RESEARCH PROGRAM ON Livestock

More meat milk and eggs by and for the poor

Technical guideline for reproductive biotechnologies of sheep and goat in Ethiopia

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CGIAR is a global partnership that unites organizations engaged in research for a food-secure future. The CGIAR Research Program on Livestock provides research-based solutions to help smallholder farmers, pastoralists and agro-pastoralists transition to sustainable, resilient livelihoods and to productive enterprises that will help feed future generations. It aims to increase the productivity and profitability of livestock agri-food systems in sustainable ways, making meat, milk and eggs more available and affordable across the developing world. The Program brings together five core partners: the International Livestock Research Institute (ILRI) with a mandate on livestock; the International Center for Tropical Agriculture (CIAT), which works on forages; the International Center for Research in the Dry Areas (ICARDA), which works on small ruminants and dryland systems; the Swedish University of Agricultural Sciences (SLU) with expertise particularly in animal health and genetics and the Deutsche Gesellschaft für Internationale Zusammenarbeit (GIZ) which connects research into development and innovation and scaling processes.

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1 Introduction

Reproductive biotechnology in animal production is widely used to increase production, reproductive efficiency and rates of genetic improvement (Madan, 2005). According to NRC (2003), animal production biotechnology is defined as '*the application of scientific and engineering principles to the processing or production of materials by animals or aquatic species to provide goods and services for the well-being of human population*'. It could be also defined as 'that set of techniques by which living creatures are modified for the benefit of humans and other animals (Bin Abdullah et al., 2011).

Conventionally, reproductive biotechnology, applied to animal production, includes 4 generations going from artificial insemination (AI) which was the technological advance of the 1950s used in traditional selective breeding and enhancing modern industrial animal production, especially in dairy and poultry (first generation). Other related biotechnologies were adopted to improve the efficiency of AI such us estrus synchronization. After AI and estrus synchronization and with the development of new techniques, embryo transfer (ET) is the most commonly used biotechnology. Later, with the development of DNA techniques, new techniques and technologies including in vitro fertilization (IVF) and sexing embryos appears as third generation of reproductive biotechnologies. Finally a generation of transgenic animals (animals with one or more genes introduced by human intervention), using gene knockout technology to generate animals in which a specific gene has been inactivated or production of nearly identical animals by somatic cell nuclear transfer (also referred to as clones), emerges in the early 1990s (fourth generation) (Cowan and Becker, 2006). Recently, new techniques such as 'surrogate male' have emerged, which enables the sterilization of males and to make their bodies produce the sperm of other males. While the livestock could not produce enough sperm to impregnate females, 'surrogate sires could one day be an alternative to artificial insemination', says Professor Oatley (Washington State University).

Despite all listed techniques, the use of assisted reproductive techniques (ART), such as semen cryopreservation, artificial insemination (AI), estrus synchronization and superovulation, laparoscopic ovum pick-up (LOPU), in vitro maturation, fertilization and culture (IVMFC), intracytoplasmic sperm injection (ICSI), embryo sexing, embryo/oocyte cryopreservation, cloning, stem cell, embryo transfer, pregnancy diagnosis (ultrasonography/hormone essays), remain the best way to improve animal production performance in farm practices (Cowan and Becker, 2006). For example, using AI in the ovine/caprine industry, enhances genetic improvement, synchronizes the lactation period and meat production in order to overcome the market need. Pregnancy diagnosis allows detecting females with reproductive problems and a better control of management conditions such as nutrition improvement.

In countries under tropical conditions, such as Ethiopia, sheep and goats production is an important activity for smallholders, particularly as resource by augmenting income and employment and reducing the incidence of rural poverty through direct food production and a vast range of products and services such as cash income, meat, milk, skin, manure, risk spreading/ management and social functions (Adane and Girma, 2008).

In Ethiopia, animals are reared in a crop-livestock farming system, under poor management condition, severely limited resources and seasonal fluctuations in feed resources especially under prolonged drought period in the arid and semiarid lowlands of the country, uncontrolled breeding techniques are usually used especially in the absence of improved breeding techniques. Therefore, flock fertility is affected since infertile male and female goats are kept together. In addition, given the diversity of goat breeds, the risk of crossbreeding, and losses of pure-bred genotypes is increased.

Animal owners are generally smallholders with little or no land and few animals, who must get through the constraints of the local conditions. In low and middle income countries, such as Ethiopia, biotechnological applications relating to livestock are essential to improve animal production and to conserve the indigenous animal genetic resources. Therefore, they need to be simultaneously suitable for animal owners and dictated by commercial considerations and socio-economic goals (Madan, 2005).

The constraints of biotechnology applicated to animal production in such countries are lack of infrastructure, technical skills and the high cost of such techniques.

2 The Reproductive platform for the delivery of improved genetics in Community-based breeding programs (CBBPs)

Generally in developed countries and in high-input animal production systems, animal breeding sector is well structured and supported by the state through national breeding programs. This is not the case in developing countries and low-input production systems where such breeding schemes and structures are absent and livestock keepers have usually limited access to improved breeding stock or reproductive biotechnologies such as AI and rely only on their own traditional breeding practices.

Centralized breeding schemes, usually a nucleus breeding unit entirely managed and controlled by governments with minimal, if any, participation of farmers (Haile et al., 2018), have failed to sustainably provide the desired genetic improvement to small holders (Haile et al., 2020).

The import of improved lines of live animals, semen or embryos, usually crossbred with the local and 'less productive' breeds, failed also in disseminating genetic improvement since this was done without sufficient pretesting of the suitability and adaptability of the exotic breeds and their resulting crosses to local production systems or conditions, and with no clear strategy concerning what the final genotype would be.

Community-based breeding program (CBBP), using the community's own genetic resources instead of the regular use of externally produced males, was used as an alternative approach. This program:

- relate to low input systems with farmers within limited geographical boundaries;
- farmers have a common interest to work together for improvement of their genetic resources;
- takes into consideration farmers' needs, views and decisions;
- rely on the active participation of farmers from inception through to implementation;
- provide opportunities for the implementation of breeding strategies that might successfully improve livestock production in rural areas with limited resources available;
- provide the only sustainable option for conservation of local animal genetic resources by utilizing and improving them (Mueller et al., 2015);

In Ethiopia, CBBPs have been implemented since 2009 by:

- the International Center for Agricultural Research in the Dry Areas;
- the International Livestock Research Institute;
- the University of Natural Resources and Life Sciences;
- in partnership with the Ethiopian national agricultural research system;

Looking at the benefits and practical feasibility of CBBP (Gutu et al., 2015; Haile et al., 2017), the government of Ethiopia through its regional research and extension system (with ICARDA's support), established additional CBBPs and the number of CBBPs has increased from the initial six to more than 40 CBBPs having each around 80 households (Figure 1).

Breeding programs are supported by other platforms such as the reproductive platform, which is a very generic term designating the infrastructure put in place and based on the use of reproductive biotechnologies and the development of national capacities in the field of sheep and goats' rearing. This platform contributes to the data flow, establishes linkages between relevant national institutions (research centers, universities, national AI centers, private veterinarians…) and synergizes exchange of knowledge between the different project target areas. This concept, established across breeds, sites and countries, represents a dynamic space for the enhancement of CBBP's (Figure 2).

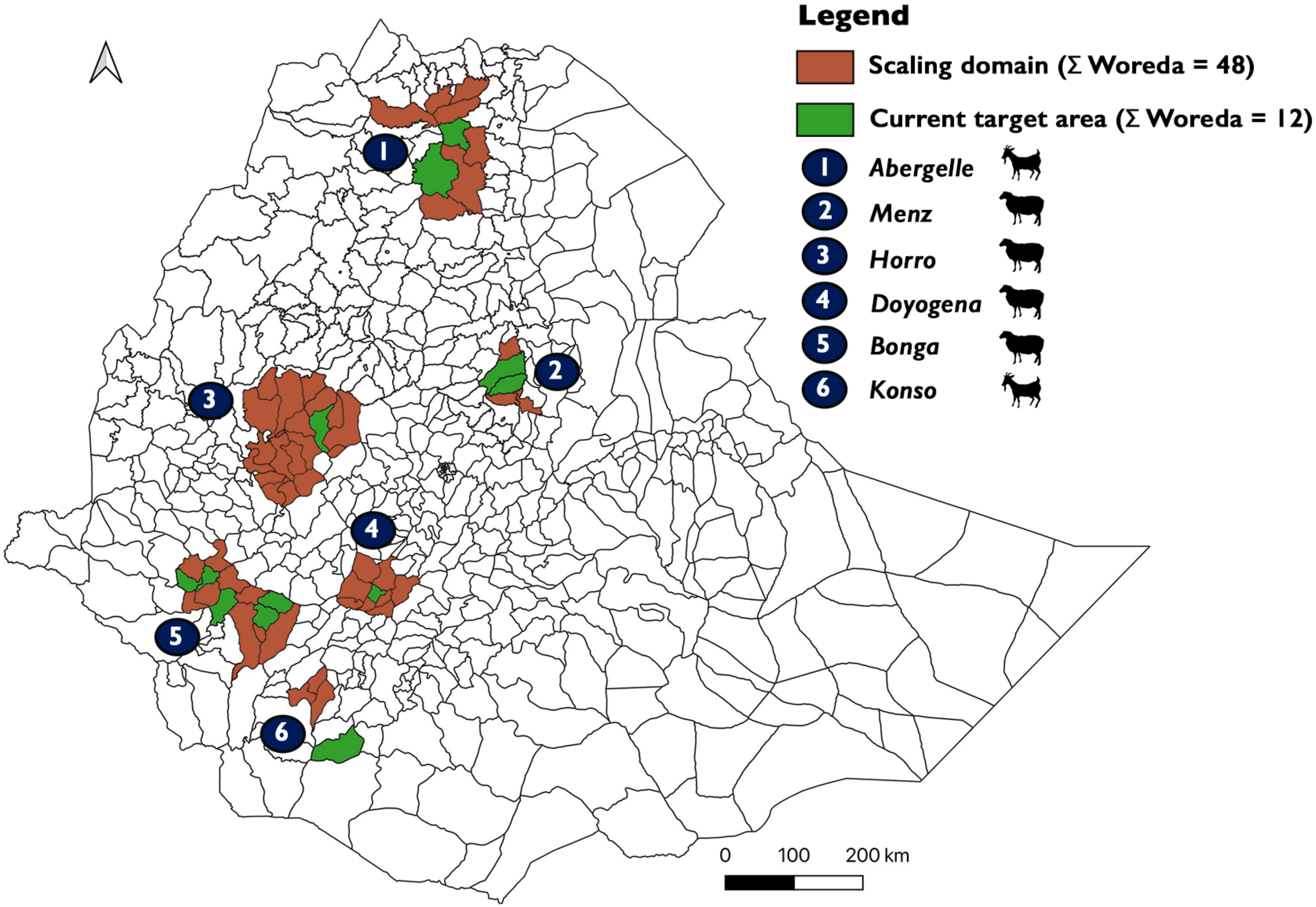


Figure 1. Location Community-based breeding programs (CBBPs) in Ethiopia

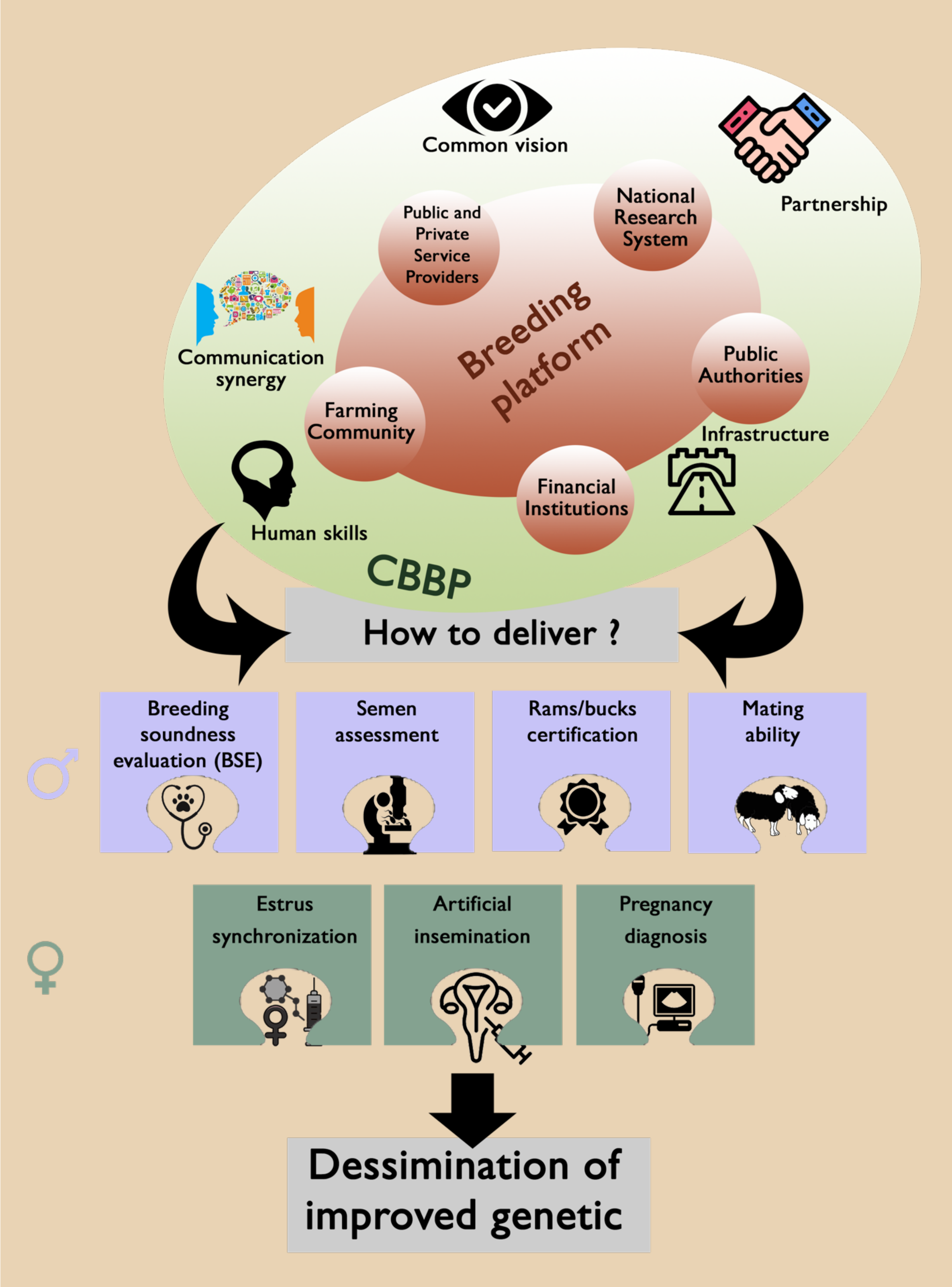


Figure 2. Dissemination and delivery system of improved genetics in the reproductive platform (CBBPs) in Ethiopia

3 How to use this guideline

Objectives of the guideline

This guideline has been conceived in order to resume all reproductive biotechnologies held in Ethiopia as part of the reproductive platform for the delivery of improved genetics in Community-based breeding programs (CBBPs).

This guideline was based on practical experiences going from male selection until pregnancy success in Ethiopia and will therefore provide guidance for extension to other regions or for other countries planning similar projects.

The principals objectives of the guideline are:

- Describe in a very simple and smart way all the steps inside the reproductive platform for the delivery of improved genetics in CBBPs;
- Give details about the success of the reproductive events;
- Take the user through the main reproductive biotechnologies used in the reproductive platform for the delivery of improved genetics in CBBPs such as artificial insemination (AI);
- Describe innovative protocols used in the East-African context such as synchronization protocols in sheep and goats;

Who will benefit from this guideline…

The guidelines are intended for use by all persons and organizations interested and involved in planning and implementing reproductive development activities, in particular research institutions, nongovernmental organizations (NGOs), private institutions (e.g. farmers' associations and livestock development projects), and government officials. In the case of the Ethiopian sheep breeding program the target group for the guidelines included livestock keepers, national and regional research institutions, the extension system, universities, NGOs, and policy makers.

When using this guideline…

Reproductive biotechnologies described in this guideline concern all animals that are involved in improved genetic programs.

Conditions under which the guidelines should be used

Most of the elements described in this guideline are applicable beyond the local Ethiopian situation and hence can be easily adjusted to specific cultural, social, economic, or ecological conditions and similar production systems in other countries.

The guidelines can be used in connection with the guidelines provided in ……

Structure of the guideline

The main parts of the guideline consists of 2 section:

- **Section 1**: Reproductive techniques used to select rams/bucks, covers all the techniques used to select the best rams and bucks through Breeding Soundness Evaluation (BSE) including visual appraisal of general health and condition, as well as a soundness check on eyes, nose, mouth, teeth, feet, legs, body score, and reproductive organs, semen assessment, certification procedure and mating ability.
- **Section 2**: Reproductive techniques used in female sheep and goat, covers all the techniques used in ewes/does going through estrus synchronization, AI and pregnancy diagnosis in order to disseminate genetic improvement.

4 Reproductive techniques used to select rams/bucks

In Ethiopia, the productivity of livestock (sheep and goats) is considered as low which is due to low reproductive performance including a low fertility rate in the breeding herds (Payne and Wilson, 1999). Even if infertility of the ewe or the doe is closely linked to this productivity problem, the fertility of the male has generally a greater influence on the herd performance than the fertility of individual females (McGowan, 2004). Therefore, as males affect the reproductive efficiency of the herds, screening breeding males is very useful for improving sheep and goat production (Chacon et al., 1999).

At mating, rams and Bucks should be in excellent condition: the examination of rams for mating ability is an important step to improve flocks' fertility and avoide disease transmission.

The reproductive performance of the male should not be addressed only prior to the mating season but should be monitored regularly during the year. and not only during the mating season.

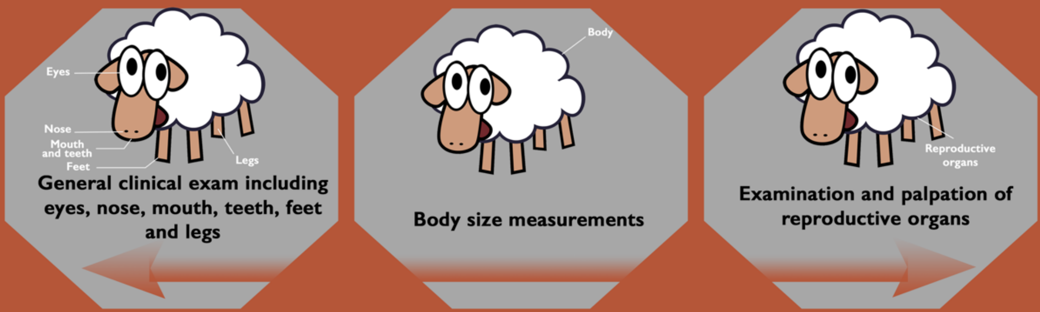
When purchasing… The breeding ram/buck should have a known origin (non-contaminated flock and certified or at least known parents). A veterinary examination for the general health and condition will be of great help to detect any health trouble. Breeding Soundness Evaluation (BSE), as well as a soundness check on eyes, nose, mouth, teeth, feet, legs, body score, and reproductive organs is essential. Where possible, males should be assessed for semen production and viability. Breeding soundness should be performed at least two months before mating to allow recovery of rams from pathologies or poor physical conditions.

When feeding… To maximize testis mass and therefore quantity of sperm produced, males need to be fed a grain-based supplement each day for 8 weeks before mating. Concentrate-fed rams are prone to the precipitation of mineral elements in their urine. An important issue here is the concept of 'fit but not fat' – males that are overweight and do not get exercise can perform poorly, even when they have maximum testicular mass.

Figure 3. Breeding A: Doyogena ram, B: Bonga ram, C: Sekota buck

4.1 Breeding Soundness Evaluation (BSE)

Breeding soundness examination (BSE) is the most practical tool which enable selecting the potentially best breeding male animal in a flock. This routine examination is done even for rams/bucks that are not implicated in genetic improvement programs and for males used or not for AI. This technique enables selection of breeding males for a reproductive purpose.



Provides an overview on other reproductive parameters (production of viable spermatozoa, the reproductive growth status, spermatogenesis output, the seminal characteristics).

Prevents the introduction of infertility problems into a flock when selecting and purchasing breeding males, the BSE also helps disclose prevalent breeding problems in a flock such as identifying subfertile males and assessing the current status of males of previously good fertility.

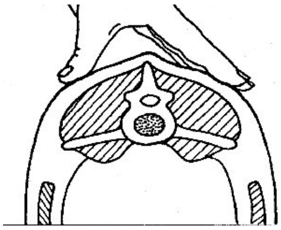
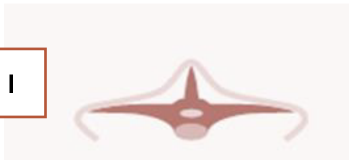
4.1.1. General Clinical exam

Table 1: When realizing a general clinical exam…

General clinical exam	*Complexity*: This exam is not difficult and could be done under veterinarian assistance or the farmer could do it by himself. *Technique*: visually and by palpation in the in the standing position, paying attention to general health (digestive disorders such as diarrhoea and meteorism, breathing distress, etc.), the hip joint, the gait and the ram/buck's conformation. *Note*: The alterations could be classified as severe or mild, taking into account the potential detrimental effect on the wellbeing or the reproductive activity of rams/bucks.
Abnormal pale conjunctiva 	**Eyes:** should be checked for any abnormality: presence of ocular lesion, anemia (pale conjunctiva), icterus (yellowish conjunctiva) or any eye discharge.
Severe purulent nasal discharge 	**Nose**: should be slightly humid with no discharge of any type. Its movements should be regular and not very perceptible.
Mouth examination 	**Mouth**: Mouth should look normal with no symptoms of hyperkeratosis, any pustules or crusty lesions.
Normal dentition 	**Teeth**: Rams/bucks with several broken teeth or abnormal dentition causing difficulties in mastication should also be culled. This is particularly important for rams grazing dry, gross material.
Feet and legs examination 	**Feet and legs**: Rams/bucks should have normal feet and legs for good standing positions during mating. Rams/bucks with lameness, foot rot, foot abscess, inter-digital growths or with non-trimmed hooves should be promptly treated.
	Neck and trunk: mainly the breastbone and the costal area, were checked for sores, wounds, dermatitis, nodules and the presence of umbilical hernia. **Limbs:** examined for evidence of inter-digital dermatitis, arthritis or poor hoof integrity. If necessary (excessive development of hooves), hooves were trimmed after examination of the limbs.

4.1.2. Body Condition Score (BCS)

Table 2: When evaluating body condition score…

	*Technique*: used to determine feeding levels for rams/bucks. Frequent scoring with appropriate feeding could minimize welfare problems, reduce losses and maximizes longevity and mating success. *Time:* The first assessment of the BCS should be carried out at least 2 months before the start of the mating season which allows sufficient time to correct the feeding regime and achieve target BCS which is 4, preferably. Rams/bucks are less hardy during the mating season and they are exposed to losses of BCS. Severe loss of condition during the mating period of up to two units of condition score endangers ram welfare and may predispose animals to respiratory disease and other infections.
	It is a simple effective technique that should be done by an experiment technician which is able to feel for fat cover with his hands
1 	**Score 1:** Spine prominent and sharp, fingers easily pass under horizontal processes (HP). Too thin, possibly diseased.
2 	**Score 2:** Spine prominent and smooth, fingers go under with pressure. Too thin, hard used rams, needs supplementary feeding.
3 	**Score 3:** Spine smooth and rounded, fingers need pressure to find ends. Acceptable condition for mating under good feeding conditions.
4 	**Score 4:** Spine detected as a line, HP are not felt. This is the optimum body condition score at the start of mating season
5 	**Score 5:** Spine not detectable, fat dimpled over spline, HP not detectable. Grossly overfat,

4.1.3. Examination and palpation of reproductive organs

In order to assess whether the rams/bucks are able to correctly serve ewes/does, it is necessary to examine in the standing position:

- Scrotum
- Testicles
- Epididymis
- Prepuce

Figure 4. Examination and palpation of reproductive organs

After each clinical examination, rams/bucks are classified as suitable or unsuitable breeders, according to the severity of the alterations recorded:

- Rams without lesions or exhibiting only mild lesions are classified as suitable breeders;
- Rams showing severe alterations are classified as unsuitable breeders;

4.2 Semen collection and quality assessment

The fertility of males, including the ability to produce normal spermatozoa and to mate, has a great influence on herd performance and livestock productivity (Gebre, 2007). Therefore evaluating the fertility of a breeding male is very relevant and could be done:

- Directly by evaluating the ability of a rem/buck to produce offspring
- Indirectly by evaluating its semen characteristics which is a shorter technique allowing the elimination of males presenting a bad quality semen

This evaluation technique is adopted for males that are generally used in AI among genetic improvement programs.

- Evaluating the fertility of a breeding male indirectly by evaluating its semen characteristics;
- Providing an important indicator of the breeding potential of males predicting breeding problems and eliminating infertile males from the flock;

Under veterinarian assistance or a certified technician;

Semen could be collected from trained males from which semen was previously collected otherwise males should be trained especially when using artificial vagina;

4.2.1. Male training

Table 3: When training males be careful to…

Training	The training of males is not difficult and could be done by a same trained person ideally familiarized with the flock.
	Begin the training at the breeding season when males are at their maximum sexual motivation or as soon as puberty is reached for young males;
	The training should be done by the same person that will collect the semen later and in the same place;
	Rams/bucks should be placed in a waiting room of about 1m^2 and are introduced one by one in the collecting room where 2 ewes/does (one free and 1 restrained) are present there;
	Females should present natural or hormonal-induced estrus behavior;

When training males different scenario could happen please try to choose the best action…

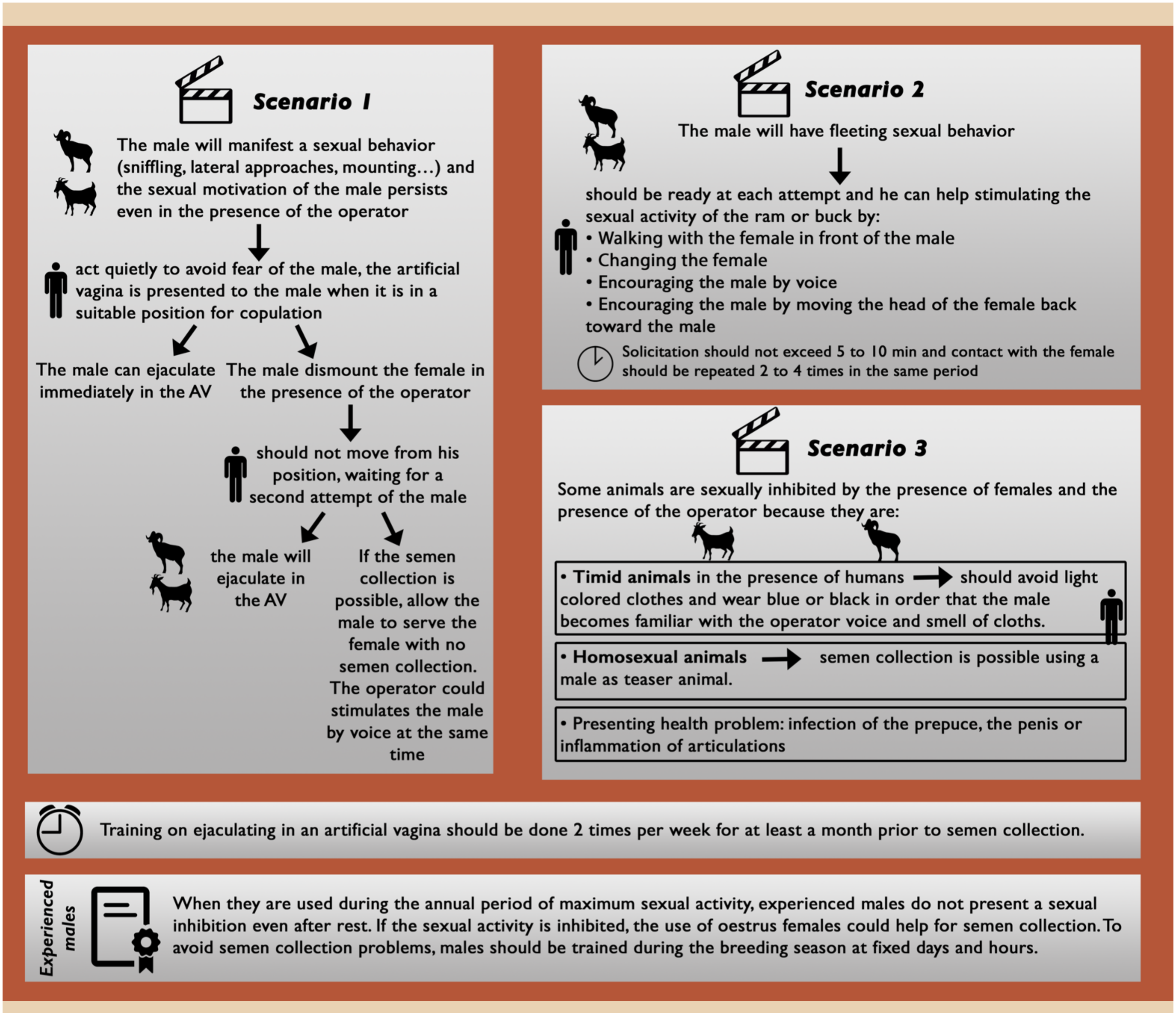


Figure 5. Different scenario taking place when training males

4.2.2. Semen collection

When collecting semen from males you will need…

The artificial vagina (AV) which is a the common and the easiest technique. This procedure is painless, quick and not stressful for the animal. The AV should be clean dried and sterilized. The inner jacked of the AV should be fitted with water at 42-50°c through the tap filled at the side. Then, pressure is adjusted by inflating air through the valve on the tap. The open end of AV should be lubricated with Vaseline up to a depth of 3 cm. The collection tube, fitted at the other end, should be maintained at temperature of 30-37°c to avoid cold shock to the collected spermatozoa;

The Electro Ejaculator (EEJ): The electrical stimulation increases the amount of seminal fluids and therefore causes a damaging osmotic influence on the spermatozoa (Axner et al.,1998). Some authors confirm that the semen have a better quality if collected by an artificial vagina (Rathore, 1970).

Figure 6. Semen collection material A: Artificial Vagina (AV), B: Electro Ejaculator (EE)

When collecting semen please follow these 6 steps…

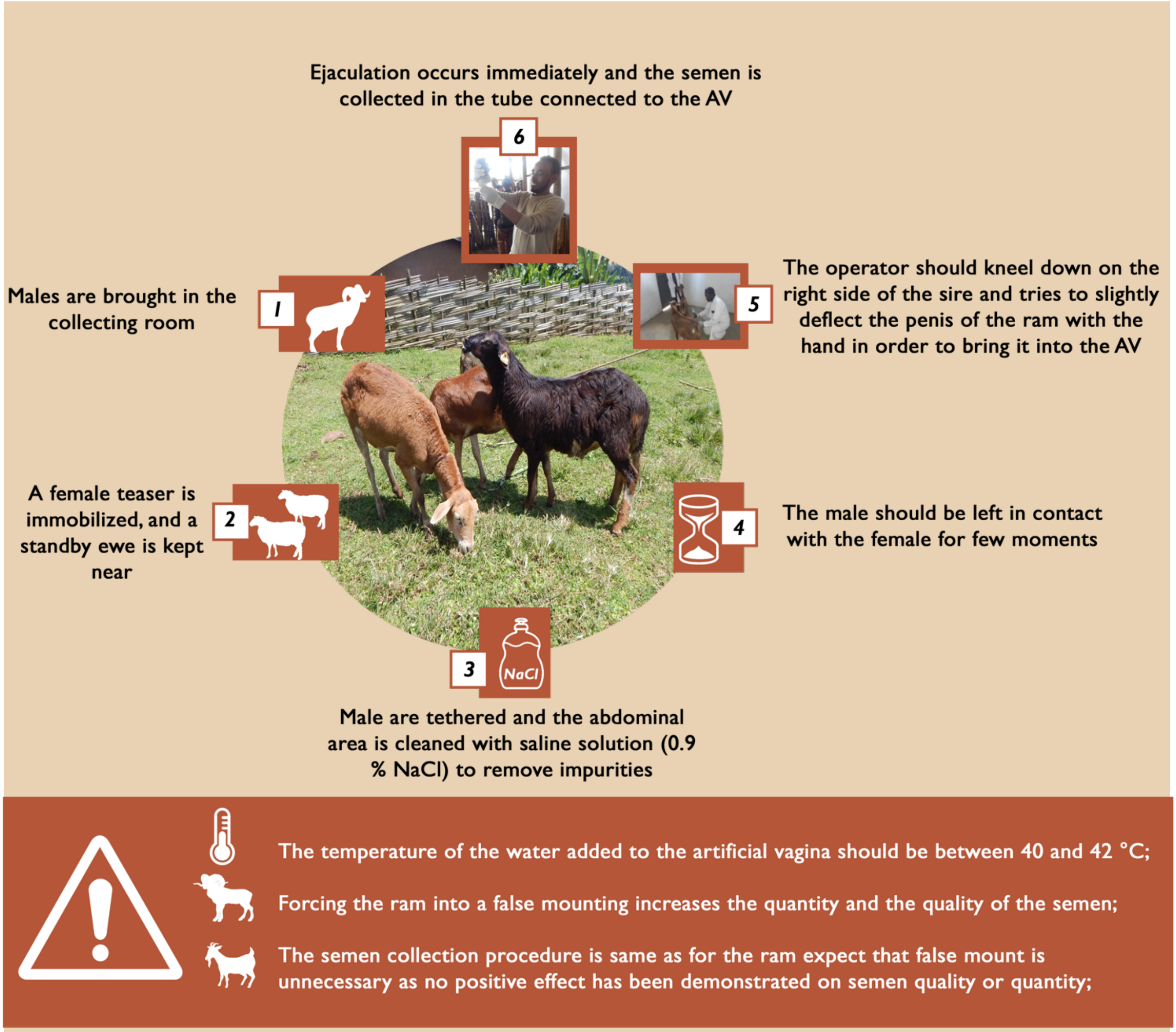
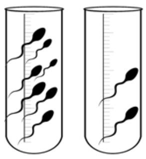
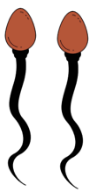


Figure 7. Different steps of semen collection

4.2.3. Quantity and quality assessment

Table 4: When collecting semen, assess quality and quantity as follow…

Method	This exam is not difficult and could be done under technician assistance or the farmer could do it by himself.
	**Volume:** The assessment of the ejaculate volume is done by directly reading the graduations on the collection tube; • The frothy part of the ejaculate is not taken into consideration; • For ram and buck an ejaculate having a volume less than 0.5 ml should be eliminated;
	**Color:** The assessment of the ejaculate color is done by directly observation of the collection tube; • White ejaculates are only used. Red, pink and brown ejaculates should be eliminated;
	**Concentration**: This is an estimation of the number of spermatozoa per ml of pure semen; • The measurement of optical density using a spectrophotometer calibrated for sheep or goat semen (at wavelength of 550 nanometers);
	**Wave motion**: Microscopic examination of the semen in order to appreciate the wave motion of the semen; • A drop of pure semen is then deposited on a glass slide and placed on a warmed stage (37-38°C) under the microscope (80 X magnification); • The score is assessed according to Annex 1;
	**Individual motility**: An estimation of individual motility done under microscope on a drop of diluted semen placed between slide and cover-slide (200 X magnification); • The dilution of the semen should be between 60 and 200 x 106 spermatozoa per ml of semen; • As for the wave motion, the score is attributed according to a scale ranging from 0 to 5 (Annex 1);
  	**Percentage of dead sperm cells and abnormalities**: This percentage is calculated using an eosin-nigrosin stain (Annex 2). • Twenty-four hours after the preparation of the stain, it is filtered and then the pH is measured. • On a slide correctly cleaned and dried on a warm stage, 3 drops of strain (~30 µl) are placed on the left-hand end of the slide. • One drop of pure or diluted semen is added and mixed with the stain for 10 seconds. • The mixture should stand for about 50 seconds then it is spread under a cover-slide by drawing a thin and regularly film. • The slide is identified (number of the male) and kept away from humidity until lecture. • The count is done under microscope using a warm stage to prevent hydration (37-38°C). • The different areas of the slide are examined until a total count of at least 150 spermatozoa taking into consideration that sperm stained pink or red-colored are dead spermatozoa. • The procedure is repeated for accurate assessment. Abnormalities are also observed under microscope and could be classified into five groups (Annex 2): - Tailless spermatozoa - Spermatozoa with abnormalities of the head - Spermatozoa with abnormalities of the tail - Spermatozoa with a proximal cytoplasmic droplet - Spermatozoa with a distal droplet

4.3 Sires certification

In livestock production, animals are certified to fit to a particular purpose. Certified sires have added value that will have an impact on the herds and should result in a monetary advantage. In addition to their breeding value for economically-important traits (growth, reproductive efficiency…) and therefore the superiority of their performances, certified sires should be healthy (ideally disease-free) with a good libido and be fertile.
After BSE and semen evaluation, rams/bucks are certified.

Breeding rams/bucks refer to:

- Rams/bucks of Ethiopian sheep breeds selected in the framework of CBBP's;
- Identified rams/bucks (ear-tags or any other official identification means);
- Having known pedigree;
- Classified, then selected among other counterpart rams/bucks based on their breeding value and phenotype description. In fact, according to the breed and the agreed breeding objectives with the community, every single ram/buck should have an estimated breeding value (EBV) for the desired trait;

Technical requirements for certification include:

Pre-breeding certification of rams/bucks should take place when they are first selected at an approximate age of 06 months and preferably at yearly intervals afterwards or prior to major mating seasons for the breed or artificial insemination campaigns.;

According to the technical requirements, animals are therefore classified as "Suitable for Breeding" or "Unsuitable for Breeding";

Examinations of rams/bucks can be performed by:
- Certified animal production technician;
- Veterinarian;
- Paramedic in veterinary medicine affiliated to an Agricultural Research Institute, Regional Extension or Animal Health Services under the authority of the Ministry of Agriculture;

The certificate of vaccination should be signed by an authorized veterinarian of the public animal health services or a registered private practitioner. Official certification of the rams/bucks should be approved and signed by the head of the animal production office of the district where the breeding program operates.

The certification process comprises 6 steps at the end of which a breeding examination certificate is issued by the competent authorities of the Ministry of Agriculture at the district level. The certification process is a cascade of action and events initiated at the flock level as early as the birth of the potential future

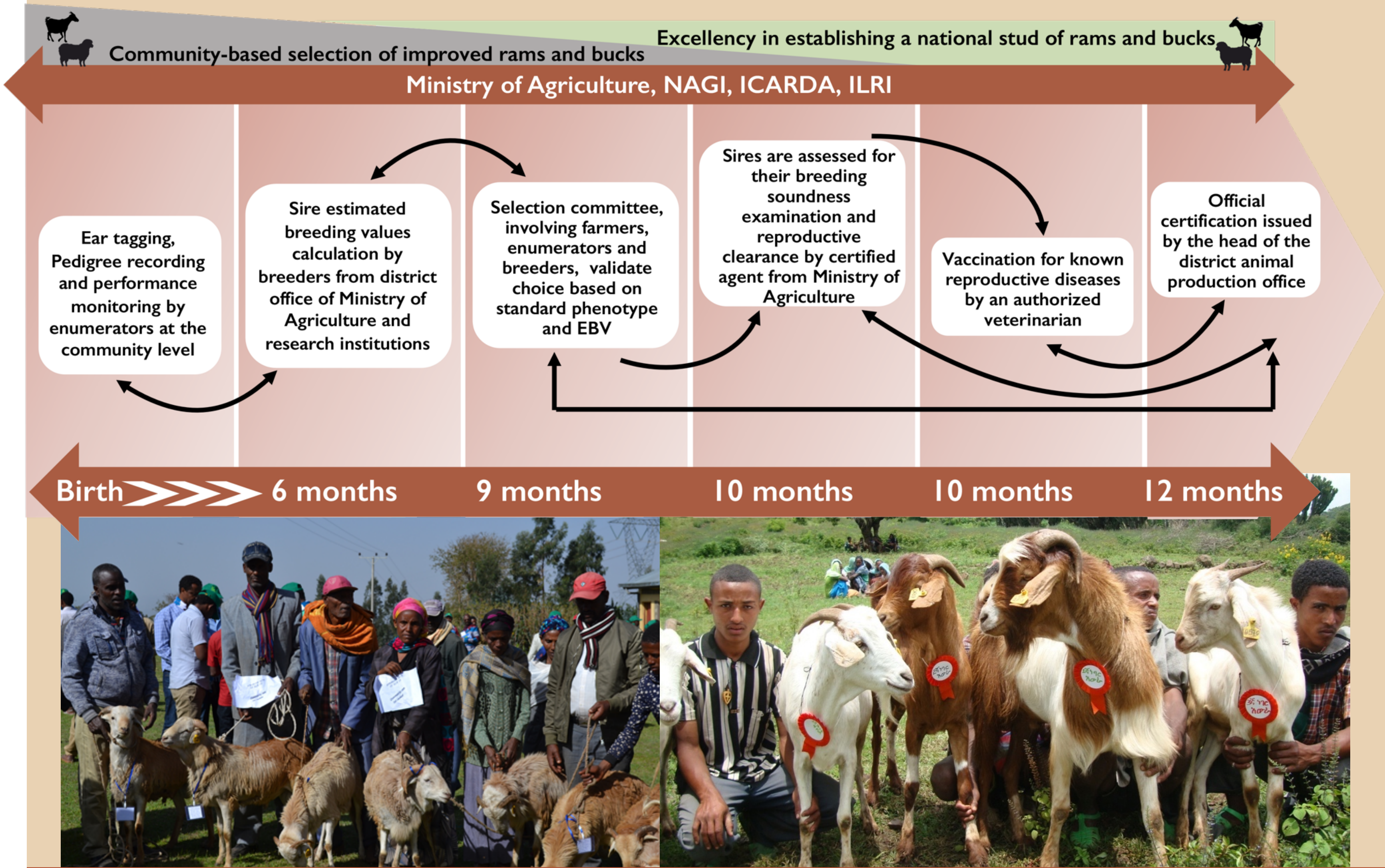


Figure 8. Sires certification

Ram Pre-Breeding Examination: On-farm Data Collection Form (Bonga CBPP)

There should be a tick, measurement, comment, or 'NE' (not-examined) in each white box.

Date of birth/Age (months)			
BCS (out of 5)			
If normal, then write N. If abnormal then describe e.g small, enlarged, soft, hard, lumpy, swollen…	Teeth		
	Feet		
	Rest of body		
	Brisket		
	Prepuce		
	Penis		
	Scrotum		
	Testicles size	L	R
	Epididymis head	L	R
	Epididymis tail	L	R
Scrotal circumference (cm)			

Semen collection method	1st collection		2nd collection	
	AV	EEJ	AV	EEJ
Volume (ml)				
Gross density 0 (clear) -5 (double creamy)				
Gross motility/ wave motion (0-5)				
Mating ability				

Vaccination	*Date*
Brucellosis	
Sheep pox	
Peste des Petits Ruminants	
Foot and mouth disease	
Enterotoxaemia	

Figure 9: Ram pre-breeding examination (on-farm data collection form) for Bonga sheep

500371	
AGE	
Estimated breeding value	

Ram Pre-Breeding Examination Certificate (Bonga CBBP)

Date of Examination: _________________

1. Physical examination

Body condition score (1-5)		
	NAD	Abnormal
Eyes, nose and mouth		
Conformation and limb soundness		
Feet		
External genitalia		
Scrotal circumference	cm	
Overall results: SATISFACTORY* / UNSATISFACTORY*		

*NAD = No Abnormality detected,*delete as required*

Tick as appropriate

2. Semen examination

Collection method	AV*	EEJ **
Appearance/density	/5	
Gross motility	/5	
Overall results: SATISFACTORY* / UNSATISFACTORY*		

**AV: Artificial vagina; ** EEJ: Electro-ejaculator*

3. Assessment of mating ability and libido

This ram has been observed exhibiting normal service behavior and mating ability	
This ram has not been observed exhibiting normal service behavior and mating ability	

Tick as appropriate

4. Classification

SUITABLE FOR BREEDING	based on meeting the requirements of section 1 only
SUITABLE FOR BREEDING	based on meeting the requirements of section 1 and 2 only
SUITABLE FOR BREEDING	based on meeting the requirements of section 1, 2 and 3
UNSUITABLE FOR BREEDING	

Name of District-Head Animal Production Office	
Date	
Certificate No	

Signature and stamp

Bonga

Figure 10: Ram pre-breeding examination certificate for Bonga sheep

Physical examination - A basic clinical examination should always be undertaken. If there are no abnormalities detected then the relevant box can be ticked. If there is an abnormality, a comment should be inserted. If that aspect was not examined, insert 'NE'.

Body condition score - Should be assessed on a 1-5 scale where 1 is very thin and 5 is obese. Rams in very thin body condition score, 2 or less, should be classified as unsatisfactory. Score 1: Spine prominent and sharp, fingers easily pass under horizontal processes (HP). Too thin, possibly diseased. Score 2: Spine prominent and smooth,fingers go under with pressure. Too thin, needs supplementary feeding. Score 3: Spine smooth and rounded, fingers need pressure to find ends. Optimum condition for mating. Score 4: Spine detected as a line, HP are not felt. Overfat. Score 5: Spine not detectable, fat dimpled over spline, HP not detectable. Grossly overfat, mating ability compromised.

Eyes, nose and mouth - Rams should be inspected for severe over or undershot jaw and gross defects, which may interfere with vision and the ability to seek out females. Eyes: should be checked for any abnormality: presence of ocular lesion, anemia (pale conjunctiva), icterus (yellowish conjunctiva) or any eye discharge. Nose: should be slightly humid with no discharge of any type. Its movements should be regular and not very perceptible. Mouth should look normal with no symptoms of hyperkeratosis, any pustules or crusty lesions. Rams with several broken teeth or abnormal dentition causing difficulties in mastication should also be culled. This is particularly important for ramsgrazing dry, gross material.

Conformation and limb soundness - Rams should be inspected for evidence of lameness whilst walking on a smooth level surface. Lame rams or rams with severe limb defects e.g. valgus deformity or elbow arthritis should be classified as unsatisfactory in section 1.

Feet - Rams should have normal feet and legs for good standing positions during mating. Rams with lameness, foot rot, foot abscess, inter-digital growths or with non-trimmed hooves should be promptly treated or classified unsatisfactory in section 1.

External genitalia - The scrotum and contents should be carefully palpated. Rams with gross physical abnormalities such as epididymitis or orchitis would be classed unsatisfactory in section 1. Testicles should have the same size (symmetric) and move freely inside the scrotal bag; their mass should be firm, but not hard, with no indication of abscesses, injuries or any other condition. Rams with only one testicle (monorchid) are sub-fertile and should not be kept for breeding even if you like their conformation and sexual aggressiveness.

Penis and prepuce *The examination is best carried out when restraining the ram at its rump. The penis and prepuce should be examined to determine if there are indications of adhesion, pizzle rot (unhealthy combination of urine scald and bacterial growth on the prepuce) or any injury. Special attention should be paid to the preputial orifice for ulceration or inflammation. There should be no adhesions to the sheath or any signs of lesions of the gland, pus and abscesses.*

Scrotal circumference *All measurements to be made in cm at the widest point of the scrotum with a tensioned measuring tape*

Equipment - *All laboratory vessels/slides used to handle semen should be warmed prior to use to 30-37°C & the microscope stage kept at 35-37 oC. AV: artificial vagina; EEJ: Electro-ejaculator*

Semen volume *should be measured by a direct reading in a collection tube graduated to the nearest 0.1 ml.*

Gross density *is not relevant when a sample is collected by electro-ejaculator. However, a figure should be given in all cases following assessment of the sample made by the naked eye.*

0	1	2	3	4	5
Clear	Cloudy watery	Thin milky	Milky	Creamy	Double creamy

Gross motility *should be assessed as a drop of semen is placed on a slide and viewed under low magnification (x10) using a phase-contrast microscope.*

0	1	2	3	4	5
Dead	No waves (very poor)	Very slow waves (poor)	Slow distinct waves (fair)	Fast waves (good)	Rapid dense waves (very good)
No sperm or all dead	Some movement at edge of drop	~20-40% live	~45-65% live	~70-85% live sperm	~90% live sperm

ASSESSMENT OF MATING ABILITY *Libido is difficult to assess and define, so this part of the examination simply confirms whether the vet/examiner has observed normal service behaviour and intromission when the ram was presented with females in oestrus. At least one successful service within 10 minutes of being presented to in-oestrous female should be expected. If this part of the examination is not carried out, then rams can still be classified as SUITABLE FOR BREEDING based on meeting the requirements of parts 1 and 2 only. The owner/purchaser is invited to observe the ram closely at the start of breeding period to monitor libido and mating ability*

Figure 11: Ram pre-breeding examination certificate for Bonga sheep

4.4 Mating ability test

Mating ability or libido test is done on the presence of 2 estrous females. The behavior of the male is recorded during 10 minutes

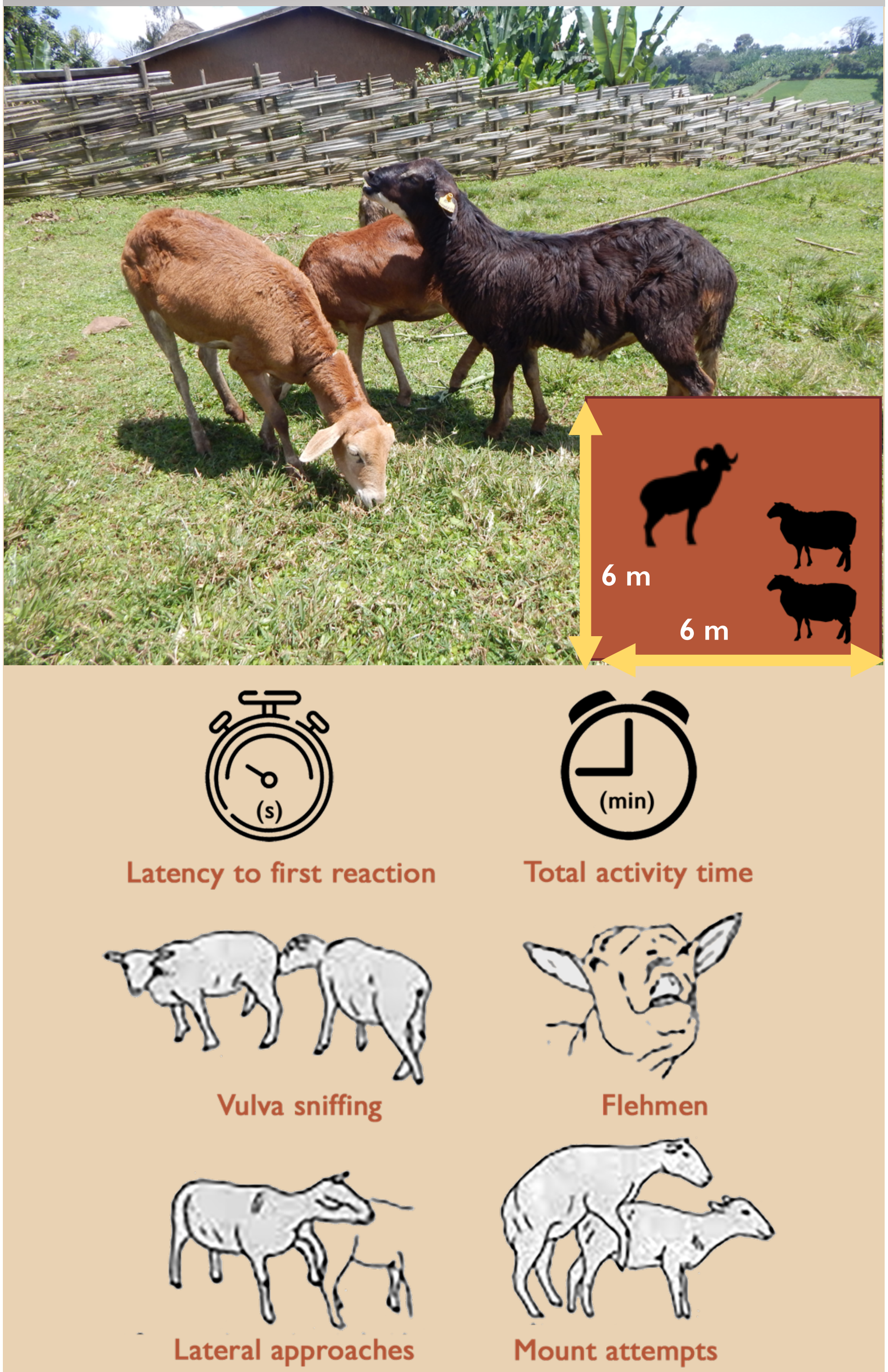


Figure 12: Libido test

5 Reproductive techniques used in females

All the reproductive techniques used to select rams/bucks are very useful but are not sufficient when used alone. When combined to reproductive techniques used in females, the potential of rams is spread out and the genetic potential is vastly spread while dissemination of sexually transmitted diseases is reduced. Therefore, reproductive techniques used in females are in a close relation with those used in males and offer a viable option for a wider and more effective use of superior rams.

- The main universal method to disseminate improved genetics in livestock species;
- Contributes to food security and improvement of genetic resources (by adjusting appropriate birth season with market demand);
- Active participation of farmers contributing to job creation with focus on youth and women.

- Various levels of infrastructure;
- Technicity;
- Field organization;

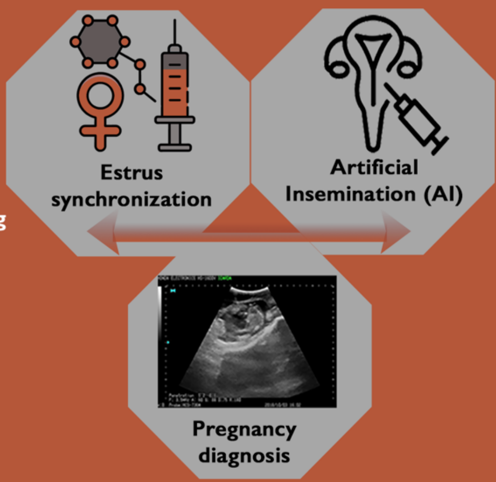


Figure 13: Breeding A: ewe and B: doe

5.1 Estrus synchronization (sheep and goats)

Even if under tropical and subtropical latitudes, small ruminants are different from temperate breeds and do not experience the total seasonal anoestrus, due to a low variation of the daylight, reproductive activity decreases at some times of the year, especially in very harsh environments. This reduced activity could be due to nutrition (Gallego-Calvo et al. 2014), sociosexual interactions (Delgadillo et al. 2015) and climatic aspects (Silva et al. 1998; Morales et al. 2016).

Estrus synchronization is an efficient reproductive techniques that allows:

- Preparation of females goes before Artificial Insemination
- Adjusting birth season with market demand

Choosing the best technique of estrus synchronization based on a reasonable use of hormones and techniques adapted to the farmers conditions remain the key of success of reproductive biotechnologies in females.

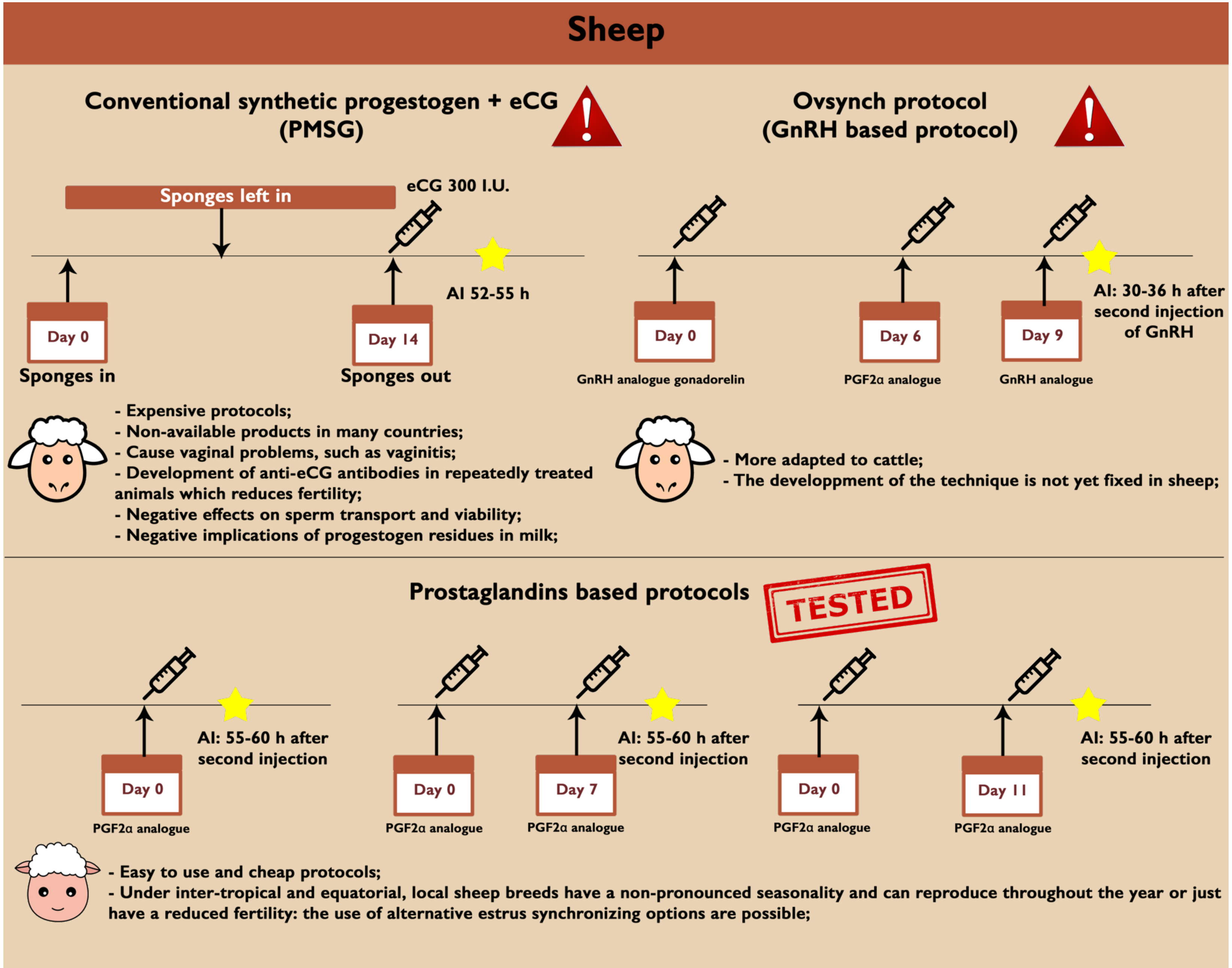
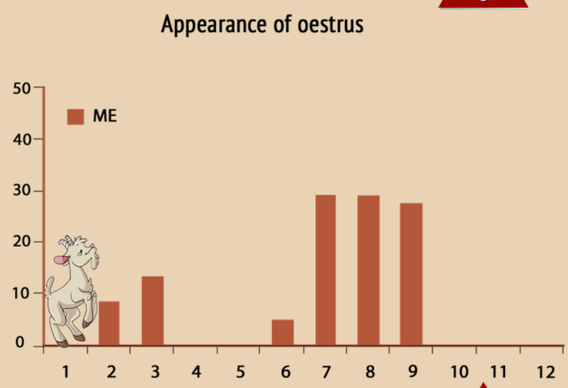


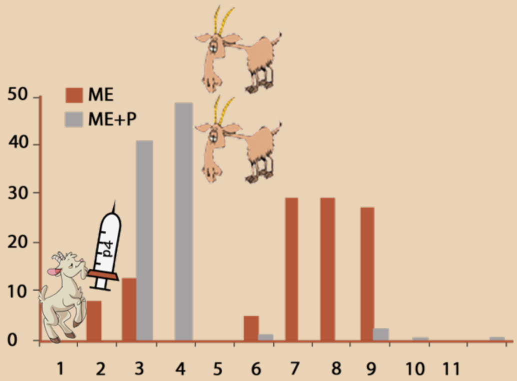
Figure 14: Estrus synchronization techniques used in ewes: strengths and weaknesses

Goats

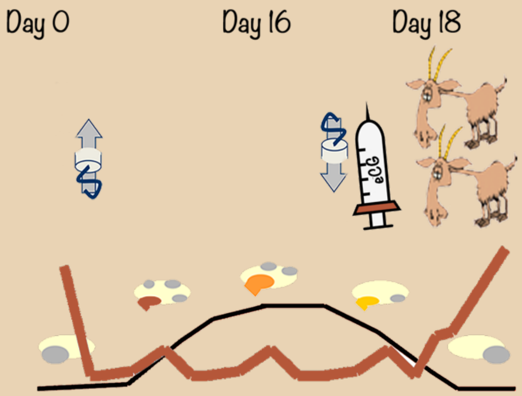
Male effect



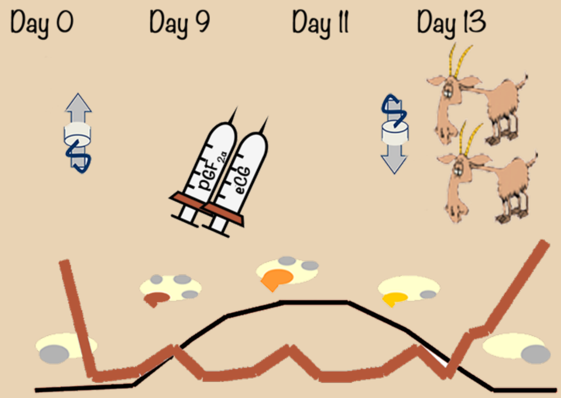
Male effect in goats + progesterone



Progestogens: Long protocol



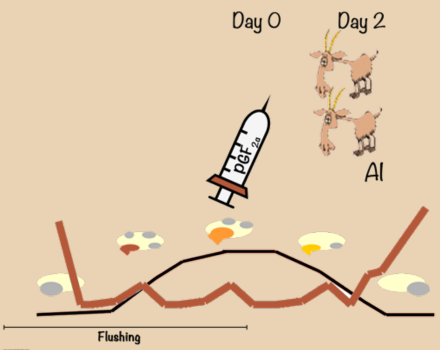
Progestogens: Short protocol



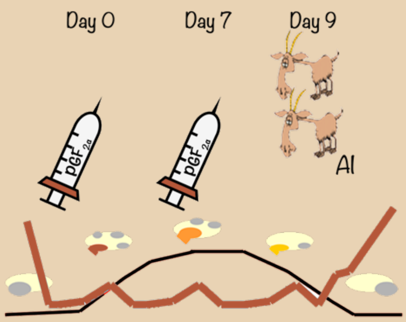
Using male effect do not allow estrus grouping and will affect AI results. The use of progesterone based techniques is not confirmed in goats.

Prostaglandins based protocols (single and double injection) TESTED

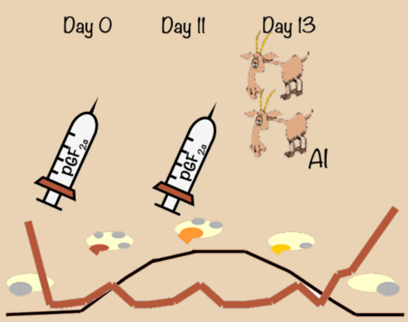
Prostaglandins (1 injection)



Prostaglandins (7 days)



Prostaglandins (11 days)



Flushing: 1 month before Artificial insemination

Fixed-time artificial insemination (48h after last prostaglandins injection) or AI 24-30h after heat detection

Pregnancy checked very seriously using ultrasound scanner before hormone administration since prostaglandin analogs cause immediate abortion in pregnant does.

As for sheep, prostaglandins based protocols are easy to use, cheaper and are adapted to breeds under inter-tropical and equatorial African condition.

Figure 15: Estrus synchronization techniques used in does: strengths and weaknesses

5.2 Artificial Insemination

When assessing artificial insemination, low-infrastructure, mobile laboratories should be operational delivering artificial insemination and other reproductive services. These laboratories are related to ICARDA and its partners jointly developed, and these structures are embedded in the national collaborating centers: Amhara Region Agricultural Research Institute (ARARI), Tigray Agricultural Research Institute (TARI) and (South Agricultural Research Institute) SARI in Ethiopia.

The national centers are engaged in providing and upgrading the physical premises to host the labs. They also provided some of the key needed equipment such as:

- a contrast-phase microscope;
- a water bath;
- a heating plate;
- a refrigerator;
- a generator (for field AI's)
- common laboratory glassware and supplies;
- electricity and running water

ICARDA investment into these labs was materialized by the supply of a spectrophotometer for the evaluation of bucks' semen, an ultrasound machine for routine transabdominal pregnancy diagnosis, precision micropipettes and specific supplies for goats' AI including artificial vaginas and accessories, AI guns, speculum, straws and sheath, extender, lighting sources, extender…

One major feature of these low-infrastructure labs is their mobility. This feature is imposed by the current structure of the AI system. So far, we are using fixed time AI's after synchronization with semen collected, processed and deposited at 35 °C which will be described later in this guideline. This requires a setting of the lab in proximity of the community superior rams/bucks and the community rams/goats to be inseminated. Out of the equipment and supplies described above, we have established a "mobile kit" which can be easily transferred, installed and used under full field conditions.

What is needed when practicing artificial insemination…

Figure 16. Low infrastructure, mobile laboratory

Figure 17. Artificial insemination techniques used in sheep and goats

Table 5. When inseminating ewes/does be careful to…

	Inseminated, females should not be exposed to stress (fights, nutritional stress….)
	Avoid any abrupt changes in the diets prior to, during and after the inseminations (up to 15-20 days)
	Do not expose females to any management stress (vaccinations, walking or grazing over long distances, transhumance...) in the first few days following AI
	Post-mating with rams at the return estrus is indispensable to keep satisfactory levels of fertility at the flock: rams should be reintroduced in the inseminated females between 7 and 10 days after inseminations
	Under routine field conditions, conception to AI is estimated by: - Transabdominal pregnancy diagnosis at 40-45 days after AI - Females are considered to have been conceived at AI if lambing date [145, 155 days after AI]

5.3 Pregnancy diagnosis

Pregnancy diagnosis through transabdominal ultrasound is a practical and efficient approach for monitoring pregnancy and fetal growth in small ruminants. Ultrasound-based solutions fit into the broader concept of clean, green management of sheep and goats reproduction. In addition they are available, easy to use, high resolution and portable machines. Their prices are going down and they are more accessible to suit even the most extensive, low input systems.

Ultrasound based solutions can be used to:

- Screening for pregnant females and litter size;
- Estimation of foetal age
- Check on repeat breeders;
- Check on females with recent reproductive pathologies;
- Discard pregnant females prior to synchronization and AI, particularly when the synchronization protocols rely on the use of prostaglandin analogues;
- Prevent slaughter of pregnant females (very common in Ethiopia);

All the gathered information through pregnancy diagnosis procedure is important taking decision regards:

- Opportunity for re-mating;
- Screening against non-fertile females and females that are reproductively problematic by spotting them for culling;
- Planning of conditions for birth;

Pregnancy diagnosis, how to realize it …

Figure 18. Ultrasound pregnancy diagnosis used in sheep and goats

Table 6. Pregnancy diagnosis, how to interpret it …

	The amnion can be first imaged at day 25 of gestation, the uterus is enlarged and the embryo is centrally located Multiple fetuses are difficult to positively identify
	The embryonic vesicles can be images at 30-35 days of gestation
	The fetus can be imagest at the begining of the fetal period (40-45 days) (head and heart)
	At 50-60 days of gestation, the heart beat is clear and bones appears highly echonic (white)
	At 75-90 days of gestation, fetuses are too large and too far within the abdominal cavity to distinguish between single and multiple pregnancies. Vertebral column, ribs, internal organs and heart are clear

6 Conclusion

Reproductive biotechnologies used in males and females should be developed across countries and covering more regions within each of the countries taking Ethiopia as example. While Sudan is not part of this MBoss project, ICARDA (within the framework of other initiatives) together with the Agricultural Research Council in Sudan have established a women-led CBBP on goats in North Kordofan and this initiative should be expanded in other countries.

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