Sperm Epigenetics and Male Fertility in Cattle: Focus on Small Non-Coding RNAs

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Équipe DREAM
Développement, Reproduction des ruminants, Epigénétique, Adaptation, Modélisation

Genetique
Genomique
Epigenetique
Evolution
GenE2

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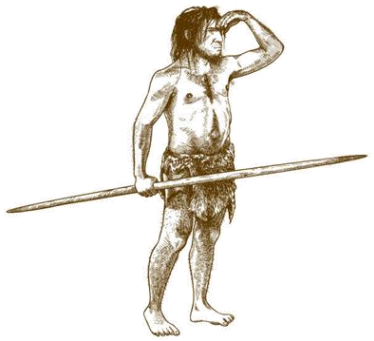
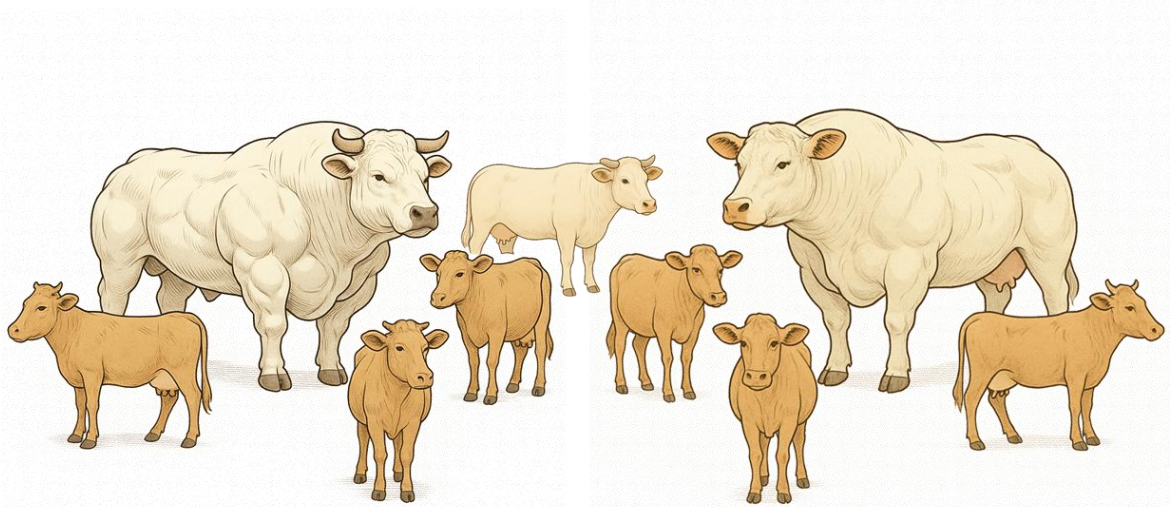
Objectives

You have to be convinced on…

- The importance to (well) manage the reproduction in any livestock
- The importance of sncRNAs for the sperm
- The sperm-borne sncRNAs are interesting fertility biomarkers
- A huge amount of work is needed to fully understand the role of sncRNAs in fertility





What's the plan?

- Introduction: reproduction management in livestock
  - Objectives
  - Animal selection scheme & Reproductive biotechnologies: some powerful tools

- The male fertility prediction
  - Sperm functionality: the main dogma of last decades!
  - Sperm Epigenome: the new approaches

- The sperm-borne sncRNAs: translating past events or future developments?
  - sncRNAs in spermatogenesis and sperm maturation
  - sncRNAs as fertility biomarkers

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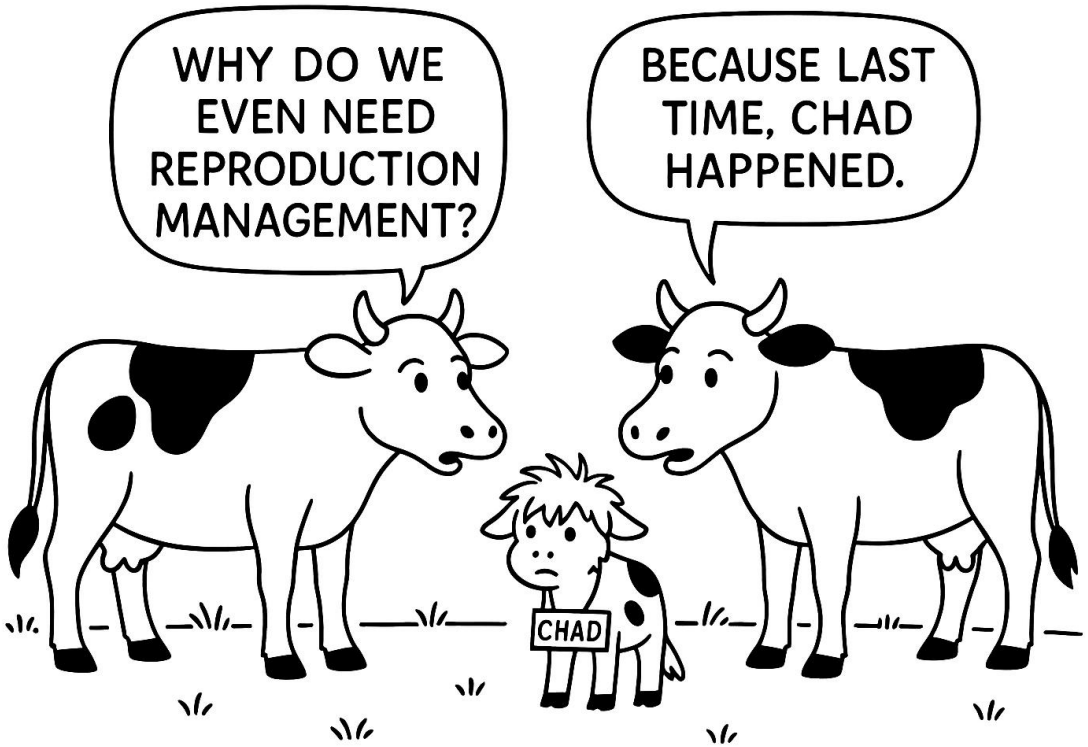
Let's start with the introduction!

Why the Reproduction management is so important for the breeding sector?



BREED

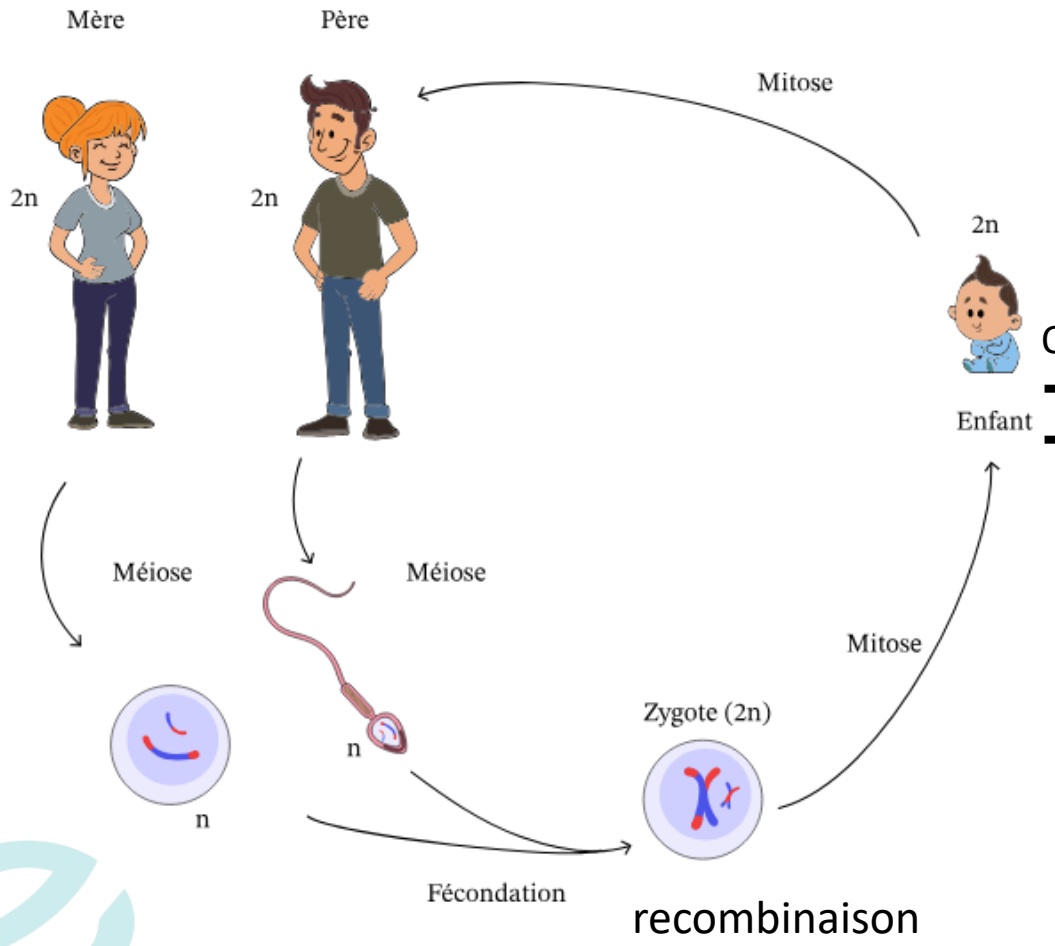
The reproduction…some general information

- A simple but vital objective!

The reproduction…some general information

Two main reproduction types

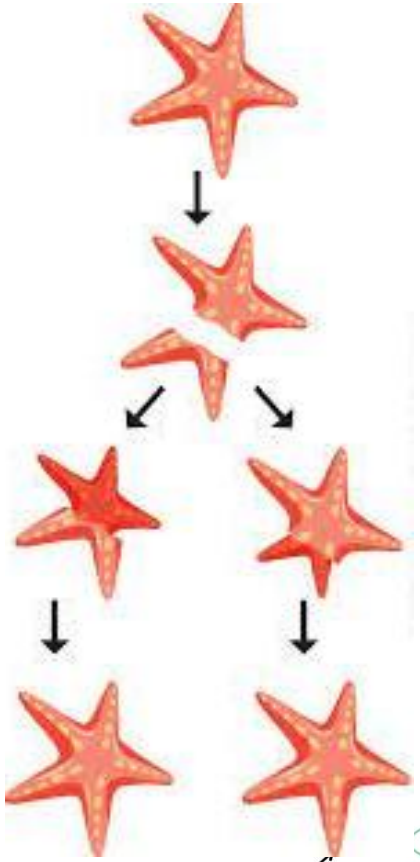
Sexual reproduction



Offspring
➔ New genome
➔ New phenotype

recombinaison

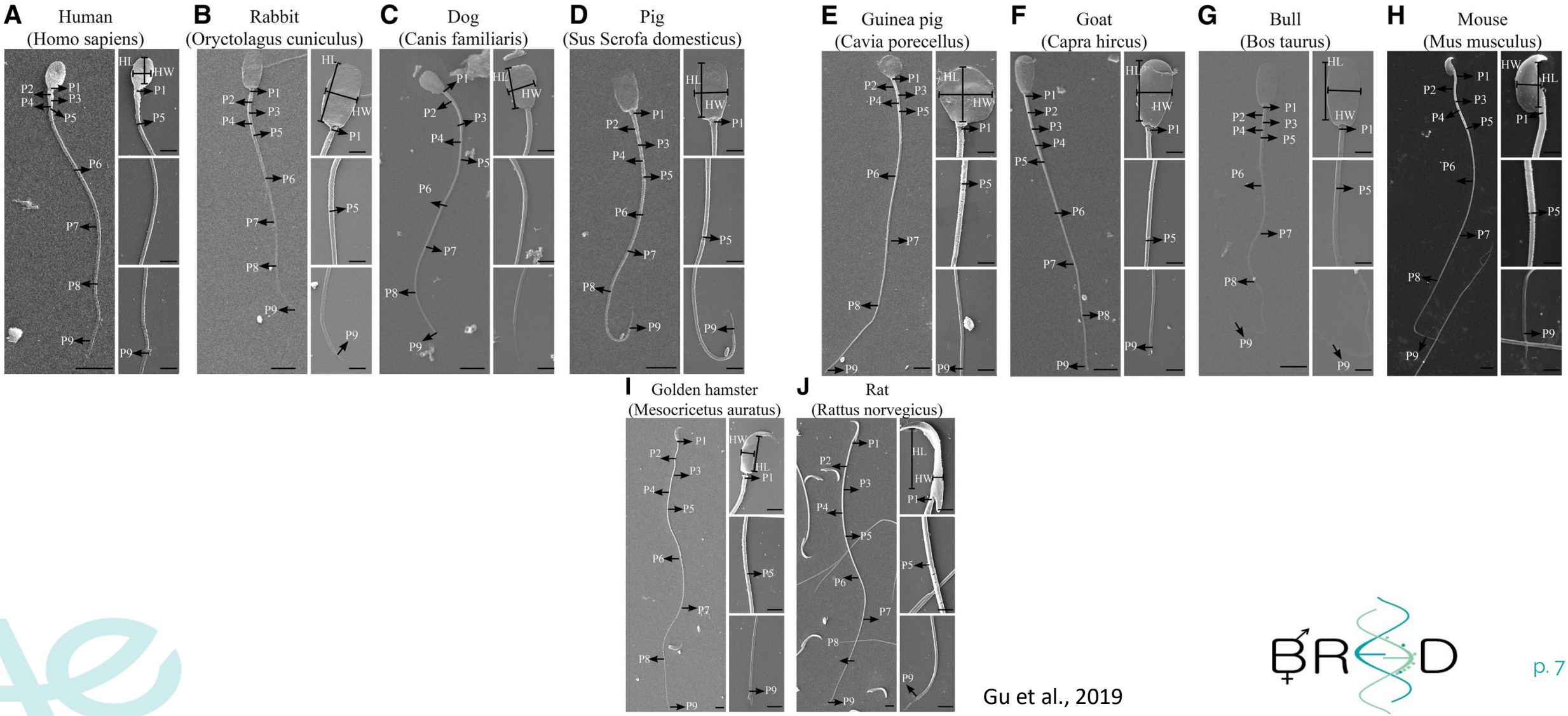
Asexual reproduction



« Clone »
➔ same genome
➔ Same phenotype

The reproduction… some general information

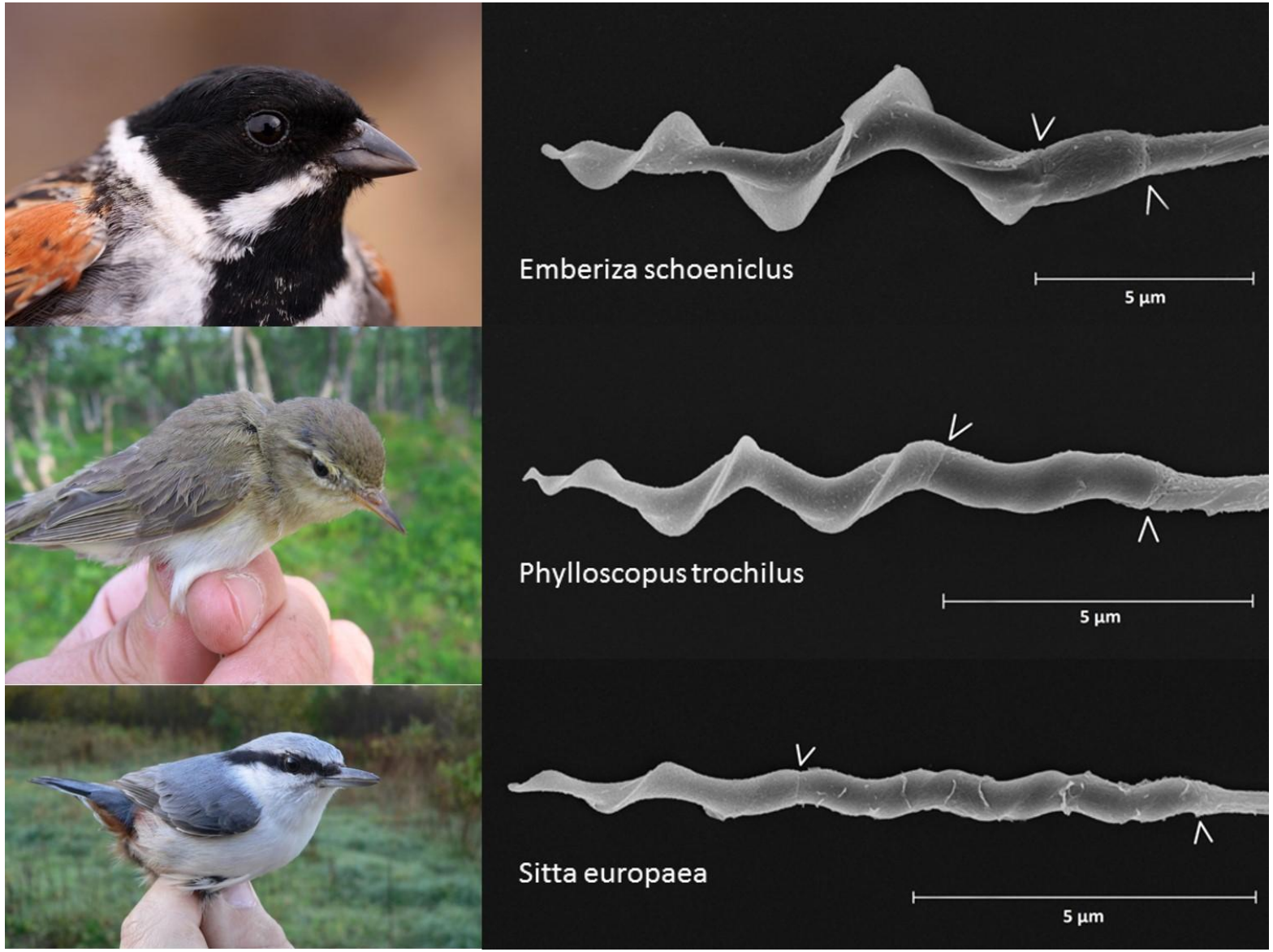
- Some important difference among the species!



Gu et al., 2019

The reproduction… some general information

- Some important difference among the species!



The reproduction… some general information

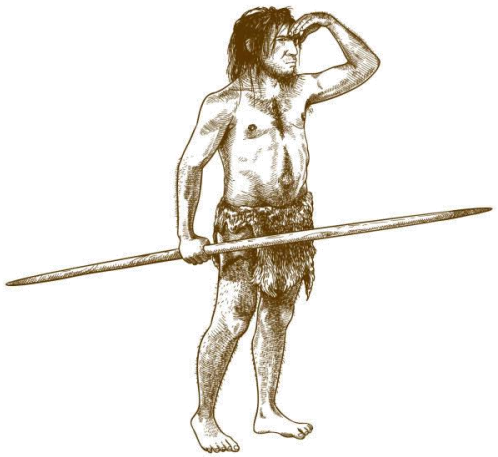
- The Livestock…an essential source of animal proteins

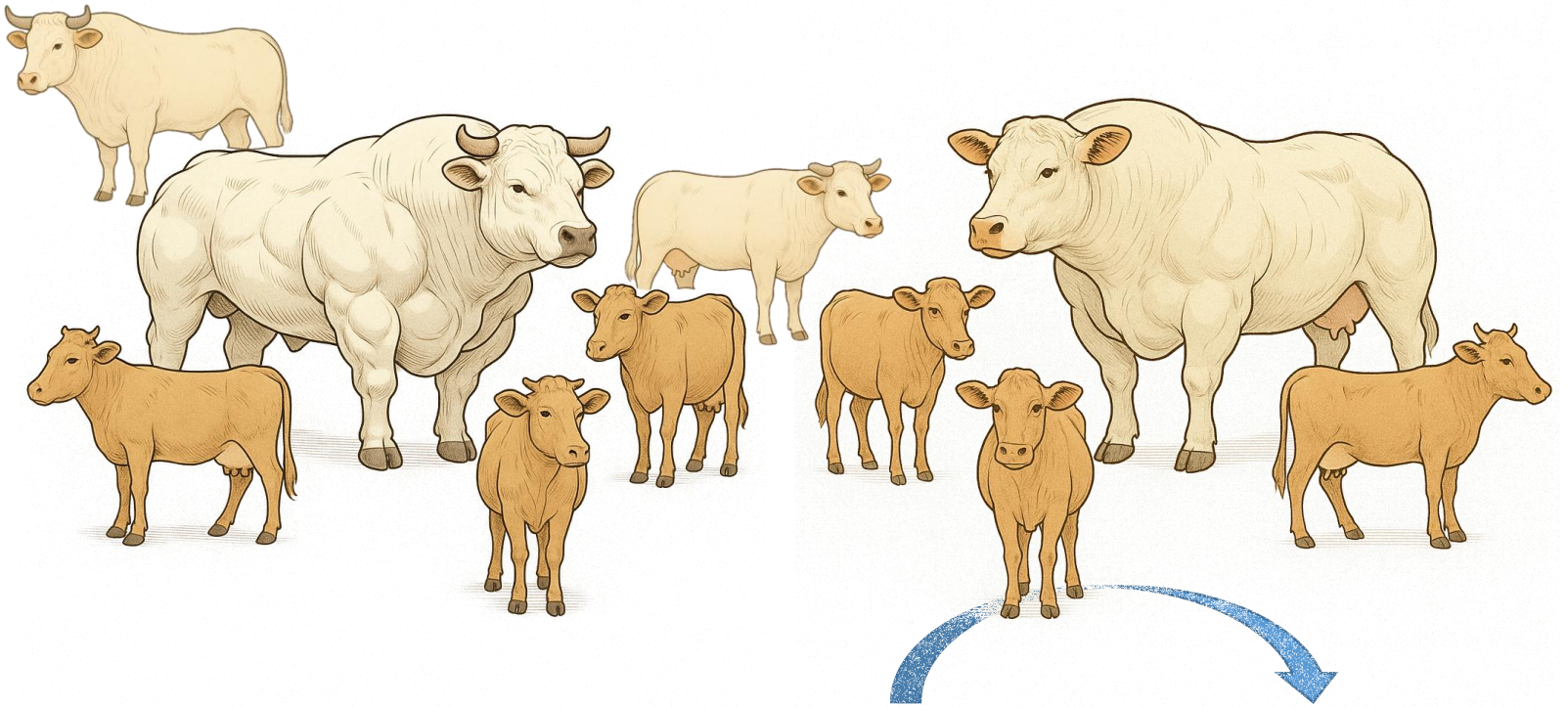
Increasing needs

Selection scheme !

The reproduction…a key stone for breeding industries

The principle of animal selection





Definition of a trait of interest - Identification of the best ones - reproduction

The reproduction…a key stone for breeding industries

The principle of animal selection

- Trait of interest?

  Health, longevity, reproduction, growth, FCR, disease resistance, developmental time…

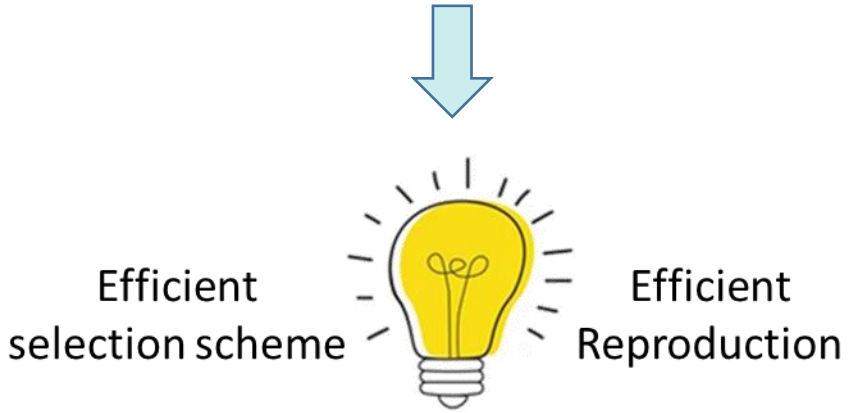
  ➔ Selection objective definition

The reproduction…a key stone for breeding industries

The principle of animal selection

The chosen one !

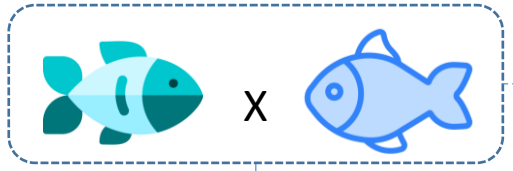
How to diffuse his advantageous phenotype in all the population?



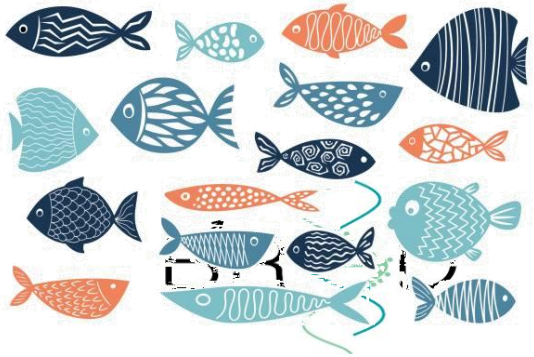
The reproduction…a key stone for breeding industries

The principle of animal selection

Selection scheme



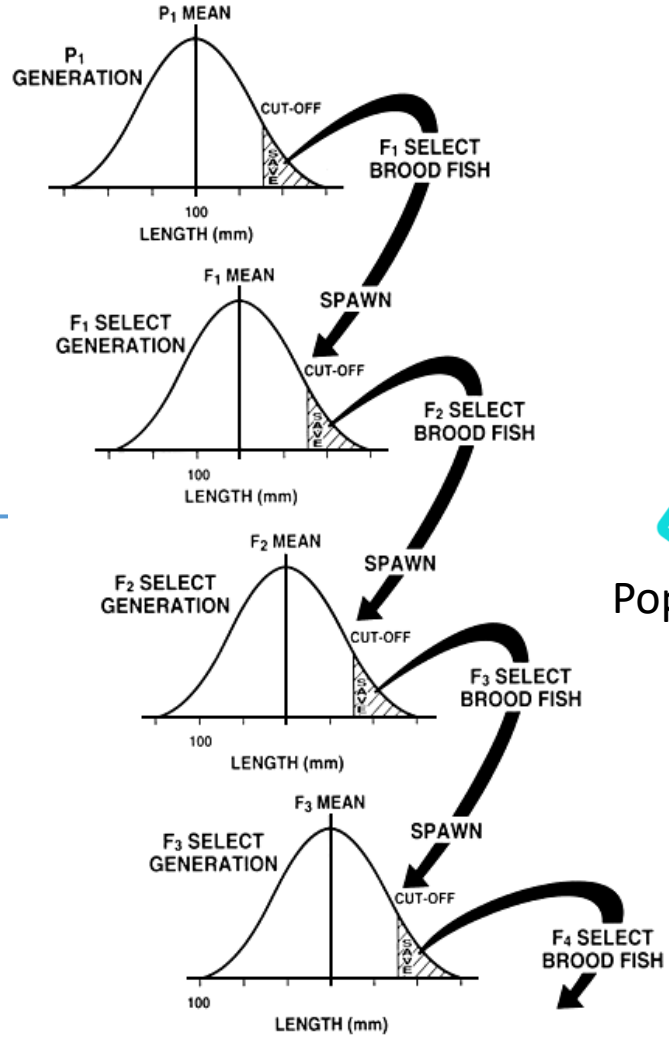
Chosen individuals



The reproduction…a key stone for breeding industries

The principle of animal selection

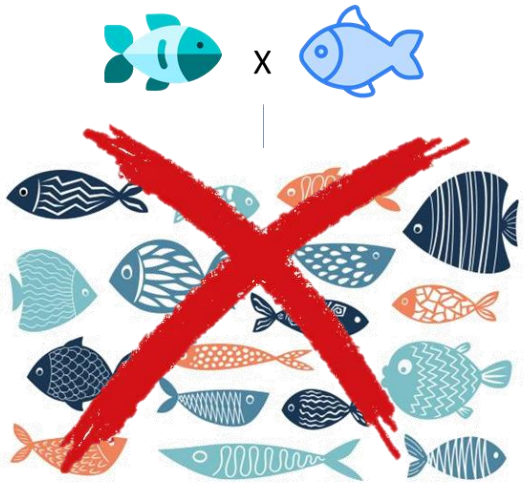
Selection scheme



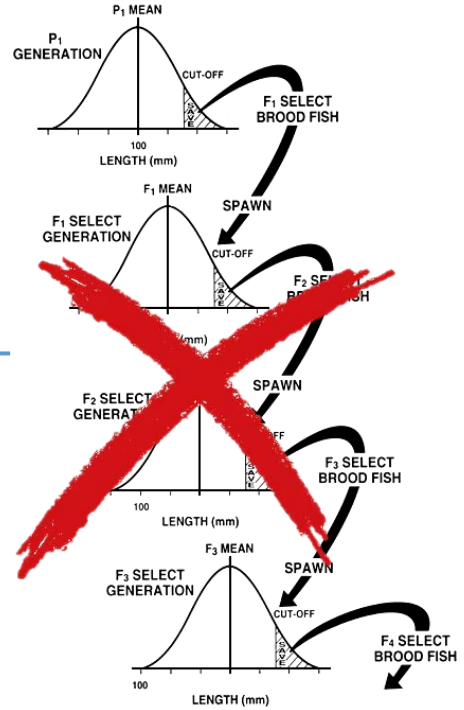
Population performance

The reproduction…a key stone for breeding industries

- Reproduction as a driver of genetic improvement



Fertility problems



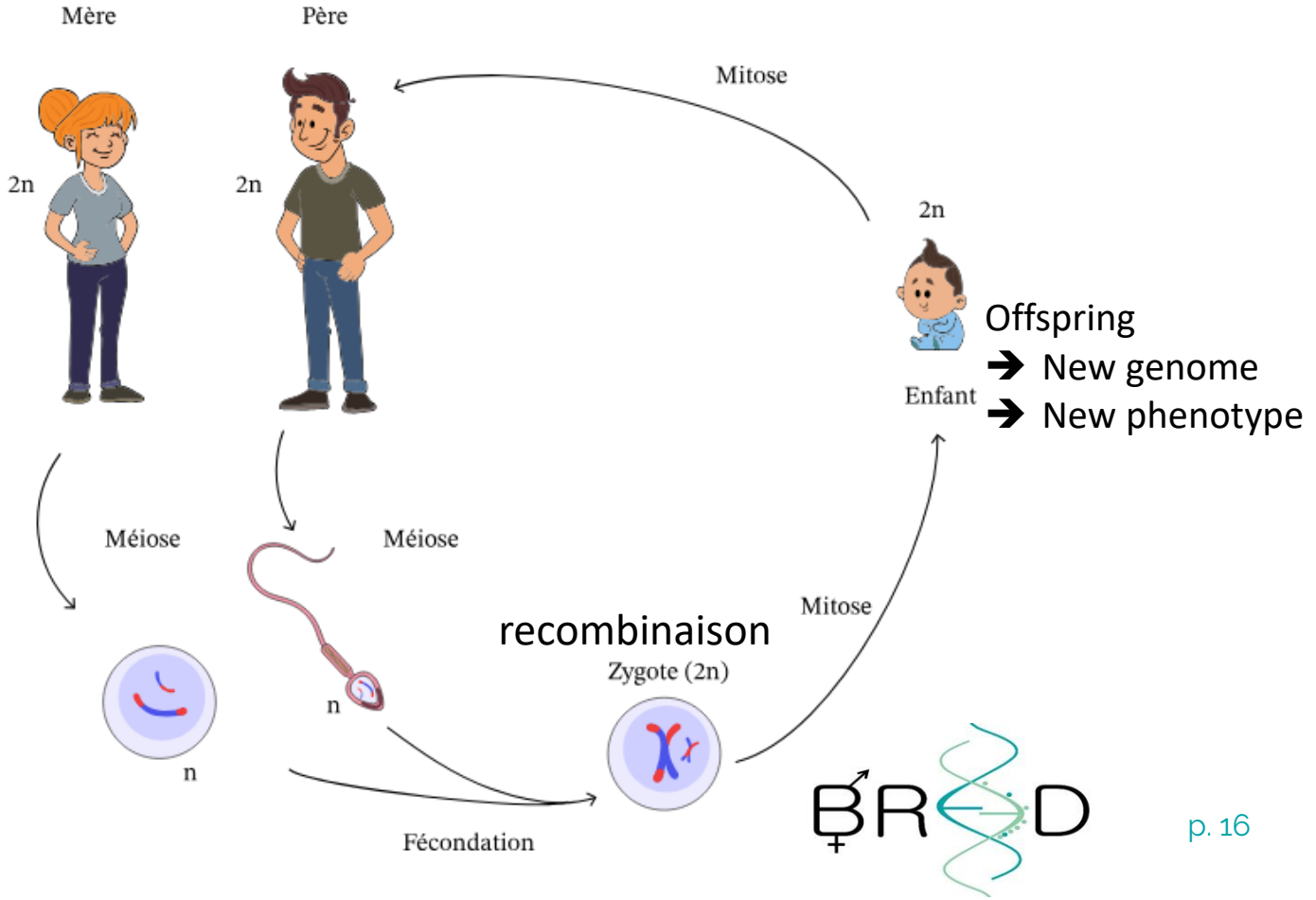
No sperm, no gain !

The reproduction…a key stone for breeding industries

- Reproduction: the pillar of improved genetic diffusion

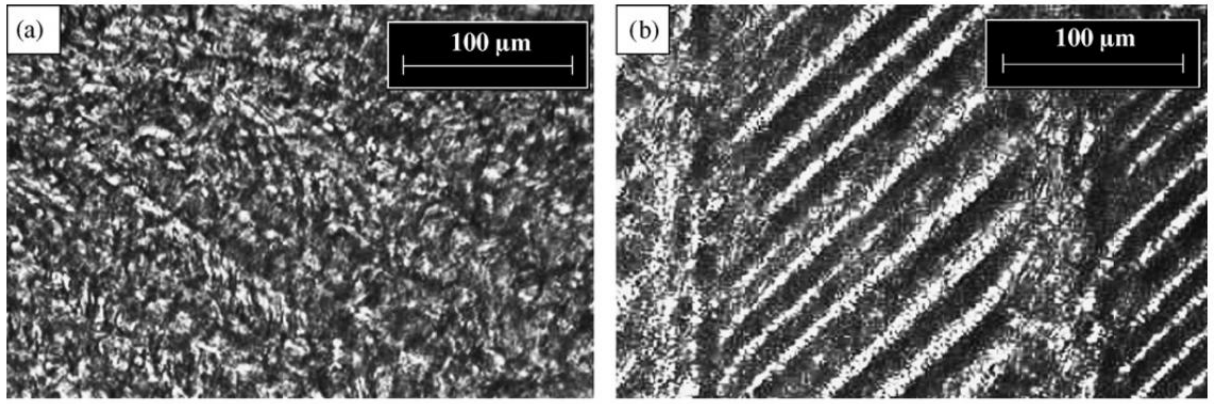
Population **renewal**

Sexual reproduction



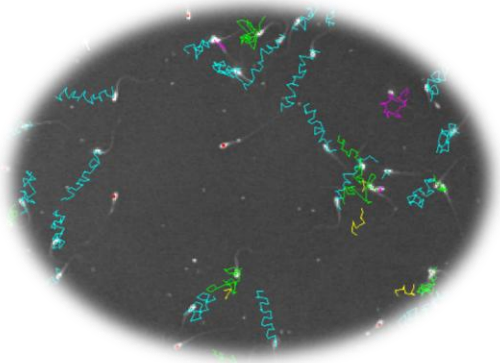
The reproduction…a key stone for breeding industries

Reproductive efficiency…how to manage it?



Frozing / Thawing conditions (kinetics, AO…)

Freeze-dried SPZ !



Prathalingam et al., 2011; Olaciregui et al., 2015/2016/2017, Keskintepe et al., 2015, Liu et al., 2014, Hirabayashi et al., 2005…

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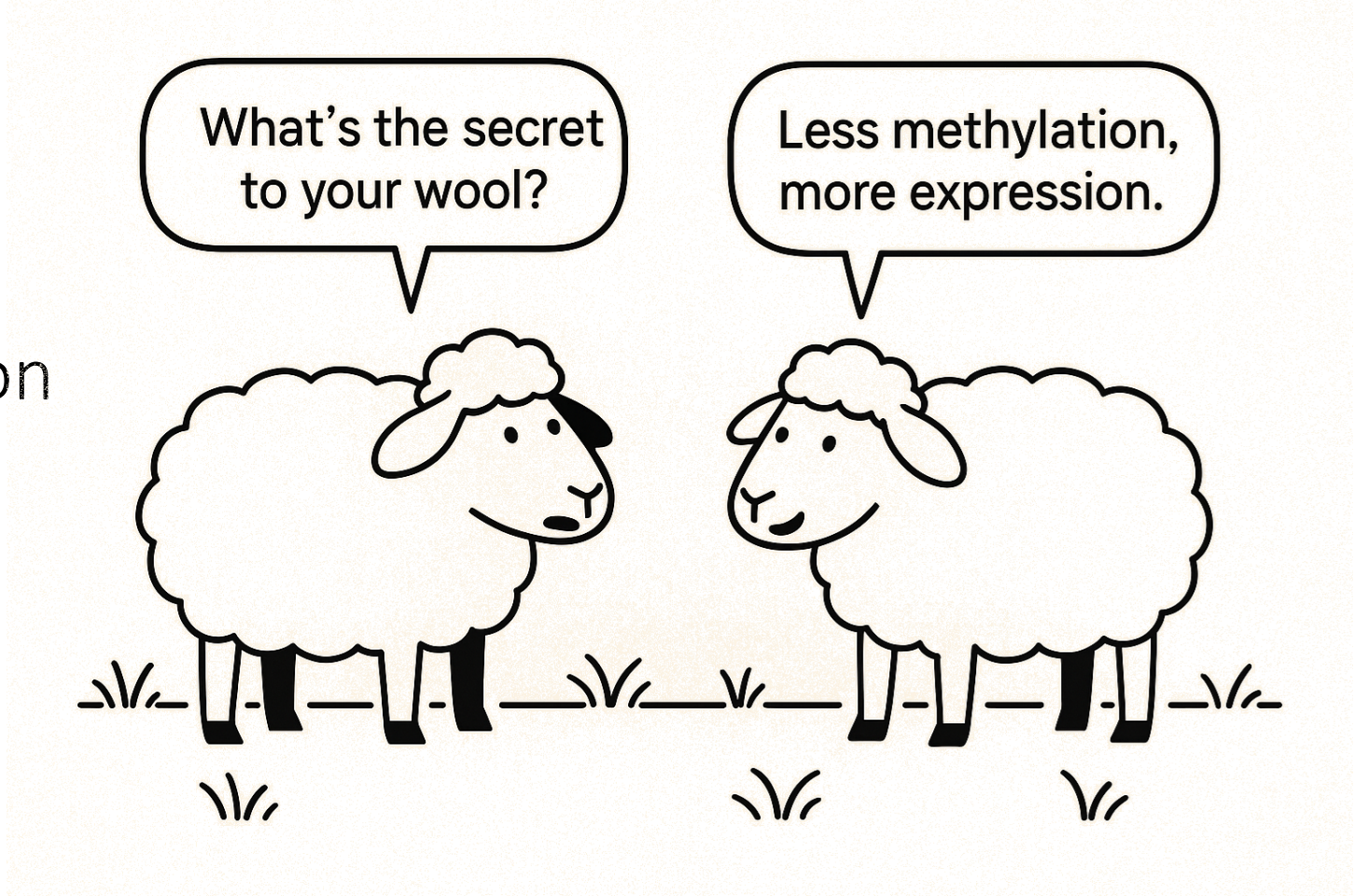
The male fertility prediction

From old dogma to the future!



The male fertility prediction

- Decades of fertility prediction approaches
  - « The biggest ones are the more fertile! »

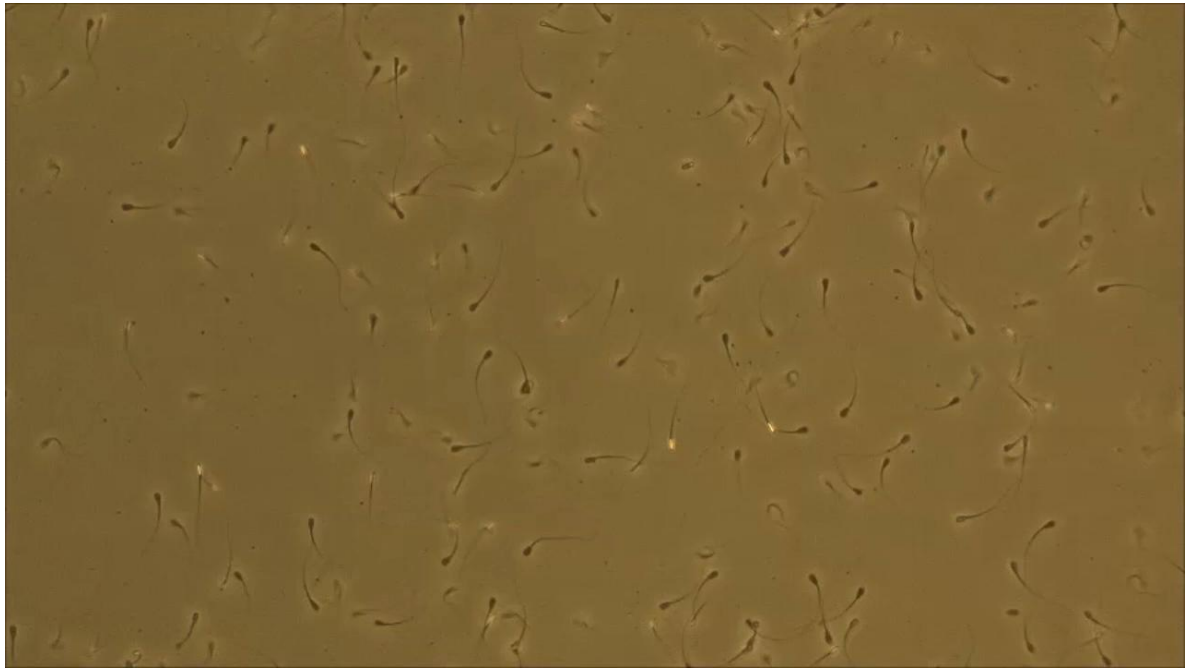
The male fertility prediction

- Decades of fertility prediction approaches
  - « I can not see through it…this ejaculate must be fertile ! »

« I can see clear enough…the sperm is gone! »

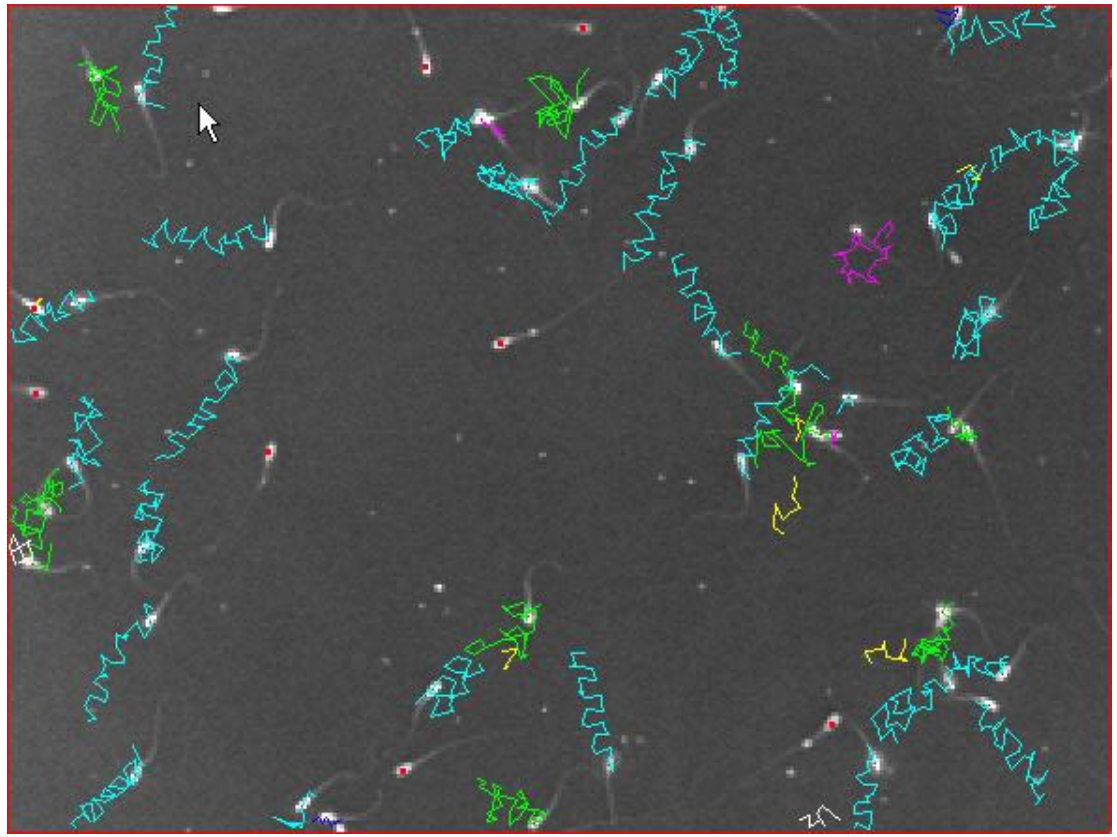
The male fertility prediction

- Decades of fertility prediction approaches
  - « the more they are motile, the more they are fertile! »



The male fertility prediction

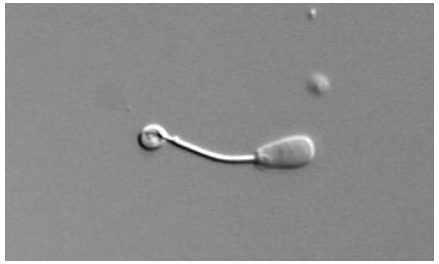
- Decades of fertility prediction approaches
  - « the faster are the fertile! »

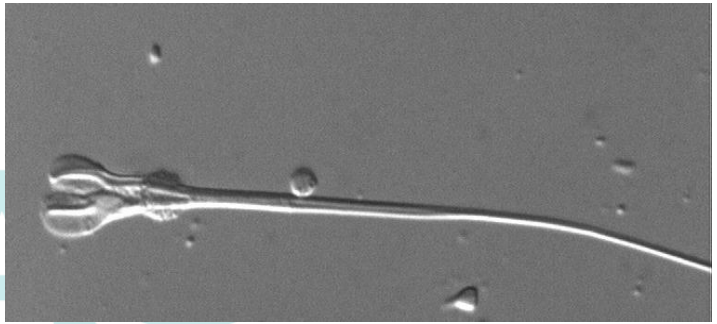
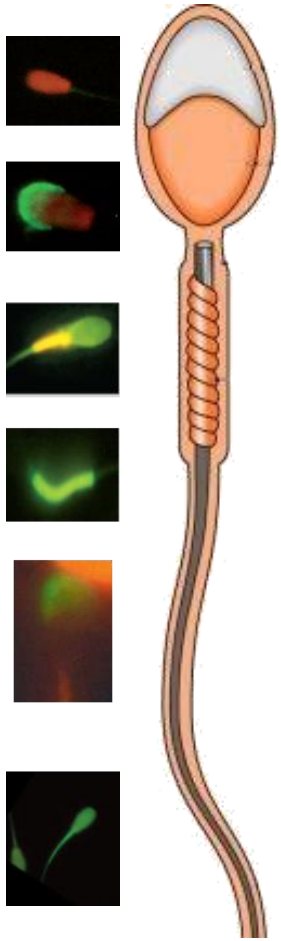
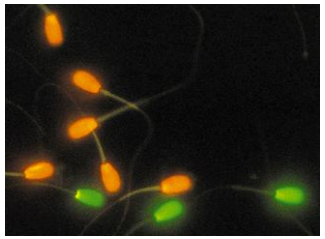


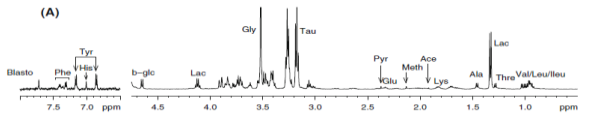
The male fertility prediction

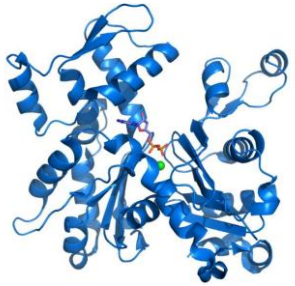
- Decades of fertility prediction approaches
  - “My compounds define my functionality…and my fertility!”







The male fertility prediction

- Decades of fertility prediction approaches
  - The fertility is a multiparametric process…the fertility prediction has to be the same!

Table 3
Simple correlations (Spearman, r) between cryopreserved bovine semen quality parameters and fertility value (n = 114 ejaculates, $P < 0.05$).

Analyses	Technology	Condition	Parameters	r	r^2
Oxidation	Flow cytometry	Ratio: 40m/10m	Viable oxidized, %	0.494	0.244
Sperm motility (speed)	CASA	T0	VSL, μm/s	−0.340	0.116
Acrosome/viability	Flow cytometry	Delta (T4−T0)	Viable with disrupted acrosome, %	0.322	0.104
Sperm morphologic abnormalities	CASA	T0	DMR, %	−0.306	0.094
DNA compaction	Flow cytometry	T4	Sperm with fragmented DNA, %	−0.287	0.082
Mitochondrial activity	Flow cytometry	T0	Polarized mitochondria sperm, %	0.271	0.073

Sellem (2015); Malama (2024)

The male fertility prediction

- Decades of fertility prediction approaches
  - The fertility is a multiparametric process…the fertility prediction has to be the same!

Table 4
Prediction models of the fertility value for the two set of ejaculates.

Models	Technologies	Protocols	r^2_{adj} (n = 114)	r^2_{adj} (n = 39)
Complete model	Flow cytometry and CASA	Oxidation, acrosomal integrity, DNA compaction, mitochondrial activity, viability, velocity, sperm morphologic abnormalities	0.40	0.39
Simplified model	Flow cytometry and CASA	Oxidation, DNA compaction, velocity, sperm morphologic abnormalities	0.34	0.37
Flow cytometry model	Flow cytometry	Oxidation, acrosomal integrity, DNA compaction, mitochondrial activity	0.33	0.22
CASA model	CASA	Sperm morphologic abnormalities, velocity	0.24	0.23

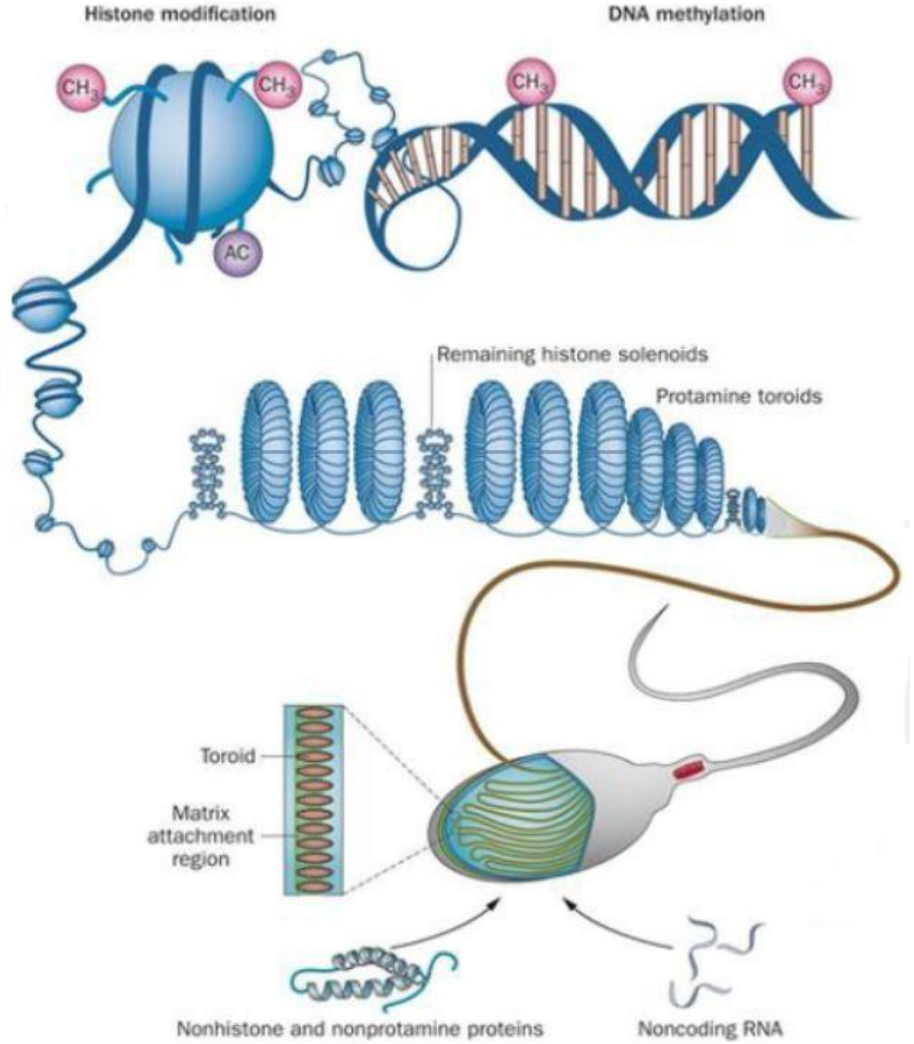
- Some enhancements….but it’s definitively not enough!
- Need another point of view!

Sellem (2015); Malama (2024)

The male fertility prediction

- New approach: Epigenome of male fertility!
  - DNA methylation
  - Histone (protamine) code
  - Small non-coding RNAs

- Sperm epigenome is essential for
  - spermatogenesis
  - Final sperm maturation
  - Embryo development



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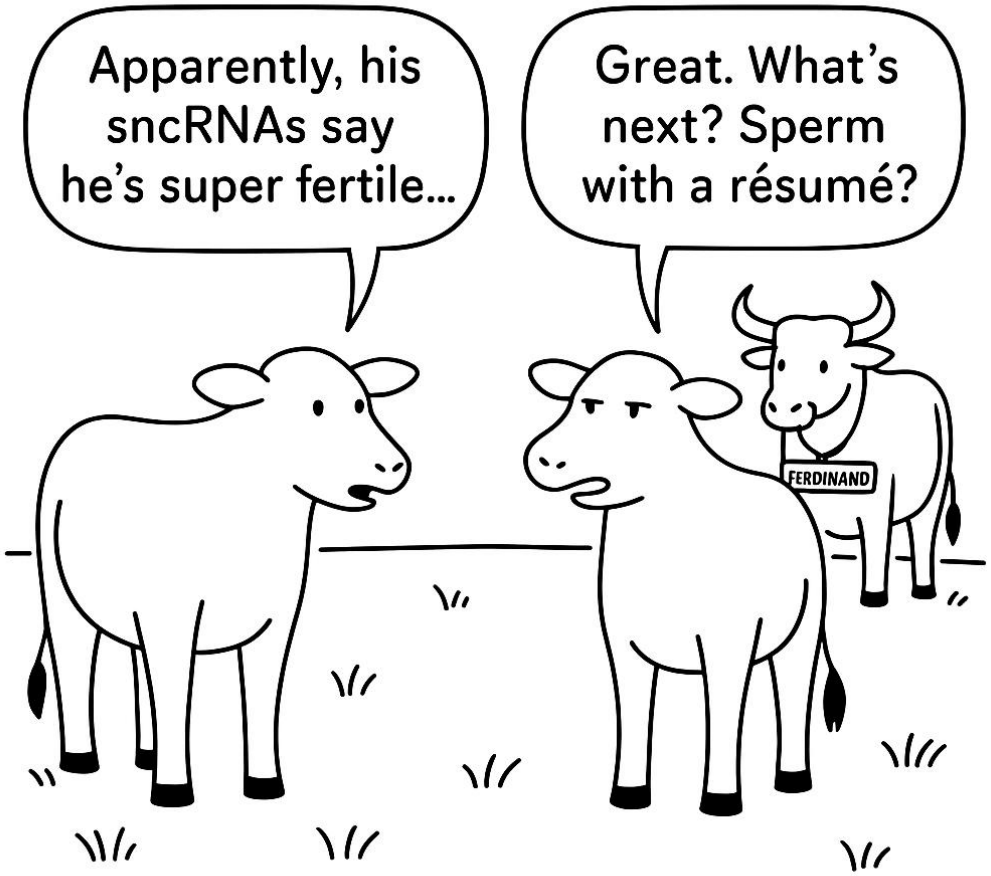
- The sperm-borne sncRNAs: translating past events or future developments?

Spermatogenesis, sperm maturation and fertility biomarkers!



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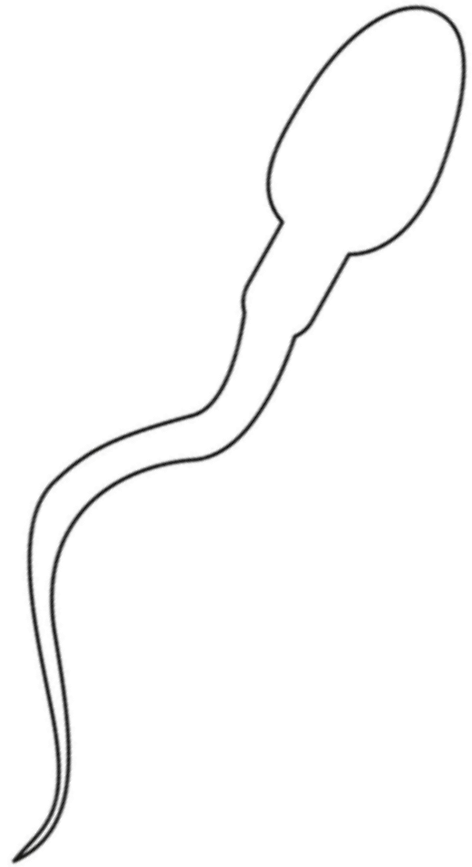
The sperm-borne sncRNAs

- The sperm is a “key element” in a breeding program



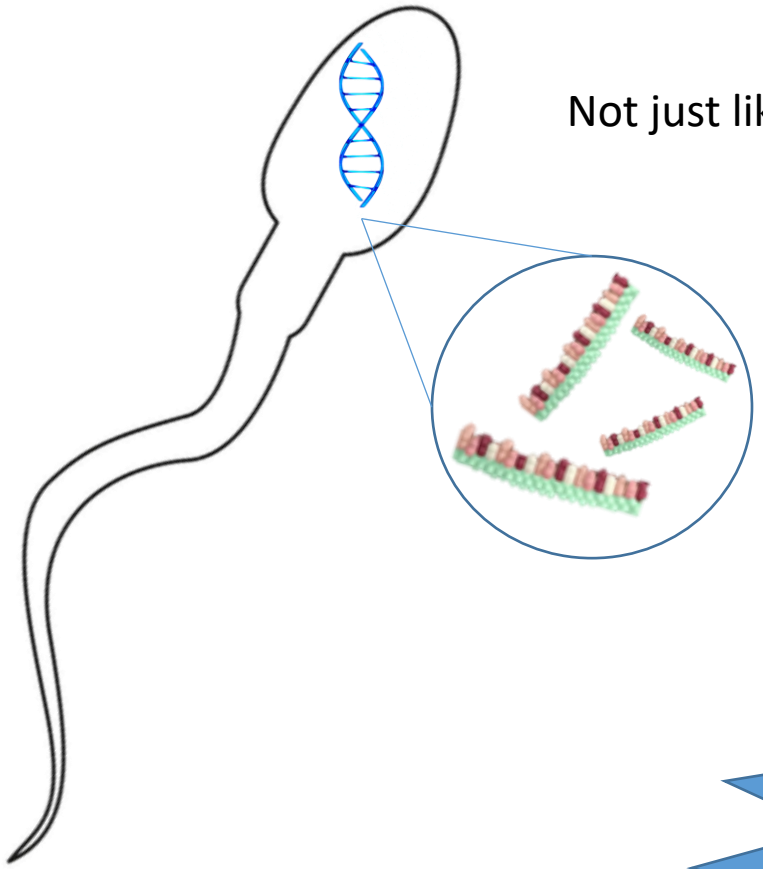
- Bull number <<<< cow number
- The semen is hugely diffused on the field
- Many cows are inseminated with the same ejaculate/bull

- Importance to well manage the sperm production/quality/fertility!

- Importance to cumulate scientific knowledge on sperm

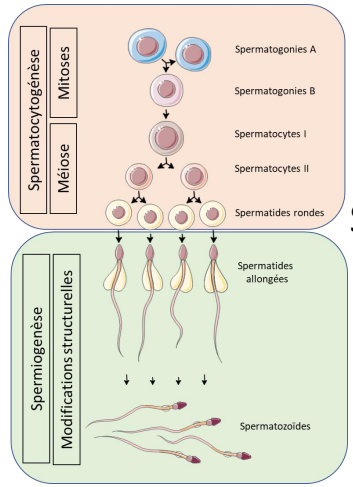
The sperm-borne sncRNAs

- Sperm is full of sncRNAs!

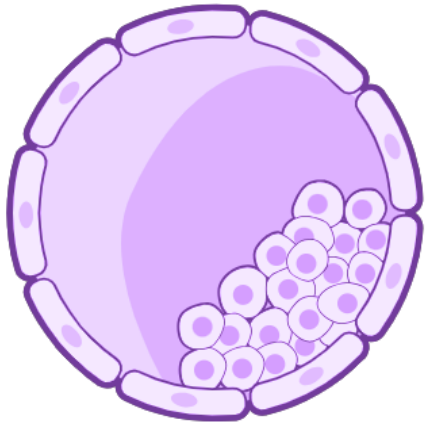


Not just like an Uber transporting DNA !!

Sperm are fool of sncRNAs



Spermatogenesis vestige?



Regulation of embryo gene expressions?

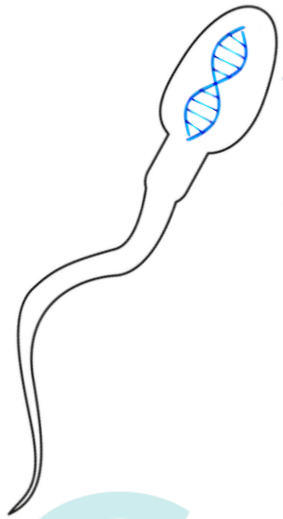
Interesting biomarkers in both cases!

Carone et al (2010); Grandjean et al (2015); Rodgers et al (2015); de Castro Barbosa et al. (2016); Sharma et al. (2016)

The sperm-borne sncRNAs

Some general information

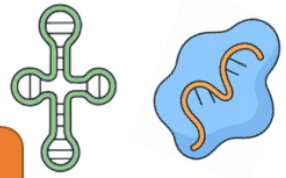
- Coding or not, short or long…



Non-coding RNAs

coding mRNA

Housekeeping (mRNA maturation & protein synthesis)



- tRNA — Protein synthesis
- rRNA — Protein synthesis
- snoRNA — mRNA splicing

Regulatory

Small RNAs

- miRNA
- piRNA
- rsRNA
- tsRNA

Long RNAs

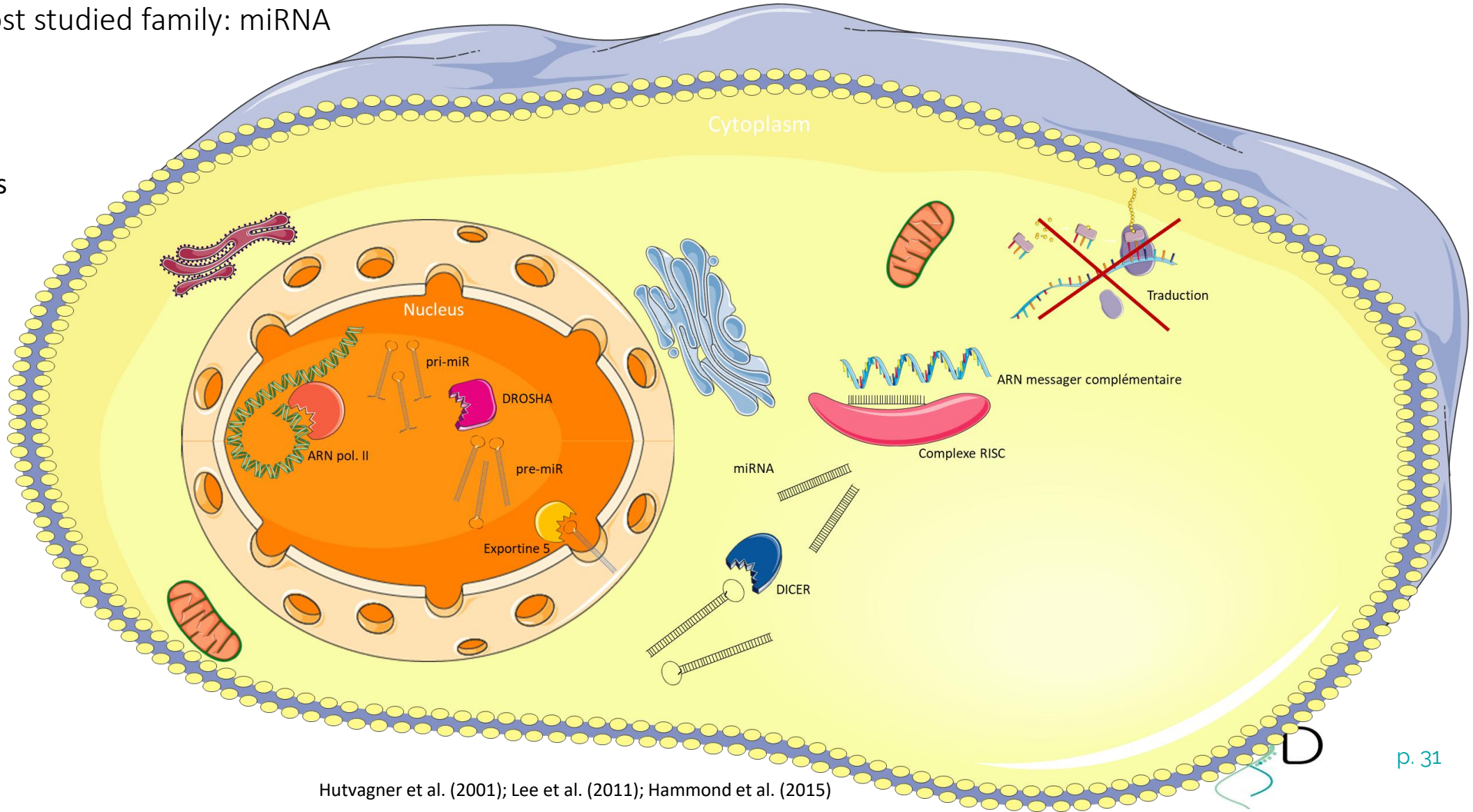
- circRNA
- lncRNA

The sperm-borne sncRNAs

Some general information

- The most studied family: miRNA

~50% intergenic regions
Alone or in clusters

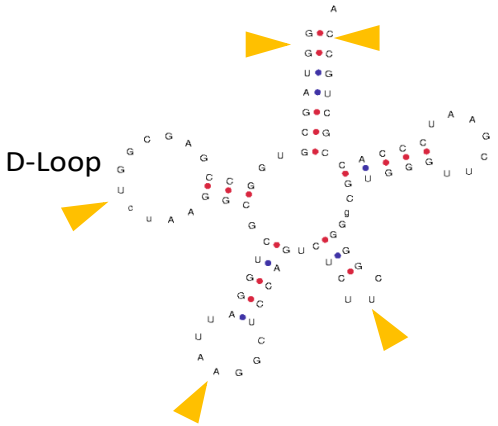


Hutvagner et al. (2001); Lee et al. (2011); Hammond et al. (2015)

The sperm-borne sncRNAs

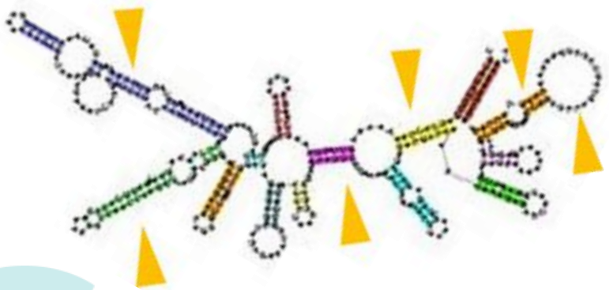
Some general information

- The tsRNAs and rsRNAs…functionally active?

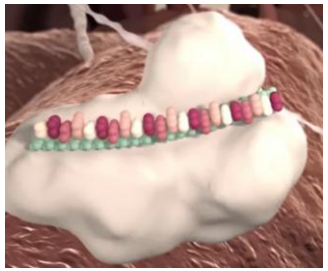


tsRNA ou tRFs
5'et 3'-tRH (30-35nt)
tRF5, tRF3 et i-tRF (18-22nt)

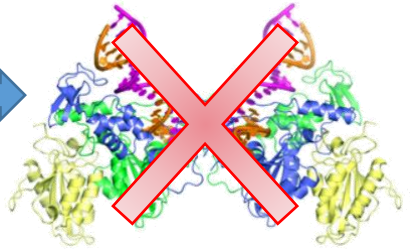
- RNAseZ, DICER, Angiogenine



rsRNA ou rRFs
5'et 3'-rRH (20-45nt)



Pas de protéine

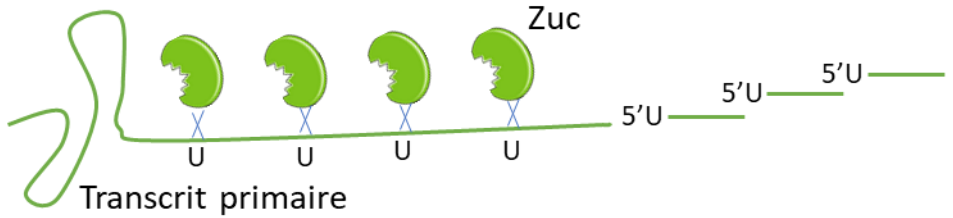


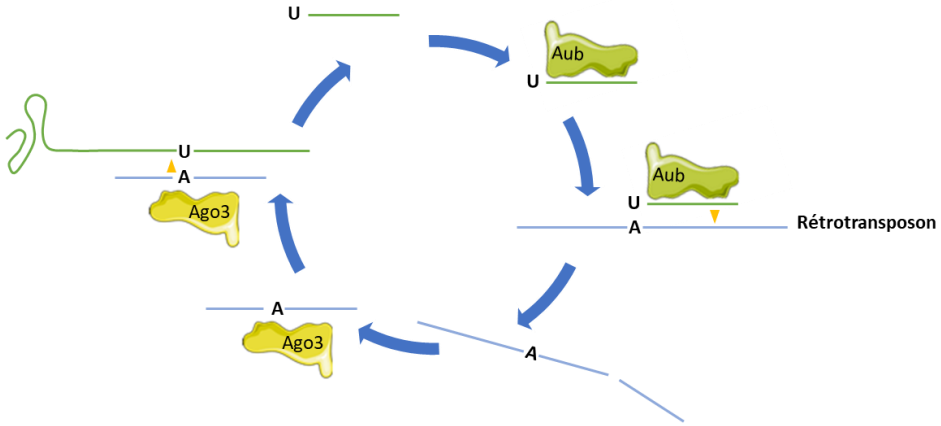
Zhang et al. (2016); Wei et al. (2013); Chen et al. (2017)

The sperm-borne sncRNAs

Some general information

- The small non-coding RNAs
  - The piRNAs, a particular family!
    - Two biogenesis pathways
      - « Primary » leading to « piRNA 1U »
      - « Ping-Pong » leading to « piRNA 10A »





- Inside in germ line…
  - Transposable elements regulation
  - Genome stability

- …but also in somatics!
  - Not complementary with transposable elements
  - Loaded in « pi-RISC » and mRNA regulation?

Aravin et al., (2008); Weick et al., (2014); Gou et al., (2014); Wu *et al.,* (2020)

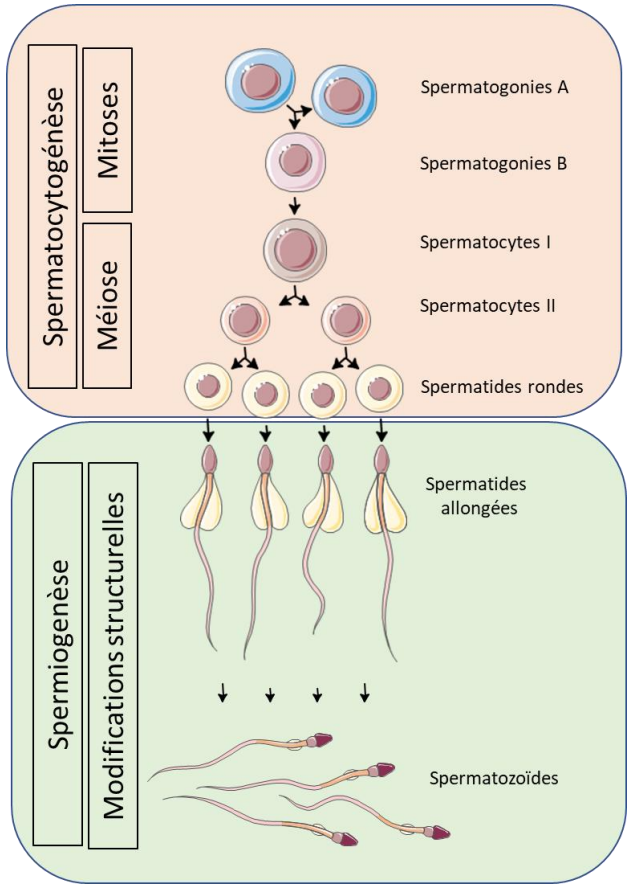
The sperm-borne sncRNAs

sncRNA in spermatogenesis

- sncRNAs are involved in germ line maturation
  - KO genes from miRNA or piRNA pathways:
    - Mitosis & meiosis disturbances (spermatocytogenesis)
    - Elongation perturbation (spermiogenesis)
    - Chromatin compaction & organization perturbation (spermiogenesis)
    - Flagella formation (spermiogenesis)

    Infertility

  - Some studied specific roles of miRNAs
    - mir-34c: Apoptosis regulation
    - mir-122a: histones -> protamines transition
    - mir-29: meiosis regulation



Bouhallier et al. (2010); De Mateo (2014); Kotaja (2014); Garcia-Lopez et al. (2015); Russel et al (2016); Zimmerman et al (2014)

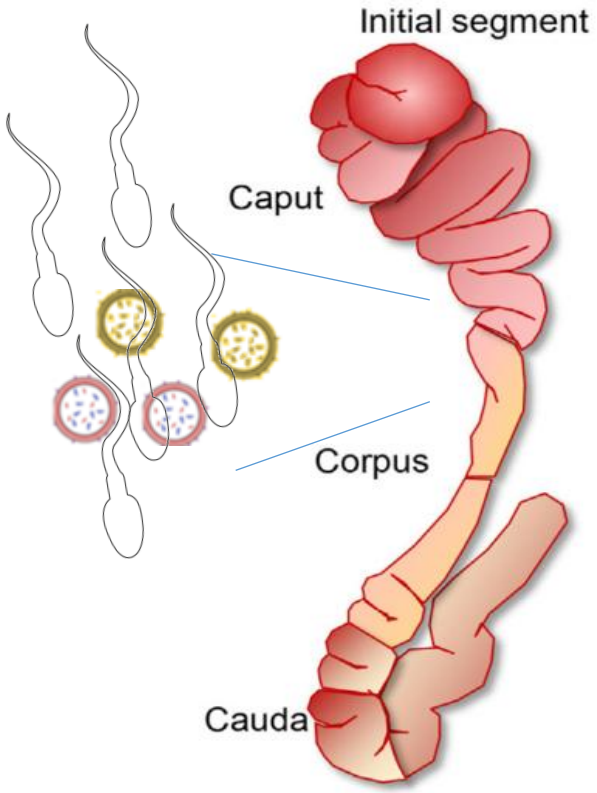
The sperm-borne sncRNAs

sncRNA in sperm maturation

- Sperm are reloaded in sncRNAs during epididymis journey: role of Extracellular vesicles!
  - Provided by somatic cells of epididymis:
    Different profiles according to the organ section
  - (re)load the sperm in
    Proteins, lipids, glycans
    sncRNAs
  - What's for ????
    The post testis sperm is transcriptionally muted

    Required for the final maturation?

    Required for normal embryo development?
    The testis is isolated from the systemic….epididymis not !
    ➔ "Open windows" to the environmental disturbances



Belleannee et al (2013); Vojtech et al. (2014); Nixon et al (2015); Sharma et al. (2016); Donkin & Barres (2018); Chu et al (2019)

The sperm-borne sncRNAs

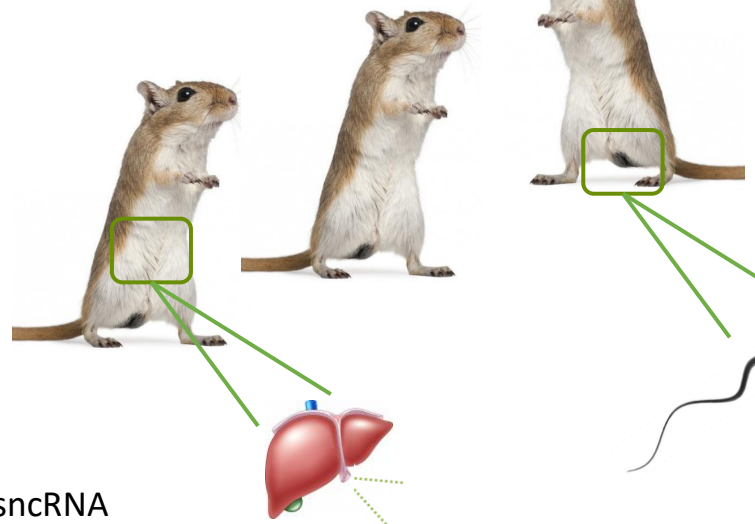
- Paternal transmission of environmental information
  - Sperm is a non-genetic inherited information vector

Fat diet

Father F0

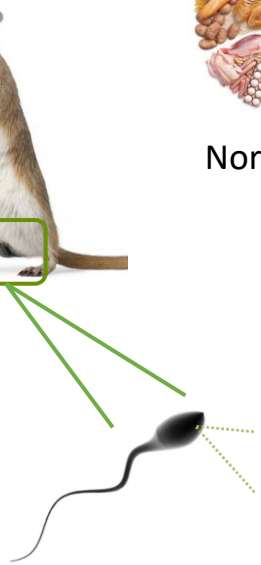
Pertubation of sncRNA expression profil (miRNA, tRF, piRNA)

Sons F1



Metabolic disorders

Normal diet



Pertubation of sncRNA expression profil (miRNA, piRNA)

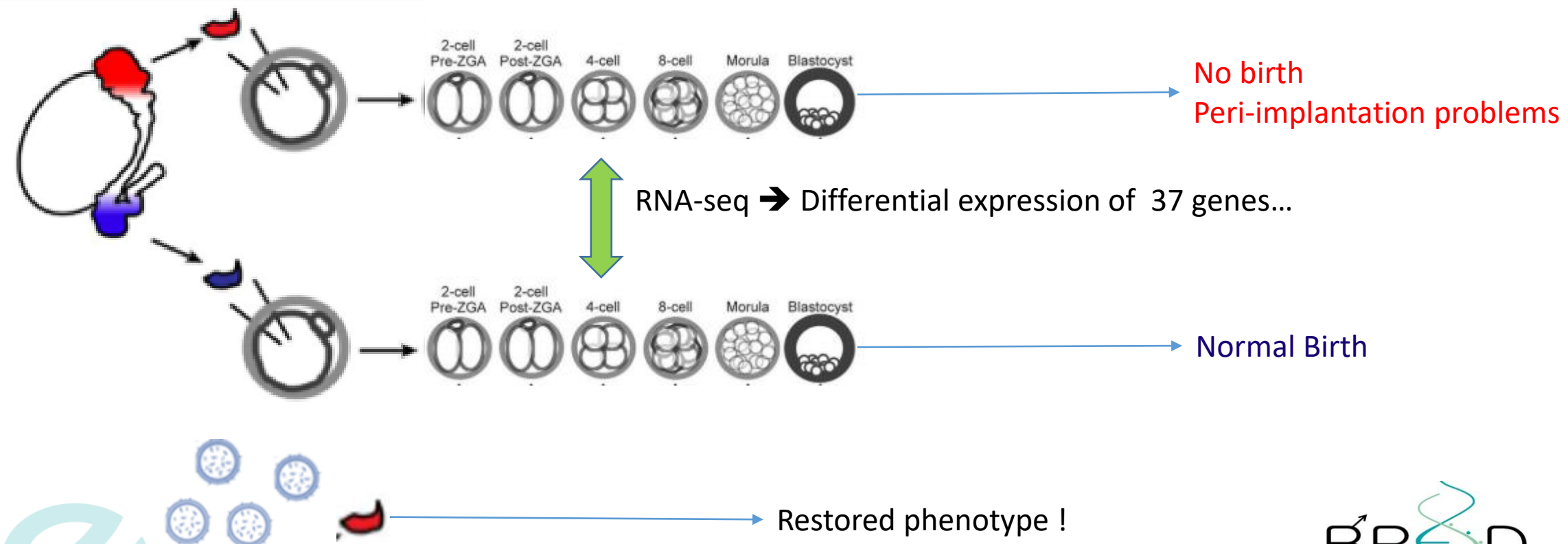
Carone et al (2010); Grandjean et al (2015); Rodgers et al (2015); de Castro Barbosa et al. (2016); Sharma et al. (2016)

The sperm-borne sncRNAs

- Paternal transmission of environmental information
  - Acquired sncRNAs are required for normal embryo development

Conine et al., 2018 : ICSI (mice) SPZ from Epididymis caput vs cauda

Identical blastocyte rate…

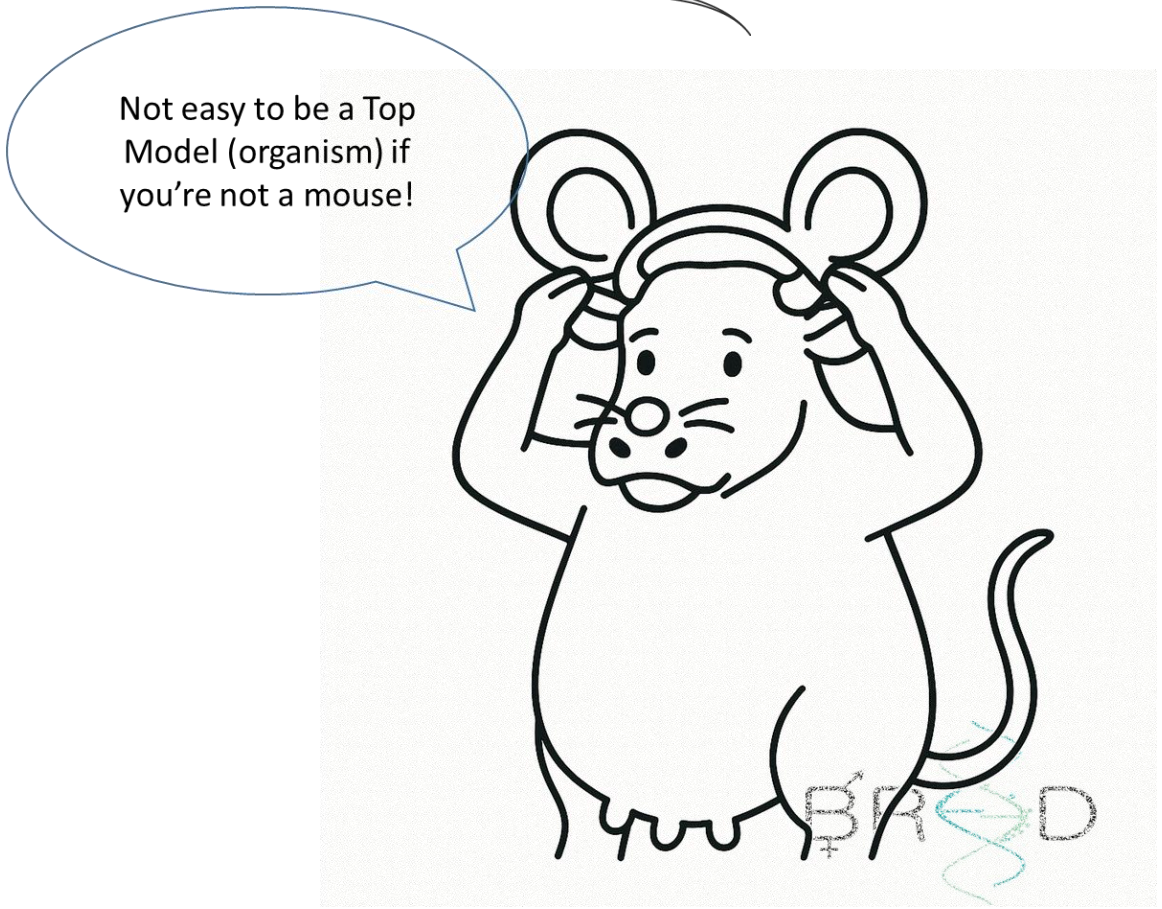


Conine et al (2018)

The sperm-borne sncRNAs

Sperm-born sncRNAs

- Absolutely required for normal spermatogenesis !
- Part of them acquired post-testis
- Involved in the first stages of embryo development
- Implied in phenotypic construction



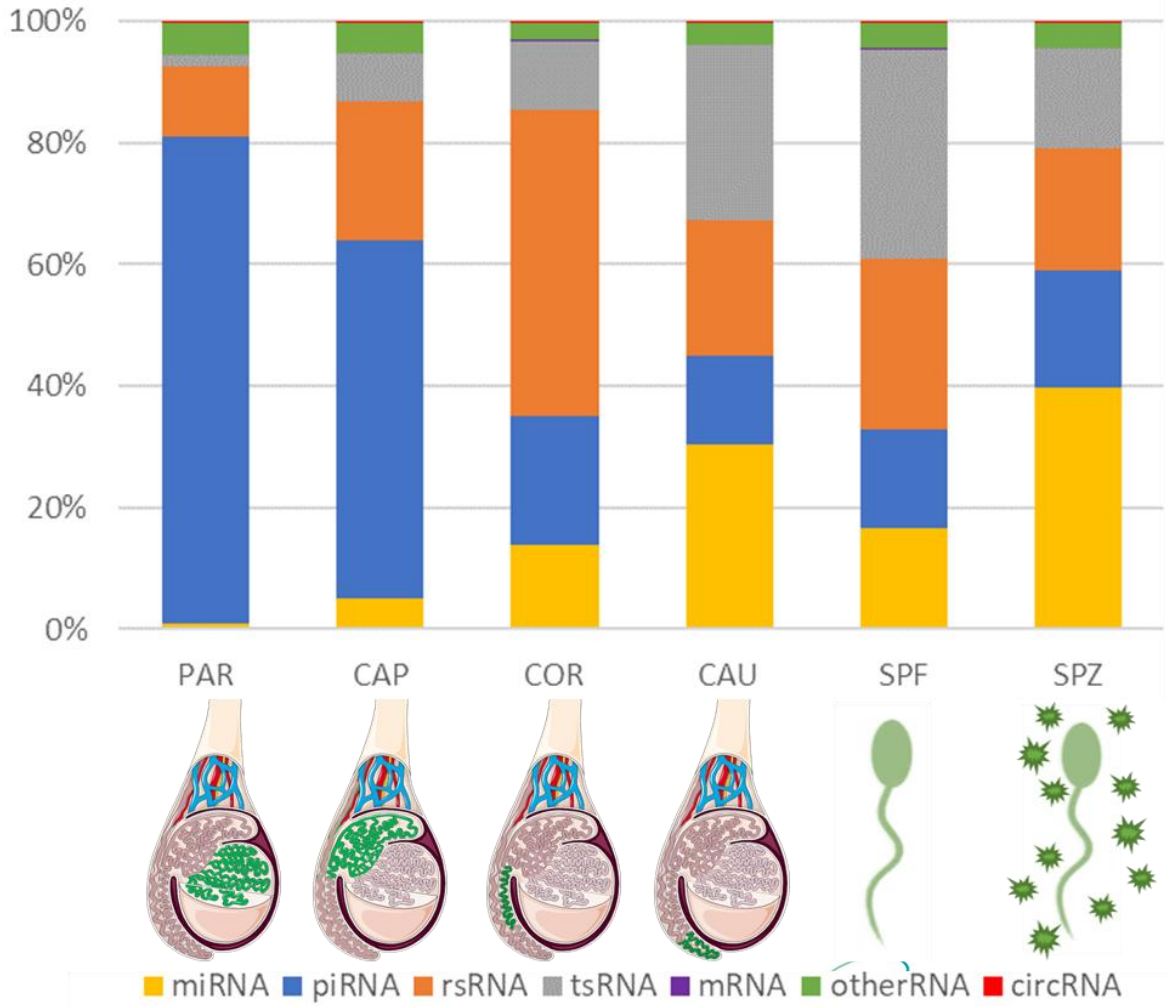
Need scientific knowledge for ruminants

- SeQuaMol project

The sperm-borne sncRNAs

Dynamics of cattle sperm sncRNAs during maturation, from testis to ejaculated sperm

- Hegemony and Decline of piRNAs
  - ~80% of the read in the testis
  - 60% and 20% upon entry in epididymis
- miRNAs and tsRNAs are post-testis acquired
- rRNAs are mainly acquired in corpus



Sellem et al., 2021

The sperm-borne sncRNAs

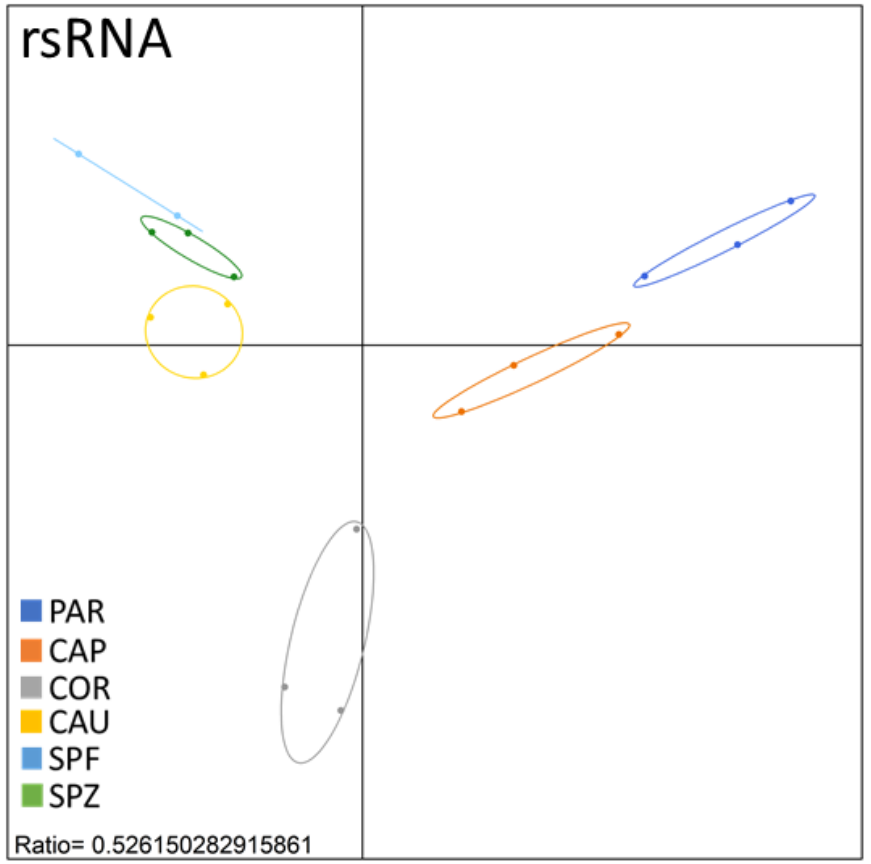
Dynamics of cattle sperm sncRNAs during maturation, from testis to ejaculated sperm

- Male tractus origin explains >50% global variability
  - miRNAs, tsRNAs and rsRNAs: same profile
  - piRNAs: only PAR and CAP are isolated from others

- Number of differentially expressed sncRNAs increase
  - With the “distance” among tractus location

(adj p values / DESEQ2)

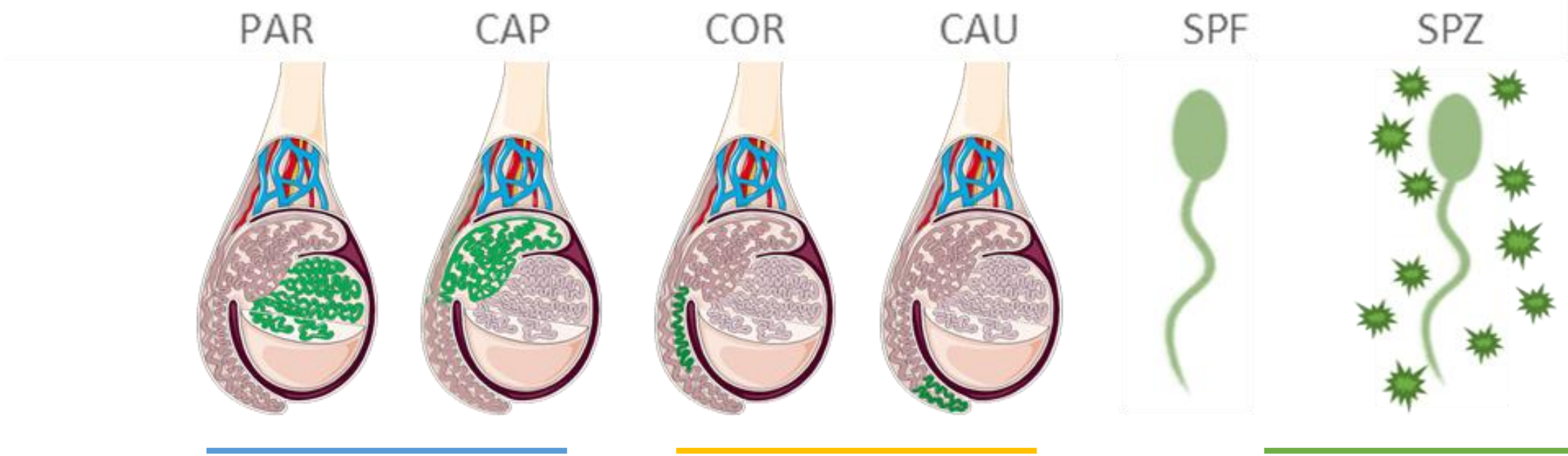
Testis	Epididymis			Ejaculated sperm	
PAR	CAP	COR	CAU	SPF	SPZ
PAR	4 436	15 6611	120 914	85 379	194 062
	CAP	52 471	77 264	65 132	82 921
		COR	5 209	9 471	21 074
			CAU	1 284	1 188
				SPF	106



Sellem et al., 2021

The sperm-borne sncRNAs

- Focus on miRNA targets according to the male tractus location



Cell cycle
Ubiquitin & ERAD pathways
➔ Organelles & compounds degradation

mTOR, PI3K-AKT & AMP
➔ Mitochondrial functions
➔ Energetic metabolism
➔ Autophagy

HIPPO, morphogenesis
➔ Mitochondrial functions
➔ Energetic metabolism
➔ Autophagy

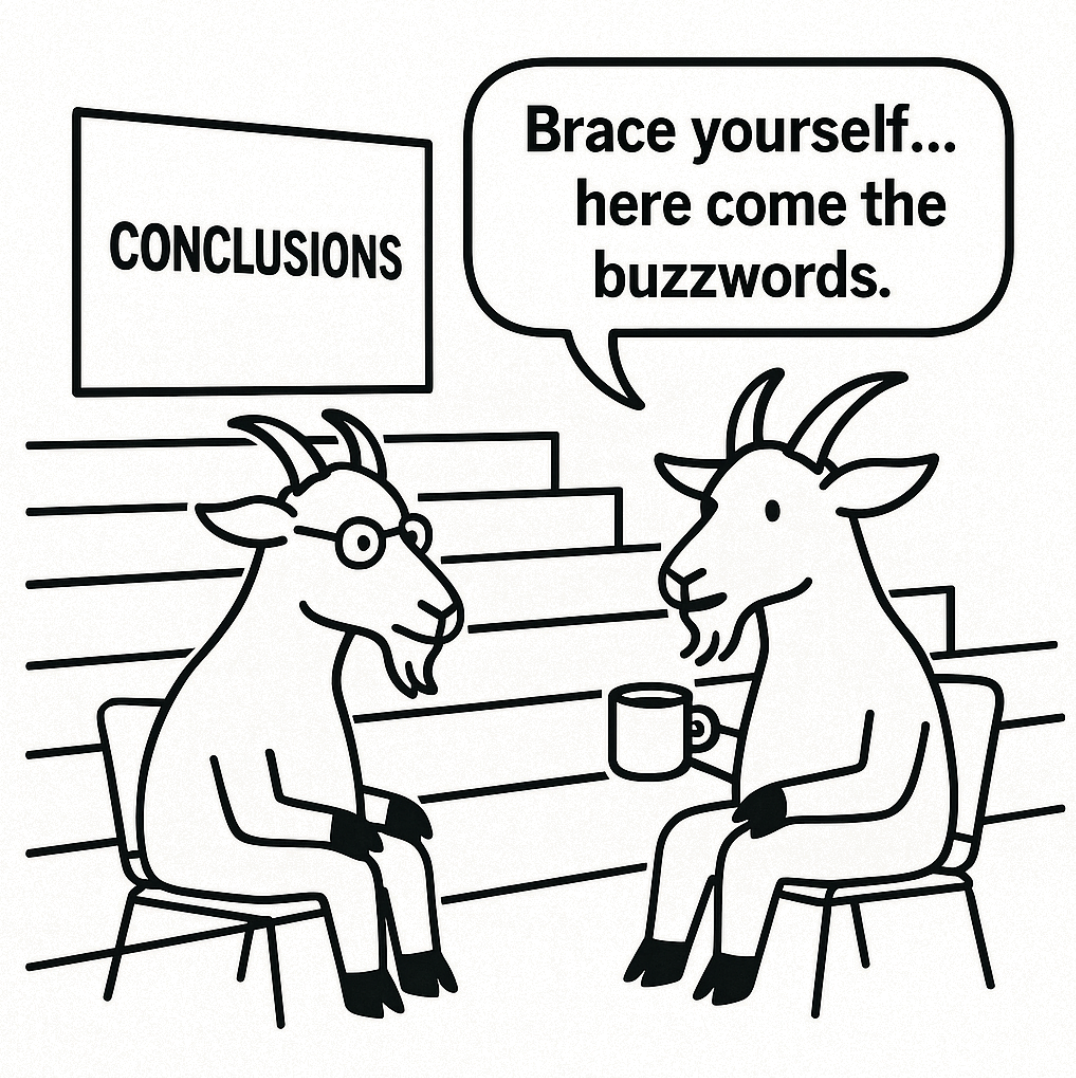
Spermatogenesis → Sperm maturation Embryo development → Embryo development

Sellem et al., 2021

Genetique
Genomique
Epigenetique
Evolution
GenE2

Conclusions

The most important points !



Conclusions

The points to keep in mind

- Sperm is highly important in the breeding sector (vector of genetic improvement)

- Sperm is not only sharing its DNA…but also a lot of epigenetic marks
- sncRNAs are required for normal spermatogenesis, sperm maturation and emb. Dev.

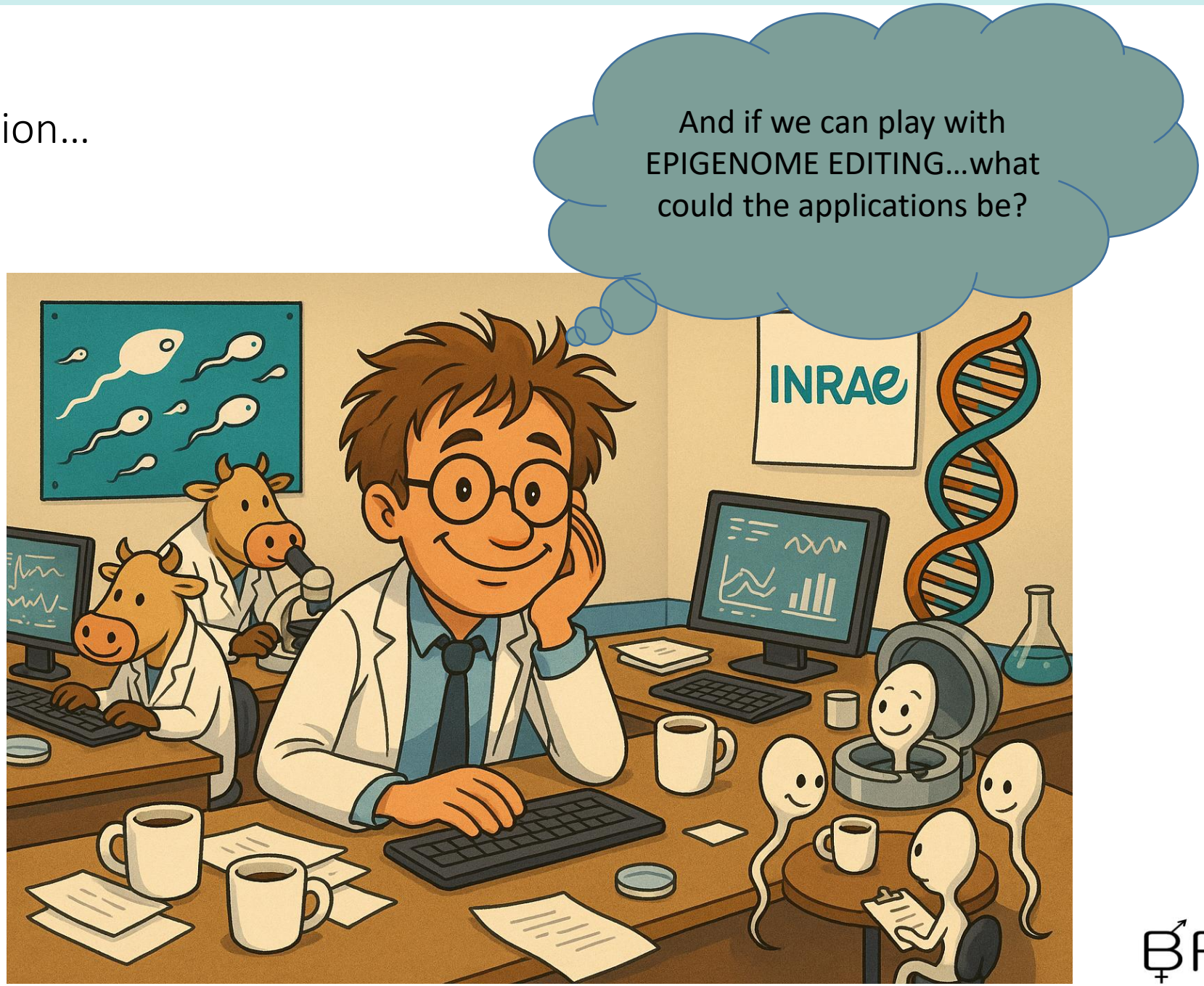
- sncRNAs are sensitive to environmental stress (modifications, perturbations…)
- sncRNA perturbations could be transmitted to the next generation

- The genetic/genomic selection has to take into account the epigenetics

- A lot of fundamental and applied science remains to do !!

Conclusions

- Open discussion…

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Merci