PHYSIOLOGY AND MODULATION FACTORS OF OVULATION IN RABBIT REPRODUCTION MANAGEMENT

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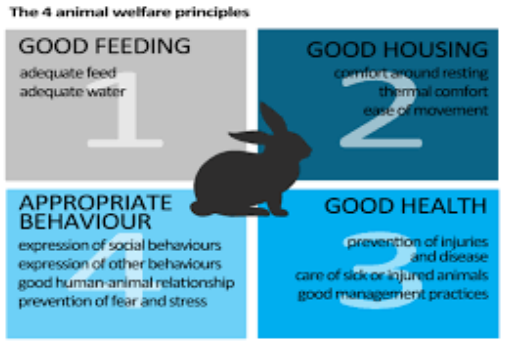
Critical points on consumer perception of animal production

« One welfare »



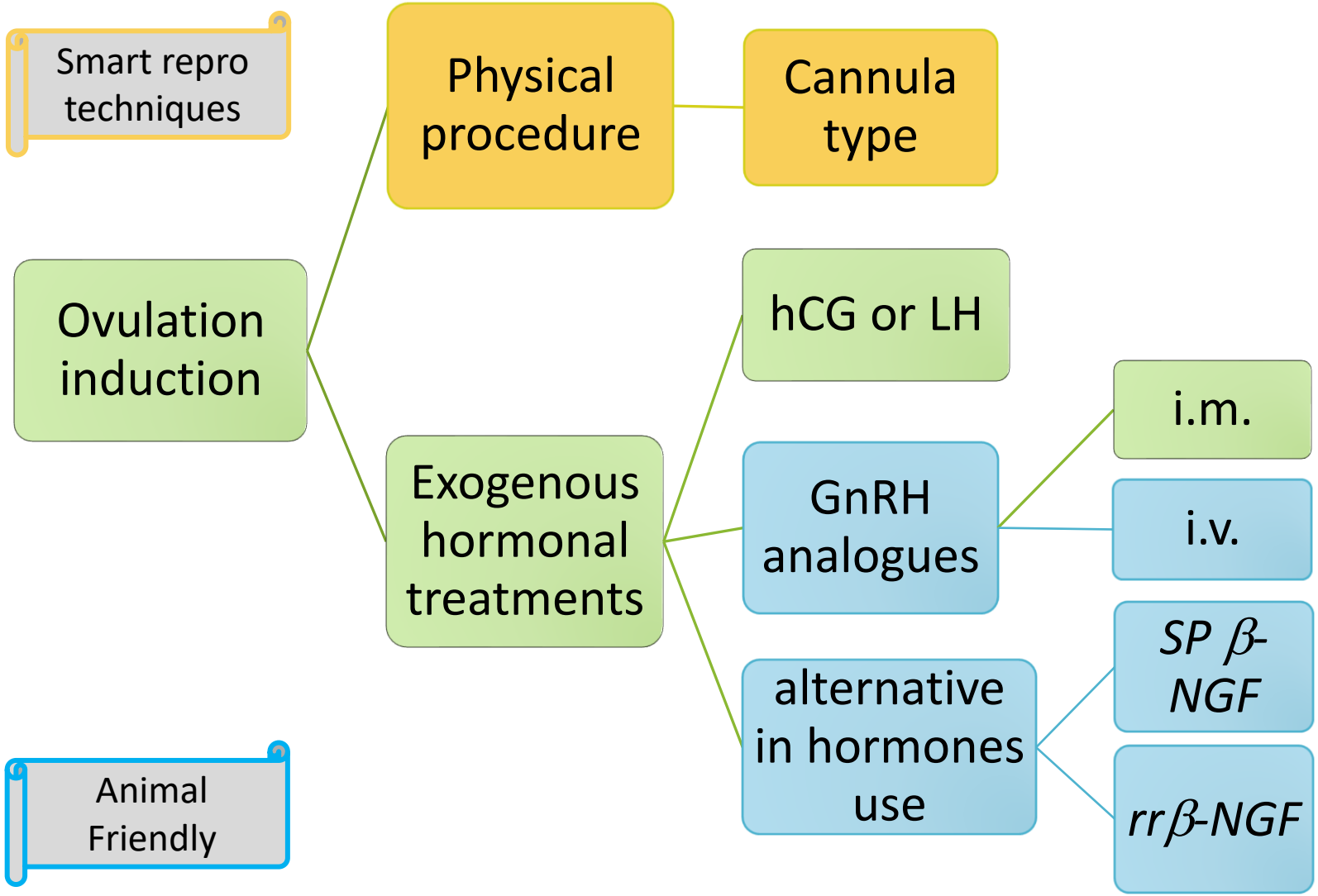
Overview report of EU commission: "Commercial Rabbit Farming in the European Union"

Objectives

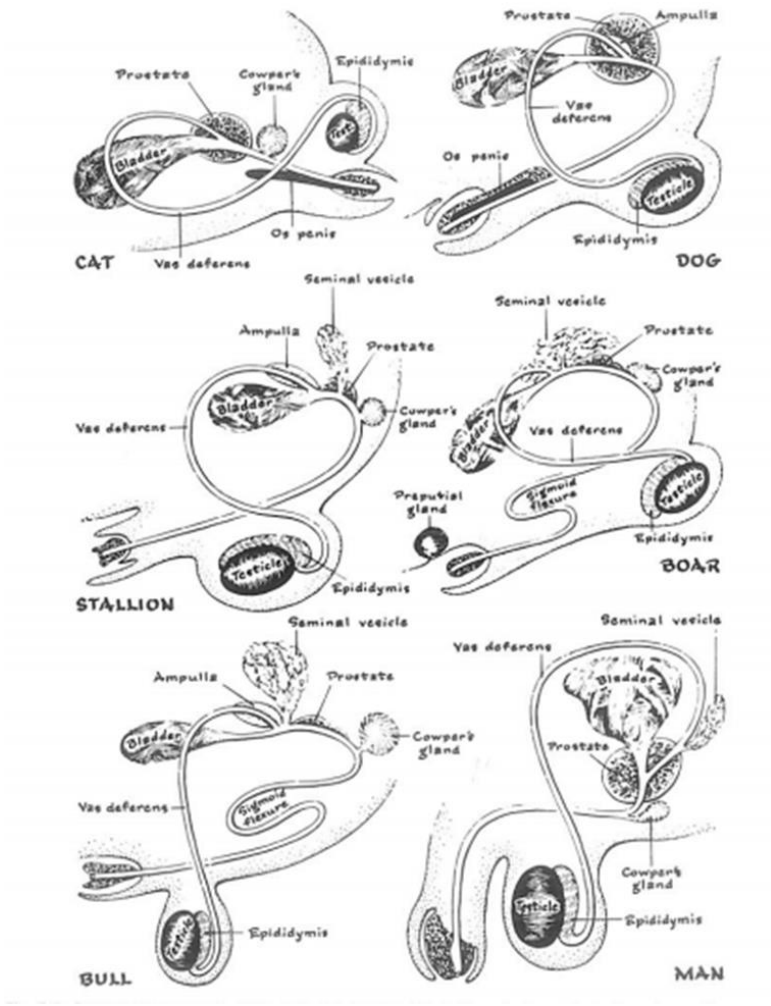
- ✓ Smart repro techniques (Repro Rithms better adapted to all in all out, high fertility, etc…)
- ✓ «Animal Friendly» repro management (health, welfare, rabbit image, antibiotic-free, hormone-free.)

Reproductive management represents one main factor which can assure productivity, health and welfare of rabbits

Current methodologies for ovulation induction

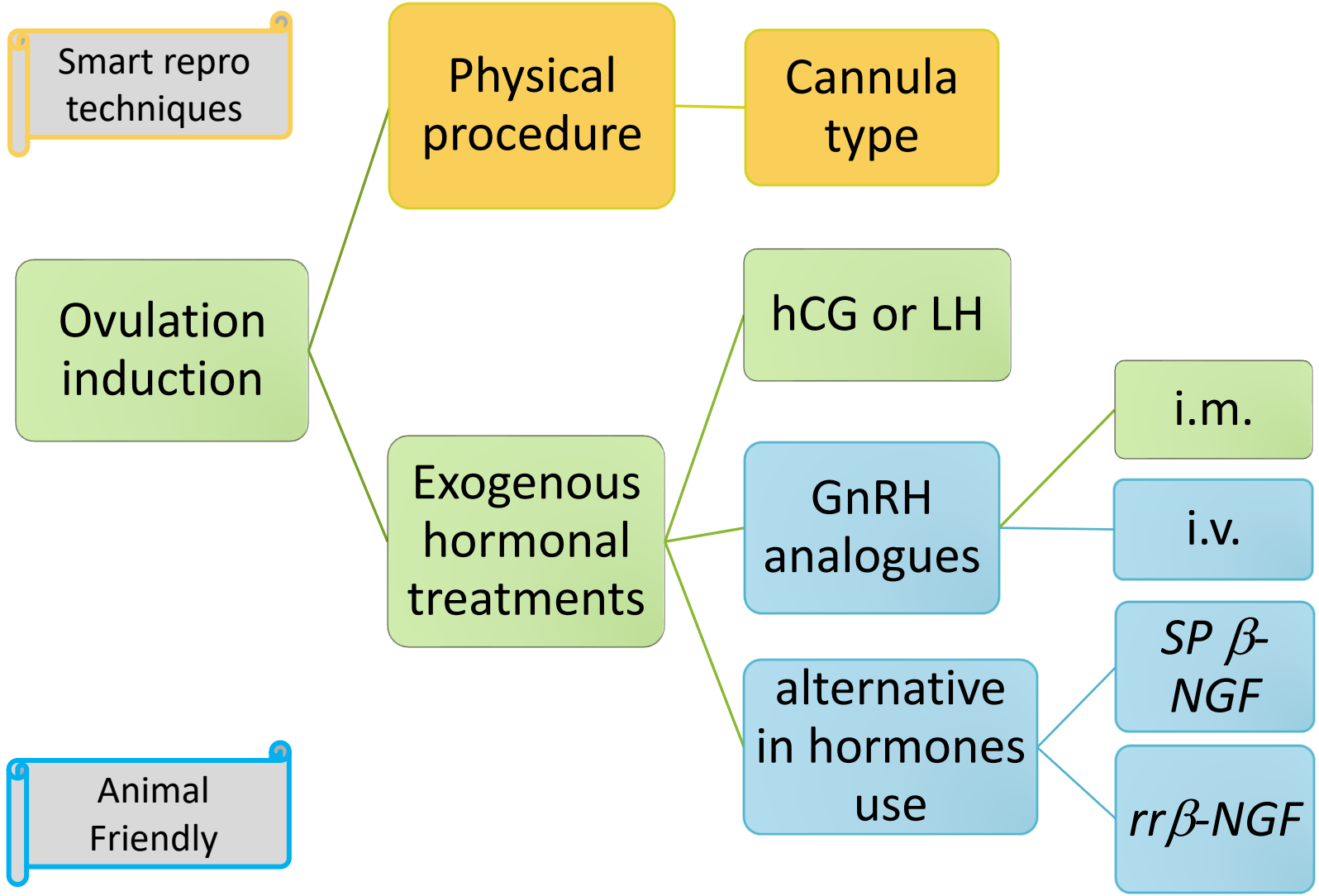


Genital accessory male glands in different species



Mysteries in reproductive biology…

Current methodologies for ovulation induction

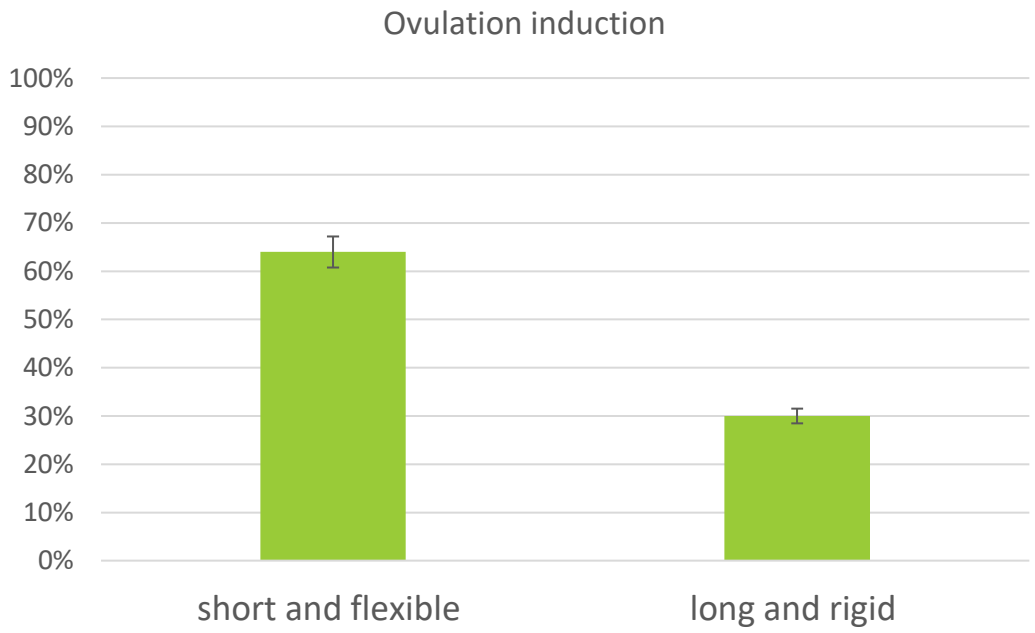


Physical procedures

- *Cannula types:*

A short and flexible cannula mimicked better the stimulation associated with the mating of the male than a long and rigid one.

when GnRH analogues is used in the semen extender (intravaginal administration), no differences in does fertility and/or prolificacy were found.



Viudes de Castro et al., 2017

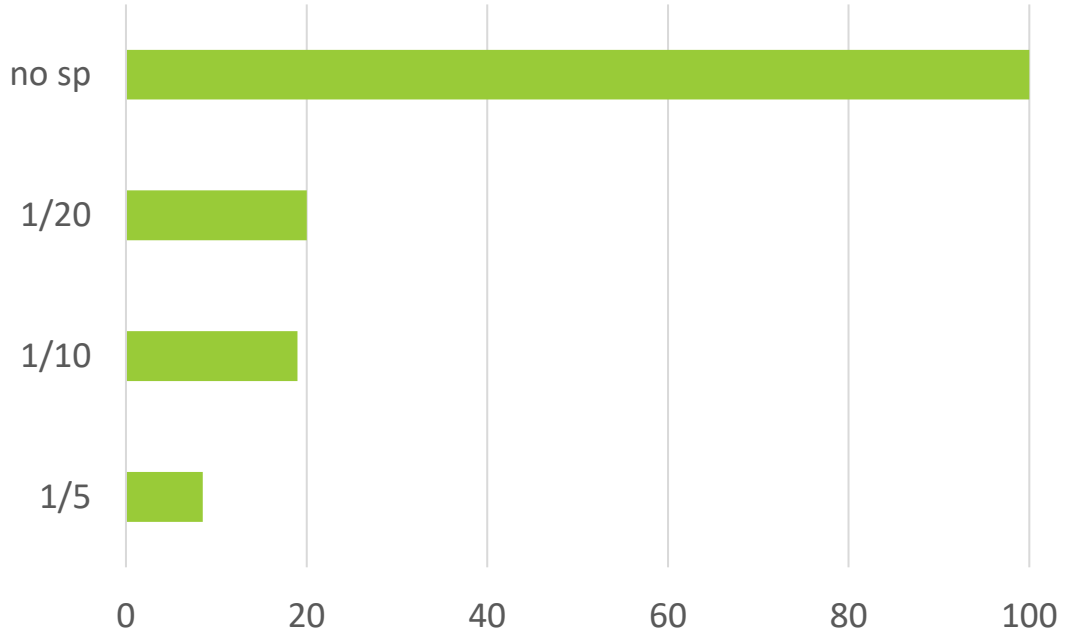
GnRH i.m. vs *i.v.*

From 15 years it is known GnRH efficacy in ovulation induction (i.m.)

- Molecular dose depends by vaginal degradative agents
- The dose required i.v. is ~10 times higher than i.m
- i.v. efficacy > affected by SR of does
- Ovulation efficiency depends on analogues (and adjuvants)

GnRH *i.v.*

GnRH (% initial dose) with different SP ratio



Addition of protease inhibitors (bestatin, EDTA, aminopeptidase) to extenders for reducing GnRH degradation

Extender	Total motility (%)	Acrosome integrity (%)	Viability (%)
CNT	75.00±4.47	86.25±4.31	64.33±5.83
Ext+GnRH analogue	78.83±4.28	86.53±4.11	68.55±5.83
ExT+GnRH analogue+ AMI	67.92±4.28	84.44±4.11	64.24±5.83

Extender: TRIS-Citric acid-Glucose; GnRH analogue: 10µg/ml buserelin acetate;
AMI: Aminopeptidades inhibitors (10µM bestatin+ 20mM EDTA)

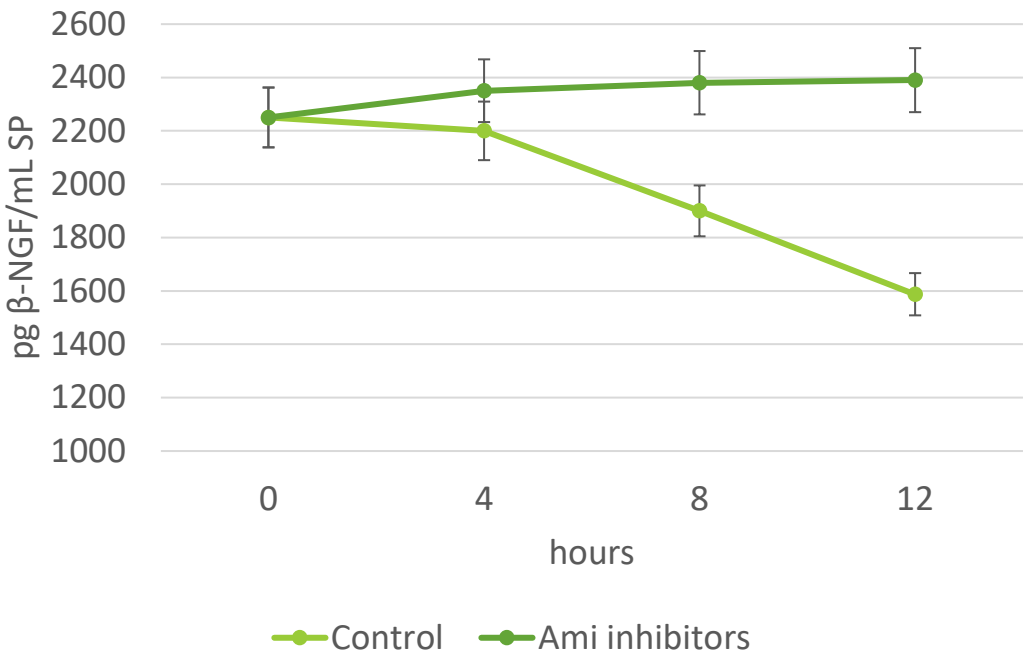
Semen quality

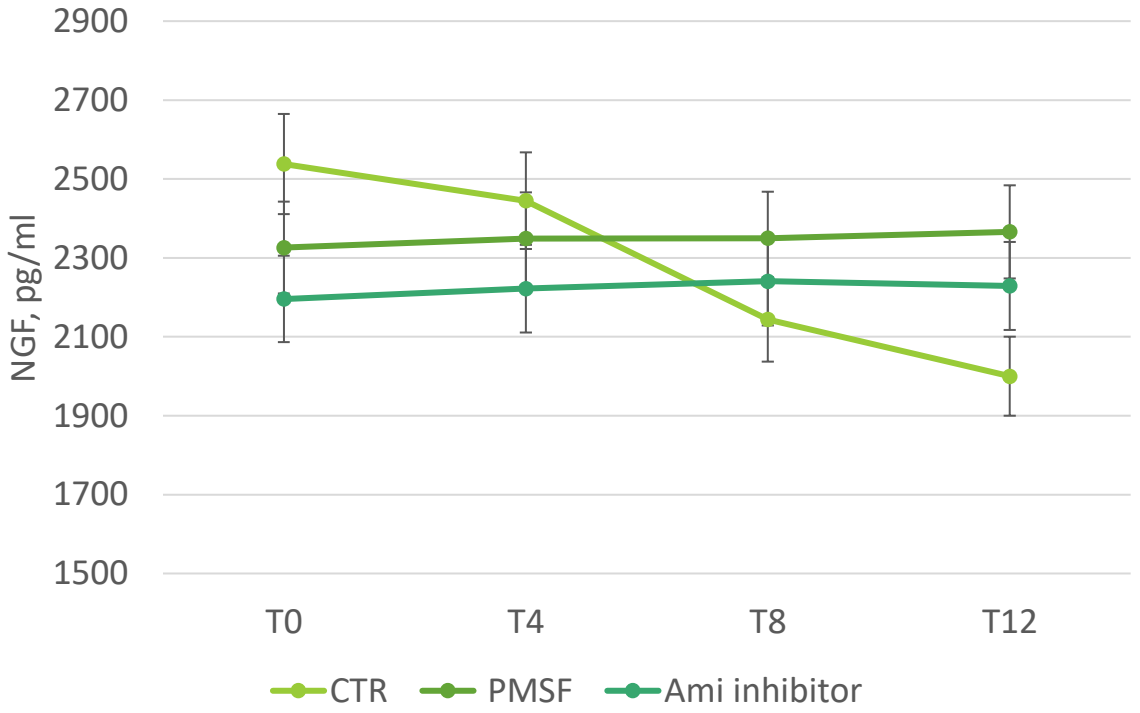
Viudes et al., 2014

Casares-Crespo et al., 2018

NGF like GnRH analogue on degradation?

alternative in hormones use





Casares-Crespo et al., 2018; inhibitor: 10 ng/ml

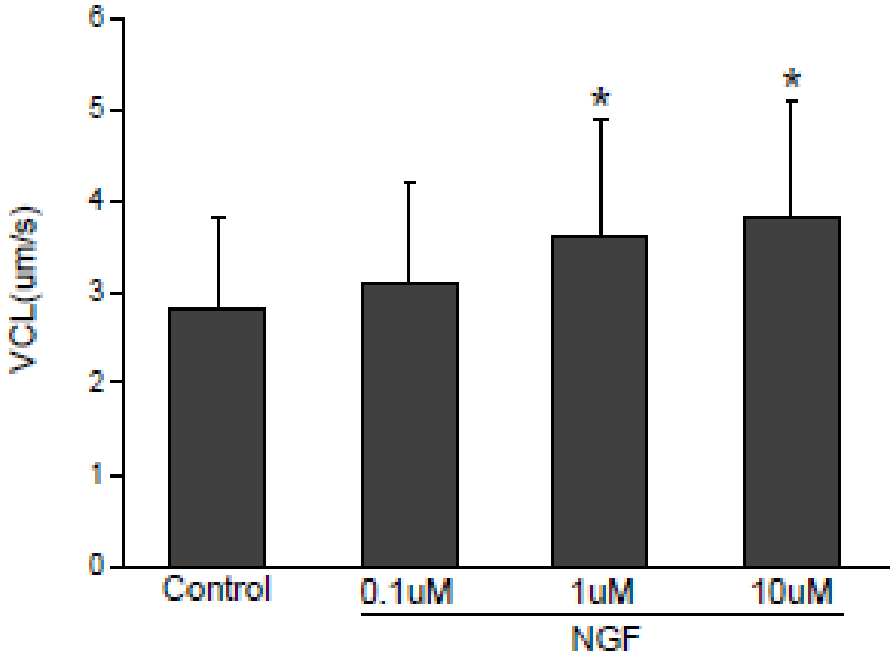
Unpublished data: Inhibitor Ami, 10 ng/ml; PMSF, 1%

β-NGF and kinetic

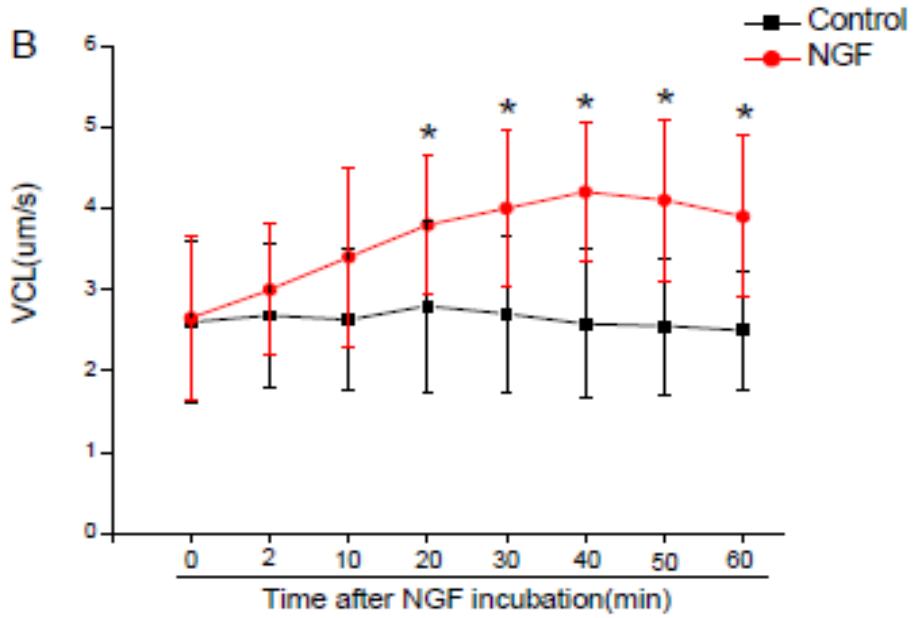
Castellini et al., 2019 - In vitro effect of nerve growth factor on the main traits of rabbit sperm, Reproductive Biology and Endocrinology

Castellini et al. 2020 - Role of NGF on sperm traits: a review, Theriogenology

Dose-dependent effect

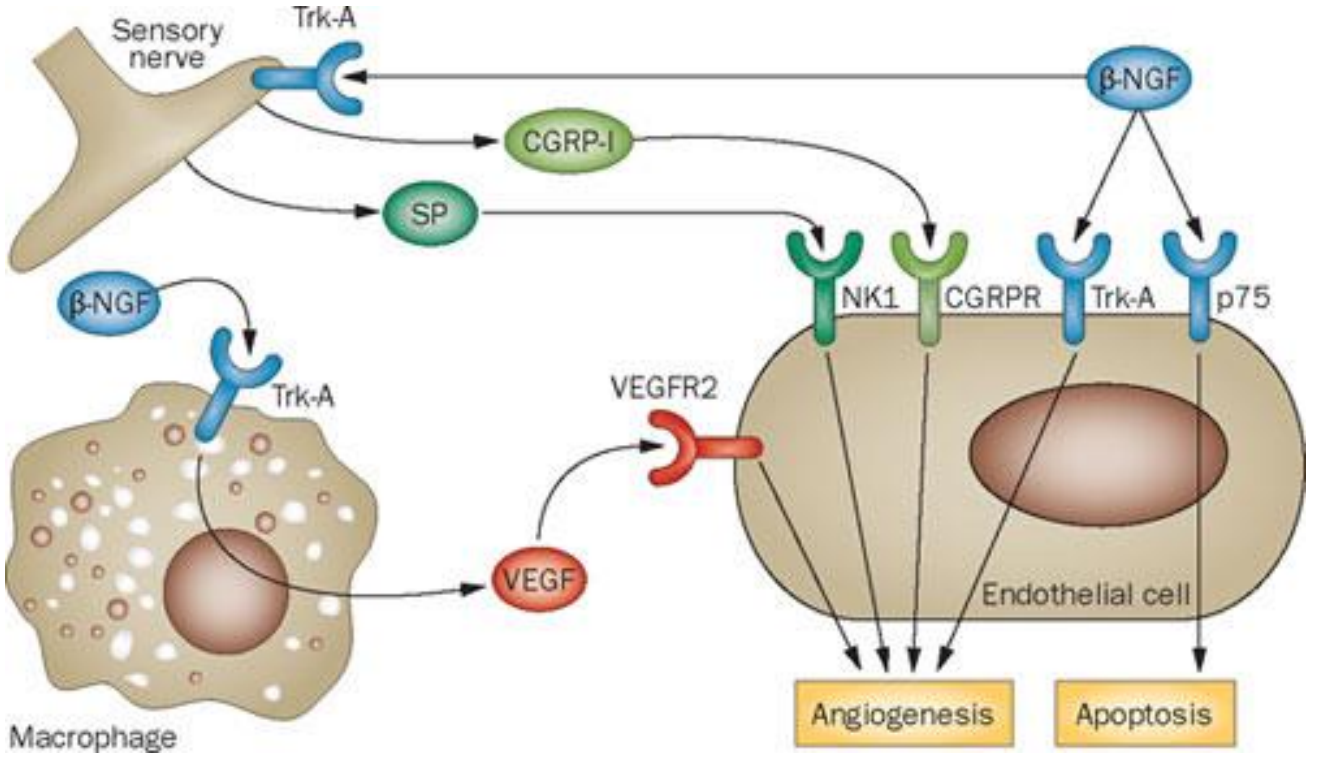


Time-dependent effect

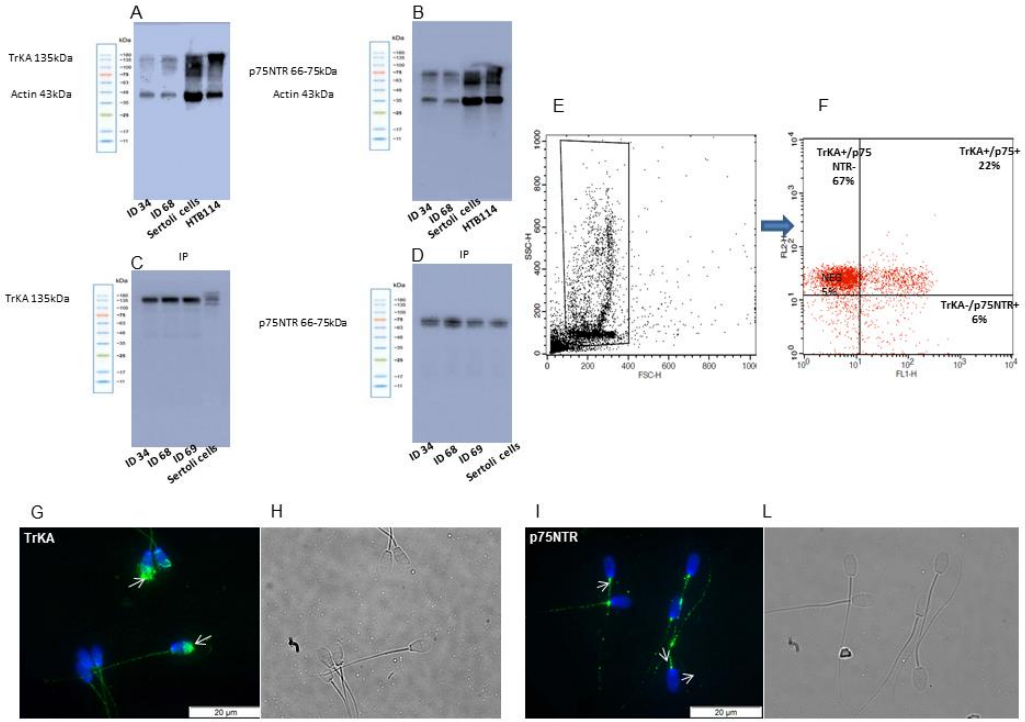


β-NGF receptors (TrKA and p75NTR) role in sperm

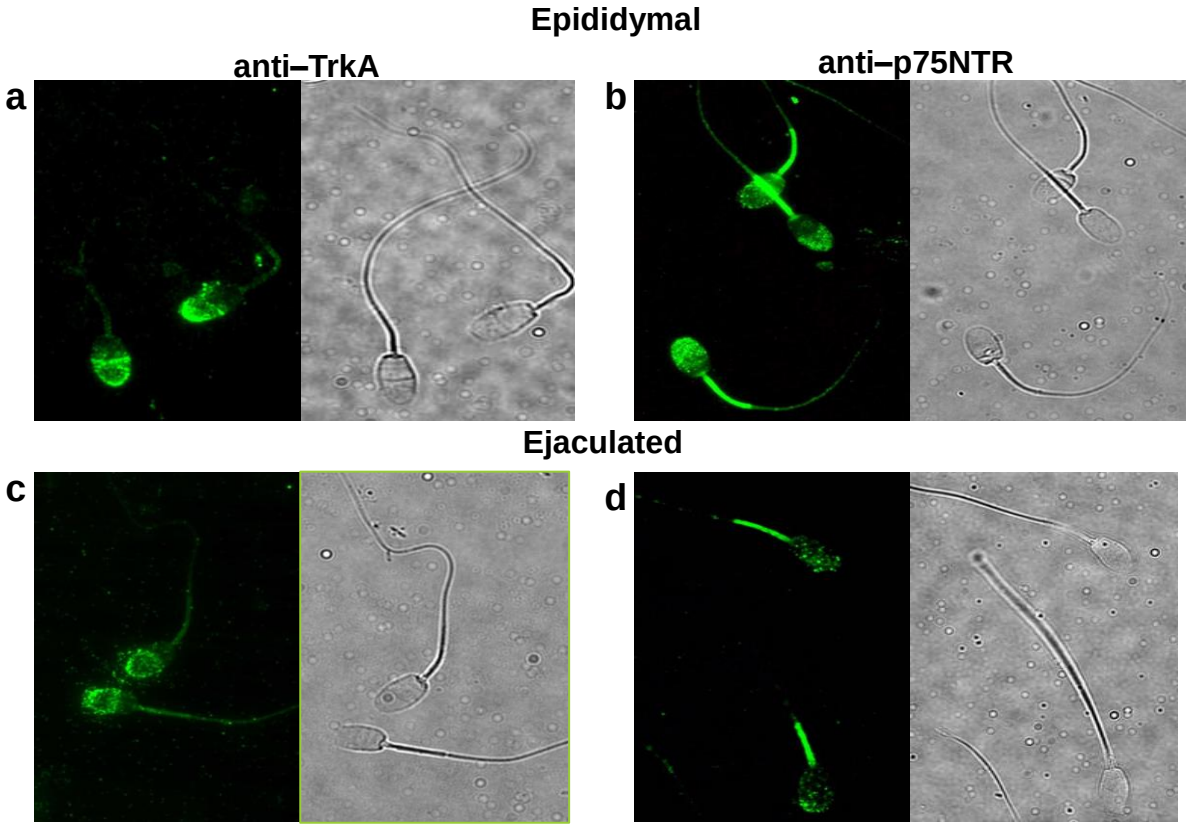
Castellini, C., Mattioli, S., Dal Bosco, A., Collodel, G., Pistilli, A., Stabile, A. M., ... & Rende, M. (2019). In vitro effect of nerve growth factor on the main traits of rabbit sperm. *Reproductive Biology and Endocrinology*, *17*(1), 1-11.



β-NGF receptors in sperm



Receptors distribution in epididymal and ejaculated semen



Castellini et al., 2019. Reproductive Biology and Endocrinology, 17(1), 1-11

NGF-rceptors interaction on capacitation and vitality

	CP %	AR %	IC %	Apoptosis %	Necrosis %	Live cells %
C	14.2 b	5.0 bc	80.8b	7.8 ab	5.0 b	87.2 a
NGF	16.3 c	6.3 c	77.4a	7.0 a	3.8 a	91.0 b
NGF+aTrKA	14.5 b	2.5 a	83.0b	9.5 b	5.5 b	85.0 a
NGF+ap75NTR	11.4 a	4.3 b	84.3b	6.0 a	4.4 a	89.7 ab

CP: Capacitated
AR: acrosome reacted
IC: intact sperm

Via p75NTR

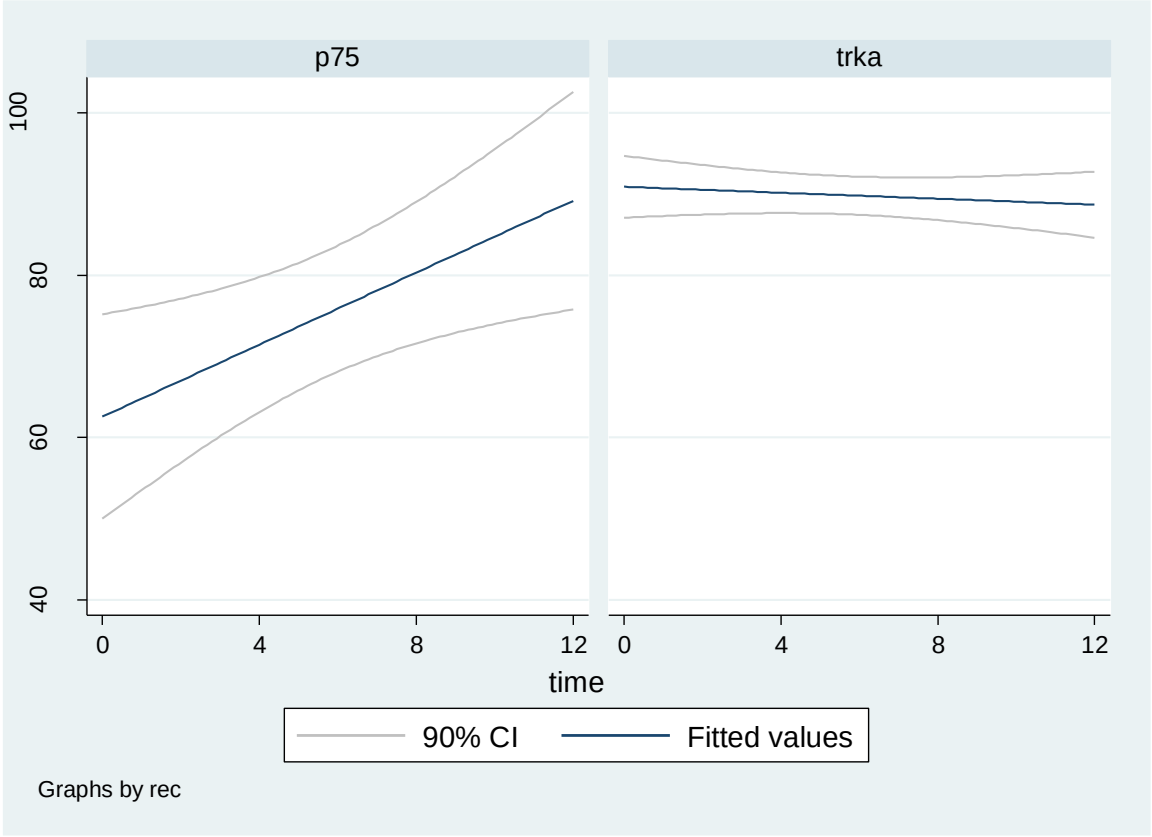
Via TrKA

Castellini et al. 2019

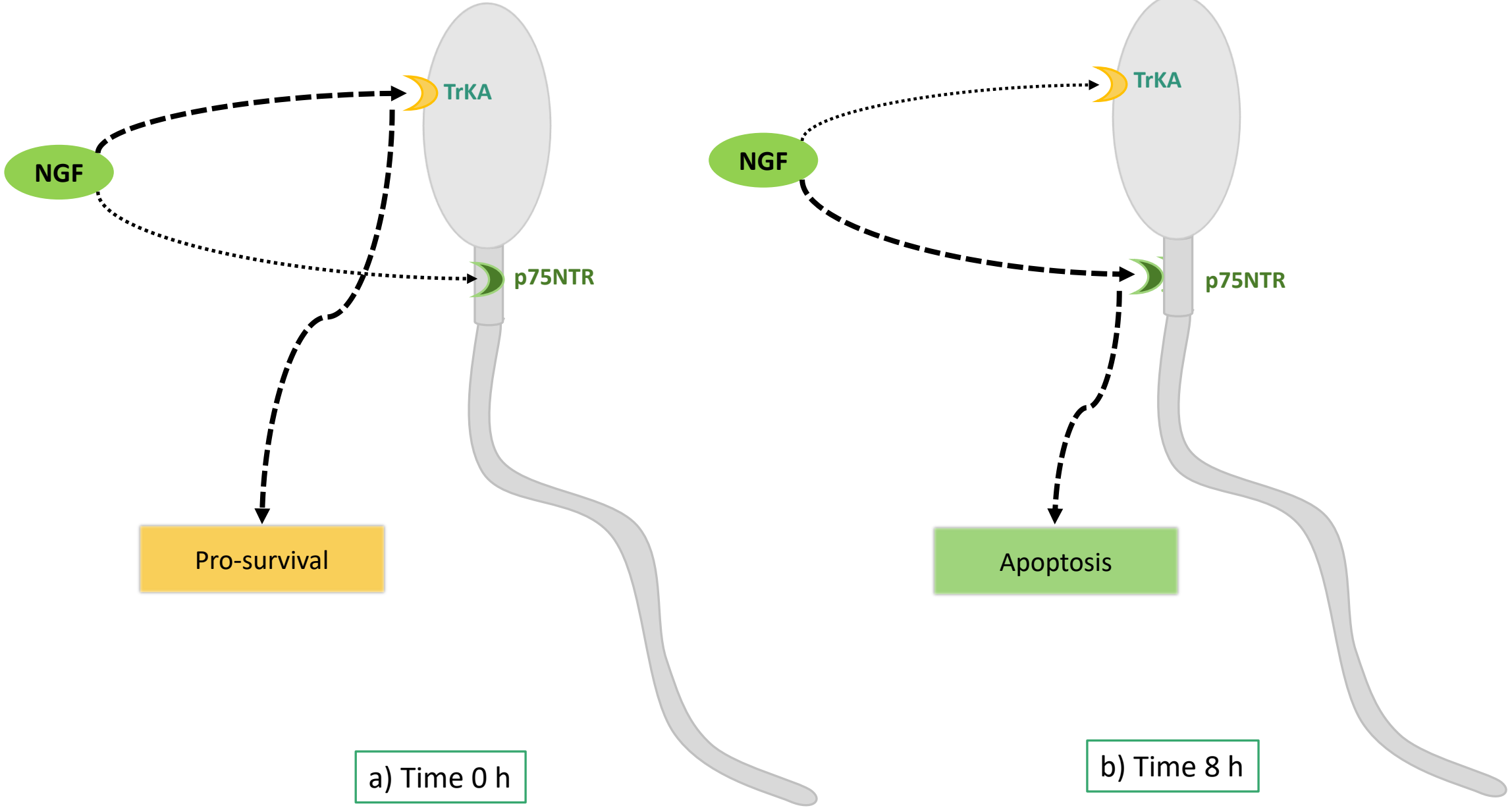
Time-dependent trend of NGF receptors

Hour of storage	TrkA	p75NTR	TrkA/p75NTR
baseline	89.30	21.83 $_{a}$	4.09 $_{a}$
4	88.30	21.64 $_{a}$	4.09 $_{a}$
8	89.47	34.31 $_{b}$	**2.60** $_{b}$
12	90.12	34.00 $_{b}$	**2.65** $_{b}$

Time-dependent trend (%) of externalised NGF receptors

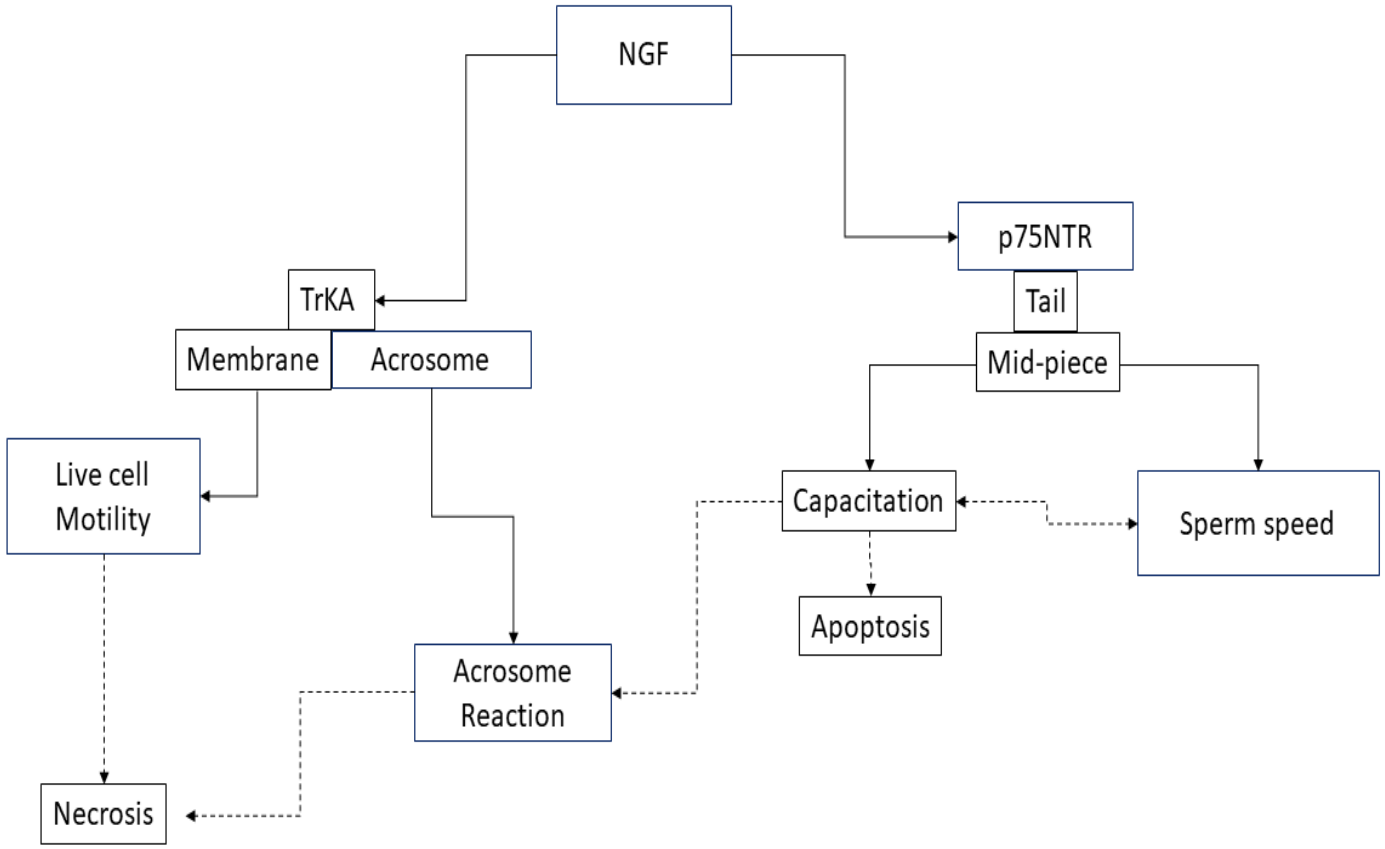


Castellini et al., 2020 - How does NGF affect sperm storage? Role of NGF-receptor interactions in rabbit sperm, Theriogenology


NGF
TrKA
p75NTR
Pro-survival
a) Time 0 h
NGF
TrKA
p75NTR
Apoptosis
b) Time 8 h

Sperm traits and NGF

(Castellini et al., 2020 - How does NGF affect sperm storage? Role of NGF-receptor interactions in rabbit sperm, Theriogenology)



IMPLICATIONS

Addition of NGF to the seminal dose would improve working time in the farm at AI moment and could be more physiological for the animals

Receptors quantity: p75NTR role

CARPE DIEM

Normal p75NTR

A

Time (h)	Motility rate (%)	Apoptosis (%)
0	44	15 a
2	57	19 a
4	58	25 ab
6	59	31 b
RMSE	4.1	2.0

B

Time (h)	Motility rate (%)	Apoptosis (%)
0	44 a	15
2	60 b	18
4	67 b	20
6	67 b	23
RMSE	4.6	2.0

+NGF

High p75NTR

C

Time (h)	Motility rate (%)	Apoptosis (%)
0	39	20 a
2	42	25 a
4	43	27 ab
6	41	37 b
RMSE	4.3	2.7

D

Time (h)	Motility rate (%)	Apoptosis (%)
0	39	20 a
2	40	22 a
4	38	29 ab
6	40	40 b
RMSE	2.9	3.0

$^{a..b}$ on the same column means $P \leq 0.05$

high (>25.6%) and normal (<25.6%) p75NTR positive cells

Take home massage

consumer perception

Smart Repro techniques

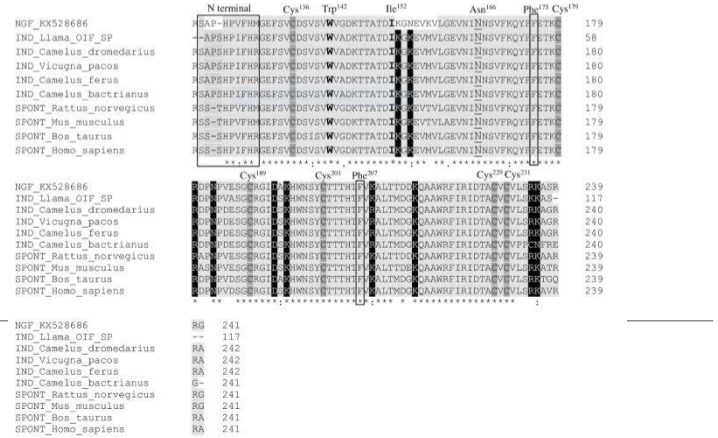
- indispensable requirement = **neural-stimuli** (Cannula type)

Animal Friendly techniques

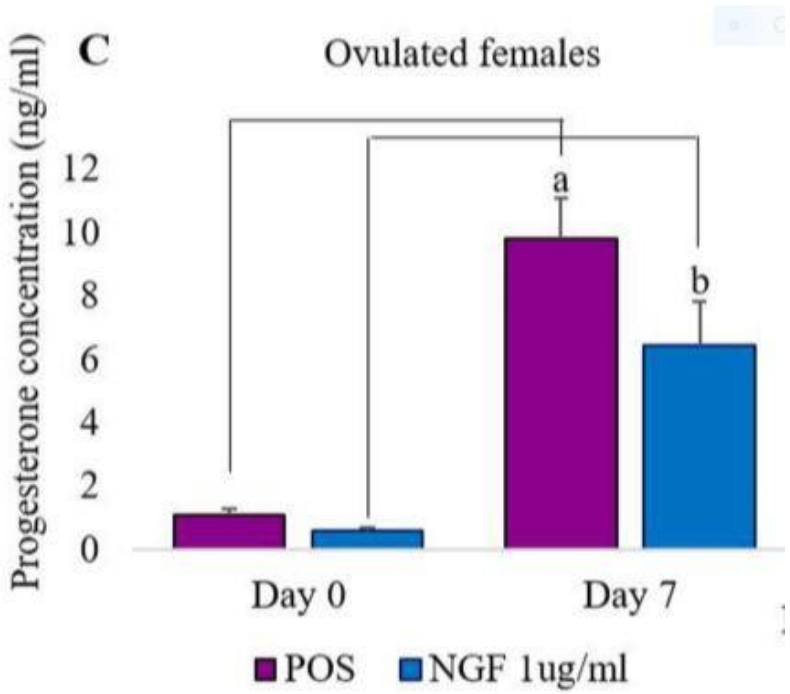
- i.v. administration vs i.m.
- SP compound protection (β-NGF)
- AI within 4 hours by semen collection

rrβ-NGF

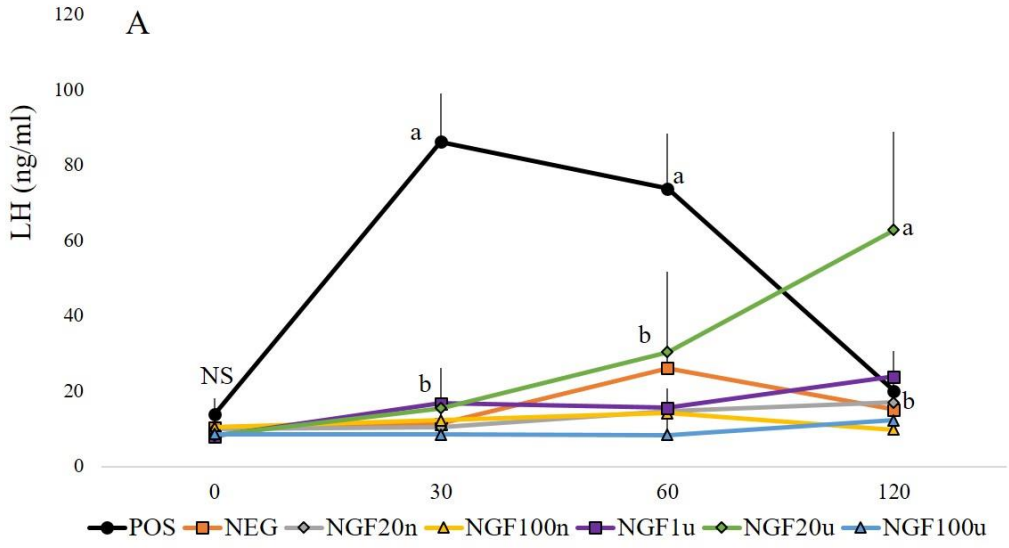
Future propective: Recombinant rabbit NGF



GnRH (i.m.) or recombinant NGF (i.v.)



Garcia et al., 2019



rrβ-NGF dos

- 20 ng/m
- 100 ng/ml
- 1 µg/ml
- 20 µg/ml
- 100 µg/ml

Sánchez-Rodriguez et al. Theriogenology 157 (2020) 327-334
Sánchez-Rodriguez et al. Theriogenology (2020)
Sanchez-Rodriguez et al. (2019) PLOS ONE 14(7)

Thank you
Any questions?

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