
<td>4</td>
<td>1.4 x 10^5</td>
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References