Vectored Contraception: Targets, Methods, and Delivery

Juice, Artie, and Romeo (FIV)

Alliance for Contraception in Cats and Dogs

Boston, July 23, 2018

Ricki Lewis www.rickilewis.com @rickilewis

Coming full circle: dogs essential to gene therapy in humans

Nature Genetics 33:119 (Feb 2003)

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

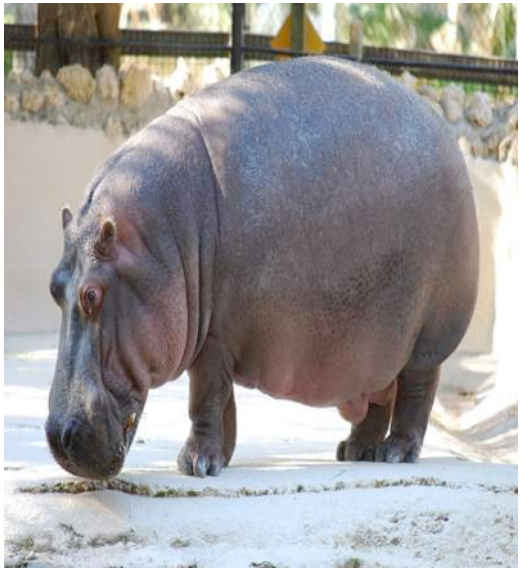
Nibs carries a natural mutation ("wasting puppy syndrome") and founded the dog dynasty that led to gene therapy, now effective in children.

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

"Vectored contraception" targets

pet

farm



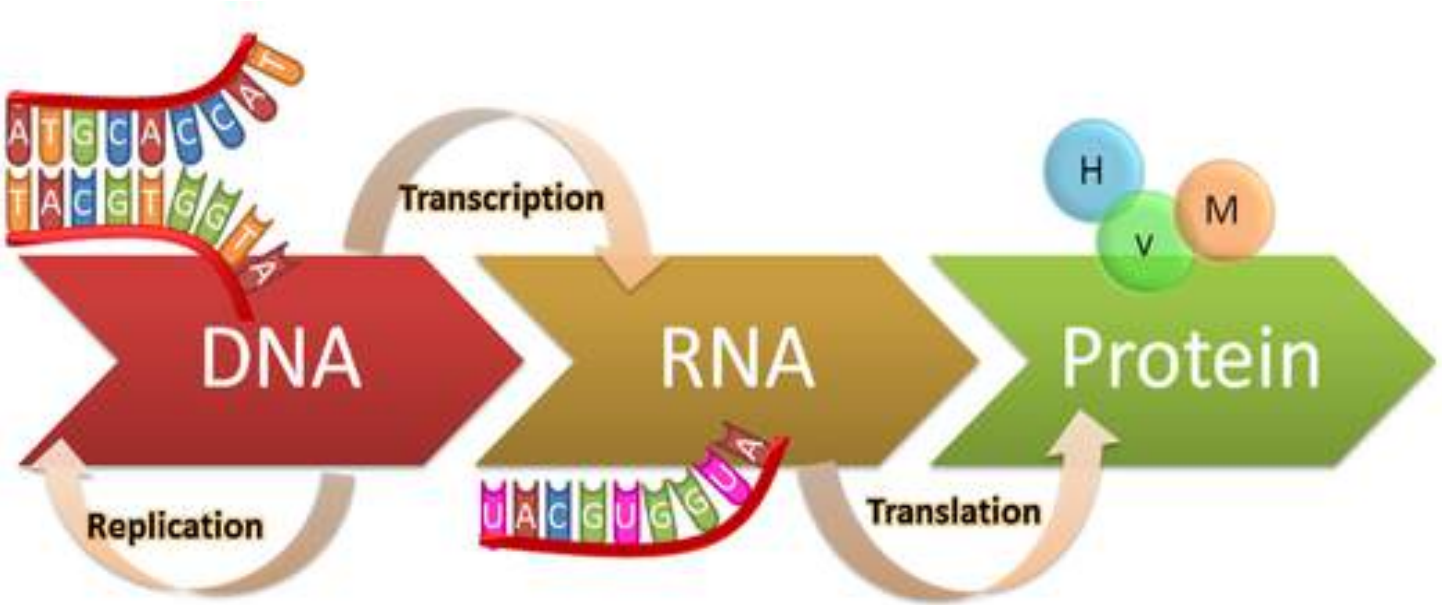
zoo

wild

The Central

Of Molecular Biology

Dogma

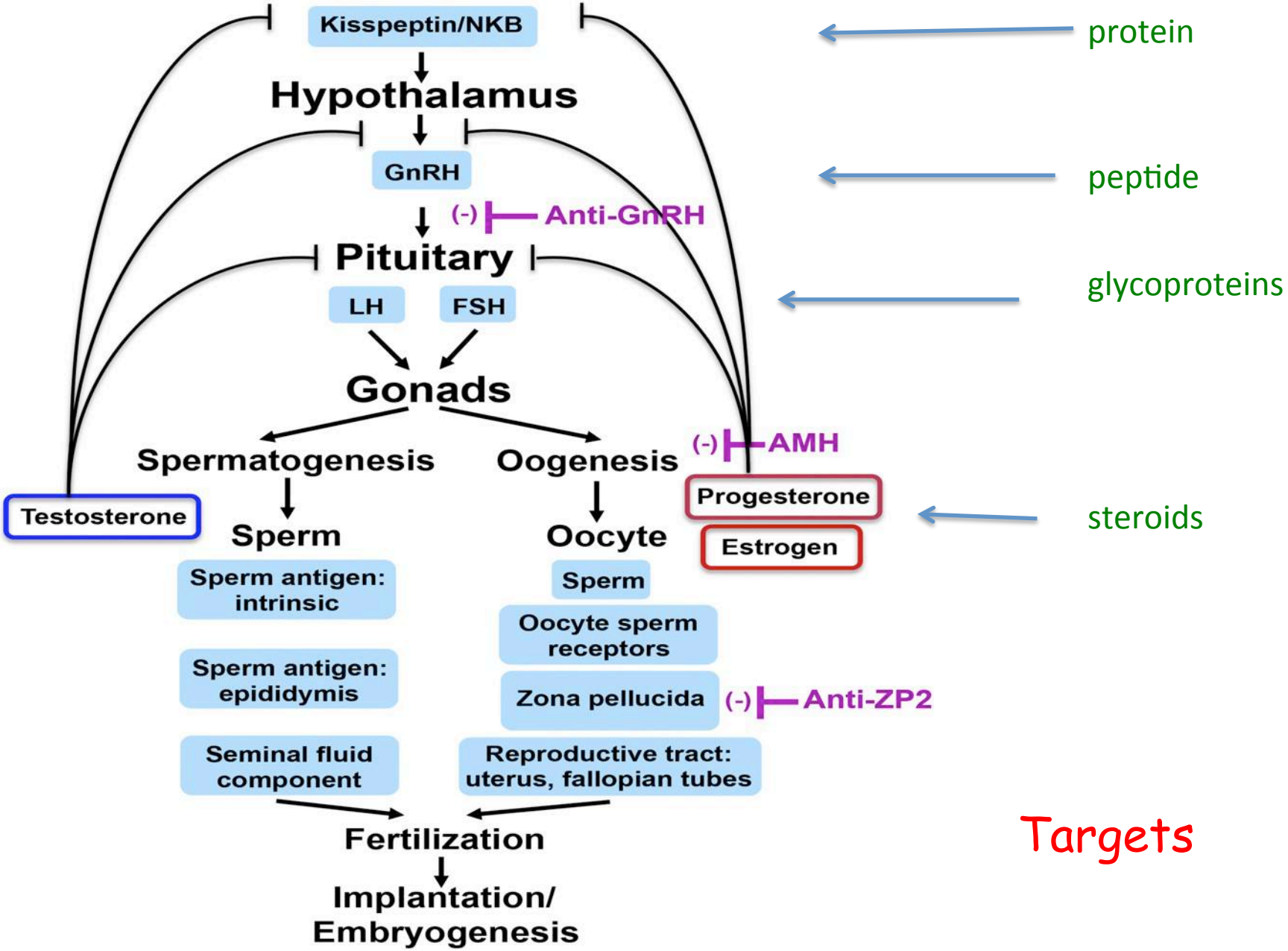


Phenotype
(effect or trait)

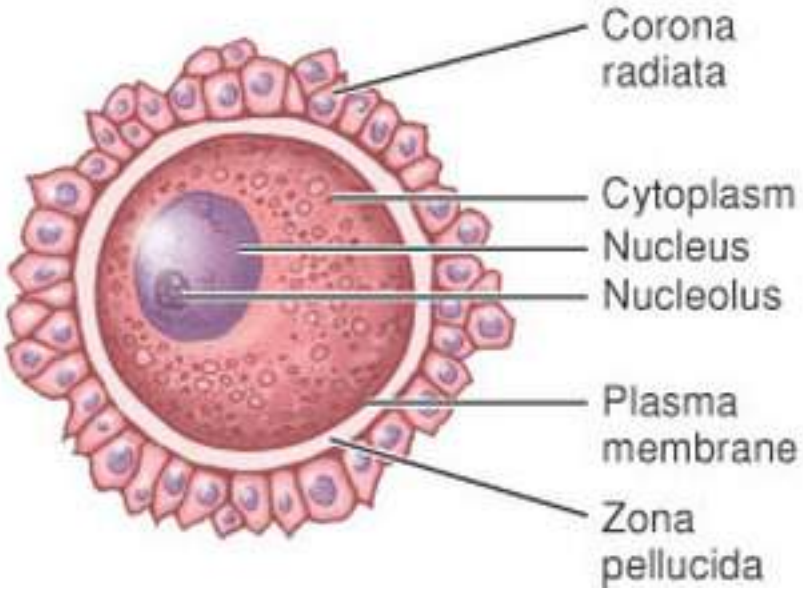
Base pairing is critical! A with T G with C

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


Kisspeptin/NKB
Hypothalamus
GnRH
(-) Anti-GnRH
Pituitary
LH
FSH
Gonads
Spermatogenesis
Oogenesis
(-) AMH
Testosterone
Progesterone
Estrogen
Sperm
Oocyte
Sperm antigen: intrinsic
Sperm antigen: epididymis
Seminal fluid component
Sperm
Oocyte sperm receptors
Zona pellucida
(-) Anti-ZP2
Reproductive tract: uterus, fallopian tubes
Fertilization
Implantation/ Embryogenesis
protein
peptide
glycoproteins
steroids
Targets

Target

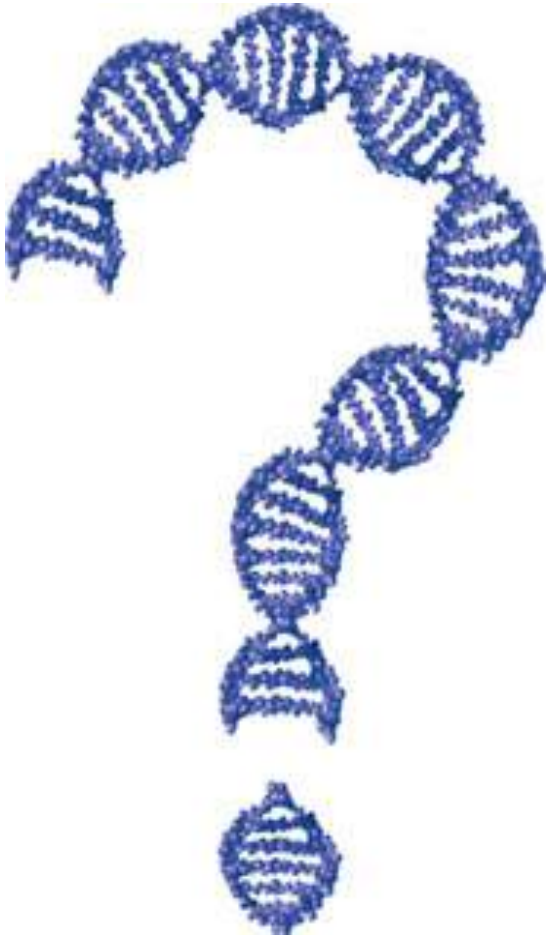


Glycoproteins (not cells)

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

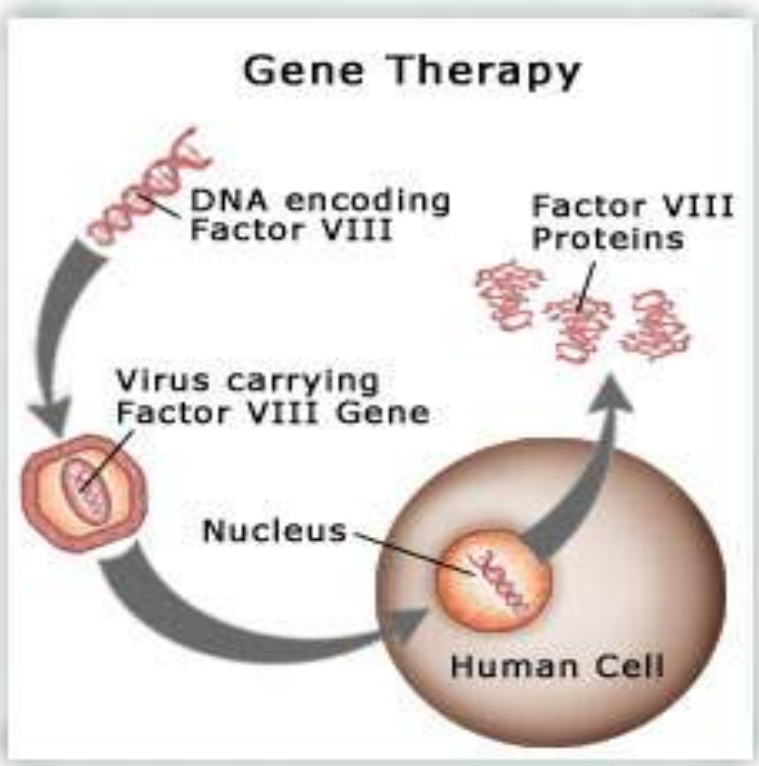
Methods

Gene therapy: adds a gene

Gene silencing: prevents expression (making protein)

Gene editing: adds, removes, or replaces gene

Method



Gene therapy only *adds* DNA

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

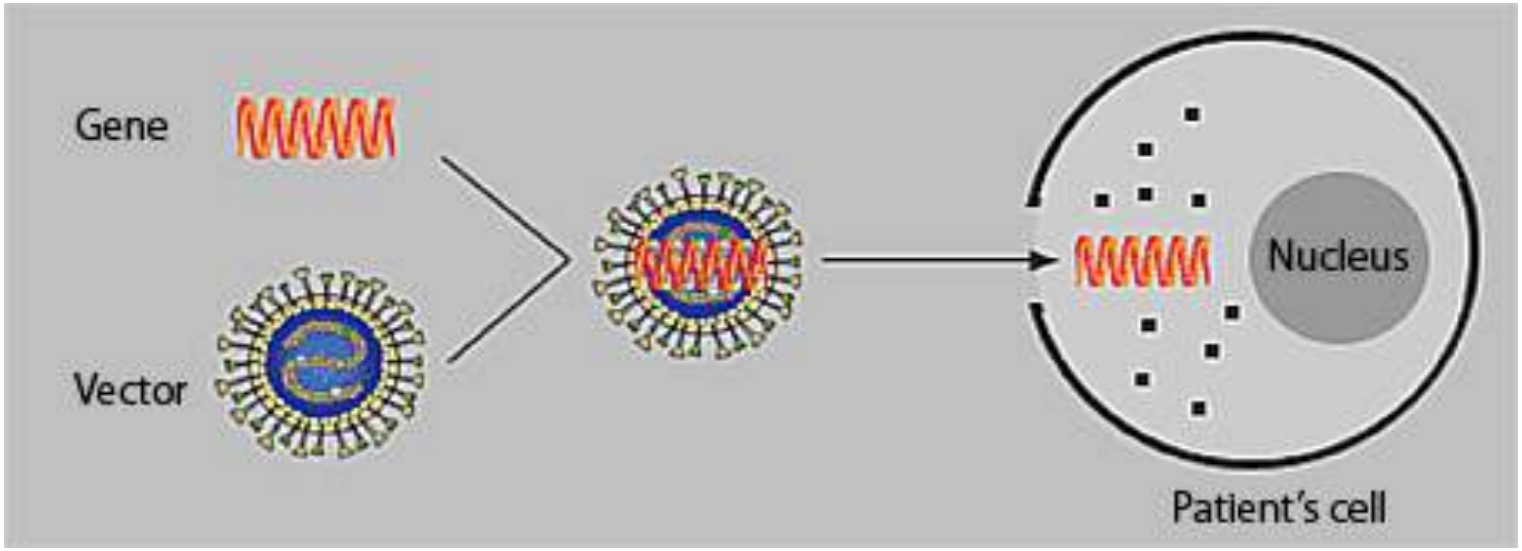
1rst gene therapy clinical trial: 1990

1rst gene therapy FDA approvals: 2017

DELIVERY

Gene Therapy

adenovirus (AV)
adeno-associated virus (AAV) ***
retroviruses
lentivirus (HIV)



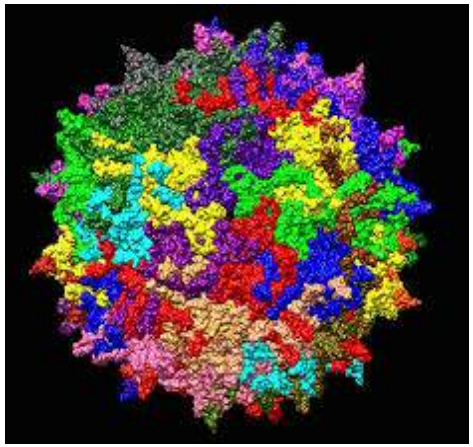
Challenges:

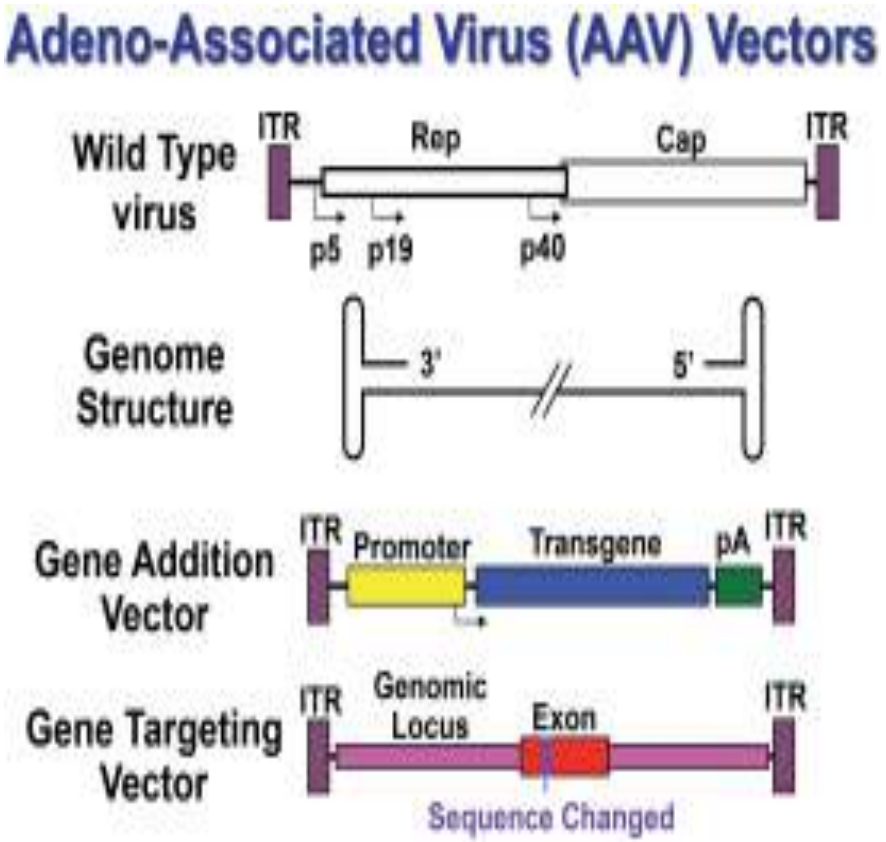
- Enter right cells
- Enter nucleus of those cells
- Gene transcribed + translated into protein
- Avoid immune response, nucleases

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*

6/13/17

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

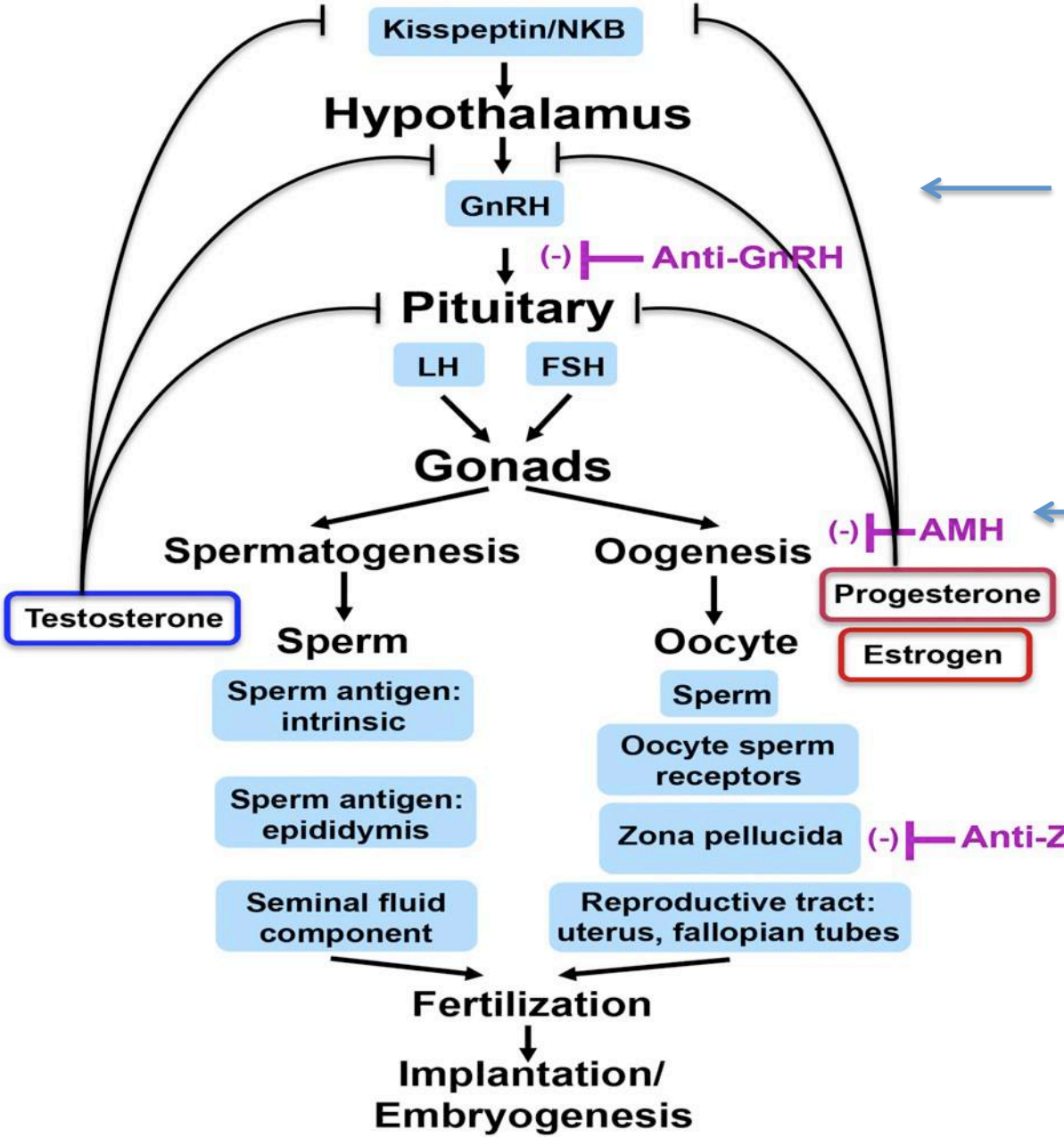
Eliza O'Neill Sanfilippo syndrome (mucopolysaccharidosis type IIIA)

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

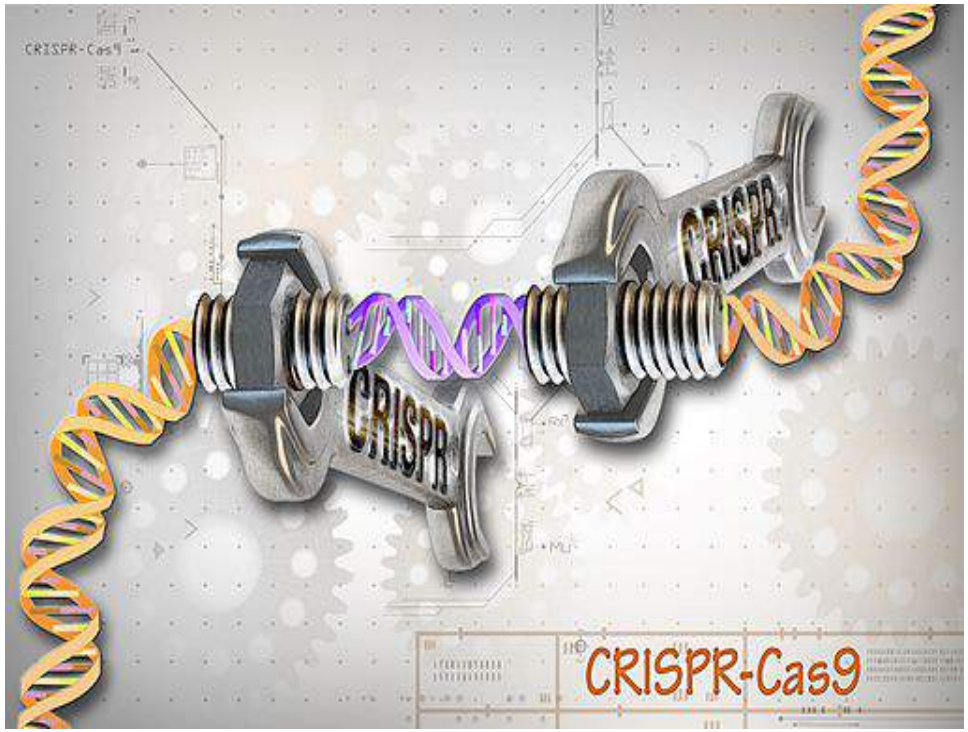


AAV introduces instructions to make anti-GnRH antibodies (IM injections lead to atrophied gonads)

Anti-mullerian protein intraperitoneal injection blocks follicle maturation

Instead of MAbs, AAV introduces instructions to *make antibodies continuously*; ragged eggs

DELIVERY: CRISPR

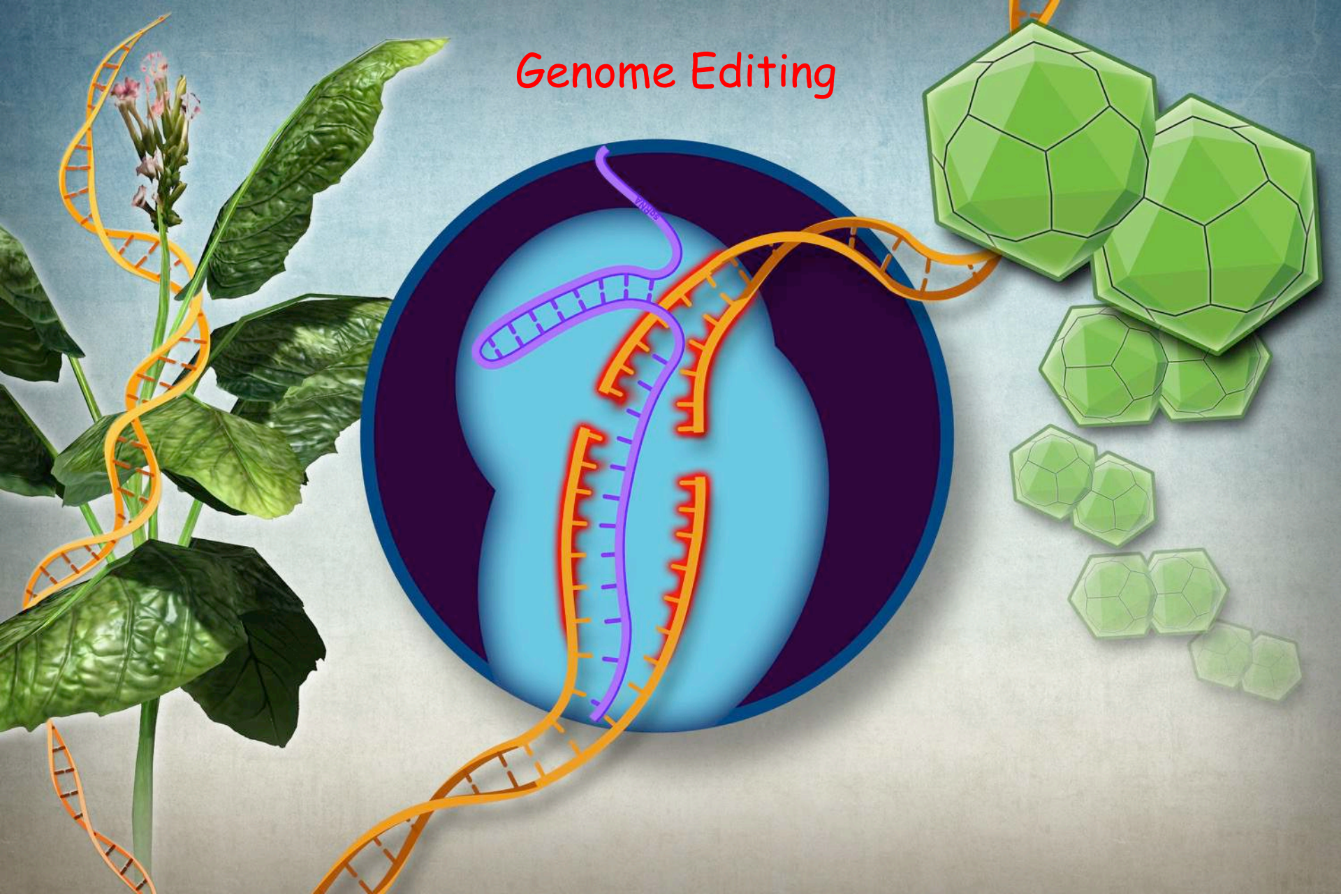


A borrowed bacterial defense against bacteria that uses short RNAs to target specific genes, then deploys DNA-cutting enzymes to remove, replace, or add a specific DNA sequence.

Limitation: off-target effects, causing cancer

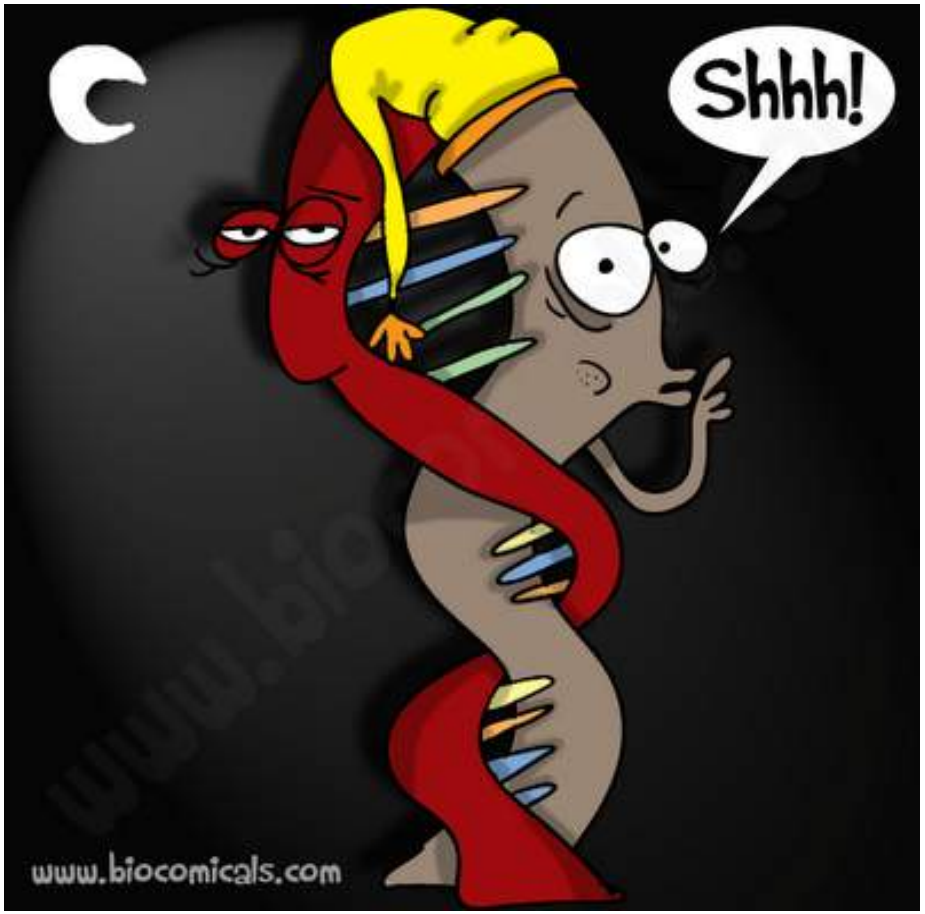
Older methods: zinc finger nucleases and TALENs

Genome Editing



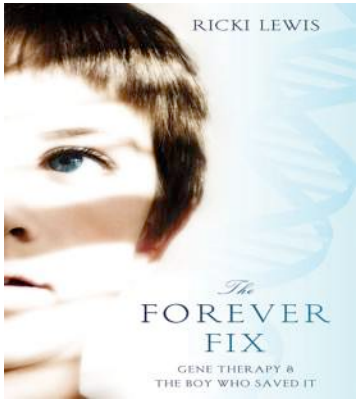
Clustered Regularly Interspaced Short Palindromic Repeats = DNA velcro

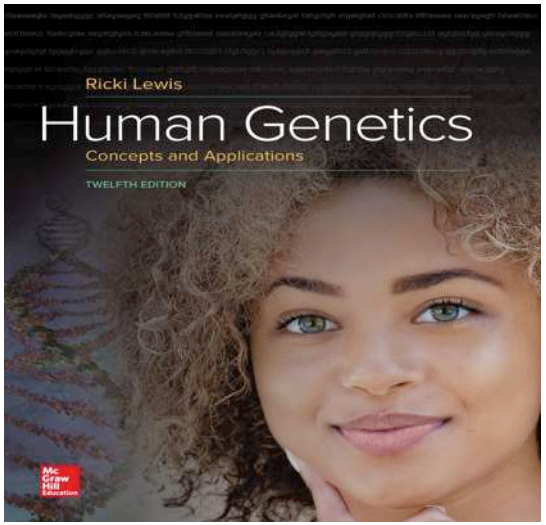
Gene Silencing

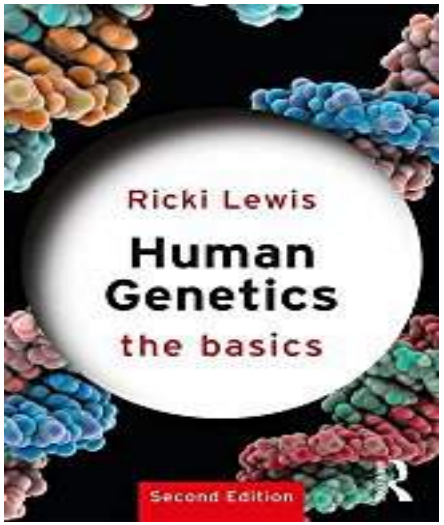


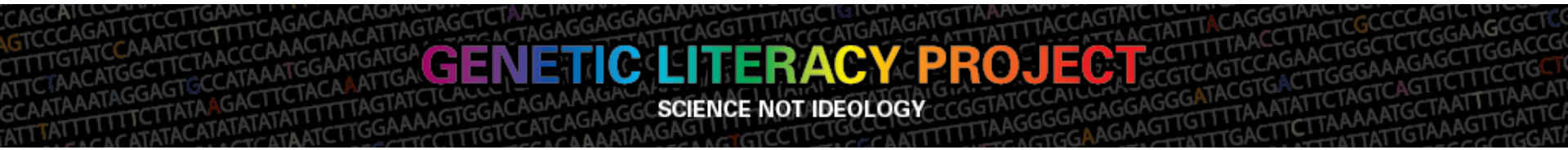
- Antisense RNAs bind to and block mRNA
- Synthetic RNAs (morpholinos) (25 bases + organic group) to treat DMD blocks splice site
- RNA interference (RNAi): short double-stranded RNAs (siRNAs)
- MicroRNAs (21-22 bases) bind mRNAs, block translation into protein
- Monoclonal antibodies (Mabs) bind proteins,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

Current work on delivery of vectors (siRNAs to cats)









Medscape from WebMD

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