GENE THERAPY WITH AAV9 DELIVERY OF AN MIS TRANSGENE INHIBITS ESTRUS IN FEMALE CATS

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Mullerian Inhibiting Substance (MIS) is a TGF-beta superfamily ligand secreted by granulosa cells which inhibits the activation of primordial follicles. Gene therapy using an adeno-associated virus (AAV9) vector to express superphysiological levels of MIS is sufficient to induce lifetime contraception in female mice with no detectable adverse effects. To evaluate the contraceptive potential of exogenous MIS in cats, we engineered a novel MIS transgene. This new construct included 76% of the known cat peptide sequence, with the remaining 24% gap in the cat genome reconstructed by *carnivora* homology and additional sequencing. The resulting chimeric MIS transgene was confirmed to be biologically active in 1) the rat Mullerian duct regression bioassay, and following incorporation into an AAV9 vector, shown to be competent for 2) in-vivo expression and 3) ovarian inhibition in mice.

Three female cats were injected with 5E12 particles/kg in the caudal thigh muscles. Cats remained healthy during the study with no meaningful alterations in blood parameters (CBC/biochemistry), nor any adverse injection site reactions; blood and fecal samples were collected prior to and following treatment to monitor transgene levels, and reproductive hormones. All cats had detectable endogenous MIS prior to treatment (8.7ng/ml +/- 4.7). Following gene therapy, MIS levels peaked at one week (9545ng/ml +/- 183), and then slowly decreased to a variable range (465ng/ml +/- 429) with one cat returning to basal levels at 9 months post-injection.

Ovarian activity was monitored via fecal estradiol (E2) and progesterone (P4) metabolites, and daily assessment of behavioral estrual signs. Fecal samples were collected (3X/week) from 6 months prior to treatment (pre) until 9 months post-treatment. All three cats demonstrated a transient period of suppressed (sup) ovarian activity ~2 months post-injection (mean, 56 days; range, 50-66 days), in which no estrus peaks occurred and E2 levels were significantly repressed ($P < 0.01$) compared to pre-injection levels (E2-sup: 55.7ng/g dried feces, E2-pre:114.2). Duration of ovarian suppression averaged 58.3 days (range, 51-64) before all cats resumed cyclicity (E2-post: 96.0ng/g).

In conclusion, administration of AAV9-MIS intramuscularly in cats generated circulating MIS levels in the contraceptive range, and temporarily suppressed feline estrus. We have identified immune reaction to the synthetic transgene as a possible barrier to long-term contraception, which is explained by 30 amino-acid differences with the latest genome build (9.0). Durable contraception may be achievable with second-generation vectors that match the cat sequence

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