Vectored Contraception: Targets, Methods, and Delivery

Alliance for Contraception in Cats and Dogs

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Coming full circle: dogs essential to gene therapy in humans

Efficacy assay: spinning pups!

Lancelot was just beginning to see, after gene therapy, when Corey was born.
Myotubular myopathy (X-linked)

Alison, Paul, and Joshua Frase

Nibs carries a natural mutation (“wasting puppy syndrome”) and founded the dog dynasty that led to gene therapy, now effective in children.

Nibs begat Rocky, the first surviving male, which led to affected females and doubled the speed of breeding dogs.
"Vectored contraception" targets

- Pet
- Farm
- Zoo
- Wild
The Central Dogma of Molecular Biology

Base pairing is critical! A with T  G with C
Types of Targets

- Peptide/protein/glycoprotein hormones
- Enzymes to make steroid hormones
- Molecules to help sperm mature and swim
- Receptors (sperm binding)
- Maternal-embryo interaction for implantation of fertilized ovum
Targets

- protein
- peptide
- glycoproteins
- steroids

Diagram:
- Kisspeptin/NKB
  - Hypothalamus
    - GnRH
    - Anti-GnRH
      - Pituitary
        - LH
        - FSH
          - Gonads
            - Spermatogenesis
              - Sperm
                - Sperm antigen: intrinsic
                - Sperm antigen: epididymis
                - Seminal fluid component
            - Oogenesis
              - Oocyte
                - Oocyte sperm receptors
                - Zona pellucida
                - Reproductive tract: uterus, fallopian tubes
              - Progesterone
              - Estrogen
              - Anti-ZP2
            - Fertilization
            - Implantation/Embryogenesis
Glycoproteins (not cells)
Species-specific
Sperm bind to receptors
Why target DNA?

• Conventional vaccines have variable effects and may require boosters
• RNAs are transient
• DNA change persists as cells divide
Methods

*Gene therapy:* adds a gene

*Gene silencing:* prevents expression (making protein)

*Gene editing:* adds, removes, or replaces gene
Gene therapy only adds DNA

Gene editing (CRISPR) adds, replaces, or deletes DNA

1rst gene therapy clinical trial: 1990

1rst gene therapy FDA approvals: 2017
DELIVERY

adenovirus (AV)
adeno-associated virus (AAV)***

Gene Therapy

retroviruses
lentivirus (HIV)

Challenges:
• Enter right cells
• Enter nucleus of those cells
• Gene transcribed + translated into protein
• Avoid immune response, nucleases

Concerns:
• Capacity
• Tropism (homing)
• Integration or episome?
AAV: leader, but capacity ~5,000 bases

AAV2 to muscle and liver
AAV6 to airways
AAV8 to liver
AAV 1 + 5 to blood vessels

All go to the brain.
Christian Guardino on America’s Got Talent

Luxturna: FDA approved 12/18/17 to treat “vision loss due to biallelic RPE65 mutation-associated retinal dystrophy”
Hannah Sames: giant axonal neuropathy

Gene therapy July 2016
Eliza O’Neill  Sanfilippo syndrome  (mucopolysaccharidosis type IIIA)

Treated May 2016

These kids all got a protein that their bodies couldn’t make.
Vectored contraception has a different goal than gene therapy:

*Blocking a natural function, not providing a missing protein*
Instead of MAbs, AAV introduces instructions to make anti-GnRH antibodies (IM injections lead to atrophied gonads)

Anti-mullerian protein intraperitoneal injection blocks follicle maturation

Instead of MAbs, AAV introduces instructions to make antibodies continuously; ragged eggs
DELIVERY: CRISPR

A borrowed bacterial defense against bacteria that uses short RNAs to target specific genes, then deploys DNA-cutting enzymes to remove, replace, or add a specific DNA sequence.

Limitation: off-target effects, causing cancer

Older methods: zinc finger nucleases and TALENs
Clustered Regularly Interspaced Short Palindromic Repeats = DNA velcro
Gene Silencing

- Antisense RNAs bind to and block mRNA
- Synthetic RNAs (morpholinos) (25 bases + organic group) to treat DMD blocks splice site
- RNA interference (RNAi): short double-stranded RNAs (siRNAs)
- MicroRNAs (21-22 bases) bind mRNAs, block translation into protein
- Monoclonal antibodies (Mabs) bind proteins, such as hormone receptors

Current work on delivery of vectors (siRNAs to cats)