

Gene Therapy Delivery of Anti-Müllerian Hormone in Kittens Induces Long-term Contraception Without Impairing Puberty

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The uncontrolled reproduction of free-roaming domestic cats is the major driver of stray and feral cat overpopulation. Without proper access to veterinary care and with the inherent risks of living outdoors, these unowned felines typically experience a shortened lifespan. The most recent estimates indicate that feral cats have contributed to 13.9% of worldwide insular bird, mammal, and reptile extinctions. Surgical sterilization of females is a lengthy procedure requiring highly trained personnel. For this reason and because of logistics and economic constraints, the current Trap-Neuter-Return approach cannot scale to control the reproduction of the hundreds of millions of stray and feral cats worldwide. We recently reported long-term contraception in adult female cats receiving an intramuscular injection of an anti-Müllerian hormone (AMH) transgene delivered with an adeno-associated viral vector. The objective of this study was to evaluate the safety and efficacy of this novel non-surgical contraceptive, termed AAV9-fcMISv2, when administered to prepubertal male (M) and female (F) domestic cats. We injected 2-3 months old kittens with either 5×10^{12} (1M, 4F) or 1×10^{13} (1M, 3F) viral genomes per kilogram of body weight (vg/kg) of AAV9-fcMISv2 or 5×10^{12} empty AAV9 particles per kg (vp/kg) (1M, 2F). Injection site monitoring, regular physical examinations, and hematologic and biochemical analyses did not reveal any sustained abnormalities. Supraphysiological (>100X basal) levels of AMH persisted throughout the 16-month-long study. Importantly, every monthly AMH reading of a treated kitten was higher than the lowest reading that induced contraception in our previous adult study. The average adult body weight of treated females (3.07 kg) was similar to that of controls (2.76 kg). In males, testosterone production and spermatogenesis were unaltered indicating that testicular function is unaffected by supraphysiological AMH. Monthly ultrasonographic measurements revealed that the prepubertal injection of AAV9-fcMISv2 impacted uterine growth as treated cats had a significantly lower average uterine cross-section area compared to controls (4.4 and 7.8 cm², respectively. $P < 0.0001$). Average fecal excretion of estrogen metabolites was lower in females from the higher dose group when compared to controls (398.4 and 523.5 ng/g of feces, respectively. $P < 0.0001$). Compared to controls, serum LH levels were higher in females from both the lower and higher dose groups (5.27, 7.23 and 7.68 ng/ml, respectively. $P = 0.0494$ and $P = 0.0134$). All but one treated female bred repeatedly during a mating trial that spanned the last four months of the study. Although the six females that bred did it significantly more often than controls (bred on 48.2 and 18.5% of days in trial, respectively. $P = 0.0286$), none of them achieved clinical pregnancy while both controls did. They presented an altered estrous behavior with

multiple occurrences of single-day breeding and prolonged breeding bouts lasting >20 days, while every breeding bout in controls lasted 7-10 days. Consecutive days of steady breeding in treated females never resulted in a luteal phase indicating that ovulation was impaired. Together, health, hormonal, behavioral, and fertility data indicate that the vectored delivery of an AMH transgene prevented ovulation and luteal phases and is a safe and effective contraceptive strategy in prepubertal female domestic cats.

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