Annex 7: Summary working document from Work Package 3

1. Present stocks of cryopreserved semen

Ram semen from heritage sheep breeds is stored in the Netherlands, France, Greece and UK.

Table 1. Overview of available semen stocks in gene banks per breed.

Name of breed	Organisation[1]	single campaign / ongoing	When [2]	No of rams	No of doses	Dose size [3]	Ejaculated or epididymal	No. of storage sites [4]
Kempen Heath	CGN	Single	2001	16	2234	200	Ejaculated	2
Veluw Heath	CGN	Single	2001	7	715	200	Ejaculated	2
Veluw Heath	CGN	Ongoing	2004-2007	26	3036	200	Epididymal	2
Drenthe Heath	CGN	Single	2001	2	44	200	Ejaculated	2
Drenthe Heath	CGN	Ongoing	2004-2007	40	3101	200	Epididymal	2
Mergelland	CGN	Single	2001	21	2538	200	Ejaculated	2
Mergelland	CGN	Ongoing	2004-2007	4	498	200	Epididymal	2
Schoonebeker	CGN	Single	2001	10	1096	200	Ejaculated	2
Black Blazed	CGN	Single	2001	7	168	200	Ejaculated	2
Basco Béarnaise	ORDIARP Cryobanque	Single	2005	16	2032	100	Ejaculated	1
Manech tête noire	ORDIARP Cryobanque	Single	2005	20	2263	100	Ejaculated	1
Manech tête rousse	ORDIARP Cryobanque	Single	2005	53	6017	100	Ejaculated	1
CHIOS	NAGREF-Veterinary Research Institute		2006-2007	15	1000	100	Ejaculated	0.25-0.5 ???
Herdwick					6257			
Shetland					17824			
Rough Fell					2584			
Derbyshire Gritstone					308			
Lonk					0			
Romney					2742			
South Welsh Mountain					3971			
Welsh Hill Speckled					10714			
Dalesbred					1153			
Exmoor Horn					1824			
Devon Closewool					1475			
Cheviot					6416			
Brecknock Hill Cheviot					3499			
North Country					28999			

Cheviot								
Clun Forest					1567			
Southdown					3567			
Poll Dorset					8061			

[1] Organisation that houses and manages this gene bank/semen stock.
[2] Year, or years during which the semen stock of this particular breeds was collected and stored
[3] Number of sperm per dose, in millions of sperm. Give range if this is variable.
[4] The semen stock may be split and stored at more than one site as insurance against unforeseen events

UK	Total Number of Doses Stored for NSP 2007	Total number of doses	Number of alleles
Herdwick	5227	6257	42
Shetland	12517	17824	
Rough Fell	2584	2584	19
Derbyshire Gritstone	308	308	2
Lonk	0	0	0
Romney	2115	2742	14
South Welsh Mountain	3533	3971	21
Welsh Hill Speckled	10303	10714	21
Dalesbred	1153	1153	8
Exmoor Horn	1305	1824	9
Devon Closewool	1475	1475	6
Cheviot	5986	6416	56
Brecknock Hill Cheviot	2671	3499	22
North Country Cheviot	10776	28999	48
Clun Forest	1567	1567	8
Southdown	3567	3567	20
Poll Dorset	8061	8061	43

Table 2. Criteria that were used for prioritizing the breeds in different countries.

	The Netherlands	France	Greece	UK
Degree of endangerment	+++	++	not	Not
Disease outbreak	++++	++++	+++	Scrape genotype
Adaptation to a specific environment	+++	+	not	Not
Traits of economic importance	+	++++	++++	Not
Unique traits	+	not	not	Not
Cultural and historical value	++	++	not	Not

Importance of criteria (++++ very important, +++ important, ++ …, +…, not)

There is genetic material from other (e.g. commercial) sheep breeds (i.e not heritage sheep breeds) stored by these same or other organisations in the Netherlands, France, UK. Not in Greece.

Material is stored by farmers in the Netherlands:
Farmer Mr. Wijnker (Swifter)
Farmer Mr. Bosgoed (Swifter)
Farmer Mr. Verduyn (Milk Sheep)

In France genetic material is stored mostly for meat breeds. The organisations involved are: Confédération de Roquefort, CIA OVI-TEST, CRIOPYC, INSEM-OVIN, CIA GIE U.S. R.O.M., CIA de Verdilly, CIAP (cf. table next page).....
For some commercial breeds, there is also semen that is cryopreserved in the "Cryobanque Nationale" : the doses are kept in 2 sites for patrimonial purpose.

In the UK Innovis holds NSP, RBST, HGB. Archives held at Aberystwith 01970-828236
Also private stores at Dan Fawcett, South West Sheep Breeders. Michelle Hamilton.
Also stored for NISp in Northern Ireland. Two sites for security in general.

More generally, who in each country is knowledgeable about cryopreservation procedures, either by using it (in HSB or in commercial breeds), or by doing research in that field.
Veterinary Faculty Utrecht Mr Rijneveld
Farmers Mr. Wijnker

Research is done by INRA of Tours: Pascal Mermillod, Xavier Druart
Gilles Lagriffoul and Jérôme Raoul are involved in the general management of sheep AI for the "Institut de l'Elevage"

UK: Bill Holt.

2. Procedures for selecting donor animals

Genetic/phenotypic/morphology considerations, reproductive/health considerations. Practical and costs considerations.

The Netherlands
I. Ejaculated semen
Rams were selected by a breeding inspector of the Rare Breeds Foundation (SZH) together with CGN/ASG. The rams were chosen from redundant rams offered after completion of the breeding season. The genetic diversity and requirements of the herd book were the primary selection criteria used.
II. Epididymal semen
Rams were accepted when they were culled for other reasons than fertility problems or genetic problems.
Ik weet niet of er nog instructies waren ten aanzien van gewenste genotype. Volgens mij is er niet aangedrongen op een bepaald Scrapie genotype.

In France for HSB breeds, the "elites" rams are selected for genetic considerations, essentially milk production (EBV values). It is just checked that they do not have any morphologic defaults and that their sanitary status is compatible with the EU regulations of ram collection in an AI center.

In Greece the breed was selected for genetic, phenotypic and morphology considerations. Further for reproductive and health considerations.

UK Innovis: 1)Financial, 2)Performance and 3)Rarity

3. Logistics of semen collection

Was the semen of the Heritage Sheep Breed frozen specifically for gene banking purposes, or for regular use (AI) in a commercial setting
Specifically for gene banking purposes.
Specifically for gene banking purposes.
For gene banking purposes and for regular use.
For gene banking purposes and for regular use.

a) What are the (practical or financial) reasons why the gene bank collections have been made in a single campaign are updated and extended continuously?
Funding was made available by Dutch Ministry of Agriculture, Nature and Food Quality (LNV) after FMD outbreak in 2001. After making a 'start collection' of a number of Heath sheep breeds, we got additional funding from LNV in the following years for extending the collection (more breeds, more males/breed).
So far a single campaign was done for only three breeds for practical and financial reasons. It takes time to cryoconserved semen, and it requires a specific funding.....
Gene bank collections are updated and extended continuously.

b) Ejaculated semen: Is semen collected on site, or are rams brought to a central facility?. If the latter, is this for practical reasons. Or in order to comply with EU regulations.
Ejaculated semen: Central facilities, i.e. The experimental farms of Animals Sciences Group, Lelystad, and Faculty of Veterinary Sciences, Utrecht, respectively. For practical reasons: That is where the expertise and equipment are available.
Rams are brought to a central facility (an AI centre that has a European agreement) in order to comply with EU regulations.....
Semen is collected on site.
Ejaculated semen: Central facilities

c) In what season/months is collection of semen performed
I. Ejaculated semen
Directly following the breeding season (the females in the herds have all been serviced and the males have become redundant and could be made available for our use), but still in the 'biological' breeding season, i.e. the rams are still willing and able to serve, Progress was slower than anticipated. We managed to start two breeds in October /November, two other breeds in November-December, and two more breeds as late as January-March.
II. Epididymal semen
Most rams were done between August-February. However, in other months it seems to be possible to collect epididymal semen just the same.
It is performed particularly in summer and autumn but it can be till winter
All the year.
All year but especially July through to March.

d) How many ejaculates need to be collected per ram (on average), and what time frame is needed for collecting the necessary number of ejaculates.
The aim was to preserve 100 doses per male. Thus, we estimated that approximately 7 ejaculates per male were needed.
Estimated time frame was 3 weeks per breed.
However, with the very wild heath sheep breeds this was not possible. Two collection sites (Lelystad and Utrecht) worked for 5-6 months and have only managed to collect semen from 40% of the acquired number of rams.

In one collection between 40 and 130 straws can be done with 2 ejaculates. 1 or 2 days of collect are necessary if we want a minimum of 100 doses per ram.
Three successive ejaculates are needed to be collected at a time range from 10-20´ depending upon season of collection.
Usually 200-400 doses – 10-20 ejaculations. Over 1 month.

e) Epididymal semen: Is this from slaughtered rams, or are animals castrated? Where does isolation of sperm from the testes take place. How is the availability of rams /testes and transport of the testes to the site where sperm isolation takes place organised?
Slaughtered rams.
Testes are brought to the laboratory of the Animal Sciences Group in Lelystad for isolation of epididymal semen.
We have sent out advertisements in a sheep magazine and letters to herd books to ask herdsmen to notify the CGN (Centre for Genentic Resources CGN (at Animal Sciences group Lelystad) when they would offer a ram for slaughter (provided that the ram is culled for other reasons than fertility problems or genetic problems). In most cases we could use rams that and were offered for slaughter. In some instances rams were not offered for slaughter because of insufficient slaughter price and we had to transport rams on our own costs to a slaughterhouse. Transport of the testes from the slaughterhouse is done by CGN personnel. This person is also present during slaughter to be sure of the origin of the testes.
No epididymal semen was collected, it is forbidden. All the semen follow the EU regulations.
No epididymal semen was collected in Greece.
Epididymal semen was collected in UK, no description.

f) Please describe other factors that you think are important for cost-efficiency or for practical reasons
We are quite sure that collection of epididymal semen from rams in the way described above is extremely cost-efficient in comparison to collection of ejaculated semen from living rams, either on farm, or at a central collection site. With epididymal semen no transport of rams was necessary (in most cases), no housing, no training, and > 100 doses of epididymal semen per ram were obtained in one single effort.

It is a big constraint to have to follow the EU regulations, particularly the fact that the collect has to be done in on AI centre, the costs are really higher than in farm collection.

For the cost-efficiency are important the semen extender, evaluation of sperm genomic integrity in frozen-thawed ejaculates. Also, ram management procedures are important to maximize sperm output and you obtain good quality ejaculates suitable for cryopreservation.

Scale, Automation, Good Technique

4. Semen collection

Describe the procedure of semen collection for the heritage sheep breeds (housing of rams, training (if necessary), actual procedure).

I. Ejaculated semen

Housing and care
The animals were housed on straw. In the period that the rams were trained, they were housed individually or with 2 or three rams in a cage. Silage and water ad lib. The sheep received ½ kg of concentrate daily (Utrecht) or concentrate was only offered for training purposes Lelystad).
If necessary, the animals received an anti worm cure. Blood samples were taken from all animals. Tests were run at the Animal Health Service for zwoegerziekte (Maedi Visna Virus), and Brucella infections (abortus, melitensis, ovis). DNA test was run for Scrapie at the Central Institute for Animal Disease Control. (CIDC, now called CVI, Lelystad). A third blood sample is stored frozen for future purposes.

Semen collection
A separate room was created in which the ram was gently ushered onto an elevation, from which he could be approached by technicians. A teaser ewe was used. Ewes were brought in heat by injecting prostaglandin (1 ml Prosolvin® intramuscular) and oestrogen (3 ml Oestradiol benzoate® subcutaneous). After this the ewes received again 2 ml Oestradiol benzoate® subcutaneous at day 3 and day 6, to prolong the heat.
Semen was collected using an artificial vagina filled with hot water (40-45 °C).
Semen collection was done twice weekly, collecting two ejaculates per ram per session.
Depending on the breed, it took 3- 15 sessions to collect enough semen doses.

II. Epididymal semen

Collection of testes
Directly after slaughter, the testes are removed in the presence of CGN personnel in order to verify the animal ID of the testes. The testes are packed together with the label with the animal ID code in a polythene bag and placed in a coolbox on top of ice or freezing elements, but isolated from that by a towel. Thus, the temperature of the testes will decrease during transport (1½ h) to approximately 15 °C.

Isolation of epididymal semen
The further processing is performed the same day. All further processing is done at 15 °C. The cauda of the epididymis can be easily dissected from the testis and the rest of the epididymis with one single cut by cutting with a slaughter knife through the epididymis at the transition between corpus and cauda and proceed cutting, while sliding the knife downward (distally) along the side of the testis, thus freeing the cauda from the testis, separated proximally from the corpus and distally from the ductus deferens. In this way, the cauda contains very little blood. This procedure is done with both caudae. The caudae are rinsed with Tris-egg yolk freezing medium, blotted dry with tissue paper, and placed in a 15cm Petri dish. Approximately 13 ml of freezing medium is poored over the cauda. Then the cauda is cut many times (more or less 'minced') with a scalpel. One can see the thick yellowish very concentyrated semen oozing out of the incisions. The cauda is 'washed in the medium by moving it about using a pair of tweezers. Washing while massaging the cauda is repeated twice in a new volumes of freezing medium. The semen/medium from the first volume is sieved through a 212 µm screen, which is then washed with the second and the third volume. The second cauda is treated in the same way. In total we

now have approximately 80 ml of semen in freezing medium. Sperm concentration is estimated turbidimetrically with an adapted calibration curve and sperm motility is assessed microscopically. The semen is then diluted with freezing medium to 400 x 10^6 sperm/ml.

In France the housing of the ram is done by group of rams of the same age. There is a training period before the real collection. The procedure for the collection is the following: cleaning of the abdomen of the ram, letting the ram 5-6 minutes with the dummy, doing 1 or 2 false mounts (it improves the quality and quantity of the semen) and then collection of 2 ejaculates (few minutes are spent between the collection of each ejaculate

In Greece the rams are housed in separate group pens, tied in standings with natural lighting and ambient temperature. The pens are bedded with straw. The ram is taken to the ewe who can be in natural heat or brought into estrus using progestagen sponges and PMSG. The ram is then bred with the worker standing at the right side of the ewe and with the left side of the ewe and ram against a wall or gate. Finally, the ram is willing to mount and breed with the worker kneeling at the right side of the ram with a hand gently rubbing his underbelly. Once the ram mounts, this procedure is repeated as many times as possible until the ram permit semen to be collected into an artificial vagina. The artificial vagina is used most commonly for collection of ram semen. It is prepared for collection by the introduction of warm water (40-42°C) and air between the outer casing and soft inner sleeve, lubrication with gel in the end where intromission of the penis occurs, and attachment of a graduated collecting glass tube at the opposite end.

UK: A-V Collection requires training but used 95% of time
EEJ – if Rams are too wild and resist training__ – 4%
Epididymal harvest_____ 1%

5. Procedures freezing of semen

a) Is a single step or multi step dilution used? Single step means that the semen is diluted after collection with glycerolated freezing medium. Multi step indicates predilution with an extender without glycerol and later addition of a medium with glycerol.
Netherlands **Single step** / Multi step

Ejaculated semen
Ejaculated semen diluted rapidly after collection with freezing medium with glycerol. The semen collection tube with semen is placed in a 30 °C water bath. Subsequent ejaculates of the same ram are pooled. Semen concentration is measured turbidimetrically. Semen extender (Triscitrate, Fructose, Yolk + glycerol) at 30 °C is added within 5 minutes after collection to a sperm concentration of 400 x 106 sperm/ml. The percentage motile sperm in a is estimated microscopically. The tubes are placed at 5 °C. When all the rams are done, the tubes are transported from the barn to the laboratory.
Epididymal semen
Epididymal semen is collected from the caudae directly into freezing medium with glycerol, as described under point 8.

France: Single step / **Multi step**
Greece **Single step / Multi step**
UK **Single step** / Multi step

b) What type of diluent(s) (extenders, media) are used?
Freezing medium
Tris-egg yolk freezing medium, using Tris medium concentrate, from Gibco BRL Life technologies, Breda, The Netherlands, and pasteurized egg yolk from Eiproma, Wormerveer, The Netherlands

Ingredient	grams/l	M.W.	mol.l^{-1}
Tris(hydroxymethyl)aminomethane	24.22	121.14	0.2000
citric acid . 1H_2O	13.44	210.14	0.0640
D- fructose	10.0	180.16	0.0555
glycerol (>99.5%)	70.56		0.7660
Tylan	0.05		
Gentamycin sulphate	0.25		
Lincospectin 100 lincomycin/spectinomycin)	0.676		
Egg yolk	200 ml/l		

1st : lactose + pasteurised egg yolk, 2nd : skim milk + glycerol
Egg-yolk based extender (home-made), milk based (home-made), soybean lecithin-based extender (commercial media)
IMV Commercial Sheep or Tryl.

c) What is the sperm concentration after initial dilution?
400 x 10^6 sperm/ml
Initial concentration divided by 2
For laparoscopic Insemination: 100x10^6 spermatozoa
For cervical insemination : 800x10^6 spermatozoa

400×10^6 sperm/ml

d) For the cooling from the temperature shortly after collection to 5 °C, please describe the cooling rate and cooling procedure.
The tubes with extended semen (in Tris-egg yolk freezing medium) are simply placed in a 5 °C thermostatted coolbox, which has low-intensity forced ventilation. Thus, the effective cooling rate of the semen is <0.2 °C/min.
10 minutes after collection the tube is placed in a glass full of water and put in a refrigerator
The semen after dilution is stored at a cold cabinet for a 2 hrs cooling period at a rate of 0.5°C/min
30→4°c @ 2 hours, 1 hour stabilise, 4°c→70°c in 3 minutes, plunge to –196.

e) What is the temperature at which glycerol is added? What is the final glycerol concentration
Added at 30 °C
Glycerol concentration is 0.766 mol per litre of medium, or 5.6 % (v/v)
The temperature is 4°C, and the final concentration is 4%
During the multi step dilution glycerol is added at the temperature of 4°C
30°c.

f) Is there a 'holding period'? At what temperature? Before or after addition of glycerol?
Holding, in the sense of long contact with egg yolk and glycerol during cooling and after reaching 5 °C (approximately 1-2h).
Yes, before addition of the glycerol there is a holding period of 2H20 at 4°C.
Yes, After addition of glycerol there is a holding period of 1 hr at a temperature of 4°C.
Yes, 3 hours minimum.

g) What is the final sperm concentration?
400 x 10^6 sperm/ml
100.10^6 spz/ml
For laparoscopic Insemination: 100x10^6 sperm/ml
For cervical insemination : 800x10^6 sperm/ml
400×10^6 sperm/ml

h) What type of straws are used (volume/brand)?
IMV, 0.5-ml
0,25 ml, IMV
Straws of 0.25 and 0.5 ml are used
IMV 0.25ml

i) Describe the identification of the straws
We use an ink-jet straw printing machine. Straws are identified by:
Breed; Country code (528); Farm number (UBN); Animal ID code; Date; CGN
On each straw, the collect centre, the identification of the ram ad its breed are indicated.
The identification of the straws is done according to the ram, date of collection and the colour of the straw.
Ram No., Name, Breed, date, collection centre.

Describe the procedures for freezing the straws of semen
0.5-ml straws are printed, filled, sealed, and placed on racks (30-40 straws/rack) and placed in a nitrogen vapour freezer with forced ventilated air/nitrogen vapour at –80 °C during 10 minutes. After freezing the straws are plunged in LN2 and transferred to storage tanks.

It's either automatic (there is a machine to control the decrease of the temperature from 4°C to –196°C) or when there is no machine, the straws are kept 8 minutes 20 cm above the liquid nitrogen in the vapour (~-75°C) and then put directly in the nitrogen
Cooling of semen from 5°C to -25°C at a rate of 5°C/min
Cooling of semen from -25°C to -130°C at a rate of -50°C/min
Liquid nitrogen directly
30→4°c @ 2 hours, 1 hour stabilise, 4°c→70°c in 3 minutes, plunge to –196.

How is the post thaw quality of the semen determined?
From every ejaculate, or epididymal semen sample, one straw is thawed and % motile sperm is assessed microscopically. In case of apparent poor quality, a second straw is done. We do not discard the semen but will note the quality in our Cryo Information System (Cryo IS) database.
The semen is put at 38°C and after 2 hours the number of alive spermatozoa and their motility are estimated. The semen is rejected if less than 10% of the spermatozoa are alive.
The post thaw quality of the semen is determined depending on sperm motility (CASA), evaluation of membrane integrity, mitochondrial membrane potential, capacitation status, genomic integrity, sperm-oocyte interaction and ultra-structure with electronic Microscopy.
Progressive motility assessed visually by experienced Technician using contrast TV monitor and heated stage.

6. Insemination results

There are insemination results with the cryopreserved ram semen in the Netherlands, France, Greece and UK.

If yes, please describe breed of ewe, method of synchronization/natural heat/heat detection, method of insemination (cervical/laparoscopical), and results obtained

We have done one insemination trial with heath sheep breed semen

We have used frozen-thawed semen from Veluwe sheep for cervical and laparoscopic insemination on synchronised Swifter ewes, comparing epididymal semen from 4 males and ejaculated semen from 4 (different) males.

Synchronisation protocol:

Day 0. Progesterone sponge for 12 days

Day 10. Prostaglandins

Day 12. Sponges removed + eCG injection

Day 14. HCG + antibiotics (52 ± 1 hours after removal)

Day 14. Cervical AI after 57 ± 1 hours

Day 14. Laparoscopic AI after 59 ± 1 hours

	Ejaculated		Epididymal	
	% motile	% live	% motile	% live
	42.0 ± 4.5	48.5 ± 2.1	60 ± 0	62.3 ± 5.6
Inseminations	pregnant	lambs/ewe	pregnant	lambs/ewe
Cervical	0/11	- - -	4/10	2.0
Laparoscopic	6/10	2.3	7/10	3.1

In later insemination trials with frozen thawed ram semen (from commercial breeds) we initially also had bad results with ejaculated semen (12% pregnancy) but in later years we obtained better results, up to approximately 30% pregnancies. Because of the initial poor results with ejaculated semen from commercial breeds we do not think that the poor results with ejaculated semen in the table above were due to the Veluwe breed. We think that it may reflect at least in part that we had little experience in semen handling, freezing, and insemination protocol, while we improved on these point in later years (2004-2006). Anyway, the above results show that pregnancies and lambing is possible with the Veluwe sheep semen in the CGN gene bank and that epididymal semen performs at least as good as (or better than) ejaculated semen.

The heat synchronization is done with sponge. The method of insemination is always laparoscopical for the cryoconserved semen (cervical for fresh semen). In 2004, the fertility measured on all the breeds using cryoconserved semen (almost 3 700 AI) was 63.3%.

No description from Greece.

Various Innovis:
Synchronisation with Progestagen pessary 12 days and PMSG at sponge withdrawal
Laproscopic AI at 52 – 60 hours
Approx. 10,000/year at 65% pregnancy /conception.

7. Veterinary/ Sanitary issues

1) Do you follow EU semen regulations?

No

Yes, the EU semen regulations were transcribed in the French Legislation

Yes

There is no legislation apart from that covering intra - community trade
Under the Balai Directive. See:
http://www.defra.gov.uk/animalh/int-trde/imports/iins/livebalai/bal_live_1.htm
http://www.defra.gov.uk/animalh/int-trde/imports/iins/livebalai/bal_live_7.htm

Statutory Instrument 1993 No. 3248 The Artificial Breeding of Sheep and Goats Regulations 1993

2) Are there additional national regulations that you (need to) follow?

No

No

Yes

3) What diseases do you routinely check for before collection?

In the Netherlands

- Maedi Visna
- Scrapie
- BTV (Blue Tongue) since 2007

In France

- Brucellosis
- Contagios agalaxia (Mycoplasma agalactiae)
- Paratuberculosis
- Caseous lymphadenitis
- Epididymitis (Brucella ovis)
- Scrapi
- Pulmonary adenomatosis
- Maedi-visna

In Greece there are diseases that are checked for, no description.

NSP we routinely blood test for Border Disease after the first collect has passed our post thaw test. This is the only test we do, if we have a ram in for private collection then we take an A. Seminus test on the semen sample.

For the bluetongue animals we can only take rams from the surveillance zone (rams from protection zone are not allowed to move for 2 yrs). With these animals we are taking a blood sample for bluetongue testing on the day of the last semen collection. There is a repeat test 21-60 days after this test.

8. Costs of ex situ conservation and use of genetic material of heritage sheep breeds

Costs for collecting and freezing

In the Netherlands CGN (subsidised by Dutch Ministry of Agriculture, Nature and Food Quality) pays for collecting and freezing semen of heritage sheep breeds.

The average costs in the past 5 years (per breed) were:

Epididymal semen
We assume a cost of €500 per ram, leading to 12,500€ per breed.

Ejaculated semen (2001-2002).
According to the budget of the project, the costs of housing, training, collection and freezing of semen, were €260,000 for five breeds, or €52,000 per breed. However this was only partly successful because the rams were very wild and it took a lot of effort to train them, or training failed altogether: Semen from only 55 out of the planned 129 rams was collected and cryopreserved (7000 straws). Per ram this is almost €5000, and per straw this is €37

In France The ONILAIT (Interprofessional national organisation for milk and milk products) and ORDIARP (the AI centre) pay for collecting and freezing semen of heritage sheep breeds.
The average costs in the past 5 years (per breed) were: The cost for this campaign for the 3 breeds in 2005 was 25 000€ (89 rams collected)

The organization or through any other projects that are running

Personal funds or NSP for selective breeds + genotypes.
The average costs in the past 5 years were: Approx £300/ram

Costs for storing semen

In the Netherlands CGN (subsidised by Dutch Ministry of Agriculture, Nature and Food Quality) pays for storing the semen of heritage sheep breeds. The average costs in the past 5 years (per breed) were: Assuming 25 rams with 100 doses per ram = 2500 straws = 8.3 goblets = € 100 per breed per year.

In France The National Cryobanque pays for storing the semen of heritage sheep breeds. The average costs in the past 5 years (per breed) were: The tank cost itself is almost: 1 000€ and an additional cost have to be paid for the liquid nitrogen…(1 200€/year)

?

Personal funds for personal clients + DEFRA for NSP
The average costs in the past 5 years were: Approx 25p/dose/year.

Who is the owner of the stored semen of heritage sheep breeds?
CGN
The organisation which puts semen in the Cryobanque remains the owner of the stock. The AI centre are owners of the stored semen.
The owner is the NAGREF-VETRINARY RESEARCH INSTITUTE.

Personal,
Heritage Gene Bank – Sheep Trust.
NSP – DEFRA currently but the future ownership is undecided.

Is the semen of heritage sheep breeds made available to be used or is it just preserved for possible long term future use? If so, what are the conditions. (For example, it can be that the semen may be used to support breeding schemes for small populations in order to minimise inbreeding).
Just preserved for long term future use.
There are 2 types of storage :

- an active stock which can be used in any case (sanitary problem…), in order to have semen of rams still used by the farmers
- a stock in the Cryobanque: it can be used for example to add genetic variability in a breed, or for research purpose.

?
Personal
10% of NSP and HGB Theoretically belongs to owner of ram However It is never used.

9. Documentation

Table 3.Data is recorded of the donor animal

	Netherlands	France	Greece	UK
Breed	Yes	Yes	Yes	Yes
Date of Birth	No	Yes	Yes	No
Collection date	Yes	Yes	Yes	Yes
Identification number of donor animal	Yes	Yes	Yes	Yes
Breeder	Yes	Yes	Yes	Yes
Owner	Yes	Yes	Yes	Yes
Pedigree	No	Yes	Yes	No
Veterinary status	Yes	Yes	Yes	Yes
Location of the sample (storage)	Yes	Yes	Yes	Yes
Year of birth	Yes	?	?	?
Quality of semen (post thaw)	Yes	?	?	?
Number of cells/dosis	Yes	?	?	?
Color of sheep	Yes	?	?	?

Other: type of material (rare breed, genetically original, representative of the animal selected at a moment
Other.....see completed form.....??????

Annex 1

Other organisations in France that keep semen.

Organisation	Address	Telephone	Breeds of the rams (number of doses in stock when it is known))
CIA Verdilly @ : stverdilly@wanado.fr	Verdilly 02400 CHATEAU THIERRY M. LEMAIRE (Chief Centre)	03.23.69.15.94 Fax : 03.23.83.33.62	Ile de France (11 266) Berrichon du Cher (10332) Hamsphire (535) Est à Laine Mérinos (6 027)
Confédération @ : confederation-roquefort.cia@wanadoo.fr @ : confederation-roquefort.elevage@roquefort.fr	Le Bourguet Vabre l'Abbaye 12400 SAINT-AFFRIQUE Siège social : Confédération de Roquefort - BP 348 36, avenue de la République 12103 MILLAU CEDEX M. BRIOIS (Dir.)	05.65.98.10.80 05.65.59.22.00 Fax : 05.65.60.28.58	Lacaune lait Lacaune viande Charollais Rouge de l'ouest Suffolk
CIA OVI-TEST @ : unotec@unotec.net	La Glène 12780 SAINT LEONS Siège social : OVITEST Les Balquières Route d'Espalion 1285O ONET LE CHATEAU M. BELLOC (Director) M. ALBARET M. GIROU (Chief Centre)	05.65.61.86.22 05.65.67.89.40 Fax : 05.65.67.89.48	Lacaune lait (14 180) Lacaune viande Berrichon du Cher (6 393) Charollais Rouge de l'Ouest (6 298) Suffolk
CRIOPYC @ : criopyc@caramail.com	Route de Langlade 31450 POMPERTUZAT M. BELLIURE Director CIA M. RICHARD (Preparation)	05.61.81.75.88 Fax :	Berrichon du Cher Romane Tarasconnaise
CIA - GIE U.S. R.O.M. @ : giebmc@wanadoo.fr	Paysat Bas Mazeyrat d'Allier 43300 LANGEAC Siège social : IMACO Route de Thiers - BP 13 63370 LEMPDES M. PERRIN (Director) M. BOYER (Chief Centre)	04.71.77.14.14 Fax : 04.71.77.08.02 04.73.92.74.07 Fax : 04.73.92.76.87	Blanc Massif Central
CIOP @cdeo.ordiarp@wanadoo.fr	CDEO – Quartier Ahetzia 64130 ORDIARP M. SOULAS (Dir.) M. CACHENAUT (Chief Centre) M. FIDELE (Chief Centre)	05.59.28.05.87 Fax : 05.59.28.19.90	Berrichon du Cher Charollais Suffolk
INSEM-OVIN @ : insemovin@wanadoo.fr	Maison Neuve ; 11 allée du Breuil 87430 VERNEUIL/VIENNE Siège social : idem Siège adm. : Toutejoie 86500 MONTMORILLON	05.55.00.14.62 Fax : 05.55.00.12.04 05.49.83.30.46	Berrichon du Cher Charollais (11 031) Charmoise (1 850) Ile de France Rouge de l'Ouest (5 029) Suffolk (6 007)

	M. KUPPEL (Dir.) M. FERNANDEZ (Chief Centre)		Texel (8 873) Vendéen (8 702)

Gilles Lagriffoul and Jérôme Raoul are involved in the general management of sheep AI for the "Institut de l'Elevage".

Annex 2

States

Approval of centres and teams

2.—(1) The appropriate Minister, upon being satisfied that a semen collection centre complies with the provisions of Council Directive 92/65/EEC[2] laying down animal health requirements governing trade in and imports into the Community of animals, semen, ova and embryos not subject to animal health requirements laid down in specific Community rules referred to in Annex A(1) to Directive 90/425/EEC (in particular Chapters I, IIB and C and III of Annex D) (so far as it relates to ovine and caprine semen), shall, for the purposes of export of ovine and caprine semen to another member State, approve the semen collection centre.

(2) The appropriate Minister, upon being satisfied that a collection team is capable of complying with the provisions of Directive 92/65/EEC in relation to the collection of ova and embryos of sheep and goats may approve that team for the purposes of these Regulations.

And

Intra Community trade

3.—(1) No person shall collect, process, store or transport for the purpose of export to another member State any ovine or caprine semen, ovum or embryo unless it meets the conditions laid down in Article 11 of Directive 92/65/EEC.

(2) No person shall use semen for the insemination of any sheep or goat for the purpose of production of ova or embryos for export to another member State unless—

(a) the sheep or goat meets the conditions laid down in Chapter IV of Annex D to Council Directive 92/65/EEC, and

(b) the semen meets the conditions laid down in Article 11(2) of that directive.

(3) No person shall collect any ovum or embryo from any sheep or goat for the purpose of export to another member State unless he is a member of a team approved under Regulation 2(2) above.

For approved training centers see:

http://circa.europa.eu/irc/sanco/vets/info/data/semen/ms-sc-ov-cp.html