

Impact of a restrictive use of hormones on breeding and selection management

Small Ruminants



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1.1. AI and hormonal treatments



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Objectives of AI and HT

- Effective technique:
 - **period of AI** according to local economical situation
 - inseminate females **once** at a predetermined time
 - **Spatio-temporal dissociation** between semen collection and AI
 - Low nb of spermatozoa per female : higher **diffusion of males**
 - High **fertility** level (60% goats and dairy ewes, 50% meat ewes)
 - Low level of time-consumption
- Unsophisticated technique, high fertility and low-cost

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Objectives when using AI and hormonal treatments :

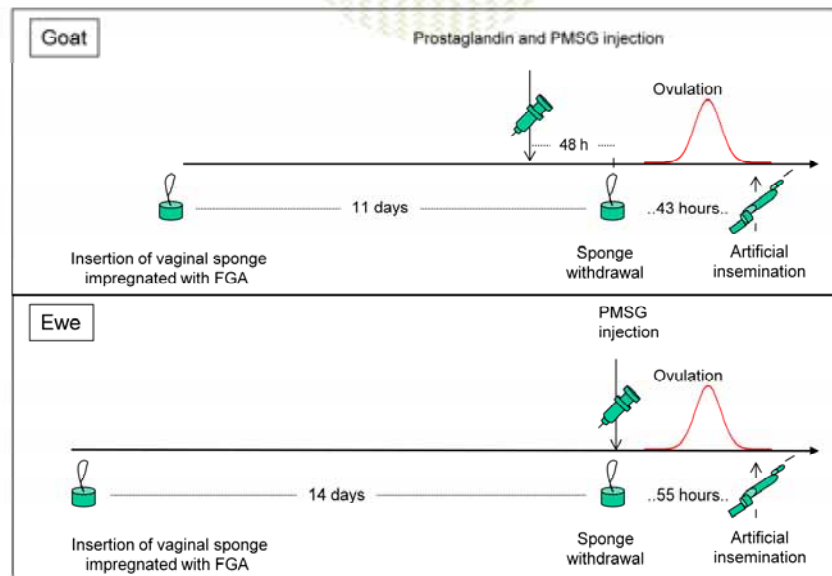
In sheep and goat, reproduction is seasonal, AI is mainly performed out of the breeding season. So breeders seek an effective technique so as to **choose the period of reproduction according to their local situation**. Actually, hormonal treatments allow to induce and group ovulations so as to inseminate only **once** at a predetermined time. This can be done at **any time of the year**.

The **spatial and temporal disruption between semen collection and its use for AI** is very interesting for males diffusion, particularly when semen is frozen. Also, the fact that you use only a **low number of spermatozoa** per female increases the **diffusion** of particular males as compared to natural mating.

This technique gives rather **good fertility results** with approximately 60% lambing or kidding rate in dairy goats and ewes and about 50% in meat ewes. It is not a very time-consuming technique since it implies **few interventions** on the animals.

Overall, the objective of breeders when they choose AI and hormonal treatment is to use a **non-sophisticated technique**, giving **good fertility results** and **at low-cost**.

Hormonal treatments used for ewes and goats



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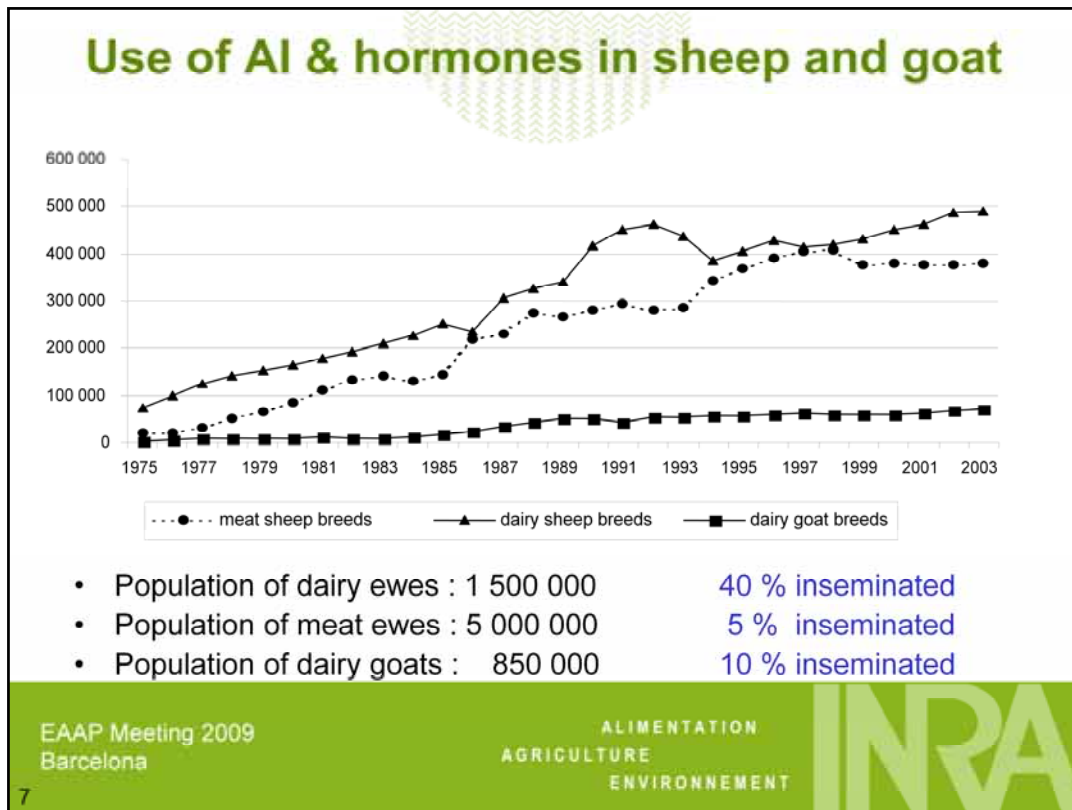
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Hormonal treatments used in sheep and goats are quite similar.

They consist in the deposition of a **vaginal sponge** impregnated with a progestagen (Fluorogestone Acetate is used in France) for 11 days in goats and 14 days in sheep to synchronize them.

Then, **PMSG** (Pregnant Mare Serum Gonadotrophin, also called eCG) is injected to induce ovulation, this injection is coupled with prostaglandin in goats to ensure luteolysis.

Artificial insemination is performed 43 (goat) to 55 hours (sheep) after sponge removal.



Here is the **evolution of the use of AI** following hormonal treatment in France :

Overall, there is a slow progression up to now.

In **dairy ewes** : the use of AI is more developed with 40% of total population inseminated. This is due to the fact that the population is concentrated geographically. Meaning, the travel costs are lower than in other productions that are more spread out. Also, the use of fresh semen is rather cheap but implies to realize the AI within 8h from collection

In **meat ewes** : AI is considered too expensive with regards to breeders income and obtained level of fertility is not satisfactory. This is why such a low proportion of the population is inseminated

In **dairy goats** : with only 10% of total population inseminated, the diffusion of genetic gain is mainly made through exchanges of males. Frozen semen is used which allows longer transports but increases the costs of AI. So, in this production AI is used mainly to create the genetic progress but not for its diffusion.

Interests of AI Genetics

- Particular **males diffusion** with AI > natural mating (NM)
- **Progeny testing**
 - ↗ index precision and genetic progress
- **Genetic connections** between flocks
 - ↗ relevance of genetic values
- Fast diffusion of a **particular genotype** (i.e. PrP)
- **Cross-breeding**

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What are the interests of using AI ?

From the genetics point of view :

- AI **increases the diffusion of particular males** as compared to natural mating
- AI allows to perform **progeny testing** which increases indexes precision and overall genetic progress
- AI also allows **genetic connections between flocks** which guarantees the relevance of individual genetic values
- using AI permits the **fast diffusion of a particular genotype** of a gene of interest : it was used in France to spread the PrP genotype for scrapie resistance for example
- and AI is also an interesting tool to manage **cross-breeding**

Interests of AI Breeding

- ↘ **nb of males** needed to serve females
and ↗ **selection pressure**
 - No need of physical presence
 - Diffusion of the proven males themselves
- ↘ **risk of disease spreading**
 - Sanitary check up during the quarantine period
 - Annual sanitary control
- **breeding management**
 - Births grouping
 - Out of season reproduction

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From the breeders' point of view :

- AI requires **less males** to serve females, since no physical presence is needed. Diffusion and therefore selection pressure are also increased.
- A major advantage of AI is of course **sanitary**. Using AI lowers the risk of spreading diseases, since males in AI center undergo sanitary check-ups during quarantine and annual controls.
- Finally, the main interests for breeding management rely in **births grouping** and the possibility to perform **out of season reproduction**



1.2. Selection programs



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AI in selection programs

Breeding scheme

= network of breeders working together to improve some characters in a population

Two main selection steps using AI :

creation of **genetic progress** & **diffusion**

- **planned mating** with best females
- **progeny testing** (dairy/meat characters, maternal abilities)
- **diffusion** of best males in and out of the selection nucleus

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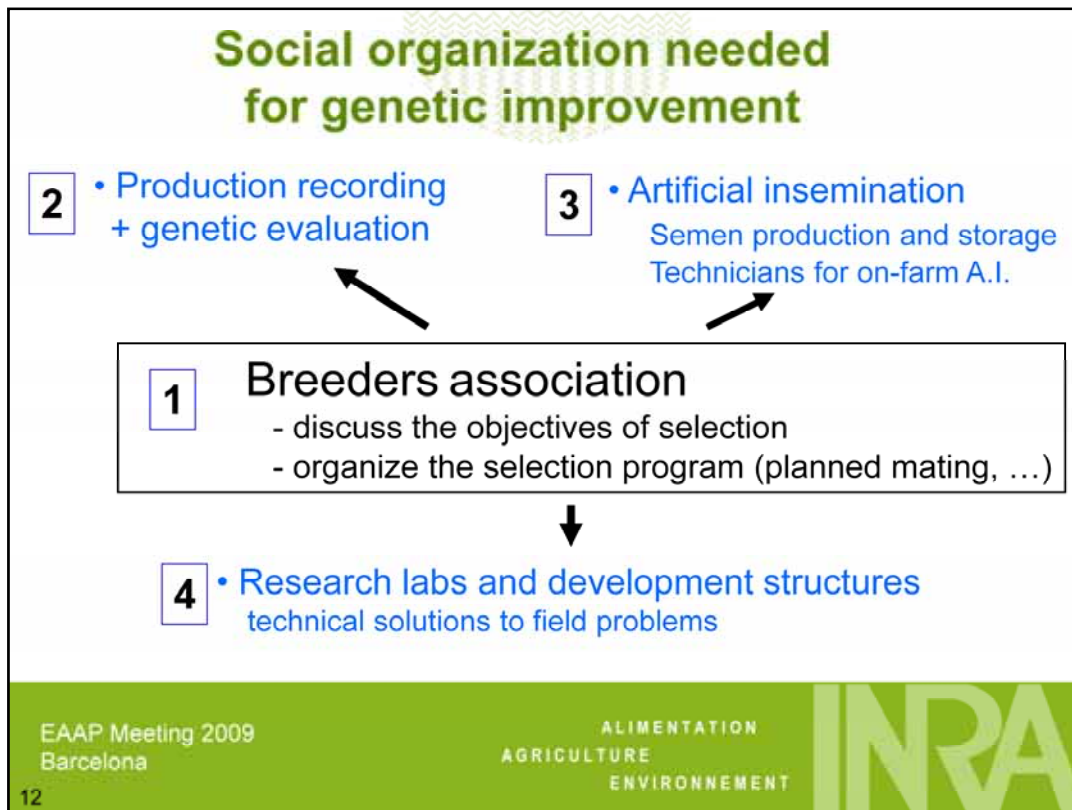
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Before describing their organization, let's define what a breeding scheme is :

A **breeding scheme** is a network of **breeders** working **together** to **improve** some characters in a population.

There are 2 main steps in selection that involve using AI :

1. the **creation of genetic progress** in the selection flocks : this is achieved mainly through planned matings and progeny testing
2. the **diffusion of genetic progress** by inseminating semen from proven sires both within and out of the selection nucleus

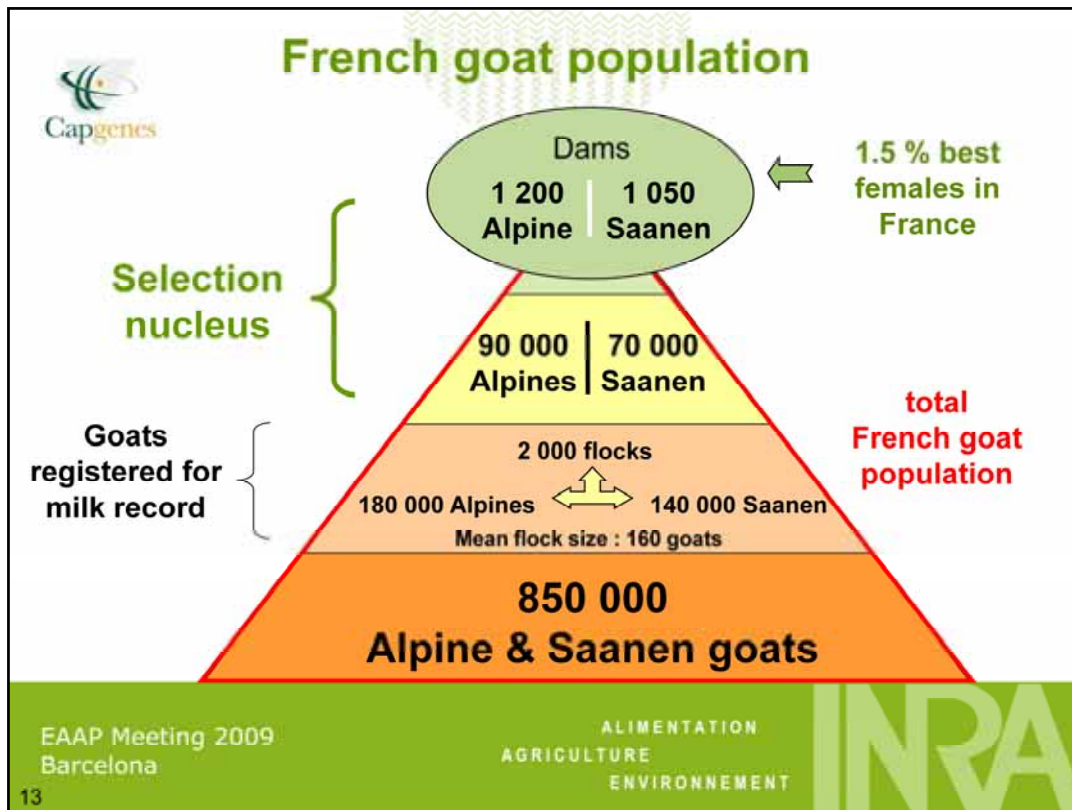


To implement such breeding schemes, the first step is to gather interested breeders in an association to establish **common objectives of selection** and organize the selection program.

The second step is to create **performance recording structures** either for milk , meat or maternal characteristics records and for the **genetic evaluation** of recorded animals.

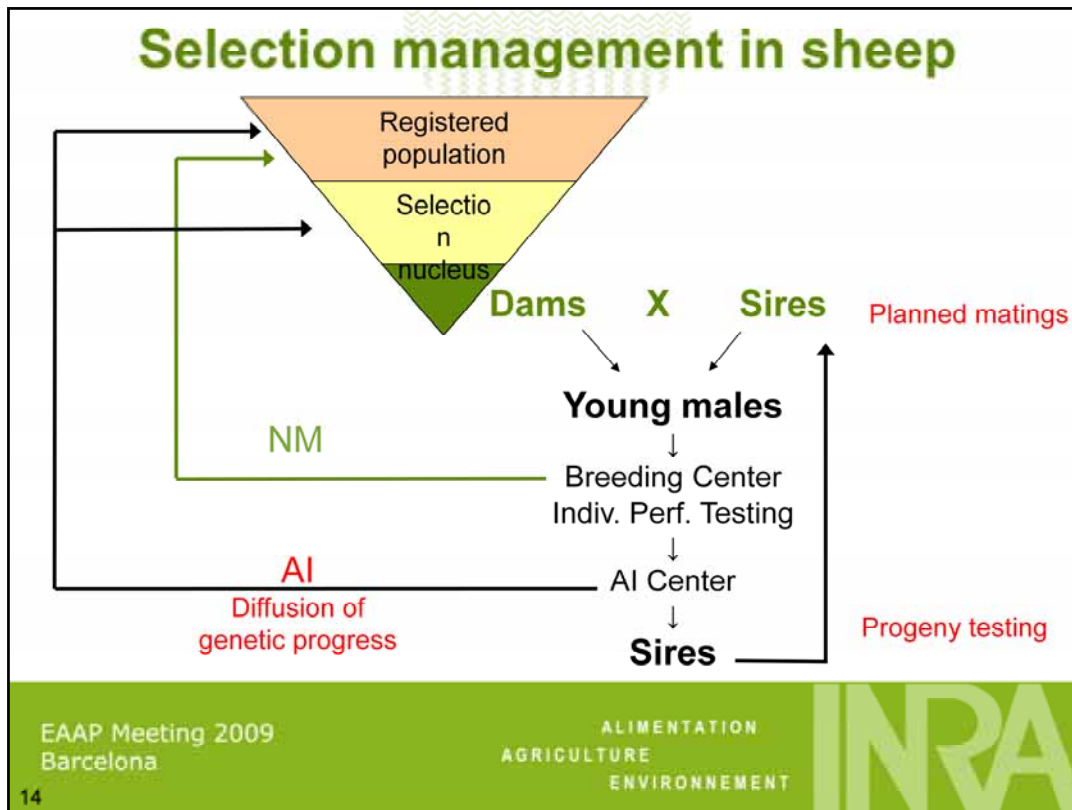
Also **artificial insemination centers** need to be created to perform semen processing and on farm AI.

Finally, interactions with **research and development structures** are highly important to **adapt and improve** the techniques used



This is how the **French goat population** is organized :

- there are 2 main breeds : Alpine and Saanen which represent approximately 50% of the population each
- the whole triangle represents the **total population** (red)
- and within the population, a certain number of animals are **registered for milk record** (light orange)
- among these, **selection flocks** participate in genetic progress creation through planned matings
- of which the **1.5% best dams** will be selected to create males that will enter artificial insemination center



There are numerous **breeding schemes in sheep productions** in France, with about 21 meat breeds and 5 dairy breeds having a specific breeding scheme.

Therefore, this graph is only a very general view of their organization. Still, they are very similar to that of goats, with only part of the total population being registered for **performance record** (either milk or meat) and only a part of the registered population being part of the **selection nucleus**.

From the selection nucleus, **best dams and sires** are paired to create young sires that will undergo different **testing steps** : individual performance testing, progeny testing and, if they become **proven sires**, then AI is used for their diffusion both in and out of the selection flocks.

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1.2. Current situation



European Regulations

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Drawbacks of hormonal treatments

- **Risk of inter-species contamination**
- **Preference for natural methods**
 - animal welfare
 - market requirements (Protected Designation of Origin)
 - organic farming
- **Residues in animal products**

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The critical drawbacks of hormonal treatments are the following :

- the risk of **interspecies contamination**, especially for PMSG, which is extracted from pregnant mare
- the social pressure for natural methods with the rise of awareness concerning **animal welfare**, some market requirements now imply hormone-free breeding to obtain certain labels not only in **organic farming**
- and of course the potential presence of **residues in animal products** (milk or meat)

European regulations & strategy

Directive 81/602 prohibits the use in stockfarming of certain substances having a **hormonal or thyreostatic action**

- estrogen, androgen, gestagen or thyreostatic.
- prohibits injection, slaughter and trade of animals treated with these substances

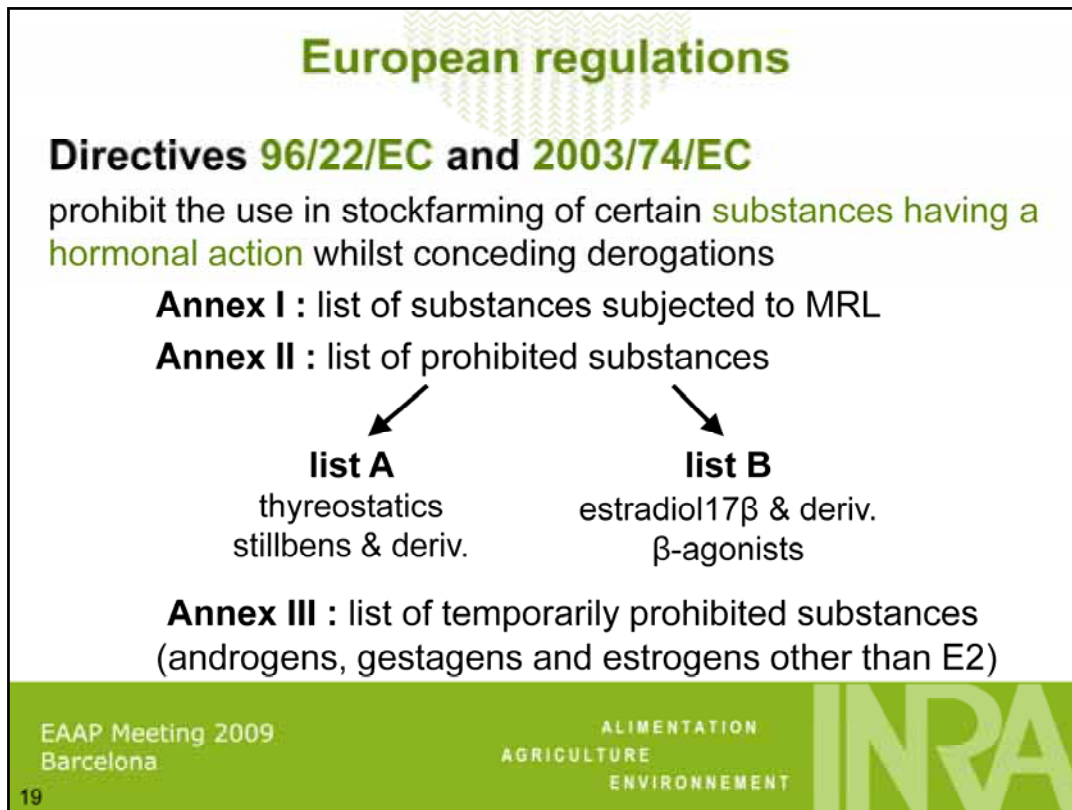
Directive 85/649 tries to limit derogations to previous directive, imposes control of substances at the production stage and controls of MRL / delay

Directive 88/146 stops all previous derogations

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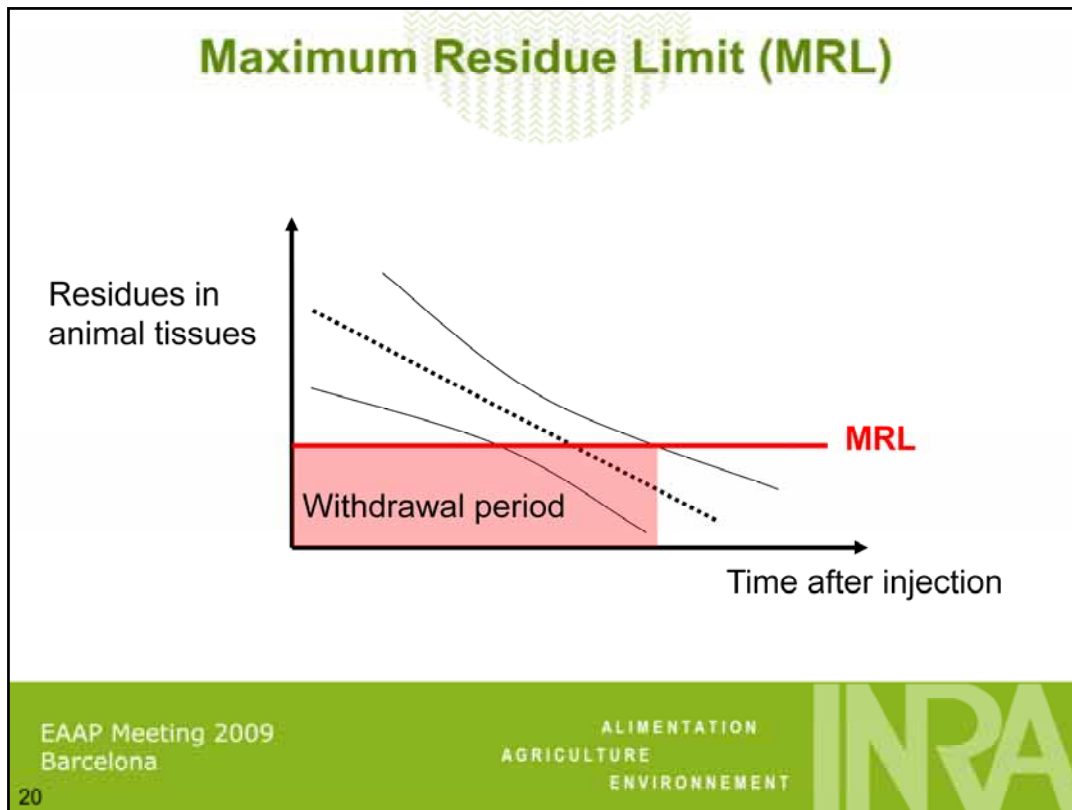


The most recent directives from 1996 and 2003 listed those substances in different Annexes.

First is a list of substances subjected to a **Maximum Residue Limit** (or MRL). This is the case of progestagens, in particular Fluorogestone Actetate (FGA) that is used in vaginal sponge in sheep and goat.

Then a list of **prohibited substances** such as estradiol and temporarily prohibited substances.

As a last example, let's mention prostaglandins which are fully authorized for now.



What is a **Maximum Residue Limit** ?

It is the maximum concentration of residues of a given substance in animal products (milk or meat) that is tolerated for consumption by human.

As shown on the graph, as long as the concentration of the substance residues in animal products is higher than the MRL, the products are subjected to a **withdrawal period** : meaning the milk has to be discarded or the animal can't be slaughtered and its meat sold until residues have decreased below the MRL.

So, for each substance, a particular withdrawal period is determined after injection.

So far, residues in urines and feces are not taken into account, but we shall be aware that this might change !



1.2. Possible evolution



European Regulations

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Possible evolution of European regulations on the use of hormones in animal productions

Management of a toxicological risk

- ✓ political and societal factors
- ✓ consumers pressure (organic, welfare, PDO)



Precautionary Measures

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In order to manage a **toxicological risk** (like a new substance or hormone to be used in stockfarming), Europe has to take into account a number of **political and societal factors**, and **consumers pressure** to make a decision.

This will lead, in most cases, to **precautionary measures**... sometimes not scaled with objective risks.

Possible evolution of European regulations on the use of hormones in animal productions

Current tendency to restrict the use of hormones :

- new vaginal sponges impregnated with FGA :
MLR => discard milk during 36h following insertion in goats
- previous formula still sold but revision of MLR expected

Risk of a progressive suppression of all hormonal treatments

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The current tendency, that we observe, is to **restrict** the use of hormones.

Actually, a new vaginal sponge impregnated with a low FGA concentration is now being sold with **a more restrictive MRL** than the previous formula.

Milk has to be discarded for **36h** following insertion of this pessary in goats (meaning 3 milkings) whereas in sheep, no withdrawal period is required.

As the previous formula is still sold, it is expected that its MRL might be revised soon compelling goat breeders to discard milk when using hormonal synchronization before AI. This might lead them to make an economical choice between the benefit of AI and cost of discarded milk...

This example shows that there is a **real risk** that hormonal treatments might progressively become fully prohibited.

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1.2. Impact on AI activity



Consequences of a ban of hormones

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Available breeding methods without HT

- **AI on natural heats**

less synchronicity
difficult estrus detection
low fertility with single AI

} implies multiple AI dates in one flock

↘ **efficiency**

↗ **cost of AI**

- **Natural mating / male exchanges**

slow diffusion
sanitary problems
lower or inexistent genetic progress

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Currently, the available breeding methods that do not require hormonal treatments are the following :

- **AI on natural heats** (induced or not by male effect) can be used but its low synchronicity and the difficulty to detect estrus properly implies to perform **multiple AI** otherwise fertility is drastically affected.

Therefore this technique is less efficient and has an increased cost as compared to a single AI after hormonal synchronization.

- **Natural mating** and **exchanges of sires** are the simplest available way to diffuse genetics.... **but** it is a very slow diffusion path that implies important **sanitary risks**. Also, the lack of genetic connections between flocks will lead to **lower or inexistent genetic progress**.

Consequences on AI activity

- **Endangered economical situation of AI centres**
 - ↘ **AI activity** in and out of the selection nucleus
 - ↗ **insemination cost**
and ↘ **efficiency of breeding scheme**
 - ↘ nb of progeny-tested males

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From the economical point of view, a total ban of hormones would lead to an **endangered situation for AI centers**.

The global AI activity would largely decrease both in and out of the selection nucleus.

Therefore, the **cost of AI** would increase and the overall **efficiency of breeding schemes** will drastically decrease with the decrease of the number of progeny-tested males and the decrease in genetic connections between flocks.

Consequences on the sheep and goat breeding schemes

- **The number of inseminations would decrease to what is « absolutely necessary »**
 - **Planned mating**
 - **Progeny-testing** for young sires evaluation
 - **Diffusion of proven sires** in selection flocks for creating a minimal connection

→ **Decrease or stop of AI for diffusion out of the nucleus**

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1.2. Genetic progress & selection



Consequences of a ban of hormones

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Definition of the genetic progress

$$\text{Annual genetic progress} = (i \times \rho \times \sigma_G) / IG$$

- **i** = selection intensity ↗ with AI
- **ρ** = index precision (\sqrt{CD}) ↗ with AI
- **σ_G** = genetic SD for a given character indep. of AI use
- **IG** = generation interval ↗ with progeny testing
↘ with AI without progeny testing

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Before going into details of the impact of hormones prohibition on genetic progress, let's define it precisely.

Annual genetic progress for a given character is a combination of :

- **selection intensity** is dependent on the number of proven sires for a given generation. The less proven males, the higher the selection intensity. AI allows a greater diffusion of a small number of sires.
- **index precision** : progeny-testing and genetic connections between flocks (via diffusion AI) increase the relevance of indexes
- **genetic standard deviation** : independent from the selection scenario (AI or not)
- **generation interval** : if progeny-testing is used, IG will increase → compromise between the latter and the increase of ρ using progeny-testing . If AI is used only for diffusion purposes (no progeny-testing), IG could decrease since males can breed earlier.

Simulations on the meat sheep selection scheme

- **Estimation of genetic progress in different selection scenarios**

using AI or not :

for creation of genetic gain

for progeny-testing and diffusion

from Bouix *et al.* unpublished data

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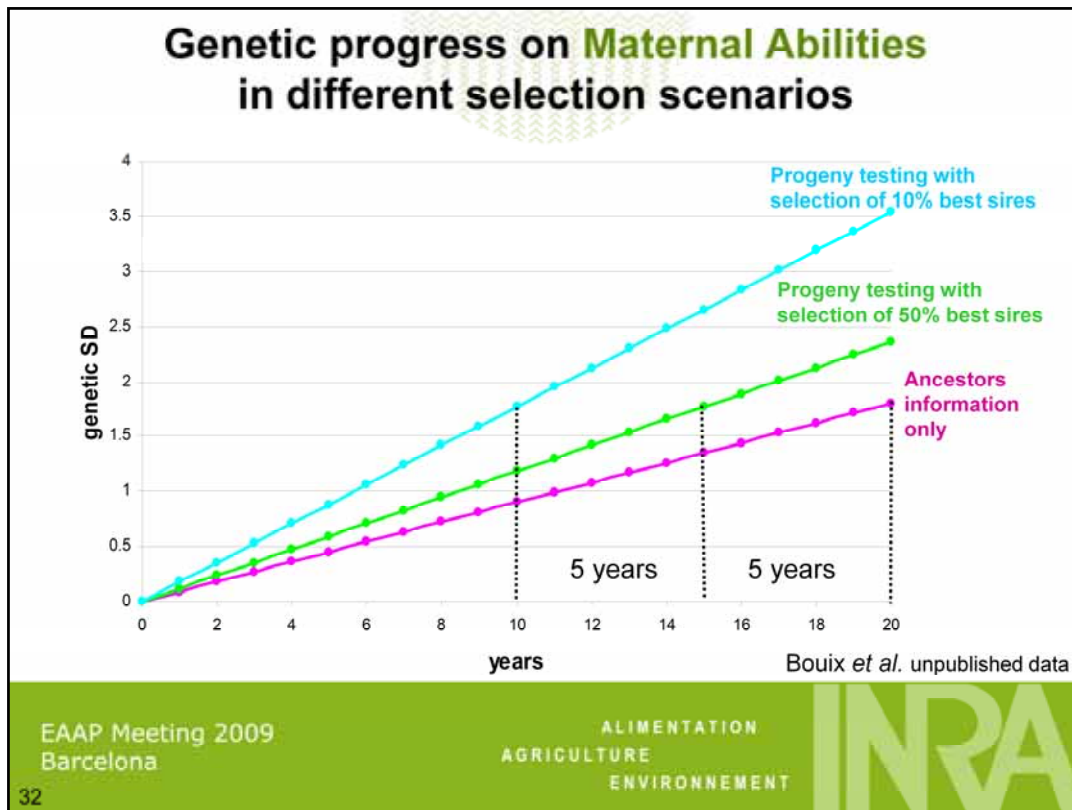
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A global reflection has started on the consequences of a ban of hormones on selection in dairy goat and sheep breeds and meat sheep breeds with **theoretical models of genetic gain**... but so far, only estimations for meat sheep are available.

Hereafter are presented **simulations** of what would be the **genetic progress** in different selection scenarios **using AI or not** in **meat sheep production**.

This study led by Jacques Bouix, from INRA Toulouse has not yet been published. For further information, please contact :

jacques.bouix@toulouse.inra.fr



This graph shows a theoretical evolution of **genetic progress** on **maternal abilities**, which is a synthetic index, in 3 different selection conditions :

- first, in the case of a selection based only on **ancestors information**, the genetic progress is figured in **pink**
- then, in the case of a selection based on ancestors information + **progeny-testing with a selection intensity of 50%** in **green**
- last, in the case of a selection based on ancestors information + **progeny-testing with a selection intensity of 10%** in **blue**

As shown on this graph, for a given genetic gain of **1.7** , the different selection options will require 5 to 10 additional years to reach the same level.

Here, when progeny testing and selection pressure of 10% best males reaches 1.7 genetic SD **within 10 years**, the option of a **selection intensity of only 50%** will require **15 years** and **ancestors information** will allow to reach the same level only after **20 years of selection**.

Genetic progress and generation intervals in selection schemes using AI (progeny testing) or not				
		Genetic progress	Generation interval (yrs)	Annual genetic progress
Maternal Abilities	1. Ancestors information (average EBV of parents)	1.12	12.3	0.090
	2. Progeny testing	1.89	16.0	0.118
	Difference between 1 and 2	+69% (+0.77)		+30% (+0.028)
Meat characteristics	1. Individual performance testing	0.874	12.3	0.071
	2. Progeny testing	2.18	13.3	0.163
	Difference between 1 and 2	+149% (+1.306)		+130% (+0.092)

Bouix *et al.* unpublished data

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Here is a general table of genetic progress and generation intervals in different selection scenarios with progeny-testing or not.

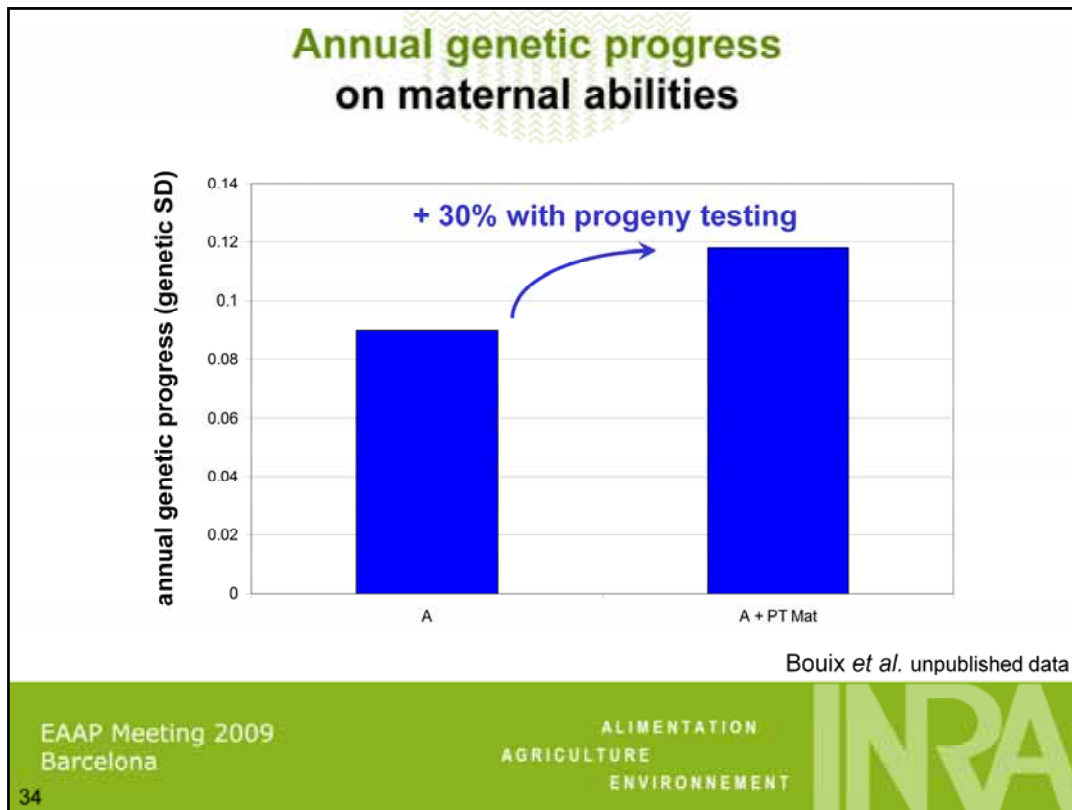
Those parameters were compared between a selection on ancestors information only or individual performance testing only and in the case of a selection with **progeny testing with a selection pressure of 50%**.

Genetic SD and generation intervals were estimated for 2 different characters : a synthetic index on **maternal abilities** and a second index on **meat characteristics**.

The generation interval is estimated as the cumulated generation intervals of all 4 selection paths : sire - son, sire - daughter, dam - son, dam – daughter.

→ Whatever the considered character, progeny testing, and therefore, the use of AI, allow to increase the annual genetic progress but also increases a little the generation interval.

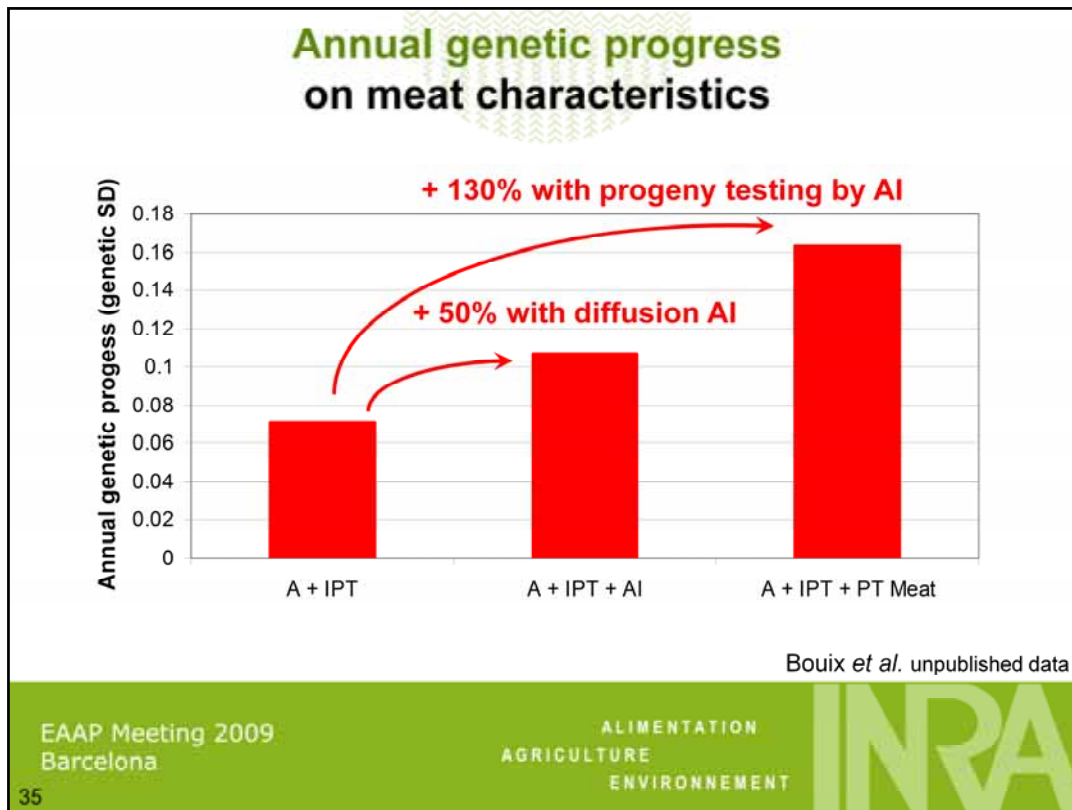
→ The difference is even more drastic for meat characteristics where progeny testing by AI increases genetic SD by +149%.



A = ancestors information

PT Mat = progeny testing on maternal abilities

More precisely, the annual genetic progress on maternal abilities will be increased by 30% when progeny testing (selection intensity 50%) is used as compared to a selection based on ancestors information only.



A = ancestors information

IPT = individual performance testing

PT Meat = progeny testing on meat characteristics (with selection intensity of 50%)

Here is represented the estimated **contribution of AI** to annual genetic progress on meat characteristics :

- using AI for **diffusion** allows an **increase of 50%** of annual genetic progress

- using AI for **diffusion** and **progeny-testing** increases annual genetic progress on meat characteristics by **130%**

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Main alternative to HT

Male effect

- **Synchronizes ovulations in +/- 70 % females**
 - over a 24h-period for 1st induced ovulation
 - over a 48h-period for 2nd ovulation following a short cycle
- **Protocol : using a teaser male with apron**
 - 1 male / 10-20 females, permanent contact until ovulation
 - at least 2 inseminations with a 24 hour interval

time-consuming and expensive

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Currently, the main alternative to hormonal treatments to synchronize ovulations out of the breeding season is the **male effect**.

Using the **male effect** to synchronize females allows ovulation synchronization in about **70% of treated females**.

When coupled with progesterone or prostaglandins, the male effect allows a **grouping of fertile ovulations over a 24h period**.

When using **male effect alone**, short cycles or silent ovulations can happen, in which case induced **fertile ovulations** will **spread over a 48h period or more**.

Performing a male effect consists in placing a **teaser male wearing an apron** among a female flock (1 male per 10 to 20 females). Males will remain in **permanent contact** with females until ovulation (15 to 20 days depending on the species and use of luteolytic treatment).

AI will be performed within 12-24h after estrus is detected. Since synchronicity is not optimal in hormone-free protocols, **2 consecutive AI** or more should be made with a 24h interval in-between.

These constraints make this technique **time-consuming** and **expensive**. Therefore, improvements are required before this technique can be used on a large scale.

Prospects in alternative to HT Optimizing AI

- **Increase efficiency of the male effect**
 - Improve preparation of animals (flushing, photoperiod...)
 - Reduce short cycle occurrence
 - Increase the females receptive period
- **Vegetal molecules with effects on the reproductive axis**
 - Acceptation by consumers ?

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Therefore, the prospects in alternative to hormonal treatments are mainly to **optimize the male effect technique**.

Particularly, the efficiency of the male effect could be increased by :

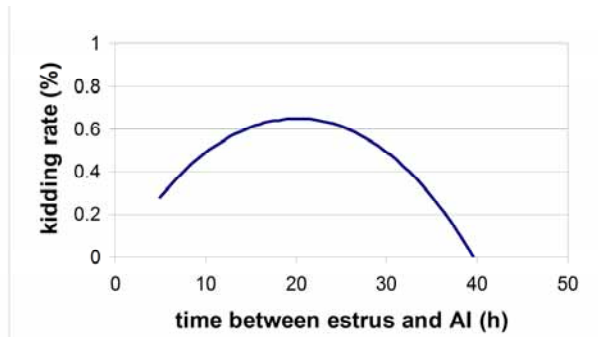
- improving the **preparation** of the animals, both male and females before performing the male effect (i.e. flushing, photoperiodic treatment...)
- reducing the occurrence of **short cycles** (alternative to progestative/luteolytic treatment ?)
- increasing the period during which females are **receptive** to the male effect, to allow its use year-round (photoperiodic treatment ?)

Vegetal substances are considered to replace synthetic or extracted hormones, but their acceptance by consumers is not obvious because they still imply an artificial manipulation of the hormonal cycle...

Optimizing AI technique

- **Oestrus detection**

- male apron marking oestrous females
- electronic devices



Bodin *et al.* unpublished data

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Research should also address to two key-points concerning AI :

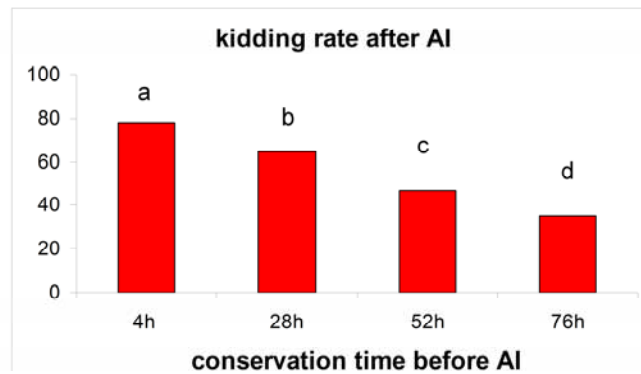
- first, the **estrus detection** : as shown on the graph, the timing of AI after the onset of estrus is quite important for fertility results. But estrus detection efficiency is not optimal (usual protocol : detection with teaser male every 4h).

The use of marking aprons and the optimization of electronic devices could lead to a more precise estrus detection.

Optimizing AI technique

- **Increase of the survival of spermatozoa :**

- increase fecundability of fresh semen up to 2-3 days
- in the female genital track



Leboeuf *et al.* 2004

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- **the survival of fresh semen** is a concern both in sheep and goat. Fresh semen has to be inseminated within 8h from collection otherwise fertility is altered.

In sheep, fertility is decreased when frozen semen is used, so fresh semen is widely used for AI.

In goats, the use of frozen semen allows more flexibility in the timing and location of AI but implies storing constraints.

Increasing the survival of spermatozoa should cover both **survival to storage** and resistance in the female genital tract but also the **maintenance of its ability to fertilize** for up to 2-3 days.

European project : FLOCK-REPROD

Hormone-free non-seasonal or seasonal goat reproduction for a sustainable European goat-milk market

- Maintain AI activity to sustain selection schemes efficiency
- Enable the European dairy-goat industry to conform
 - ✓ the existing restriction on the use of exogenous hormones
 - ✓ the expected total ban on the use of these hormones
- Offer alternatives both economically and technically viable
 - ✓ male effect + light treatment strategies + AI
 - ✓ maintain out of season production

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This major concern of **maintaining AI in selection schemes despite the risk of hormonal treatments suppression** preoccupies most European countries that breed goats.

An informal European network has recently obtained a European fund to work on this particular topic. The project was entitled **FLOCK-REPROD** standing for “**Hormone-free non-seasonal and seasonal goat reproduction for sustainable European goat-milk market**”.

Its objectives are detailed above and hereafter.

FLOCK-REPROD

Growing market demand + regulatory constraints
=> needs of the EU dairy-goat industry

- **Use sustainable and ecological breeding and reproduction methods**
- Optimize the quality and quantity of the milk produced
- Enable a year-round constant supply of goat's milk
- Ensure a high-standard of sanitary conditions
- Diversify supply via organic production

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