

A novel non-surgical method to reduce male fertility using the rat as a model

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Reducing or eliminating fertility in male domestic animals is important for food animal production, and control of wild or feral animal populations. Although surgical gonadectomies, immunocontraception vaccines, and chemical castrations are available, these procedures are not without problems including, costs, training, equipment, efficacy, and morbidity. Our laboratory is developing a non-surgical method of sterilization using an antibody-guided, lipid-nanoparticle carrying a cytotoxin that could potentially address many of these issues.

The goal of this study was to evaluate the effects of this non-surgical sterilization method on male rat fertility as evidenced by changes in testicular architecture and sperm development 30 days after administration of the nanocomplex. We hypothesized that a reduction in fertility could be accomplished by targeting the anti-Mullerian hormone receptor II (AMHR II), which is expressed primarily in the Sertoli/Leydig support cells of the testes. PEGylated lipids were used to form stable nanocomplexes tagged with a commercially available AMHR II antibody (Sigma Aldrich, St. Louis, MO) and loaded with the cytotoxin, saporin (Sigma Aldrich). If effective, injection of this nanocomplex would disrupt testicular architecture and reduce sperm development.

Eight adult male Sprague-Dawley rats were divided into two groups. Experimental animals were injected with 8.2 nmol IV (tail vein) of the nanocomplex in 0.1 ml of sterile saline followed by a 0.1 ml sterile saline flush. The controls received two 0.1 ml IV doses of sterile saline. After injections, animals were housed for 30 days, and weighed twice weekly. At the end of the experiment, all animals were sacrificed by exposure to CO₂. Left and right testes were collected and weighed. The epididymides were removed and placed in sterile saline. Epididymal sperm were evaluated for concentration and motility using light microscopy. Testes were preserved in 10% formaldehyde for histological processing. Paraffin embedded gonadal tissue from the central axis of each testis was sliced (5 µm sections), affixed to slides, and stained with hematoxylin and eosin to evaluate gonadal architecture.

There was no significant difference between control and experimental animals in weight gain, or testicular/epididymal weights. There was an observable difference in epididymal sperm samples between the experimental and control males. All experimental males had many fewer sperm than controls, and the sperm exhibited limited motility. To evaluate testicular architecture, 10 random seminiferous tubules were assessed from both the left and right testes (20 total) of each animal. Tubules were designated as either normal or abnormal. Criteria for designation as abnormal included atypical luminal contents (no sperm or undeveloped sperm, acellular material, immature germ cells), disruption of normal developmental patterns of spermatogenesis, and lack of Sertoli cells leading to random mixing of developing germ cell types. There were no differences between the left and right testes within a group, thus these data were combined. There was a statistical difference in normal versus abnormal tubules between the control animals (normal = 78 out of 80 tubules) and experimental animals (normal = 24 out of 80 tubules; chi-square test, $p < 0.001$). Further histological analyses are being performed.

This study demonstrates that 30 days after injection with the nanocomplex, fertility of male rats was reduced as evidenced by decreased sperm production, and disruption of testicular architecture. Future work will investigate the effects of increased doses, and different injection methods, as well as the effect of this non-surgical sterilization technique in other species.

Keywords: Sterilization, non-surgical, anti-Mullerian hormone, nanoparticles, nanocomplex