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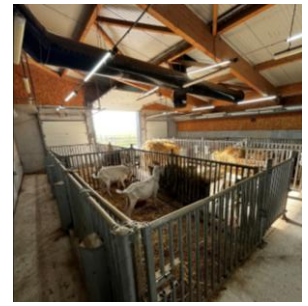
ERFP - Digital follow-up meeting on Small Ruminant Semen Cryopreservation

Impact of reduced sperm concentration in caprine AI doses :

› *in vitro* quality assessment and
in vivo fertility outcomes



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INRAE - National Research Institute for Agriculture, Food and Environment

Experimental Unit FERLUS - Lusignan
Forages, ruminants, environment

➔ *FERTICAP : Experimental facility for caprine reproduction*

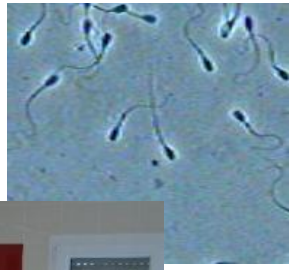






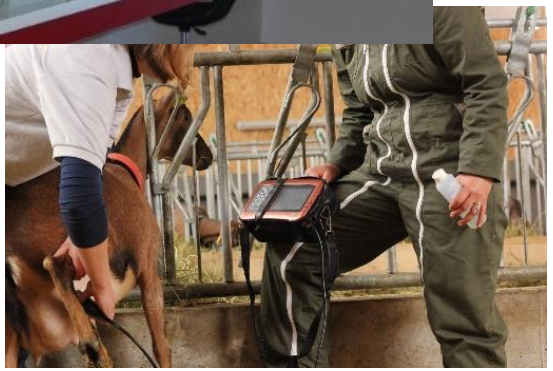
FERTICAP Experimental Facility

Objective = Design and develop sustainable goat breeding techniques



➢ Approved Artificial Insemination Center

- Bucks from the national selection scheme
- Semen analysis and conservation Lab
- Controled housing conditions (light, temperature, humidity…)



➢ Specific skills

- Insemination, ultrasonography
- Semen collection and quality assessment
- Light quality and environment measures in goat sheds
- Medical training (blood sample, semen collection)

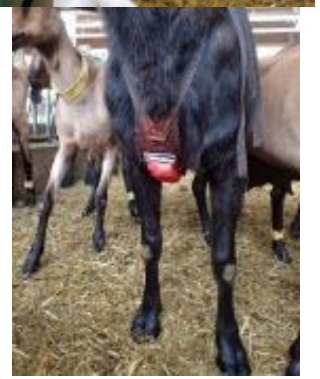
➔ Historic partnership with Capgènes

Capgenes L'excellence caprine

Only goat selection and breeding association + semen production center in France

Research Agreement on caprine **semen and embryo biotechnologies**

FERTICAP Experimental Facility



➢ **Main topics addressed in our research (aside from semen) :**

➢ How to trigger and detect heats with reduced or no hormonal inputs (photoperiodic preparation, male effect, automatized heat detection…)

➢ Better understand bucks activity (testicular function, onset of sexual behavior, efficiency in stimulating females during male effect…)

➢ Rearing of young male and female kids to prepare them in becoming efficient breeders

➔ **contributing to the design and drafting of technical cards on goat reproduction :** [GRC - Idele.fr]

Groupe Reproduction Caprine

« Routine » caprine semen processing

Semen processing steps

Buck collection

Volume / Concentration
mean 1,2mL - 3,5.10^9 spz/mL

Seminal plasma "washing"

Dilution & Cooling 4°C
Milk/Cold chamber

Glycerolization
3 steps + 1,5h equil.

Freezing
2 steps, LN_2 vapors

Quality control and storage
microscope (>30%, 3) + CASA

Production of doses at a final concentration of 500.10^6 spz/mL = **100.10^6 spz / straw**

➔ **Assessing the possibility of lowering the final dose at 50.10^6 spz / straw**

1st step : Assessing the impact on *in vitro* quality

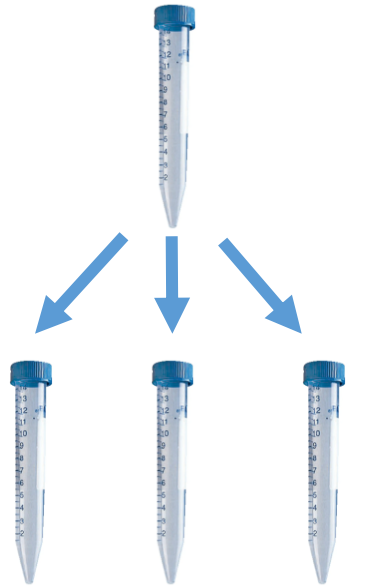
- **Study conducted in 2020**
  - 13 bucks x 3 collection dates
  - 26 treated ejaculates

- **Each ejaculate was divided in 3 fractions :**
  - T : 500.10^6 spz/mL => 100.10^6 spz/straw
  - A : 300.10^6 spz/mL => 60.10^6 spz/straw
  - B : 100.10^6 spz/mL => 20.10^6 spz/straw

- **Post-thaw assessment :**

  - % motile, motility + « technical yield » (% of frozen ejaculates that actually reach the threshold of >30% motile, 3 motility that allows use for AI) - microscope

  - concentration, % motile, % progressive - IVOS

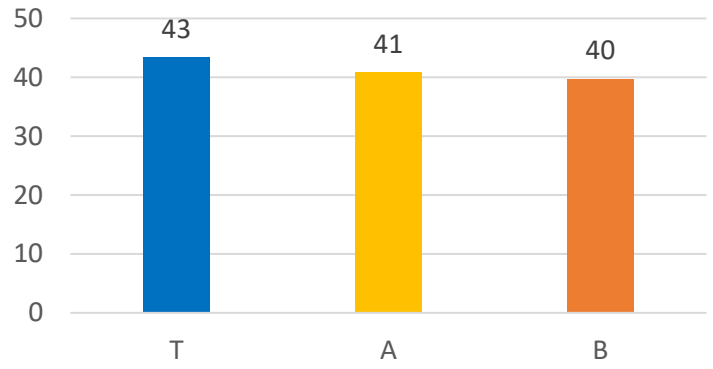


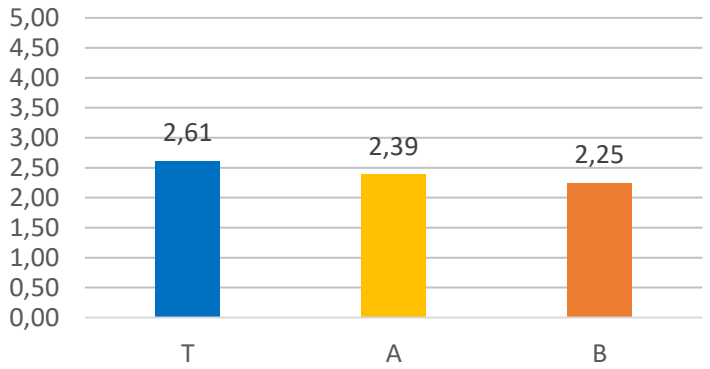
T	A	B
500.10^6 spz/mL	300.10^6 spz/mL	100.10^6 spz/mL

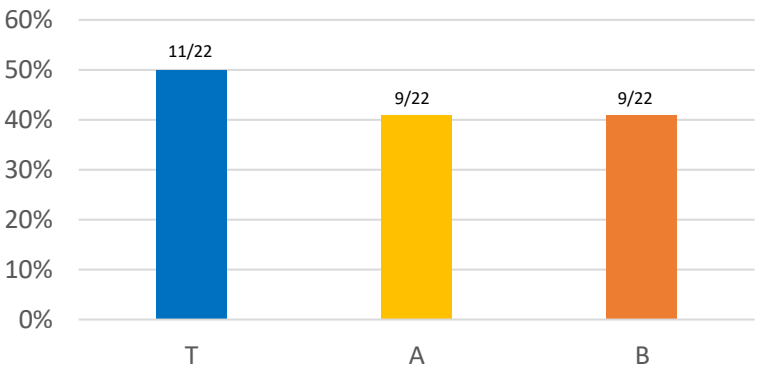
100.10^6 spz/str	60.10^6 spz/str	20.10^6 spz/str

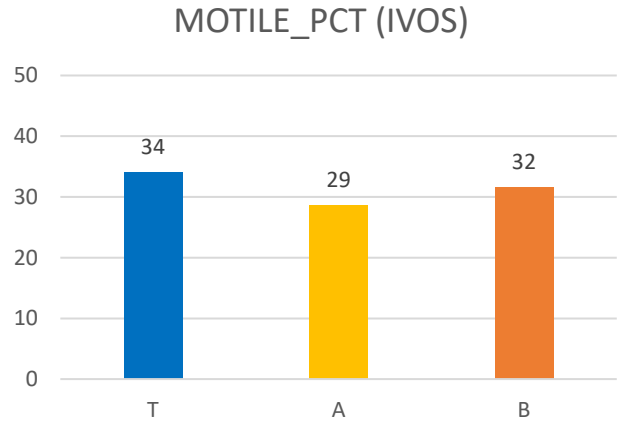
1st step : Assessing the impact on *in vitro* quality

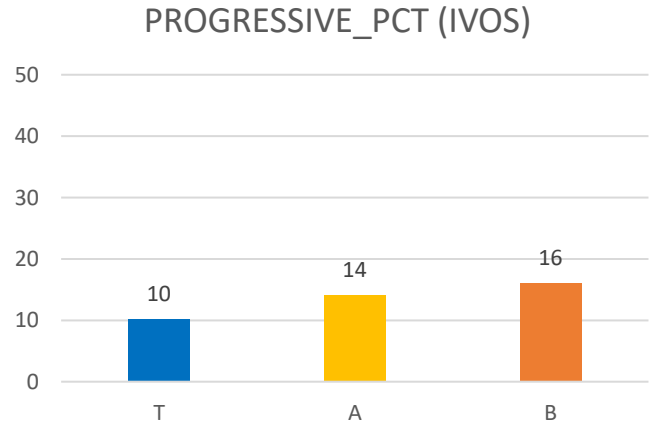

Microscope











➔ Sample size **limited**

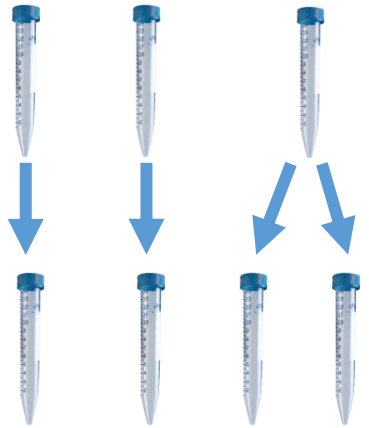
➔ No clear **positive or detrimental** trend of lowering [spz]

➔ **No** technological or feasability **constraint** identified

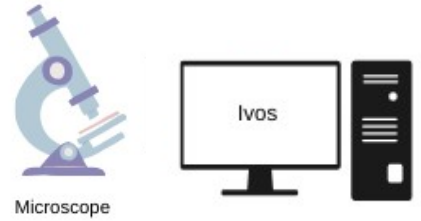
➔ Proceeding to the next step = **in vivo fertility assessment**

2nd step : Assessment of *in vivo* fertility (2021-2022)

- **Semen production in INRAE during winter 2020-2021**
  - 12 bucks x 19 collection dates
  - 264 treated ejaculates (alternate or split) – 366 fractions



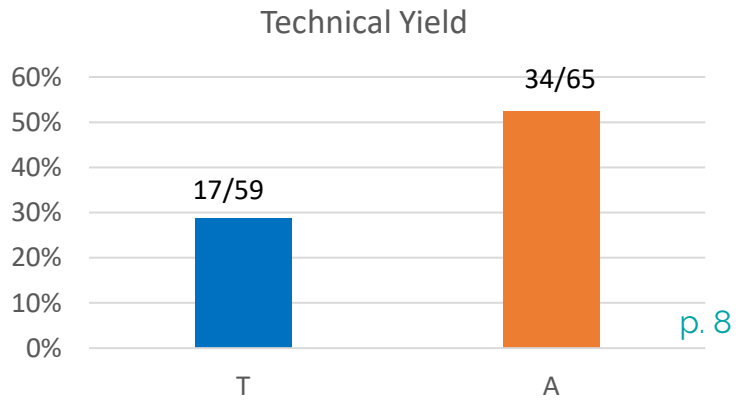
- **3 collection types :**
  - **Day 1** : Control 500.10^6 spz/mL => 100.10^6 spz/straw
  - **Day 2** : ½ Concentr. 250.10^6 spz/mL => 50.10^6 spz/straw
  - **Day 3** : Ejaculate divided in 2 fractions = C100 and C50



Post-thaw assessment – 124 fractions :

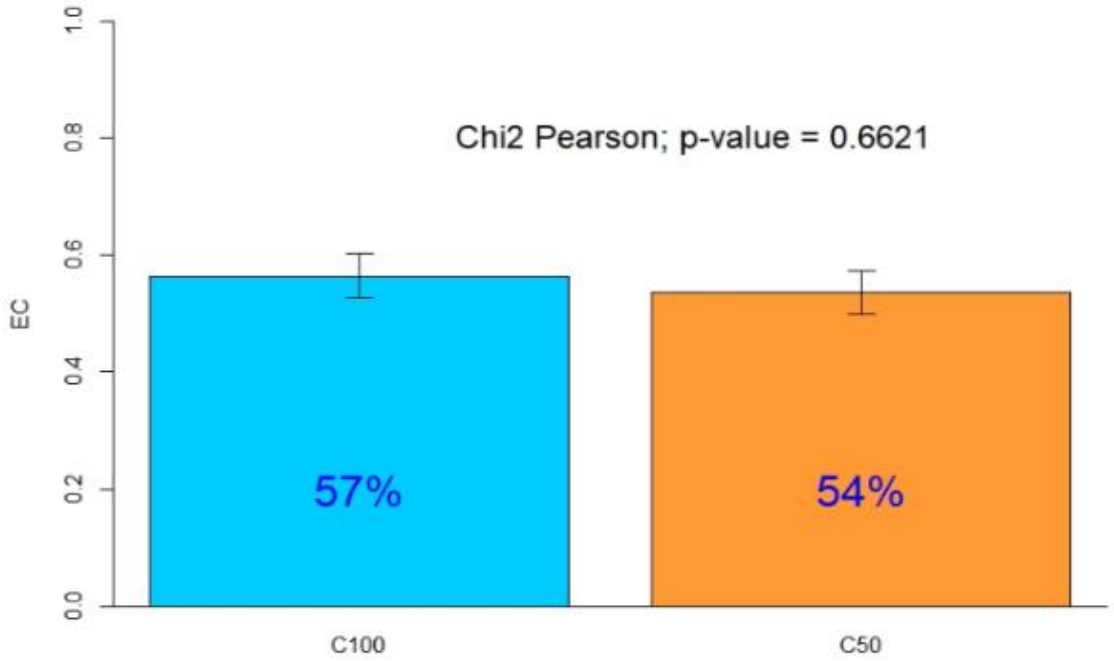
- % motile, motility + « technical yield (>30% motile, 3 motility) - microscope
- concentration, % motile, % progressive – CEROS

- **In vivo fertility = 356 inseminations in 8 farms (4 x 10 goats)**
  - 177 control AI + 179 AI with 50.10^6 spz/straw
  - 158 in Alpine breed, 198 in Saanen breed
  - 190 AI from alternate ejaculates, 166 from split ejaculates



2nd step : Assessment of *in vivo* fertility (2021-2022)

% Kidding rate depending on the final dose (Chi², NS)

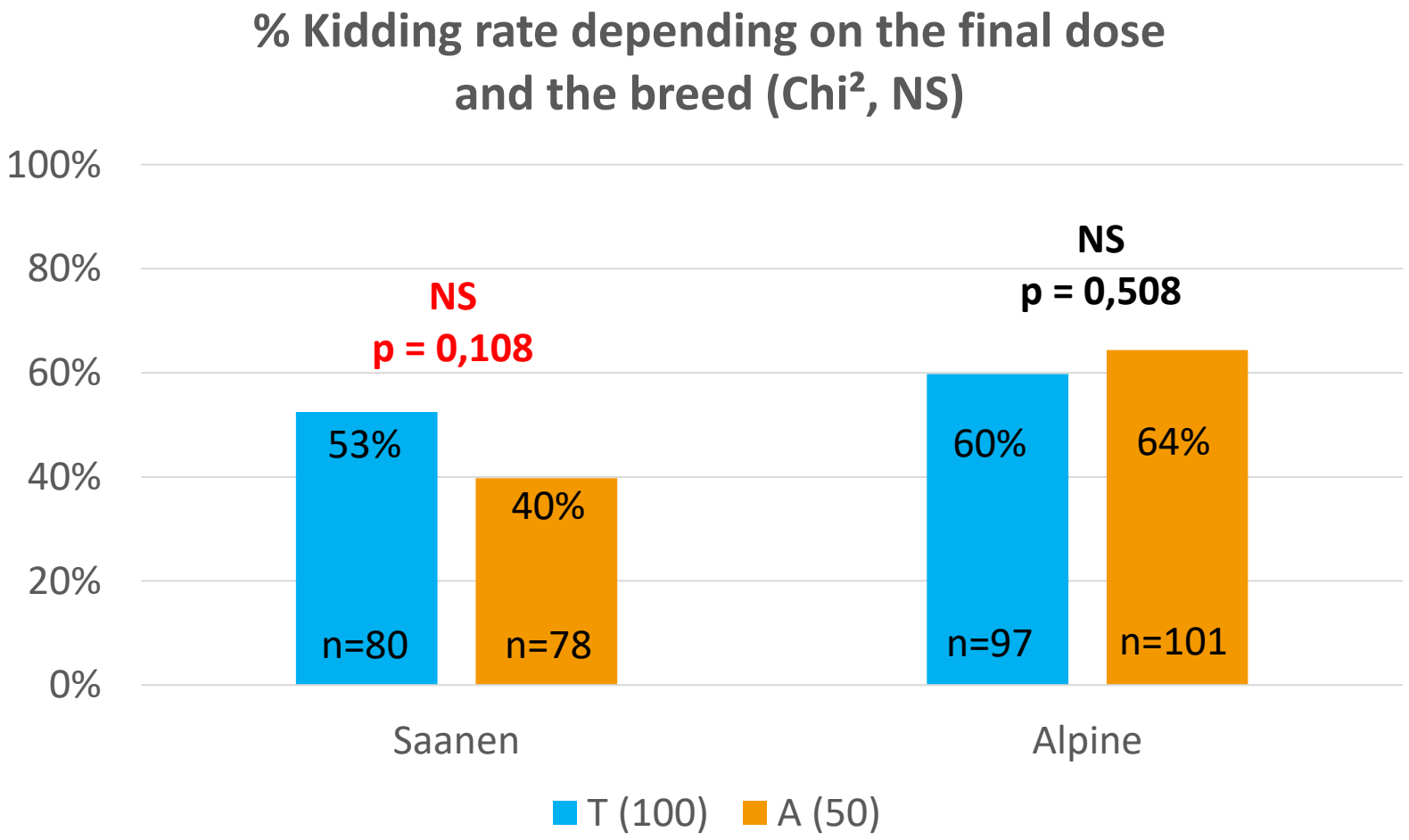


➔ **Overall, no statistical difference between the final doses of 100 and 50 million spz /straw**

➔ **However, there may be a breed effect to further explore**

➔ **Further field validation needed**

2nd step : Assessment of *in vivo* fertility (2021-2022)



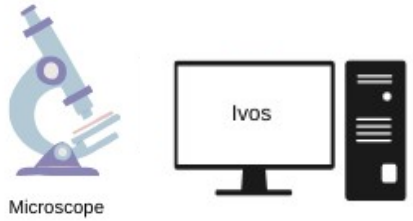
➔ **Sample size still limited (nb bucks, nb does)**

➔ In Saanen breed, a **detrimental trend on fertility** with lower concentration

➔ To be confirmed with **further field fertility tests …**

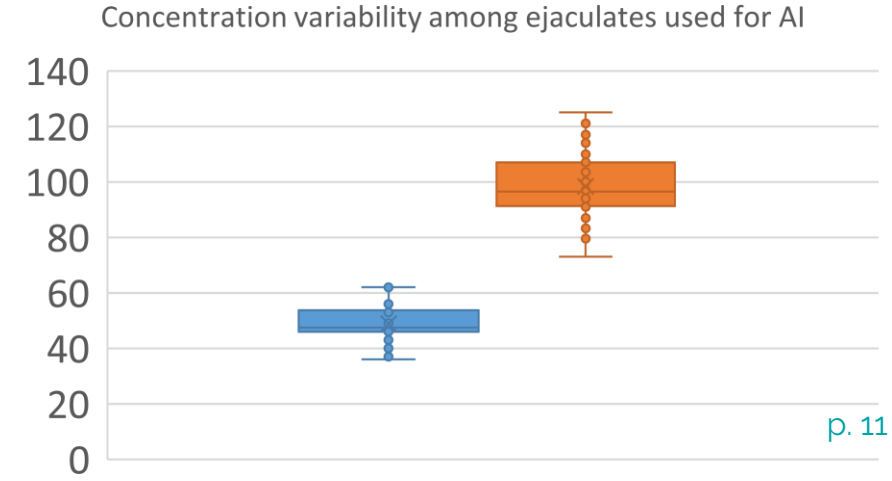
3rd step : Large scale fertility tests (2023-2024)

- **Semen production in Capgènes (« real life » conditions) during winter 2021-2022**
  - 78 bucks in total ==> to select 20 bucks x 2 breeds
  - 2 ejaculates / buck :
    - > 25 straws
    - 500.10^6 spz/mL and 250.10^6 spz/mL



- **Post-thaw assessment :**
  - % motile, motility + « technical yield (>30% motile, 3 motility) - microscope
  - concentration, % motile, % progressive – CEROS

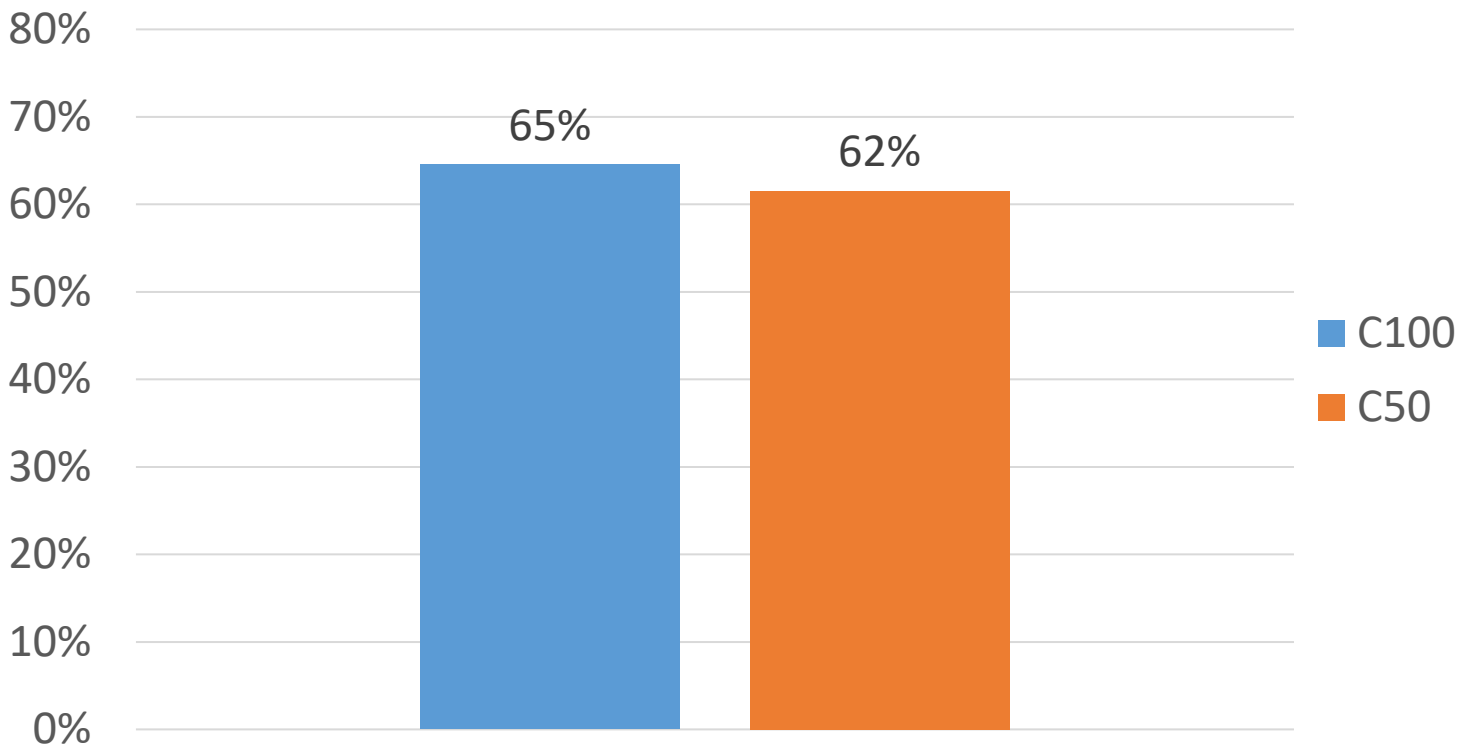
- **In vivo fertility = 1049 inseminations in 26 farms (2 x 20 goats / farm)**
  - 13 Saanen farms : 263 AI at C50 + 269 AI at C100
  - 13 Alpine farms : 259 AI at C50 + 268 AI at C100



3rd step : Large scale fertility tests (2023-2024)

Kidding rate depending on the dose of sperm/mL

(Chi² = NS, p=0,353)



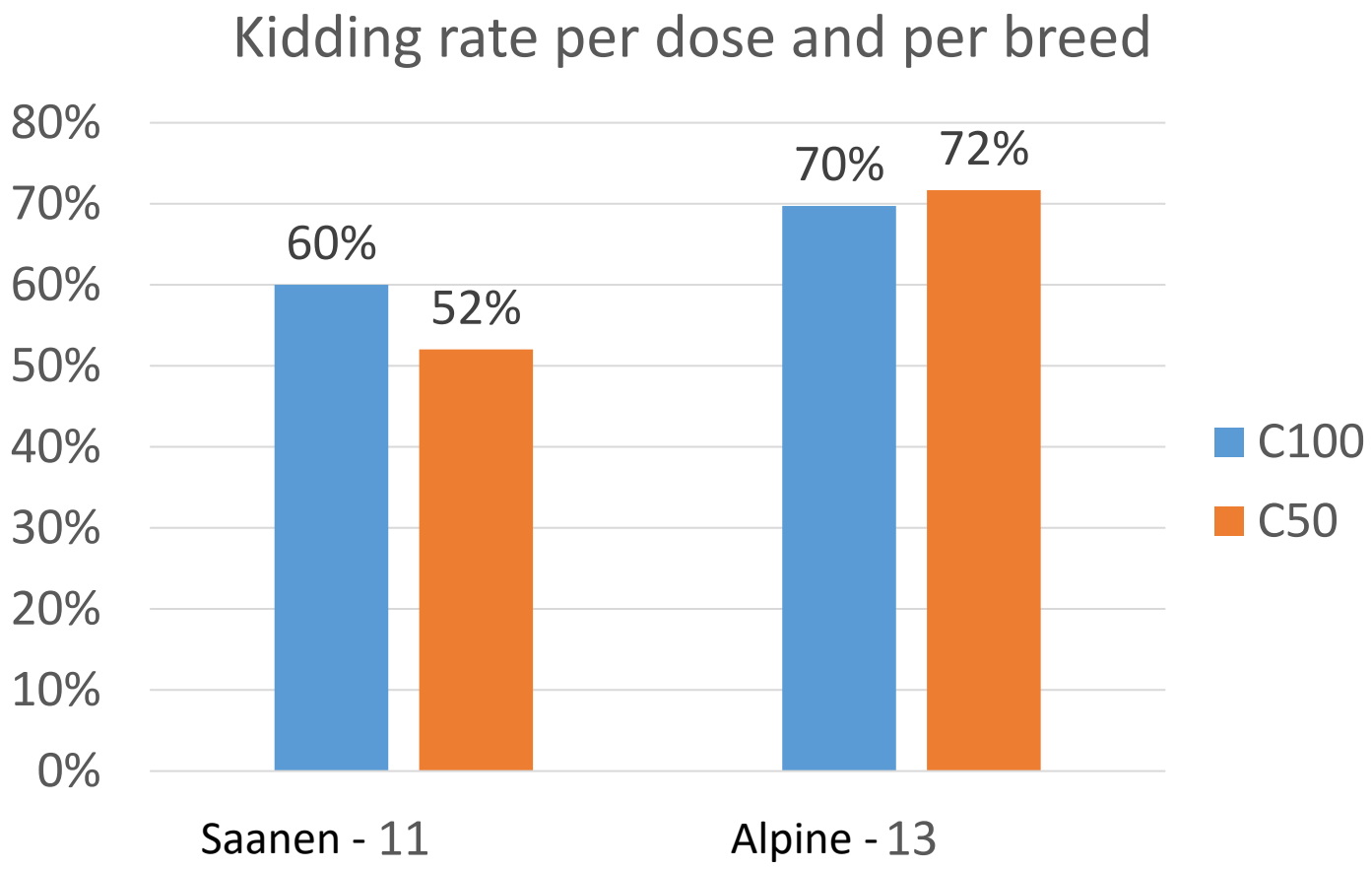
970 inseminated females still present at kidding among 1011 AI

Results from 25 farms

481 AI at C50
489 AI at C100

➔ **Overall, no statistical difference between the final doses of 100 and 50 million spz /straw**

3rd step : Large scale fertility tests (2023-2024)



Saanen breed : 13 farms
Nb inseminated does at kidding
C50 = 248
C100 = 260

Chi² NS
p= 0,07

Alpine breed : 12 farms
Nb inseminated does still present
C50 = 233
C100 = 229

Chi² NS
p= 0,597

Partial conclusions – further study

- **Possible interaction genotype x concentration (SNP known to influence semen volume) in Saanen breed**
  - 3 main genotypes for that SNP in the French population
  - highly uneven representation in our trials
  - Assessment of fertility of these genotypes

- **Last validation trial run in 2024-2025**

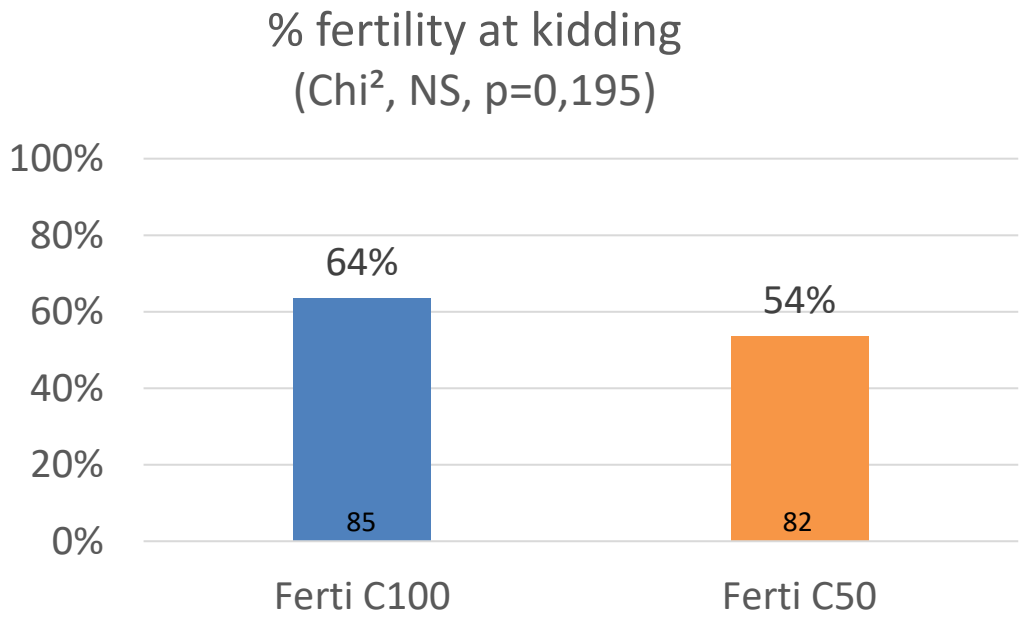
  ⇒ Final validation in Alpine breed

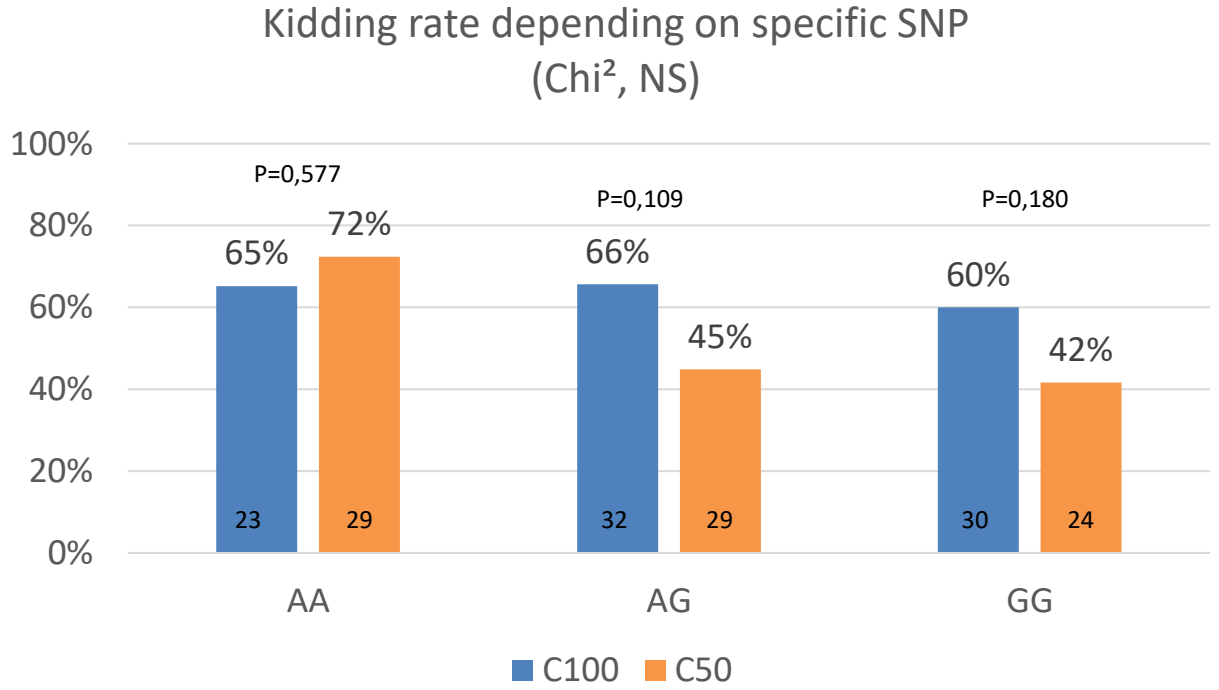
  - Objective to balance each genotype in Saanen bucks
  - Unfortunately only 4 farms could be recruited…

4th step : Fertility tests/ genotype (2024-2025)

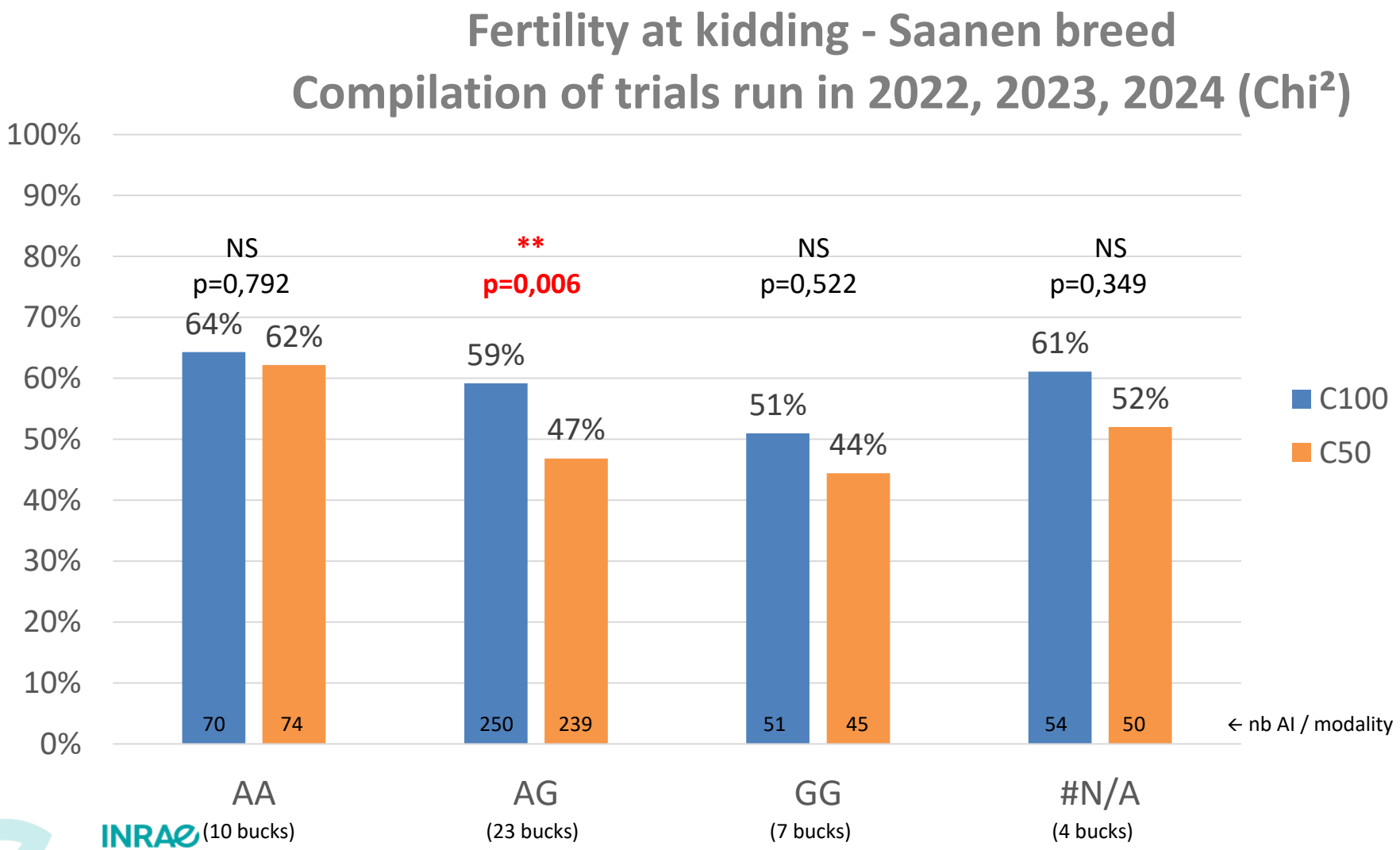
Experimental design in Saanen breed :

Results in 5 Saanen farms – **167** goats inseminated and still present at the time of kidding





Combined fertility at kidding over 3 trials – specific SNP



➔ It is **possible** that there is indeed an **interaction between genotype at considered SNPs and concentration in Saanen breed.**

However, once the results of the 3 trials are compiled :

- only one genotype shows a **significant** detrimental effect of C50
- all genotypes seem to have a trend in the same direction.

Conclusion - Perspectives

- **Capgènes is evaluating the opportunity to change the process in Alpine breed in 2026**
  - Implications in terms of equipment / lab supplies…

➔**From a research perspective,** it seems necessary to delve **deeper into the analysis using a mixed model** to assess the influence of **breed, genotype, individual characteristics, production parameters, etc.** The limited number of animals will remain a limiting factor.

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Thank you for your attention !

Open to questions and future collaborations 😊

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