ERFP Ad-Hoc Workshop
Semen Cryopreservation in small ruminants

Practical aspects of buck and ram semen cryopreservation in France

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INRAE

INRAE - National Research Institute for Agriculture, Food and Environment

Experimental Unit - FERLUS
Forages, ruminants, environment
➔ *FERTICAP : Experimental facility for caprine reproduction*

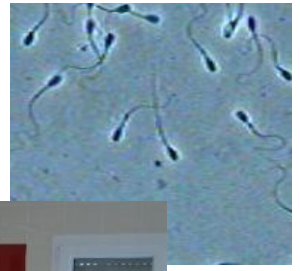


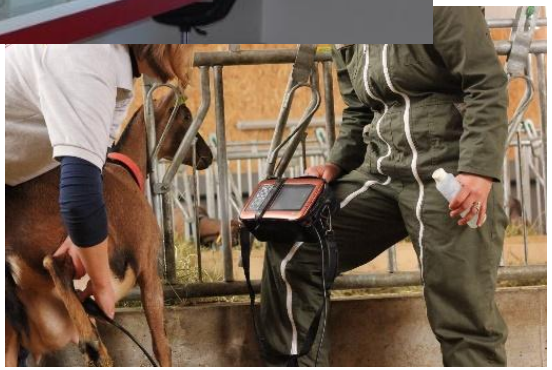




FERTICAP Experimental Facility

Objective = Design and develop sustainable goat breeding techniques





➢ Approved Artificial Insemination Center

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

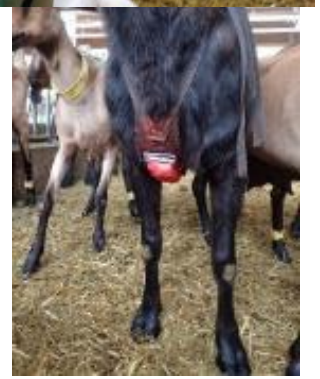
➢ Specific skills

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

➢ Very close regional and national partnership :

Membre de :

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

The dairy goat sector

- Milk production in France
- Insemination numbers
- Semen production numbers

Dairy Goats in France

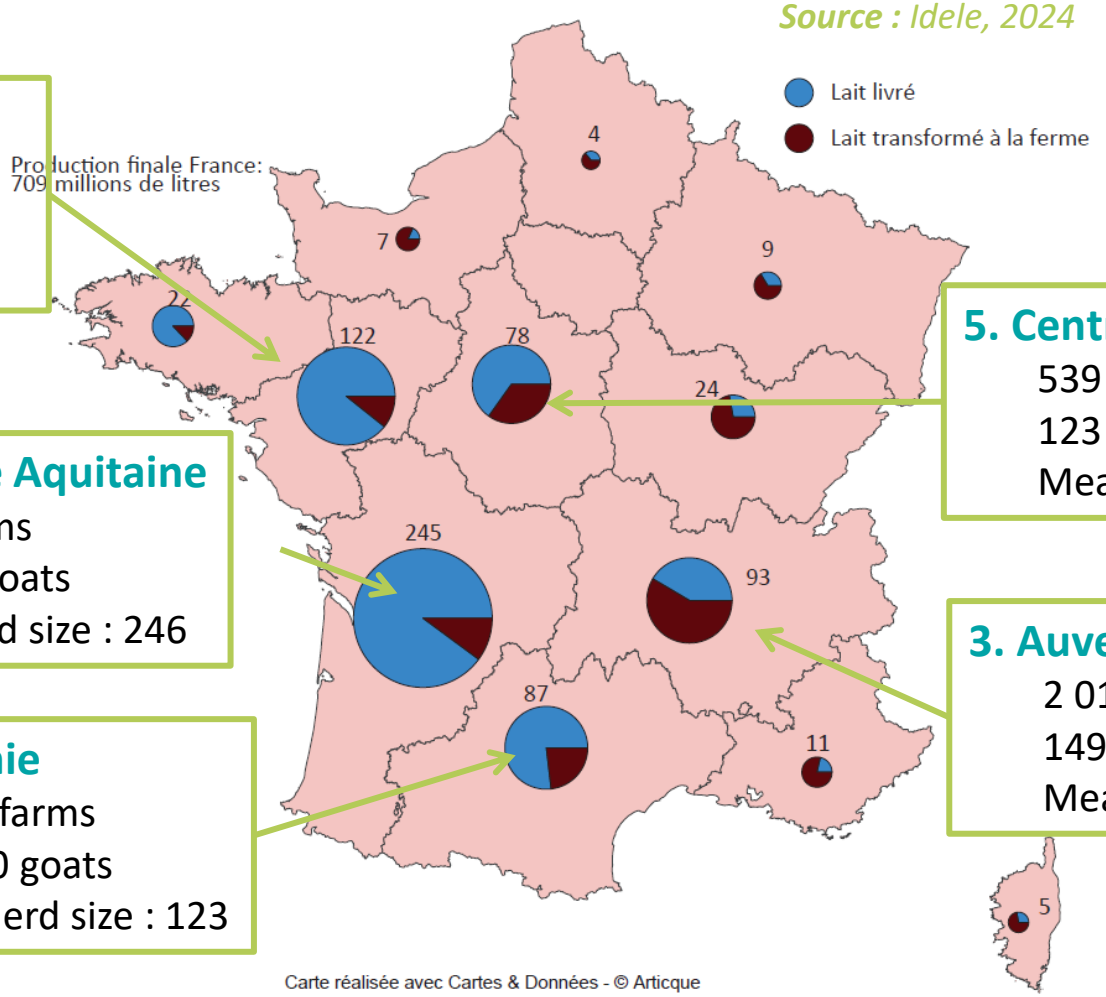
Geographic milk and cheese farms (2023 estim.)

Source : *Idele, 2024*

2. Pays de Loire
543 farms
170 400 goats
Mean herd size : 314

1. Nouvelle Aquitaine
1 476 farms
363 500 goats
Mean herd size : 246

4. Occitanie
61 364 farms
167 500 goats
Mean herd size : 123



5. Centre
539 farms
123 100 goats
Mean herd size : 228

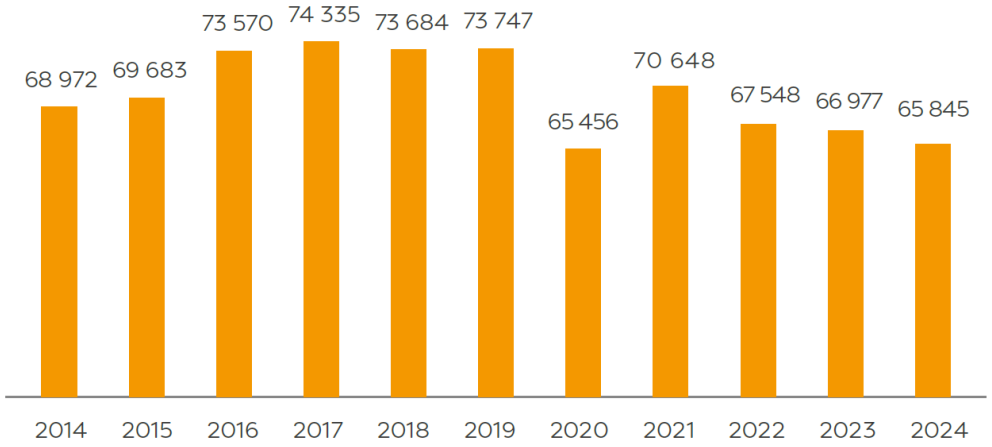
3. Auvergne-Rhône-Alpes
2 017 farms
149 700 goats
Mean herd size : 74

5 410 farms
1 250 000 goats

Among which :
- 860 000 dairy goats
- 290 000 nulliparous doe kids

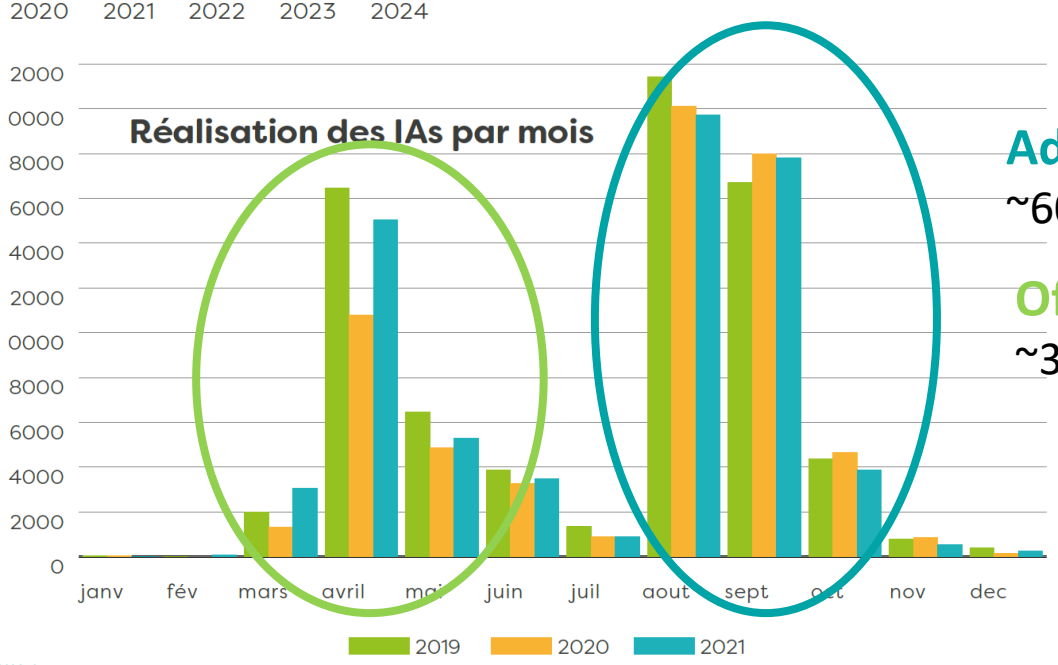
Insemination in goats

AI numbers and season
AI Geographic distribution
AI Fertility



France 2024 : 65 845 IA (5-8% total goats)
+/- stable (econom. hazards 2012, covid 2020)

Source : Capgènes, 2025



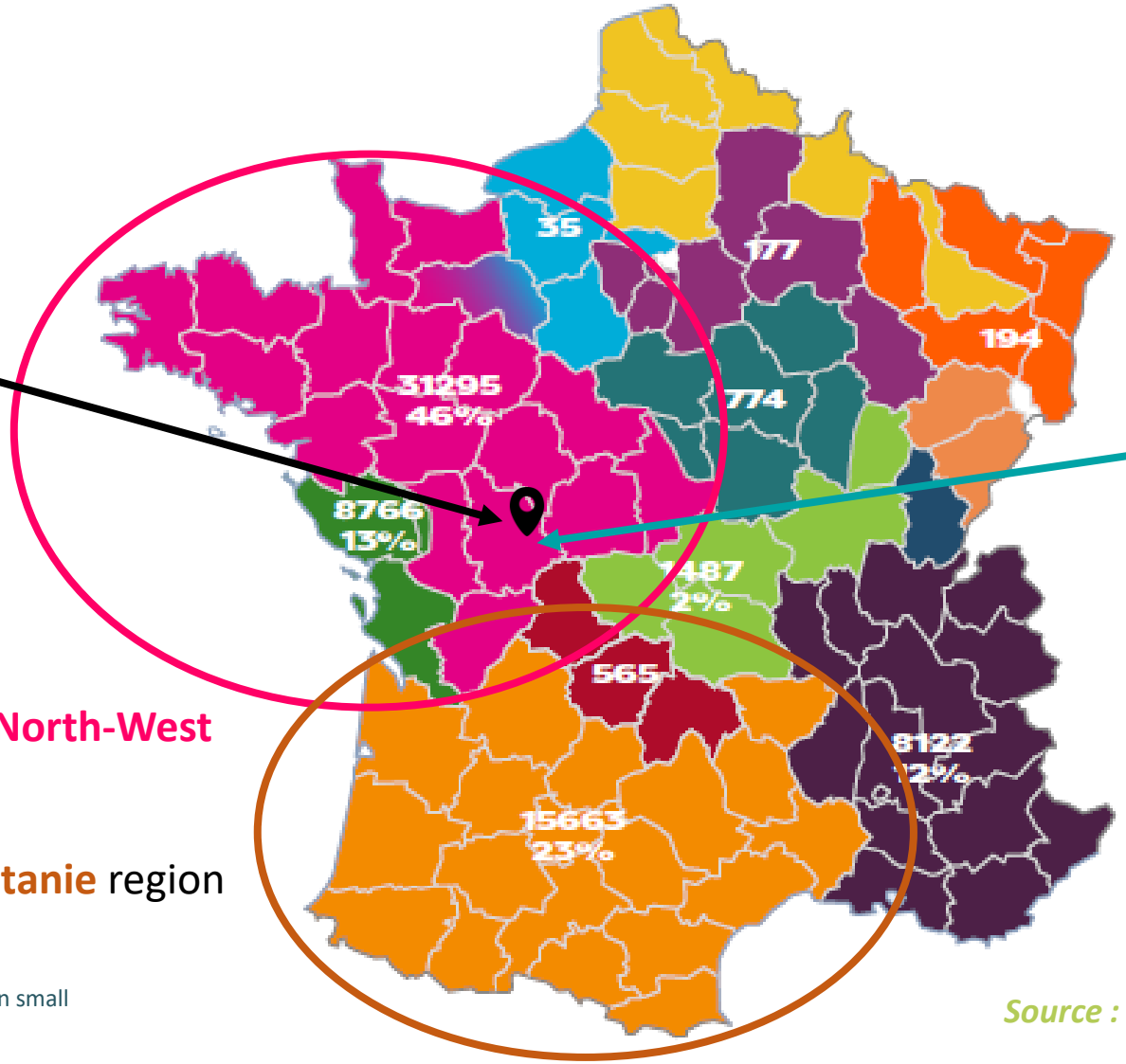
Advance of season :
~60% AI

Off-season :
~35% AI

AI in Alpine (66%) and Saanen (34%) breeds almost exclusively

Insemination in goats

1 unique semen production center for goat AI (Capgènes)



1 experimental semen production center

59% AI performed in the **North-West**
(milk industry, large herds)

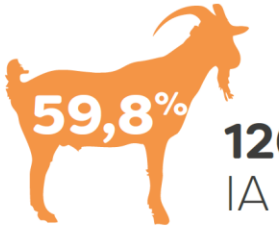
82% when adding up **Occitanie** region

Source : Capgènes, 2022

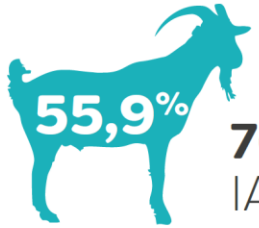
Insemination

Fertiliy per breed

% kidding / AI
over 2017-2019 period

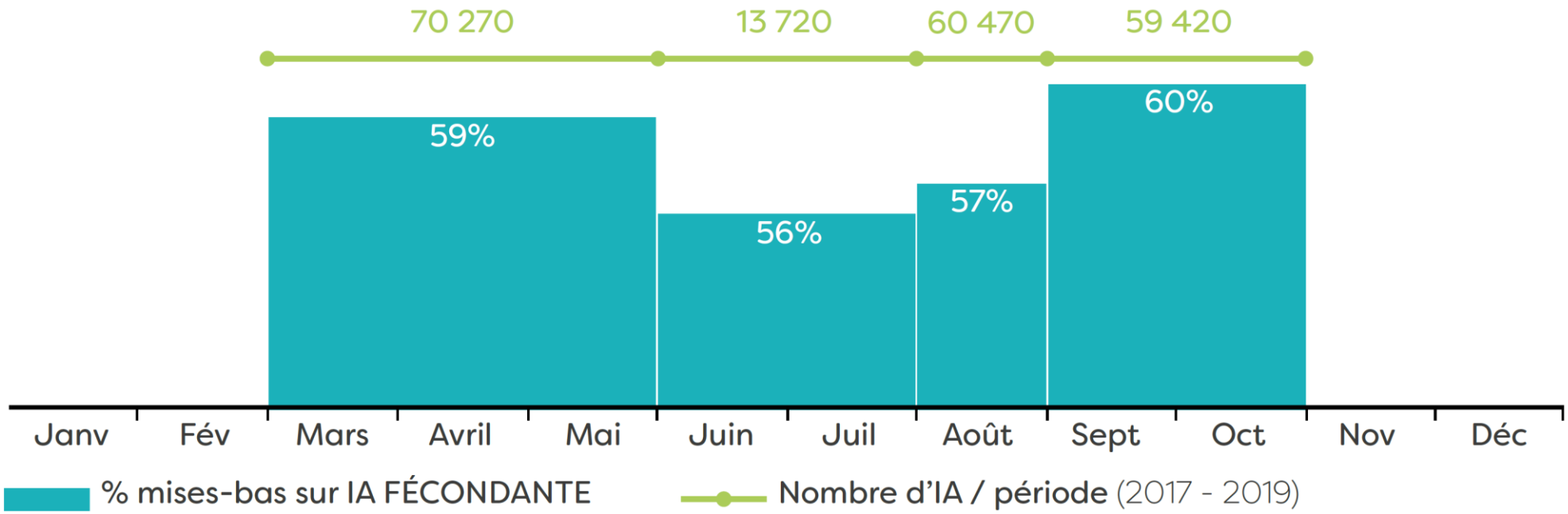


126 106
IA Alpine



76 849
IA Saanen

Fertility per season



Source : Capgènes 2022

Cryopreserved semen production

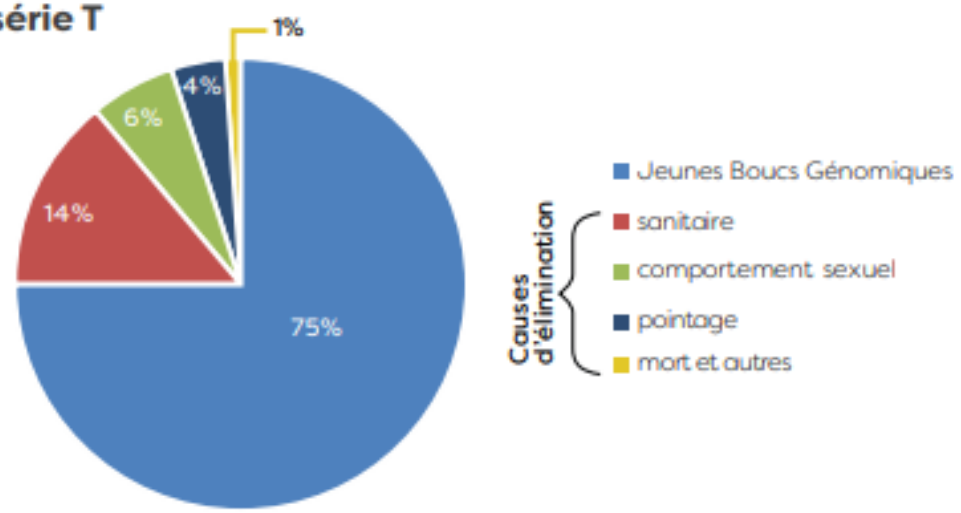
Source : Capgènes, 2024

Number of males entering Capgènes per year

(& causes for elimination)

Résultats du prétestage	Total à l'entrée	Éliminations
Quarantaine	162	31
Production de semence	131	22
Jeunes boucs génomiques diffusés	115 mâles diffusés au catalogue 2023 67 Alpins et 48 Saanen	
Boucs en production de semence	122 mâles - 72 Alpins et 50 Saanen	

Résultats du prétestage - série T



Cryopreserved semen production

Production per year (2021)

- 318 males collected
  - 92 yearlings
  - 207 adult males under photoperiodic treatment (122 two y.o. + 85 three y.o.)
  - 16 older males + 3 local breed males
- 80 males present at collection/day – 4 collections / week
- 204 collection days / year
- **246 384 AI doses** produced (stored after quality control = > 30% motile – 3 motility)

Alternance of 60LD/60SD
Production year-round 2 days/week

Objective = 2500 AI doses stored per buck within 22 months of presence

Source : *Capgènes, 2022*

CRYOBANQUE NATIONALE
Groupement d'Intérêt Scientifique

CRB ANIM

Goat breeds

- 13 different breeds
- Among which ~6 are local small-number breeds

➔**130** donor bucks
➔**13 107** semen doses

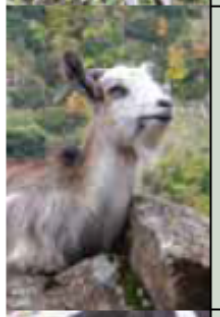
➔**206** embryons from 1 breed (Creole)

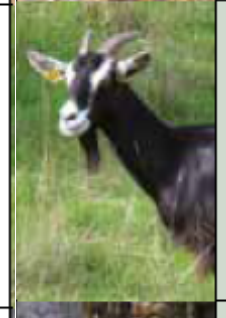
1 accredited embryo transfer team
(Capgènes + INRAE invited members)

Race	Nombre de donneurs	Nombre de paillettes semences	Nombre d'embryons
Alpine	7	356	
Angora	3	328	
Chèvre de Lorraine	12	2 043	
Chèvre des Fossés	20	2 204	
Chèvre des Savoie	2	370	
Chèvre du Massif Central	3	706	
Corse	1	20	
Créole	16		206
Poitevine	33	3 720	
Provençale	10	1 503	
Pyrénées	5	522	
Rove	6	417	
Saanen	12	918	
	130	**13 107**	206

Local breeds preservation programmes

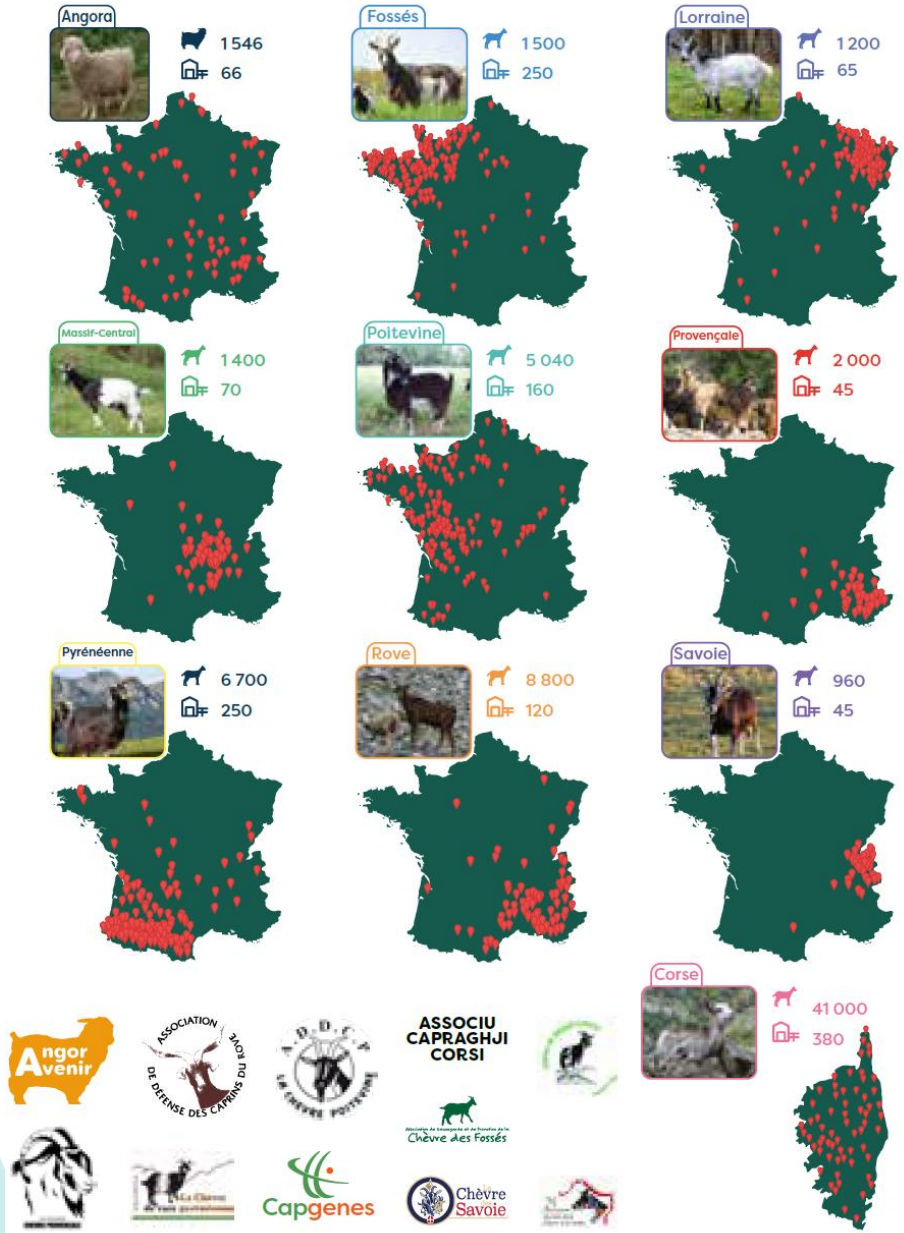
Source : Capgènes, 2025

	Races	Effectifs	Actions
	La Chèvre Poitevine	5 040 chèvres 160 élevages	• 34 boucs cryoconservés • 120 animaux génotypés • Accompagnement pour la constitution de troupeaux, achats et échanges de boucs, diffusion des résultats du contrôle laitier • Reconnaissance AOP Mothais sur feuille • Participation au SMQ Certification des Parentés Caprines • Actualisation de la note d'originalité
	La Chèvre Corse	41 000 animaux 380 élevages	• 1 bouc cryoconservé • 170 animaux génotypés • Promotion du contrôle laitier • Travaux sur IGP cabri de lait de Corse • Choix de mères à boucs à partir d'un objectif de sélection • Montage d'un projet de génotypage et de phénotypage (sanitaire)
	La Chèvre des Pyrénées	6 700 animaux 250 élevages	• 14 boucs cryoconservés • 130 animaux génotypés • Gestion des inventaires et de la consanguinité génétique via PEDIG • Animation d'une plateforme numérique pour l'échange des reproducteurs • Promotion collective de la race et de ses produits (viande, lait, fromage...)
	La Chèvre des fossés	1 500 animaux 250 élevages	• 22 boucs cryoconservés • 160 animaux génotypés • Développement de l'écopâturage • Gestion d'une pépinière de chevrettes

	Races	Effectifs	Actions
	La Chèvre du Rove	8 800 animaux 120 élevages	• 5 boucs cryoconservés • 70 animaux génotypés • Élaboration de cas-types grands pastoraux par l'intermédiaire du réseau de Référence régional (PACA) • Suivi des adhérents au contrôle laitier avec conseil de renouvellement
	La Chèvre Provençale	2 000 chèvres 45 élevages	• 10 boucs cryoconservés • 270 animaux génotypés • Affiche de promotion de la race
	La Chèvre du Massif-Central	1 400 chèvres 70 élevages	• 3 boucs cryoconservés • 160 animaux génotypés • Agrément des reproducteurs • Accompagnement des éleveurs sur la gestion de leurs inventaires • Confirmation de boucs et création d'une note d'originalité
	La Chèvre de Lorraine	1 200 chèvres 65 élevages	• 12 boucs cryoconservés • 90 animaux génotypés • Suivi des élevages et des animaux, des performances laitières et pointage • Soutien aux éleveurs engagés en contrôle de performances • Action de promotion de la race, concours racial

Local breeds preservation programmes

Source : Capgènes, 2025



Races	Effectifs	Actions
La Chèvre des Savoie	960 chèvres 45 élevages	• 2 boucs cryoconservés • 100 animaux génotypés • Soutien aux éleveurs engagés en contrôle de performances • Suivi de la pépinière de chevrettes

Capgènes s'implique également dans les programmes :

Races	Effectifs	Actions
La Chèvre Créole		• Programme piloté par l'INRAE de Guadeloupe • Soumission d'un programme sur la valorisation du cabri
La Chèvre du Péi	200 chèvres 7 élevages	• Programme de sauvegarde de la race sur l'île de la Réunion géré par l'APPER • Création d'un troupeau de 60 animaux et suivi technico-économique • 80 animaux génotypés
La Chèvre Boer	180 chèvres 9 élevages	• 11 boucs cryoconservés • Entrée de nouveaux boucs à CAPGENES pour renouveler l'offre génétique à l'international • Projet de développement de la chèvre Boer

Angora breed selection program

Source : Capgènes, 2025

The French Angora breed

Photo: CAPGENES – Copyright

35 farms

Population in France :
4,500 goats, 2,200 of them recorded.

Weight of males : 40 to 60 kg
Weight of females : 30 à 40 kg

Characteristics of the fleece :
Weight at 180 days : 2 to 2,6 kg
Average fineness: 27 to 30 microns
Length of lock at 180 days : 13 to 14 cm

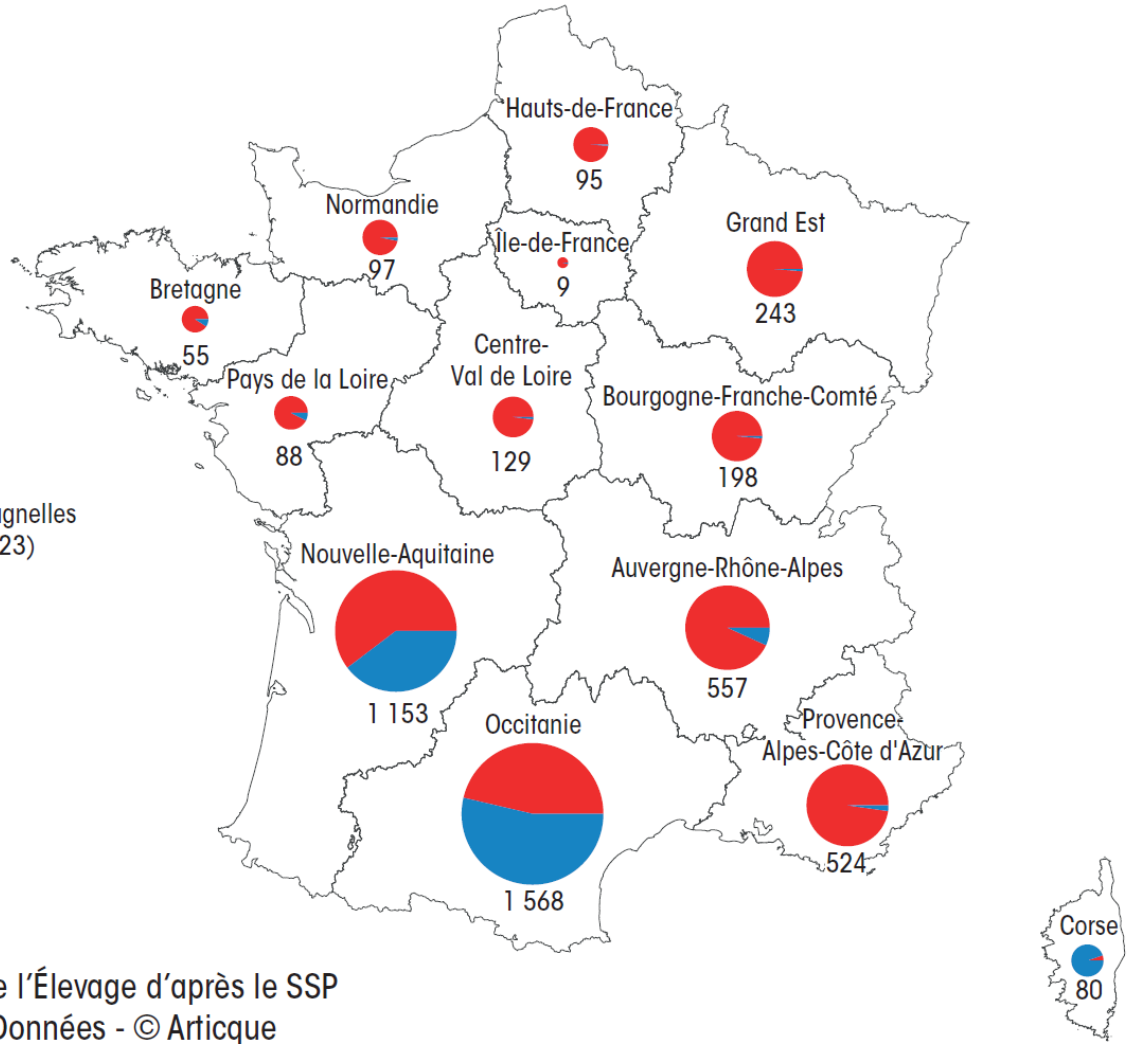
The sheep sector

- Dairy and Meat Numbers
- Insemination Numbers
- Semen Production Numbers

Dairy and Meat Sheep in France

Geographic distribution of dairy and meat sheep (x 1000 heads)

Source : *Idele, 2024*

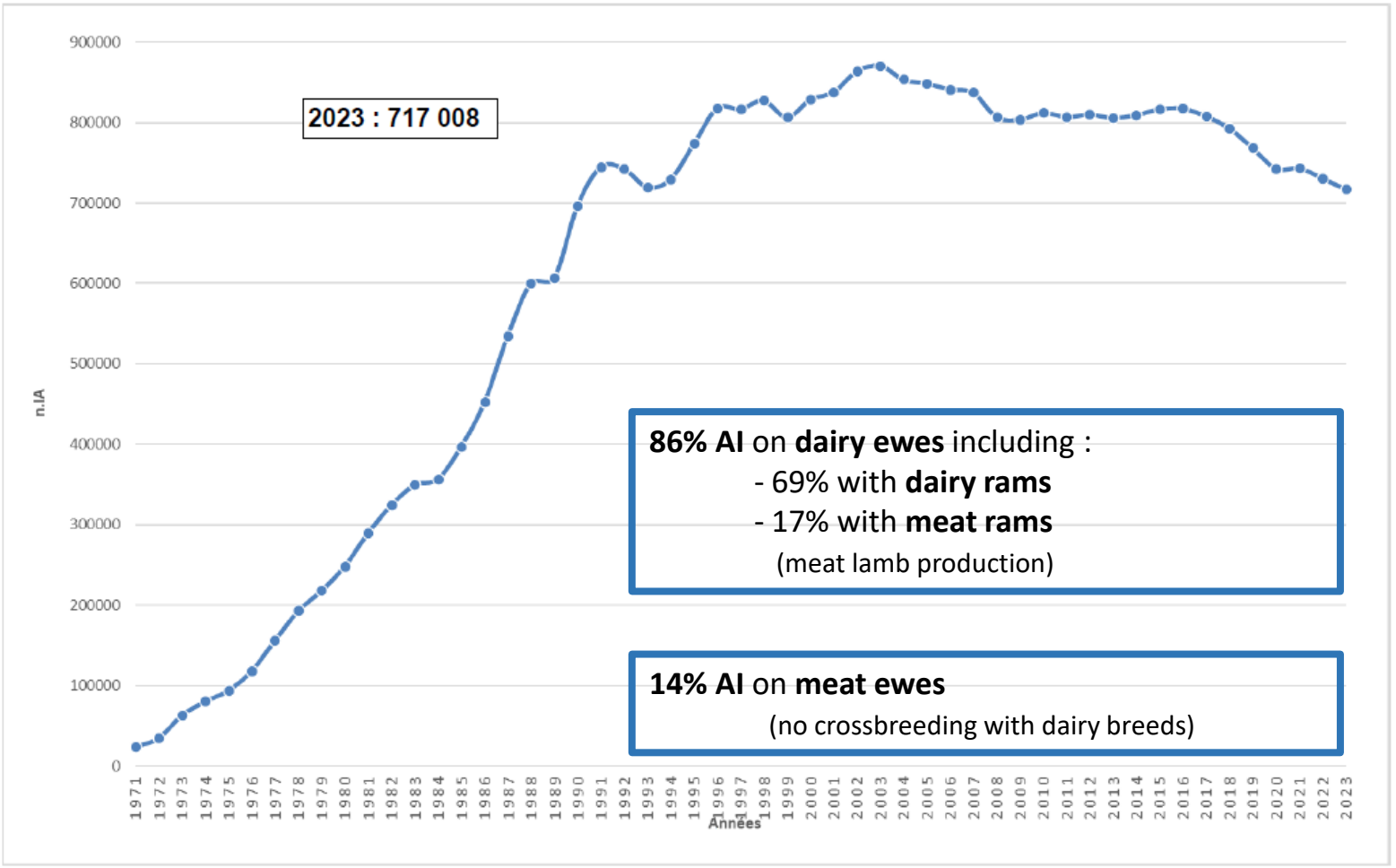


29 982 farms
6 576 000 sheep

Among which :
- 1 449 000 dairy ewes
- 3 355 000 meat ewes

Insemination

Source : ANIO, 2024



86% AI on **dairy ewes** including :
- 69% with **dairy rams**
- 17% with **meat rams**
(meat lamb production)

14% AI on **meat ewes**
(no crossbreeding with dairy breeds)

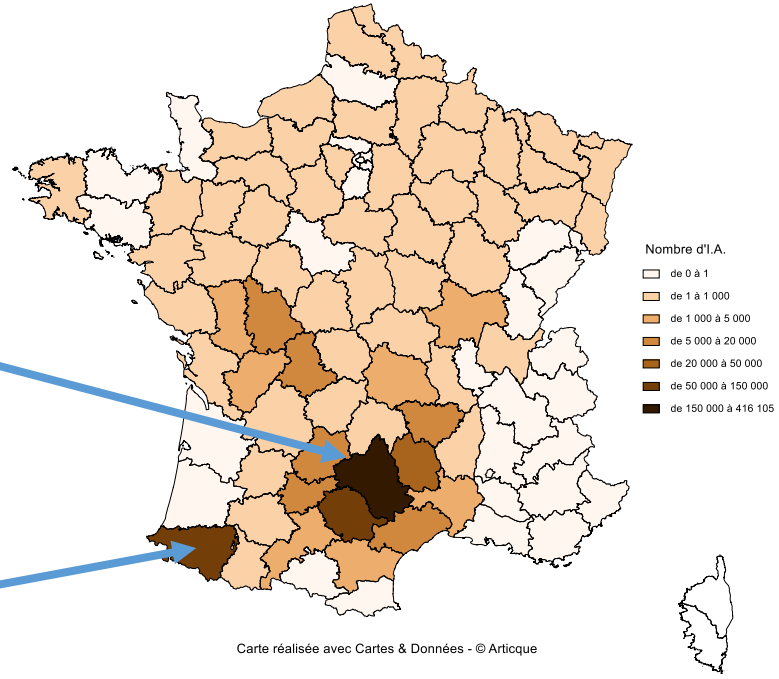
Insemination

Source : *ANIO, 2022*

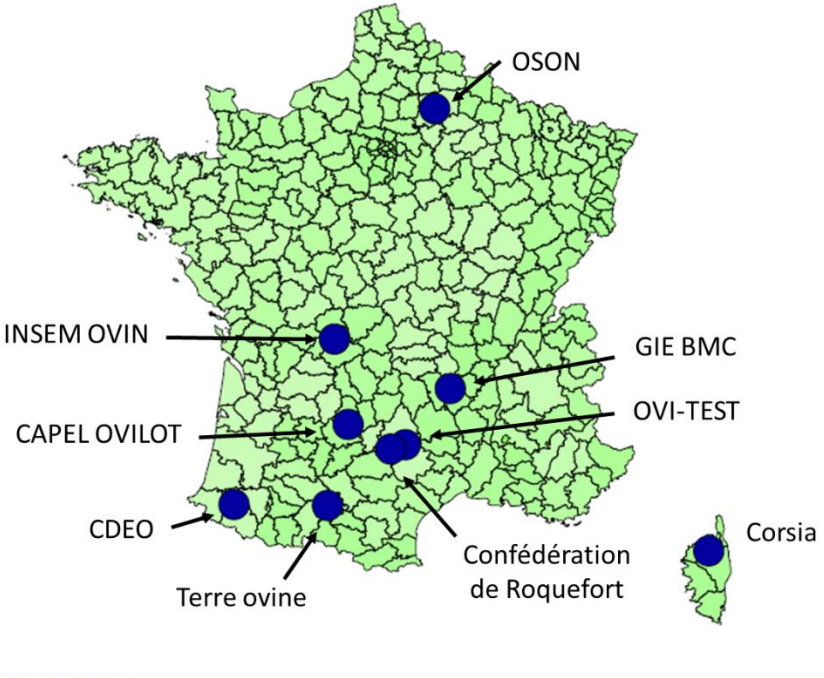
Geographic distribution of ovine AI

Roquefort DOP

Ossau-Iraty DOP

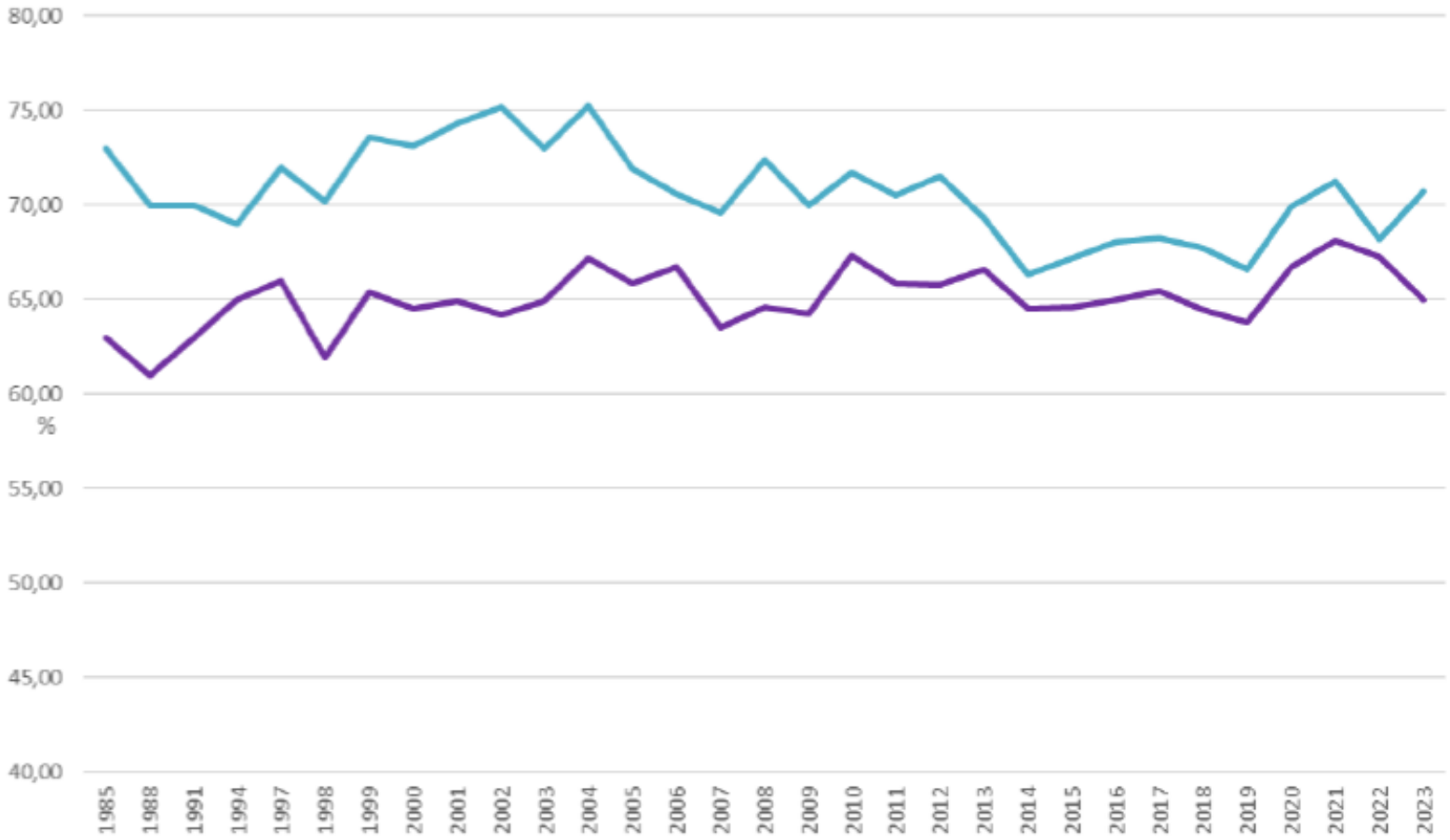


Geographic distribution od semen production centers (since fresh semen implies proximity)



Fertility after AI

Dairy



Dairy ewe lambs 70,75%

Agnelles

moyenne= 70,75
min= 33
max= 76

Adult dairy ewes 65%

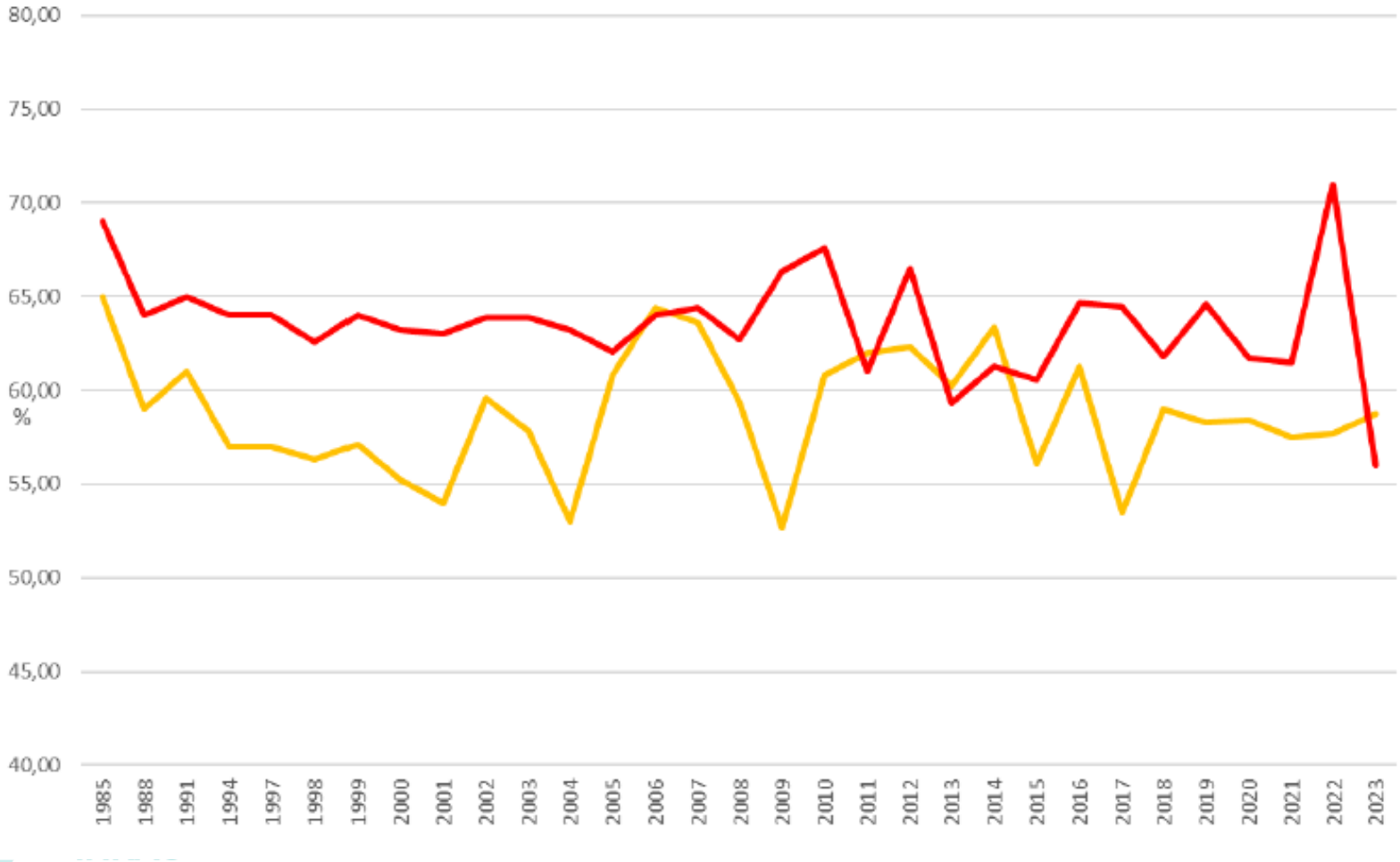
Adultes

moyenne= 65
min= 28
max= 73

Source : ANIO 2024

Fertility after AI

Meat breeds



Meat ewes 65%

Viande

moyenne= 65,63
min= 61,08
max= 66,96

Breeding ewes 56%

Reproducteurs

moyenne= 56,72
min= 49,98
max= 71,66

Source : ANIO 2024

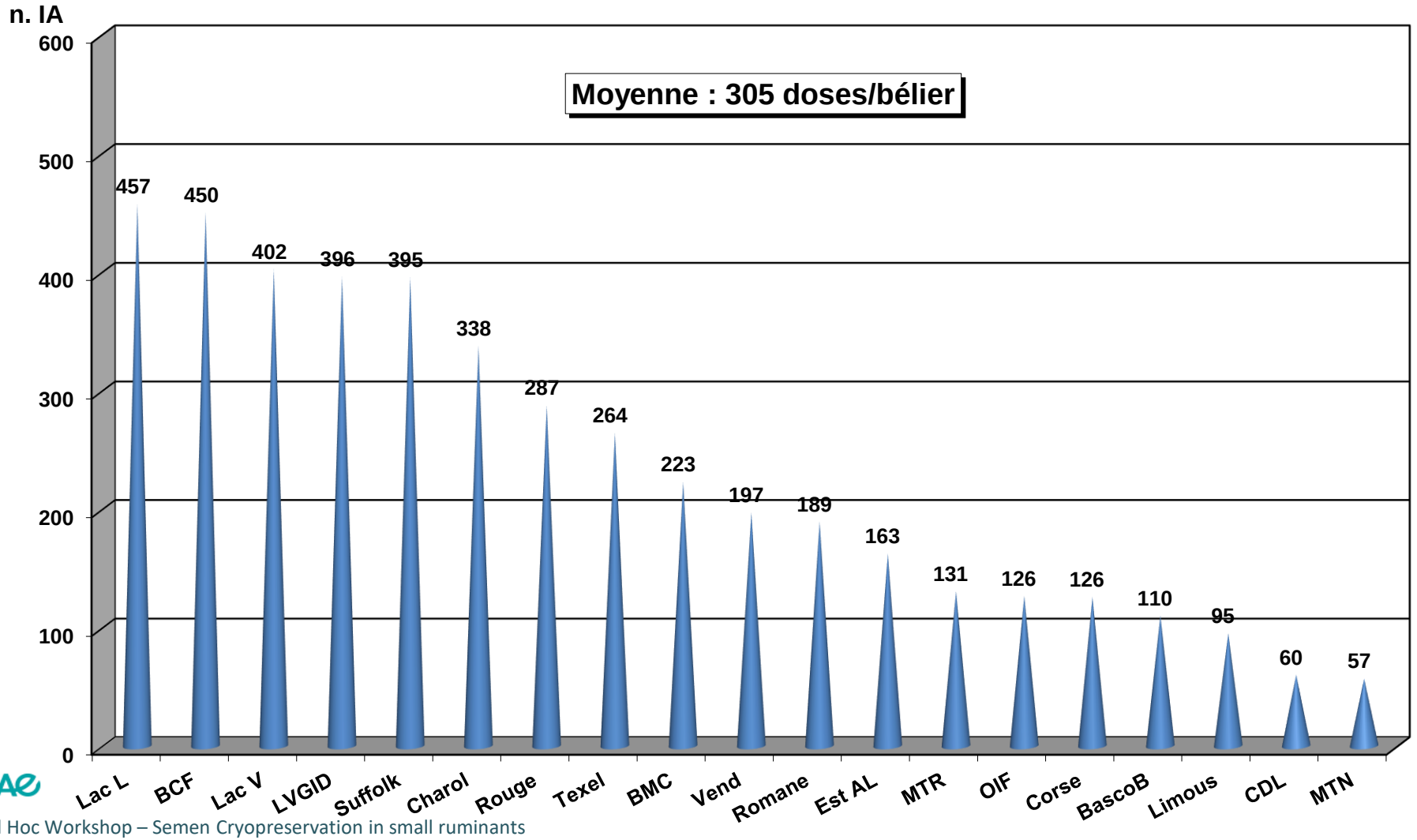
Fresh semen production

5 à 8 heures

Fresh semen production

Mean production per ram (nb AI / year)

Source : ANIO, 2023



Frozen semen production



Dilution :

- Egg-yolk-lactose + milk-glcerol for domestic use (France)
- Optixcell 2 when semen is bound for export (specific antibiotics)

Insemination done exclusively by **intra-uterine deposit by laparoscopy**
(only about 5000 laparoscopic AI/year in France, no precise fertiliy number)

Expected fertility ~55-65% (pers. communication from 1 meat SPC)

French Biological Resources Center

Sheep breeds

- 49 different breeds
- Among which 21 classified « threatened with abandonment for agriculture » by the Ministry of Agriculture

➔**1 318** donor rams

➔**132 075** semen doses

➔550 embryons from 3 different breeds (Merinos de Rambouillet, Black Belly, Booroola x Romanov) *not from local breeds*

Race	Menacée d'abandon	Nombre de donneurs	Nombre de doses	Nombre d'embryons
Aure et Campan	oui	4	317	
Avranchin	oui	16	2 560	
Baregeoise	oui	4	248	
Basco-Bearnaise		32	2 972	
Belle Ile	oui	6	1 442	
Berrichon de l'Indre	oui	18	2 711	
Bizet	oui	3	525	
Blackbelly		18	1 016	22
Blanc du Massif Central		28	3 186	
Bleu du Maine	oui	3	552	
BooroolaxRomanov		38	724	427
Boulonnaise	oui	16	2 017	
Castillonnaise	oui	13	1 057	
Caussenarde des Garrigues	oui	8	1 122	
Causses du Lot		22	2 765	
Charmoise		34	2 771	
Cotentin	oui	11	1 656	
Est à Laine Merinos		1	200	
F1 Blackbelly-Romane		7	458	
Grivette		6	601	
Lacaune lait		324	33 260	
Lacaune viande		71	4 478	
Landaise	oui	13	1 731	
Limousine		10	1 934	
Lourdaise	oui	20	2 098	
Manech tete noire		26	2 570	
Manech tete rousse		48	5 590	
Mourerous		8	1 308	
Moutons Charollais		55	5 701	
Moutons Vendeens		69	6 209	
Mérinos Precoce	oui	11	1 282	
Mérinos d'Arles		7	1 181	
Mérinos d'Arles Booroola		9	1 218	
Mérinos de Rambouillet	oui	87	2 063	101
Noir du Velay	oui	3	479	
Ouessant	oui	4	526	
Prealpes du Sud		7	1 218	
Rava		9	1 623	
Raïole	oui	12	2 157	
Romane (Ex INRA 401)		31	2 534	
Romanov		29	2 473	
Rouge de l'Ouest		30	4 027	
Rouge du Roussillon		11	1 418	
Roussin	oui	13	1 987	
Solognote		14	1 069	
Southdown	oui	6	1 345	
Suffolk		36	5 509	
Texel		64	6 002	
Xaxi-Ardia	oui	3	185	
TOTAL		**1 318**	**132 075**	**550**

Practical aspects of semen production

- Housing and photoperiod
- Training males for semen collection (Artificial Vagina)
- Semen dilution and freezing

Survey conducted 2015-2018 among 10 ovine-caprine SPC (CASDAR project Maxi'mâle)

Housing of males in Semen Production Centers

Young and adult rams

- Collective areas of 6 to 25 males (dpdt age)
- Possible presence of 1-2 female/batch

Bucks / high genetic rams

- Individual boxes

Building type

- When no photoperiodism is used = tunnel or lightweight shed possible
- Light-proof building necessary when photoperiodic treatment require shorter daylight than the natural day

Individual housing

Collective area

Photoperiodic treatment of males in SPC

Photoperiodic conditionning principle

Alternance of long days followed buy short days to stimulate the sexual activity when needed or maintain it throughout the year by inducing a mini-sexual season

➔ **Type of conditionning should be chosen depending on production needs** (production peaks / duration of activity)

Photoperiodic treatment of males in SPC

Photoperiodic program	*Production objectives*
• Reversed photoperiod • LD dec-jan ↘ SD june-jul ↗ • LD jan-feb ↘ 8h/day end of aug	• training march, coll. april-jul • training april, coll. may-sept • training april, coll. from may-june until august
• LD dec-feb followed by SD • Flashs jan until mid-march + melatonine	• coll. april-june • coll. may-june, aug-sept
• Continuous altern. 60LD/60 SD • Continuous altern. 30LD/30 SD	• Year-round • Year-round (June, Aug-sept, feb-march)

Photoperiodic treatment of males in SPC
Implementation

Light sources

- Fluorescent mainly
- Sodium
- LED

200 lux
at eye level

Flock-Reprod©, 2012

Long Days

- 16h light / day
- Fixed Dawn (6-9h) + flash 1-2h
- 7h continuous light + flash 1h

Short Days

- 8-9h light in a light-proof building (shades)
- Melatonine implants (1-3 at once or staggered)

Photoperiodic treatment of males in SPC
Special attention

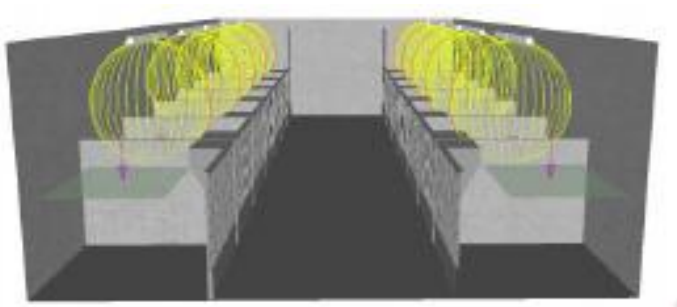
Light intensity inside the housing

→ should be well estimated before installing

→ and monitored during production

- Light source and protective translucid tend to age and opacify
- Light sources get dirty, ambiant dust contribute to lowering intensity
- Heterogeneity of light sources

→ Can lead to 30-50% loss from expected/estimated intensity



Etude de cas ENSIP 2018, installations photopériodiques du CIA de Rouillé

Training

« From a candidate lamb to a semen producing ram »

Training step by step

- Move from the box to the collection room
- Getting used to the collection room (closed / confined place)
- Interact with the female (unknown congener)
- Accept proximity with the trainer/collector
- Accept collection with the artificial vagina

The order of those steps is flexible (dpdt spatial organisation)

Variety of layouts for the collection room (ramp to have the males at height, pit allowing the collector to be below the male, restraint systems for the female…)


OSON


Conf. Roquefort


Conf. Roquefort


Capgènes


CDEO

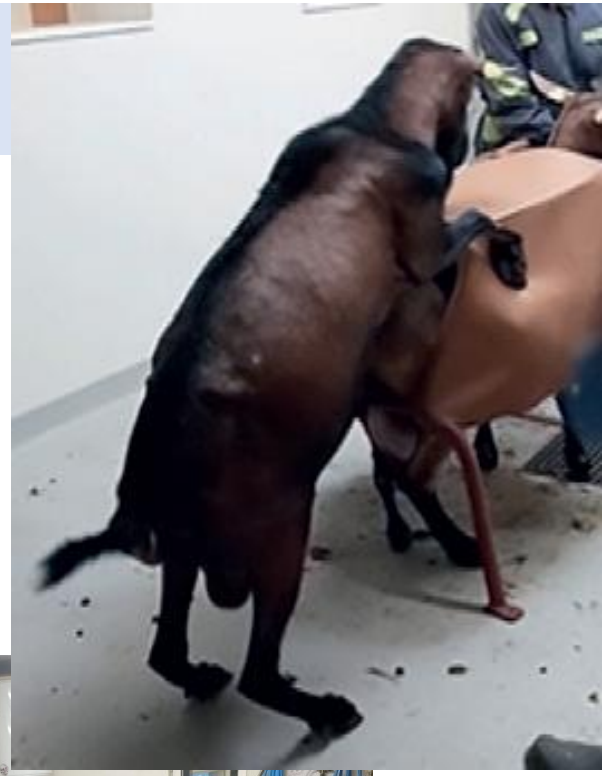

CDEO


Insemovin


Capgènes


Capgènes

Training with a dummy

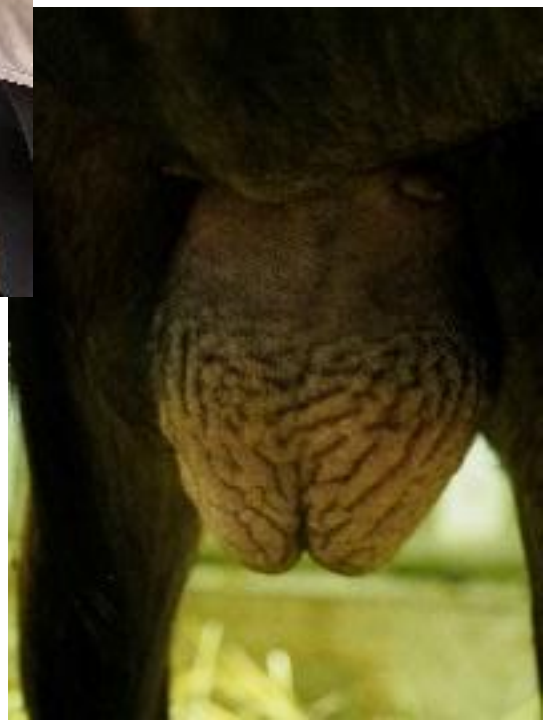


Prototyping after precise behavior observation

- Room for a female below the dummy
- Room for the collector to hold the artificial vagina
- Support points on the sides for the male

→ The idea was to have the males get used to being collected with the female under the dummy and to take her away once the conditioning is sufficient.

Medical training



Tips with reluctant males

Presence/absence of congener
(Baril *et al.*, 1993)

Teaser female
(Prado *et* al., 2003 ; Baril *et al.*, 1993)

Trainer
(Boivin *et al.*, 1998)

Teaser female

- Let the female unrestrained (moving freely around the room)
- Synchronise female with hormones
- Bring another male for them to mount
- Leave 1-2 females in the collective box where reluctants are grouped
- Add another female in the collection room (preference choice) / rotate between several females

Tips with reluctant males

Presence of a congener

- Have a few other males come to be collected before them to « set the example » or « exacerbate competition »
- Collect inside the housing area of the males
- Isolate the male with 2-3 females
- Re-arrange the group / batch of males to change the established dominance

Human-animal relationship (scared males)

- Let the male mount/ejaculate before trying to collect
- « Desensitize him from his fear of human » by interacting more / brushing sessions / feed reinforcement

Other

- Prevent him from mounting (frustration)
- Shearing

Most frequent problems during training

Linked with environment

- Difficulties in the distance from housing to collection room (visibility, corner turns, passing through the outside before entering again, female not visible before entering…)
- Changes in the collection place (in the housing *vs* specific room, access ramp) = necessary to train again

Human-anima relationship

- Few interactions with human before training (until 9 mo) except for vaccines and serologies (not very positive interactions)
- Blood samples made in the collection room

Teaser female

- For some males, estrus is obligatory (= dependent on hormonal sync) / certain female

Linked to the breeding « agenda »

- Rams in natural mating before entering SPC
- Lay-off periods (periods with irregular or no sollicitations of the males = hard to restart)

Buck semen processing

Baril et al. 1993 FAO's Training manual for insemination in sheep and goat (https://www.fao.org/4/t0121f/T0121f00.htm)

Collection using an artificial vagina (water at 40°C) /!\ Below 38°C, bucks will not ejaculate

Volume control (weighing) / **concentration** (photometer)
Mark the initial volume with a permanent marker on the tube.

Wash the semen in KRPG at 32°C (volume qsp in mL = total number of sperm x 2.5)
2 x 15 minutes at 600g (centrifuge at 20°C).
Remove the supernatant by aspirating up to the mark.

Currently running tests :
- Washing in milk diluent
- Final concentration divided by 2

Dilution (1) at 1 billion spz/mL in **milk-glucose diluent**:
Final diluted volume = (total number of spz – 15% (estimated losses during washing)) / 1.10^9
Or: Quantity of milk to be added = Final diluted volume - Initial volume

Cooling : After dilution at 20°C, place the collection tube containing the diluted semen in a beaker or cup containing water at 20°C and place in a cold room at 4°C for 30 minutes (water in the cup at approximately 10°C). Ideally, cool at a rate of 0.2°C/min. Place the cup in a tray of water at 0-2°C containing melting ice until the water in the beaker reaches 4°C (approximately 30 minutes). Then place the tube on a rack in the cold room.

Buck semen processing

Glycerolization = Dilution (2) to reach 500.10^6 spz/mL.

Add the milk-glucose with 14% glycerol diluent at 4°C in 3 portions :
To do this, divide the final diluted volume from step (1) into 3 and add each portion 10 minutes apart to allow time for the osmotic pressure to equilibrate slightly in between 2 additions.

Equilibration
Wait 1.5 to 2 hours before freezing. Fill in the straws and seal with polyvinyl alcohol.
1 straw = dose of 100.10^6 spz

Freezing
In a TA21 or liquid nitrogen tank :
- Place the freezing rack for 2 minutes at 16cm from the liquid nitrogen surface
- Then for 3 minutes at 4cm above the liquid nitrogen
- Then immerse the straws directly in the liquid nitrogen before storage.

Selection threshold : 30% motile sperm and motility > 3

Commercial diluents also tested (lab+fertility) :
- INRA96 (milk-casein)
- INRA99 (equine diluent with egg-yolk liposomes)
- Stem-alpha (lab only)
- Bioxcell (IMV)

Ram semen processing

Collection using an artificial vagina (water at 40°C) /!\ Below 38°C, rams will not ejaculate

Immediate 1:1 pre-dilution in the **lactose-egg-yolk** diluent at 32°C.

Volume control (weighing) / **concentration** (photometer).

Dilution with the lactose-egg yolk diluent at **900 million spz/mL**:
Final diluted volume = total spz / 900.10^6
i.e., Quantity of diluent to add = Final diluted volume - pre-diluted volume.

Cooling: Place the collection tube containing the diluted semen in a beaker or cup containing water at 30°C and place in a cold room at 4°C for 2 hours (until the water in the cup reaches 4°C).

Ram semen processing

Glycerolization
Add the milk - 10% glycerol diluent at 4°C in two batches :
Volume of each of the two added fractions = Final diluted volume x 0.4
Add the two fractions 20 minutes apart to allow time for the osmotic pressure to equilibrate slightly between two additions.

Equilibration
Wait 1.5 hours before freezing.
Fill in the straws and seal with polyvinyl alcohol.

Freezing
In a TA21 or liquid nitrogen tank:
- Place the freezing rack for 8 minutes, 16 cm from the liquid nitrogen surface.
- Then immerse the straws directly into the liquid nitrogen before storage.

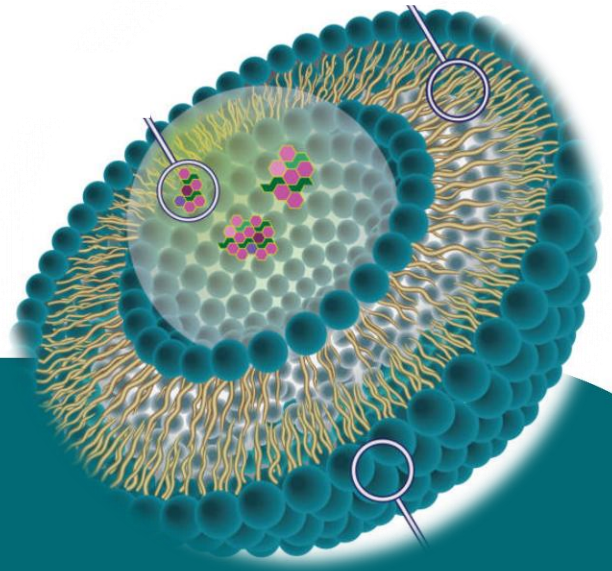
Selection threshold : 10-15% motile sperm

Commercial diluents also used :
- Optixell 2 (IMV)
- Bioxcell (IMV, in AGRISS)

RÉPUBLIQUE FRANÇAISE

Liberté
Égalité
Fraternité

VetAgro Sup



• NEXT-GENERATION LIPOSOMES
• FOR RAM SEMEN CRYOPROTECTION

Coordinator: Dr. Elodie **PILLET-MICHELLAND**
PhD Student: Elodie **MAZENOD**
Pierre **BRUYERE** and Loris **COMMIN**
Director of Research Unit: Prof. Samuel **BUFF**

Project Framework and synergies

Research carried out at VetAgro Sup - ICE Unit – Lyon
In partnership with Eliance and ROM-Selection

Funding secured :

- **LipoCryoPOC** (2024-2027) – Proof of Concept
- • Design and evaluation of tailored liposomes for ram semen cryopreservation

- **LIPINNOV** (2024-2026) – Technology platform reinforcement
- • Acquisition of advanced equipment for precise liposomes characterization

- **REPRODAV** (2025-2028, ROM-Selection Project Leader) – Field Application
- • Transfer of innovations

Objectives

From cellular mechanisms to field-ready solutions

- **TO UNDERSTAND the unique features of ram sperm and the underlying cryoprotection mechanisms**
- ■ Compare the lipid profiles of sperm plasma membranes across different ram breeds
- ■ Investigate the mode of interaction between liposomes and sperm cells: fusion, adsorption?

- **TO SYNTHESIZE liposomes specifically tailored for ram semen cryopreservation**
- ■ Fine-tune the composition of the phospholipid bilayer and encapsulated agent
- ■ Shift from manual extrusion to automated, scalable production using microfluidics

- **TO EVALUATE the cryoprotective efficacy of liposomes *in vitro* and *in vivo***
- ■ Develop a panel of functional cytometry assays suited to ram semen
- ■ Assess the impact of liposomes on post-thaw semen quality and fertility potential

INRAE

Thank you for your attention !

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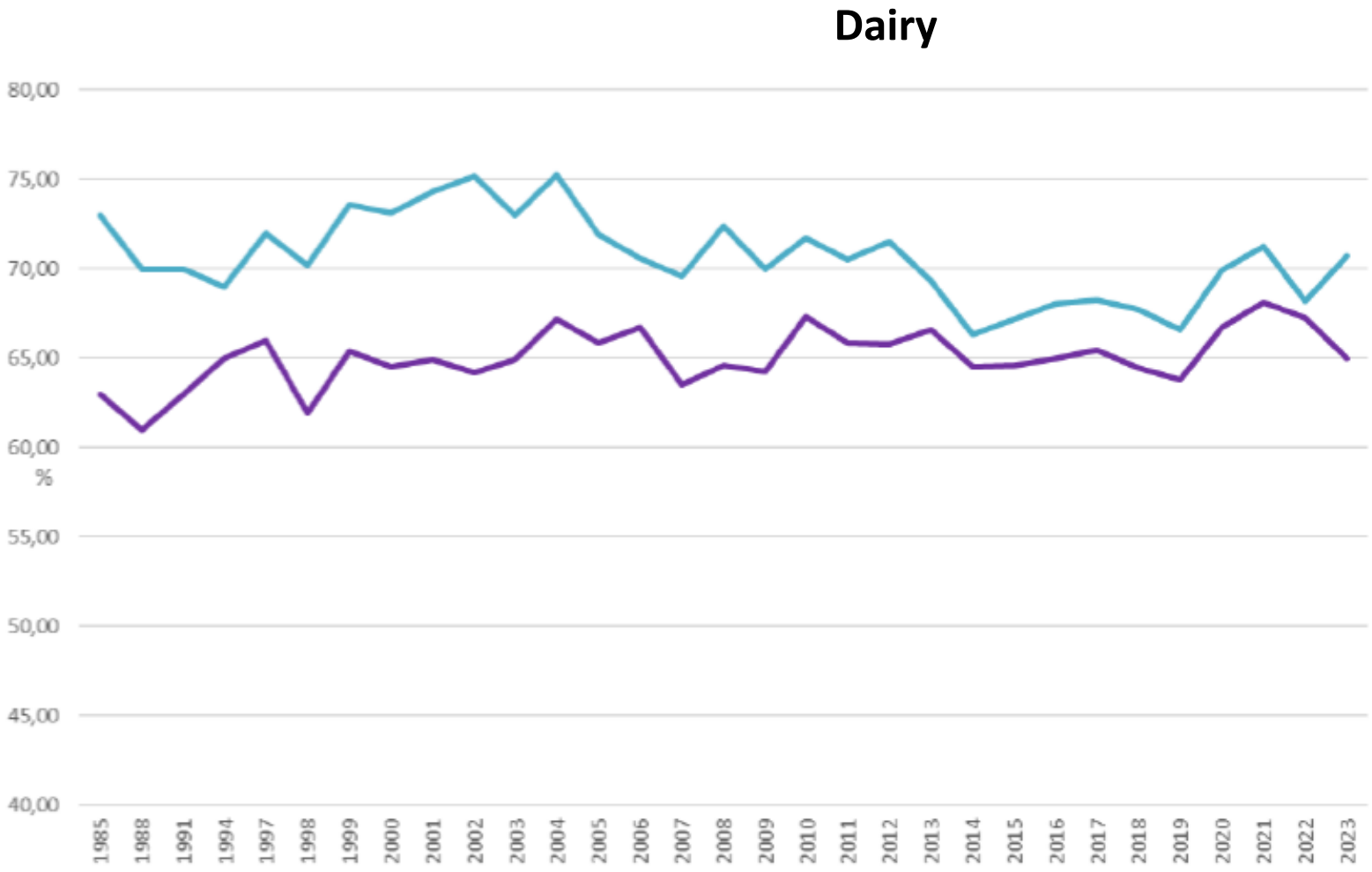
Fertility after AI



Dairy ewe lambs 70,75%

Agnelles

moyenne= 70,75
min= 33
max= 76

Adult dairy ewes 65%

Adultes

moyenne= 65
min= 28
max= 73

ANIO
ASSOCIATION NATIONALE INSEMINATION OVINE

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Source : *ANIO 2024*