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Field guideline for low-cost artificial insemination of sheep and goats in Ethiopia

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The Program thanks all donors and organizations who globally supported its work through their contributions to the CGIAR system.

Editing, design and layout—(ICARDA)

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ISBN: ……

Citation: Tesfaye Getachew, Mariem Rouatbi, Aynalem Haile, Barbara Rischkowsky, Mourad Rekik. 2019. Field guideline for low-cost artificial insemination of sheep and goats in Ethiopia. ICARDA (Guidelines). Addis Ababa, Ethiopia: ICARDA.

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1 Introduction

Currently, improved rams and bucks produced by the community-based breeding programs (CBBP's) are shared to serve the ewes/ does in the communities. There is compelling evidence that improved rams/ bucks are bringing genetic progress where they have been used. Features inherent to the production systems, in particular the small flock size, mean that the reproductive impact of the improved sires is limited. Whether a ram with a high breeding value is placed at the level of the household or when the ram is being used collectively at the level of the community under common natural mating practices, it may only mate to a very limited number of females (20-30) during the mating season. To scale up the genetic progress made and expand the use of improved rams/ bucks, reproductive options may be brought up together in specific packages to support delivering improved genetics. Artificial insemination (AI) remains the main universal method for dissemination of improved genetics in livestock species, especially in cattle and swine breeding. This technique, consisting of placing collected and fractionated semen in the female reproductive tract, can be of advantage in management of the herd. In fact, AI permits reproduction when males are unavailable for natural mating (out of season breeding, libido, limited semen quality and production). Furthermore, AI takes full advantage of the oestrus synchronization technique. From a genetic point of view, in flocks where single sire could not mate all females, this reproductive technique is a good solution for parental control. In addition, males of high genetic value could be used at any time of the year. AI avoids disease transmission since donor males are generally certified and are under health control. Using fresh cooled or uncooled semen, a ram may produce at least 300 to 400 semen doses for a mating season of 6-8 weeks. Assuming an average conception rate of 50% and a litter size of 1 for ease of calculation, then improved genetic material will be passed on to at least 150 to 200 offspring. This step will also unlock the CBBP to outside the direct participating communities bringing improved traits into the population. Artificial insemination is a staged technology with various levels of infrastructure, semen technology, technicity and field organization. Insemination using with fresh semen collected in the field and relying on basic infrastructure is regarded as a promising technology for a wider delivery of improved genetics under low input systems. This facilitates reaching more farmers within the communities and reaching out to other communities. These guidelines are aimed at providing livestock technicians and field veterinarians with the basic theoretical and practical information required to design, conduct and assess artificial insemination field campaigns in support of the sheep and goat breeding programs. These guidelines are elaborated in the specific context in which CBBP's are run in Ethiopia. Following an overview of the anatomical and physiological aspects of reproduction in sheep and goats that are important to AI, these guidelines describe the conditions for the set-up of low infrastructure, mobile laboratories, the choice of inseminated females and their oestrus synchronization and all other steps, e.g. sires' training, semen collection, evaluation and processing as well as semen deposition. Other

aspects such as the pos-insemination management and the assessment of success of AI are also reviewed.

2 Anatomy of the reproductive tract

2.1 Female

The reproductive tract of ewes and does is similar and will be discussed together in this section. The female reproductive tract consists of the vulva labia, vagina (copulatory organ), cervix, body of the uterus, uterine horns, oviduct (also called Fallopian tube) and the ovary (Figure 1).

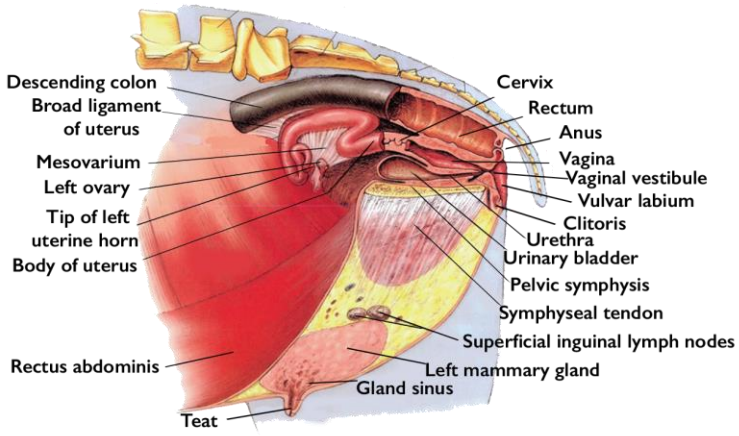

Figure 1 Left lateral view of the topographic anatomy of the female reproductive system of the ewe (McCracken et al., 2008)

Ovaries

Left and right ovaries remain in the abdominal cavity suspended by the broad ligament, they ensure both endocrine and exocrine function Indeed, the ovaries contain the ova (eggs) and secrete female reproductive hormones (progesterone and estrogens) which maintain sexual characteristics and partially control several reproductive functions.

Oviduct

This organ constitutes the initial part of the female genital tract, it opens like a funnel (the infundibulum) near the ovary. The infundibulum is the terminal end of the oviduct which connects the ovary to the corresponding uterine horn. The infundibulum is involved in the transport of ova from the ovary to the site of fertilization in the oviduct. The oviduct ensures the sperm transport to the site of fertilization, provides a proper environment for ova and sperm fertilization, and transports the subsequent embryo to the uterus.

Uterus

The uterus is the organ of pregnancy. It connects the oviducts to the cervix and consists of two separate uterine horns (coruna). In sheep and goats, the type of uterus is bicornuate characterized by having two uterine horns of intermediate length and a small uterine body. In animals with multiple births, each horn can contain one or more fetuses. The uterus plays an important role in implantation, gestation and parturition. In fact, the uterus provides the adequate environment for the survival of the embryo, ensures its development during gestation by providing nutrients, elimination of waste and its protection. It also carries the fetus out of the maternal body during parturition.

Cervix

The cervix is the gateway to the uterus, it has a structure similar to the sphincter. It provides lubrification, a flushing system and a barrier during pregnancy by forming a barrier (highly viscous mucus). The role of the cervix is to isolate the uterus from the vagina and from the outside environment. In sheep the cervix constitutes a major constraint in artificial insemination since it remains closed even in estrus and therefore it is not crossed by the insemination gun.

Vagina

The vagina is the copulatory organ and then the site of semen deposition during natural mating. It is also a passive birth canal during parturition.

Vulva

The vulva is the external part of the reproductive tract. It constitutes a barrier for preventing external contamination. It consists of two labia meeting in the medial portion of the tract forming two commissures.

2.2 Male

The male reproductive system consists of scrotum, testicles, a duct system for sperm transport (Epididymis and vas deferens), accessory sex glands, and the penis which deposits semen in the female (Figure 2).

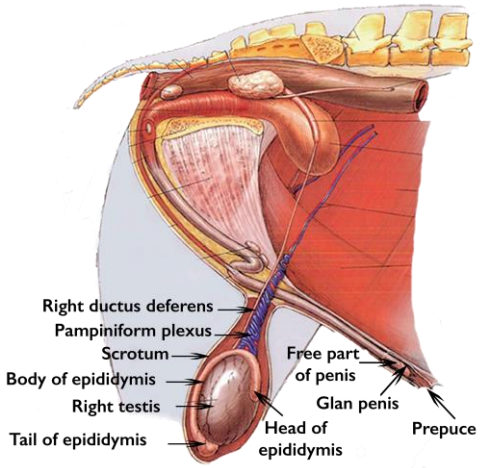

Figure 2 Topography of the reproductive organ of the ram: right lateral view (McCracken et al., 2008)

Scrotum

It is a muscular sac that supports and protects the two testicles. Each testicle is contained in a separate part of the scrotum. The scrotum plays an important role in controlling the temperature of testicles which is extremely important for their functioning. The scrotum keeps the testicle at a temperature around 32°C, 4 to 7°C below the body temperature for optimal function.

Testes

The testicles produce sperm and the male hormone, the testosterone which is essential for the development of male characteristics. The testes are paired organs which descend from the abdominal cavity during fetal development to lie in the scrotum. The activity of the testes is controlled by the gonadotropic secretions of the anterior pituitary gland, governed by the central nervous system (Figure 3).

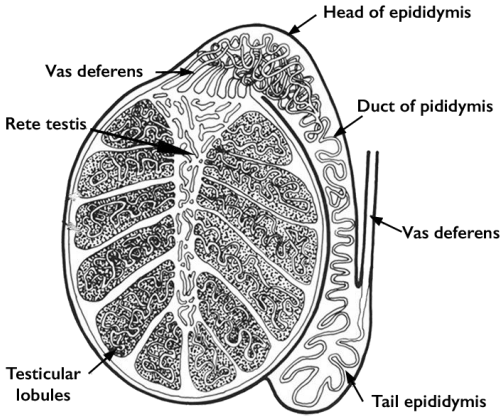

Figure 3 Main parts of a testicle (Castonguay, 2012)

Epididymis

The epididymis is located in the testes and is a long and convoluted tube. The sperm produced in the testicle are transported to the epididymis and are stored until ejaculation. The transport of sperm in the epididymis lasts between 9 and 13 days. The maturation of sperm is acquired during their transit through the epididymis and their motility increases when sperm enter the body of the epididymis with mature sperm being stored in the tail of the epididymis.

Vas deferens

The vas deferens is the duct that extends the tail of the epididymis to the abdominal cavity to reach the base of the prostate. It therefore connects the epididymis to the urethra. These channels are one in each testicle.

Accessory sex glands

The annex sex glands include the seminal vesicles glands, the prostate, and the bulbo-urethral and the ampulla. They secrete the seminal fluid in order to form the semen when combined to the spermatozoa and other secretions of the epididymis. The seminal fluid creates an alkaline environment in the vagina, favorable to the survival of sperm. It also allows the coagulation of the ejaculate and the constitution of a deposit of spermatozoa in the vagina as well as a storage of inhibitors of capacitation, activation and improvement of the motility of spermatozoa. Some of the secretions contain nutrients like fructose.

Penis

This is the final part of the male reproductive tract and the copulatory organ. Its function is to deposit semen into the vaginal tract of the female. At the end of the penis is a narrow tube called the urethral process (or ‘worm') that sprays the semen in and around the cervix of the ewe/doe. The sheath, or prepuce, is formed by an invagination of the skin that protects the terminal part of the penis.

3 Female sexual cycle

3.1 Sheep

Puberty is reached when the genitals organs are mature and when the secondary sexual characteristics are developed. At this stage, animals acquire the sexual capacity to produce fertilizable gametes and then reproduce. In lambs, the age of puberty is between 5 and 9 months depending on breed, body weight, growth rate and fat content, healthcare and season of birth. Once puberty is attained, the ewe lamb comes on heat (oestrus) at regular intervals unless interrupted by pregnancy or disease and the sexual activity is manifested by a succession of heats. The oestrus is also defined as the period of time

when the ewe is receptive to the ram and will stand for mating. Therefore, the oestrus cycle is defined as the number of days between two consecutive periods of oestrus. The average estrous cycle (time from one ovulation to the next) is typically 17 days in the ewe. The length of the cycle can vary between 14 and 19 days depending on the breed, age, individuals and the time of year. Proestrus, estrus, metestrus, and diestrus are the phases of estrus cycle. In sheep, duration of estrus is approximately 24 to 36 hours. Ovulation occurs in mid to late oestrus (~24 hours after the onset).

The sheep and goat's sexual cycle is divided into two phases (Figure 4):

- A follicular phase, which corresponds to the proestrus. This phase begins with the regression of the corpus luteum (CL) and drop in progesterone and extends to the start of estrus. Rapid follicular growth is occurring during this period leading to ovulation.
- A luteal phase corresponding to the Metestrus and Diestrus. This phase lasts from ovulation to regression of the CL (14 -15 days). Metestrus begins with the cessation of estrus, it is the period of the formation of the CL and lasts for about 3 days. Diestrus is the period when the CL is fully functional. It usually extends from day 4 to day 13-15 of the cycle.

The cyclicity of these two phases is ensured by the variations in frequency of the LH secretion pulses and the feedback exerted by the steroid hormones (progesterone, oestradiol and androstenedione) on the hypothalamus-pituitary axis.

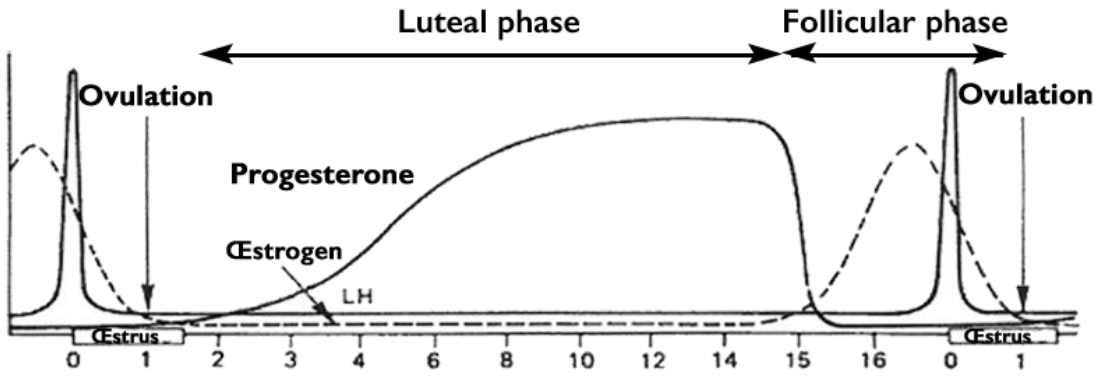

Figure 4 Sexual cycle of the ewe (Castonguay, 2012)

3.2 Goat

Young female goats reach puberty at about 6 to 8 months of age. As for the ewe, the ovarian cycle is divided into two phases: follicular phase and luteal phase (Figure 5). The follicular phase corresponds to the wave of follicular development. The beginning of the follicular phase, known as proestrus, corresponds to the period that precedes the estrual behaviour. Hormonal changes induce the goat's

estrus behaviour and the goat, unlike the ewe, is particularly sensitive to burst of oestradiol. The estrus phase includes the events of the sexual behavior of the goat until ovulation. The oestrus cycle length of the doe is 21 days in average and can range between 17 to 25. The goat is characterized by a very large variability of the length of the oestrous cycle.

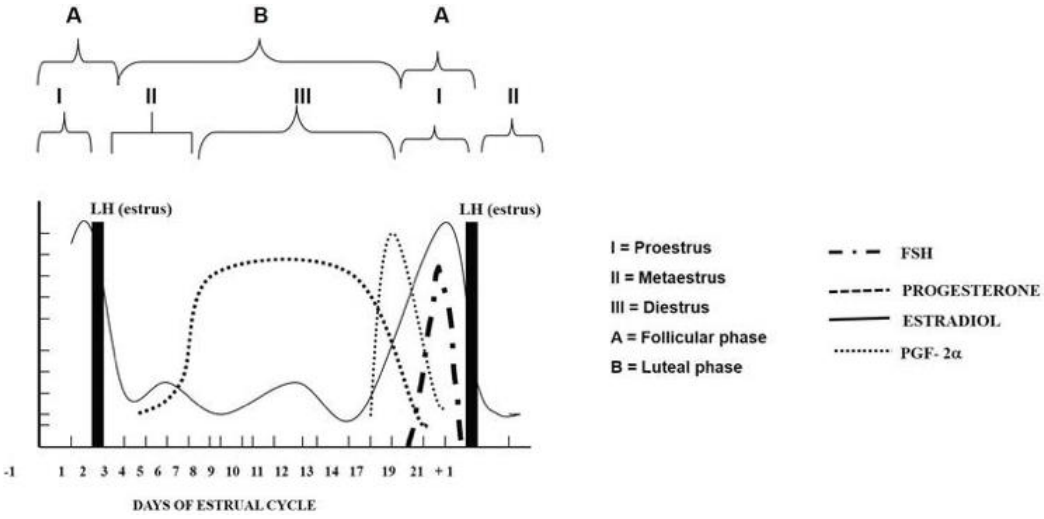

Figure 5 Sexual cycle of the doe (Dávila, 2017)

4 Hormonal control of the oestrus cycle

Estrus cycle is controlled by GnRH (Gonadotrophic Releasing Hormone) released by the hypothalamus. Just before the onset of estrus, GnRH stimulates the production of Luteinizing Hormone (LH) and Follicular Stimulating Hormone (FSH) which act on the ovaries to stimulate the growth of follicles and ensure the maturation of the oocytes to make them suitable for fertilization. On the ovaries, the largest follicles produce estrogen, which will cause a peak in LH secretion which induces ovulation of mature follicles. Estrogen brings the ewe into behavioral estrus. In the ewe, the sexual receptivity of females at oestrus is activated by the secretion of oestrogen which is facilitated by progesterone secretion under the control of the central nervous system. However, sexual receptivity of the doe is elicited by only oestrogen.

After ovulation, under the influence of LH the cells inside the ruptured follicle begin to grow and undergo changes to form the CL which produces progesterone and inhibits the secretion of GnRH and therefore prevents the onset of heat and another ovulation. The CL is destroyed if fertilization does not occur. In fact, if there is no fertilization around 14 days after the oestrus, Prostaglandin F2alpha (PGF2α) hormone produced by the inner wall of the uterus destroy CL and allows the installation of a new cycle (Figure 6). This cycle will continue throughout the reproductive life of females and will only be interrupted by pregnancy, disease, under or overfeeding.

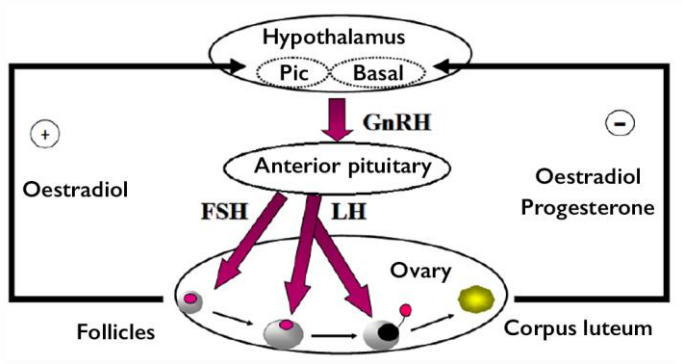

Figure 6 Hormonal control of the sexual cycle (Castonguay, 2012)

5 Oestrus synchronization

Oestrous synchronization is a method that enhances the efficiency and cost of artificial insemination (AI). In fact, all synchronized ewes/goats could be inseminated at the same fixed time. Technically and economically, this is the universal method to practice AI's in sheep and goats. This technique consists in the manipulation of the oestrous cycle of ewes/goats to come to heat within a predefined time frame. In small ruminants, oestrous synchronization is achieved either by injection of PGF2α by reducing the length of the luteal phase of the estrous cycle or by extending the cycle artificially with application of exogenous progesterone or more potent progestogens. It is very important to choose the best synchronization protocol which considers the physiology of the female (during or out of the breeding season), which leads to a synchronization that allows use of fixed-time AI (homogeneous grouping of oestrus and ovulations), which can be affordable for farmers and which use hormones that are locally available with the lowest cost. In Ethiopia, many synchronization protocols of oestrus and ovulations in sheep and goats were tested.

5.1 Sheep

The main synchronization protocols that were tested under Ethiopian conditions are schematically represented in figure 7.

Standard protocol

This protocol consists of the use of intravaginal polyurethane sponges impregnated with 30 mg fluorogestone inserted and left in the vagina for 14 days. At sponge withdrawal, ewes receive an i.m. injection of 300-400 I.U. of equine chorionic gonadotropin (eCG). Fixed-time artificial insemination is carried out at approximately 55±1 h after the end of the hormonal treatment.

Single injection of a prostaglandin analogue

This protocol is based on a singular i.m. injection of a $PGF_{2\alpha}$ analogue. The dose of the prostaglandin analogue varies according to the molecule and should be adjusted according to the manufacturer's

instructions. Fixed-time artificial insemination is carried out within 48 hours after the end of the hormonal treatment. This protocol is only applicable when the females are spontaneously cycling during their natural breeding season and most females are bearing a functional CL. Ewes should be carefully checked for non-pregnancy before the injection of the prostaglandin analogue.

Double injection of a prostaglandin analogue 7 or 11 days apart

This protocol is based on 2 i.m. injection of a $PGF_{2\alpha}$ analogue administered 7 and 11 days apart. Fixed-time artificial insemination is carried out within 48 hours after the end of the hormonal treatment. Like all prostaglandin-based protocols, this protocol is only applicable when most females in the flock are spontaneously cycling.

Studies in Menz sheep showed that the longest interval between prostaglandin injections (11 days) improved conception rate at fixed-time AI compared to the 7-days interval or the single injection. The resulting conception rate is also higher than levels obtained with the standard method associating exogenous progestogens and eCG. Therefore, the use of a prostaglandin-based protocol composed of 2 injections 11 days apart, preceded by a careful selection of non-pregnant ewes (BCS > 2) using ultrasonography for cervical fixed-time AI with fresh semen, is a feasible reproductive management option for farmers in Ethiopia.

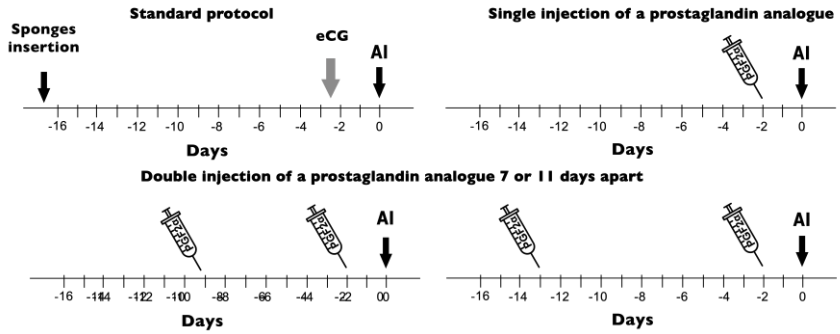

Figure 7 Various synchronization protocols of ewes

5.2 Goat

There are many synchronization protocols of oestrus and ovulations in goats which can allow fixed-time artificial insemination. Some protocols can only work when the goats are in the state of anoestrus, others are more specific to the breeding season when the goats are spontaneously ovulating and there are universal protocols which can work anytime in the year and for all breeds. When the goats are in anoestrus, goats can be induced to breed with the buck effect. However, with the buck effect, the oestrus response is usually dispersed over 9 days following introduction of the bucks and does not allow the use of AI (Figure 8).

Progestogen-based long protocol

During seasonal anoestrus (but also during the breeding season), goats can be synchronized with the progestogen-based long protocol. Progestogen-based intravaginal sponges are inserted and left in-situ for 16-17 days. At sponge removal, goats receive and I.M injection of equine chorionic gonadotrophin (eCG) and fixed-time artificial insemination is carried out within 48 hours after the end of the hormonal treatment. If the goats are in their natural breeding season, satisfactory results can still be obtained by removing the injection of eCG. However, a slight spread of the moment of ovulation is expected.

Progestogen-based short protocol

It is the most used protocol across the world. In addition to a shorter period of sponge insertion (only 11 days), the protocol associates eCG and prostaglandin at day 9 after sponge insertion therefore targeting both anoestrus and spontaneously ovulating goats in the flock. Because of acting on all goats irrespectively of their physiological status, we call it the universal protocol. The protocol is very effective in inducing a highly synchronized oestrus and ovulations and AI's are usually carried out between 43 and 48 hours after removal of sponges.

In spite of the advantages associated with the use of the progestogen-based protocols, there are many drawbacks like disturbance in the luteinizing hormone (LH) secretion patterns, altered follicular dynamics, production of eCG antibodies and impaired sperm motility inside the female reproductive tract thus reducing the possibility of achieving a good synchronization between the time of ovulation and the time of spermatozoa reaching the ovum.

Double injection of a prostaglandin analogue 7 or 11 days apart

This technique is used during the breeding season and is also valid for non-seasonal breeders at any time of the year, synchronization can be achieved by a double injection of a prostaglandin analogue 7 or 11 days apart. The most common protocol is 11 days interval between the 2 injections, but it has been shown that the quality of the growing follicles can be superior if the second injection is given after 7 days. Goats are inseminated between 45 and 48 hours after the second injection. The advantage of such a protocol is its reduced cost compared to progestogen-based protocols, the availability of the hormone (most countries in Africa have more than one prostaglandin analogue registered and available in the market). This is not the case for progestogen-impregnated vaginal sponges. In addition, synchronization with prostaglandins yields a "clean" oestrus, hence promoting higher conception rates after AI. Under field conditions, extreme care should be taken not to synchronize goats which are already pregnant. In goats, pregnancy is maintained by progesterone secreted by the corpus luteum throughout gestation. The use of prostaglandins on pregnant animals (at any stage) causes lysis of the corpus luteum and an immediate abortion ensues. In the protocol farmers should be asked if their goats

are pregnant or not before being selected for AI and then, the technical team double checks using ultrasound pregnancy diagnosis.

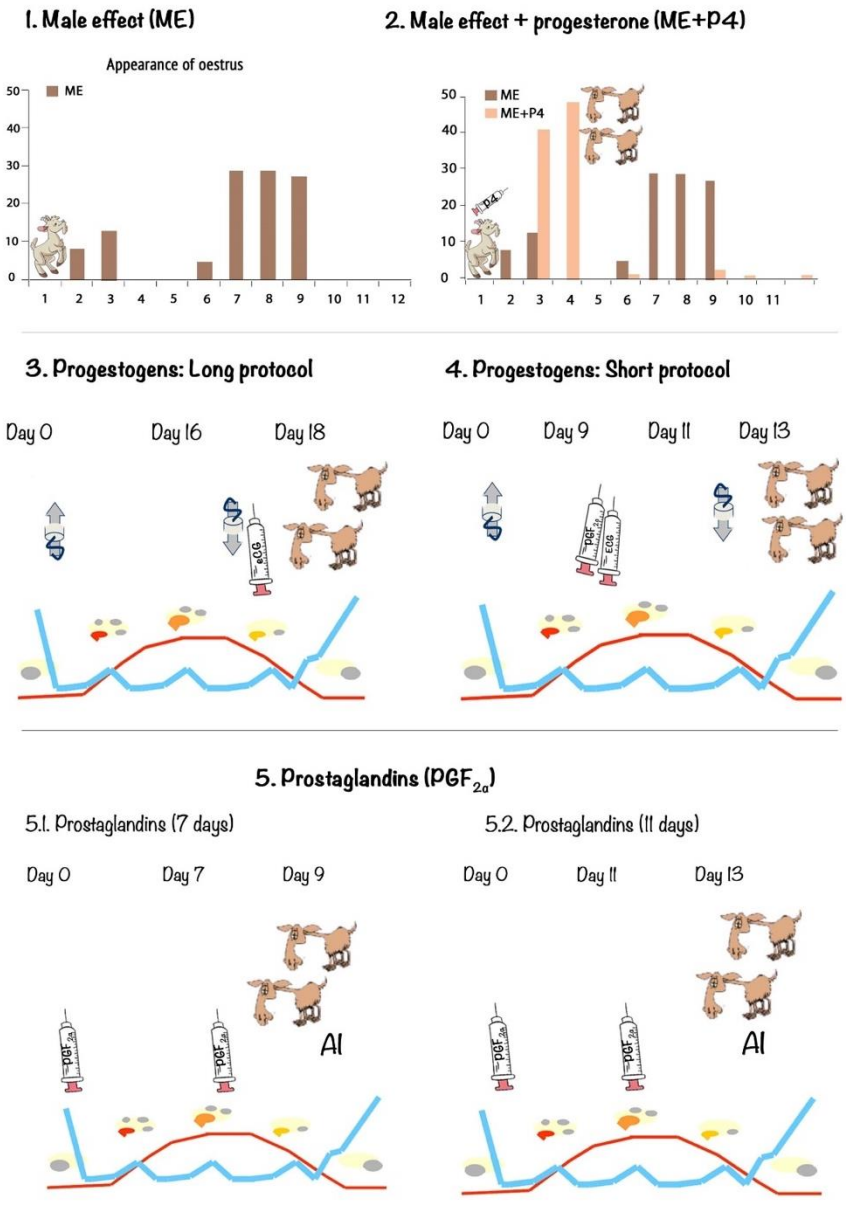

Figure 8 Various synchronization protocols of oestrus and ovulation in goats

Much of the variation in conception rates after AI is related to the choice of females to be synchronized and inseminated. This step requires good planning, sufficient time and rigor. Locally available data on the reproductive history of the females is important during selection. Key elements to observe during female selection for AI include the following:

- Plan AI's during the season when high conception rates can be achieved. Avoid periods when animals may be in anoestrus (seasonal – lactational – nutritional);

- Select only adult females with a good record of successful parturitions and a good mothering ability. The response of maiden ewes to synchronization and cervical AI is usually very low;

- Avoid selecting females still suckling their lambs. Suckling can depress conception rates after AI or natural mating;

- Females with low body condition are not fit for reproduction; care should be taken to select females with a body condition score not less than 2.5 to 3.0. Feed supplementation of the selected females is recommended if the animals are to reach a mean body condition score of 3.5 to 4.0 at insemination time.

6 Low-infrastructure artificial insemination laboratory

To proceed to AI a minimum of equipment should be present in the field. Low-infrastructure artificial insemination laboratory is related to the physical structure 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 and Tanzanian Livestock Research Institute (TALIRI) West Kilimanjaro from Tanzania (Figures 9 and 10 for laboratories serving goat CBBP's in Ethiopia and Tanzania). The key needed equipment is listed below:

- Contrast-phase microscope,
- Water bath,
- Heating plate,
- Refrigerator,
- Generator (for field AI's),
- Common laboratory glassware and supplies,
- Spectrophotometer for the evaluation of rams and bucks' semen,
- ultrasound machine for routine transabdominal pregnancy diagnosis,
- specific supplies for sheep and goats' AI including artificial vaginas and accessories,
- AI material (AI guns, speculum, straws and sheath, extender, lighting sources, extender)

These laboratories may not have electricity and running water and one major feature of these low-infrastructure labs is their mobility. This feature is imposed by the current structure of the AI system. As fixed time AI's after synchronization with semen collected, processed and deposited at 35 °C is

used, this requires a setting of the lab in proximity of the community superior rams and bucks and the community female flocks 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 (figure 11).

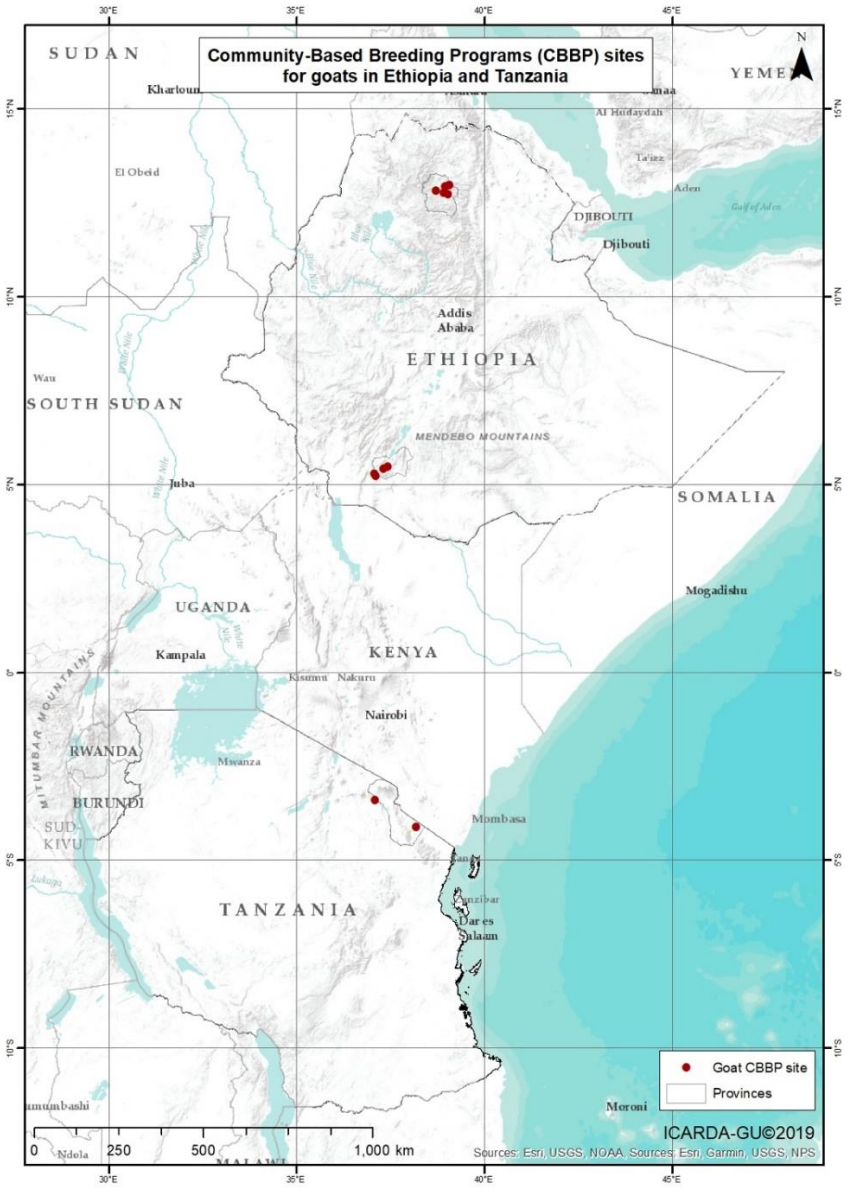

Figure 9 Project target areas in the provinces of Wag_Himra (Sekota) and Segen_Peoples (Konso) in North and South Ethiopia, respectively and Kilimanjaro in Tanzania

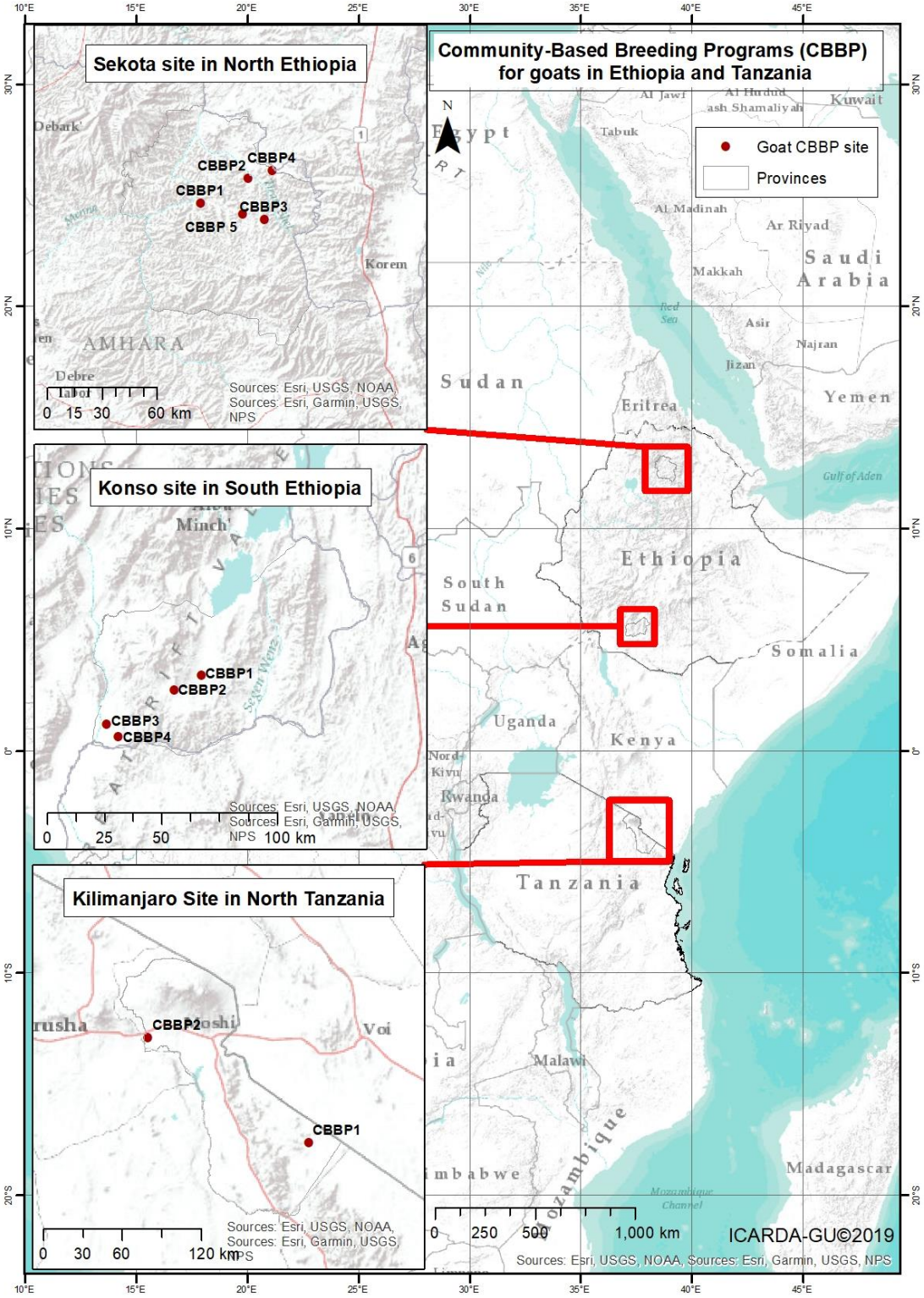

Figure 10 Location of the different project areas

Figure 11 Photos of some low-infrastructure laboratories in Ethiopia

7 Semen collection and quality assessment

7.1 Training of males

Males at first collection

It is better to begin the training at the end of summer and autumn 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. In order to prevent fights, rams should be placed in a waiting room of about $1m^2$ and are introduced one by one in the collecting room where 2 ewes (one free and 1 restrained) are present there. Females should present natural or hormonal-induced oestrus behavior:

Ewe

In Ewe oestrus behavior could be induced with different protocols:

- Insertion of a progestogen-impregnated vaginal sponge for 6 days + 1 injection of 100 µg oestradiol benzoate (48 later). Oestrus behaviour will appear 12-48h after oestradiol injection.
- To maintain the oestrus behavior, a further dose of oestradiol 17 beta could be used.

Doe

The same treatment could be used without the progestagen pre-treatment. the oestrus behavior is maintained by injections of 100 µg oestradiol three times, twice or even once a week.

In the presence of females induced in oestrus, males can adopt 2 scenarios:

1. The male will manifest a sexual behavior (sniffling, lateral approaches, mounting…).

If the sexual motivation of the male persists even in the presence of the operator, the latter should act quietly to avoid fear of the male, the artificial vagina is presented to the male when it is in a suitable position for copulation.

- The male can ejaculate immediately in the artificial vagina
- The male can dismount the female in the presence of the operator. At this time the operator should not move from his position. Generally, the male will try to mount the female again. In the second attempt, the male will ejaculate in the artificial vagina or the semen collection will not be possible. In this case, it is preferable to allow the male to serve the female with no semen collection. The operator stimulates the male by voice at the same time.

2. Some animals have fleeting sexual behavior. So, the operator should be ready at each attempt and he can help stimulating the sexual activity of the ram or buck by:

- Walking with the female in front of the male
- Changing the female
- Encouraging the male by voice
- Encouraging the male by moving the head of the female back toward the male

Solicitation should not exceed 5 to 10 min and contact with the female should be repeated 2 to 4 times in the same period. Bucks are easier to collect as they present more rapid and spectacular sexual behavior.

3. Some animals are sexually inhibited by the presence of females and the presence of the operator because they are:

- Timid animals in the presence of humans: in this case training should be done in order that the male becomes familiar with the operator voice and smell of cloths. The operator should avoid light colored clothes and wear blue or black. These males could also be used at the same time as other males for detection of oestrus. Bucks present less gregarious character and their sexual behavior is generally not inhibited in the presence of human.

- Homosexual animals: Two or three months before puberty, when males are reared together, they could present sexual activity in the presence of other males. In this case, semen collection is possible using a male as teaser animal.

- Presenting health problem: Any general infection or infections of the prepuce, the penis or inflammation of articulations could inhibit mounting.

It is preferable to select young sires at weaning to give them correct rearing conditions that will simplify semen collection later.

Males from which semen was previously collected

Experienced males do not present a sexual inhibition even after rest when they are used during the annual period of maximum sexual activity. If they are used outside this period, sexual activity might be inhibited by the season and not by the presence of man. In this case, the use of oestrus females could help for semen collection. To avoid semen collection problems, males should be trained during the breeding season at fixed days and hours.

7.2 Semen collection of trained males

Sheep

- Males are brought in the collecting room
- A female teaser is immobilized, and a standby ewe is kept near
- Male are tethered and the abdominal area is cleaned with saline solution (0.9 % NaCl) to remove impurities.
- The ram should be left in contact with the female for few moments. Forcing the sire into a false mounting and then forcing it off before ejaculation increases the quantity and the quality of the semen.
- The operator should kneel down on the right side of the sire and tries to slightly deflect the penis of the ram with the hand in order to bring it into the artificial vagina (Figure 12). The temperature of the water added to the artificial vagina should be between 40 and 42 ° C.
- Ejaculation occurs immediately and the semen is collected in the tube connected to the artificial vagina.
- It is possible to collect a second ejaculate for the same sire and with the same artificial vagina. For this the animal should be provoked to a conditioned reflex. For the first ejaculation, the operator should not wait more than one to 2 minutes and deliver the first ejaculate to the laboratory.

Goat

Bucks are led straight from their boxes to the collection room as they are reared individually. The semen collection procedure is same as for the ram expect that false mount is unnecessary as no positive effect has been demonstrated on semen quality or quantity.

Figure 12 Collecting semen from bucks for evaluation in Konso (Photo credit: ICARDA)

7.3 Quantity and quality assessment

Semen quality and quantity assessment is the same for rams and bucks and should go through:

Volume of the ejaculate

- 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.

Concentration of the ejaculate

- 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

- A 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 table 1.

Table 1 Determination of the score for wave motion of semen

Scores	Aspects of wave motion
0	Total immobility
1	Individual movement
2	Very slow movement
3	General wave motion, slow amplitude of waves
4	Rapid wave motion, no eddies
5	Rapid wave motion, eddies

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 10^6 spermatozoa per ml of semen.
- As for the wave motion, the score is attributed according to a scale ranging from 0 to 5 (Table 2).

Table 2 Determination of the score for individual motility of semen

Scores	Individual motility
0	No displacement of sperm cells
1	Very slow (or no) displacement, trembling of spermatozoa, tail oscillation
2	Slow displacement, trembling, disorganized movement, some spermatozoa move more rapidly
3	Spermatozoa follow curvilinear displacement with no trembling movement
4	Rapid displacement, some sperm cells with straight trajectory, others with circular trajectory
5	Straight and rapid displacement of spermatozoa

Percentage of dead sperm cells and abnormalities

- This percentage is calculated using an eosin-nigrosin stain (Table 3).
- 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 (Figure 13):

- 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

For sheep and goats, only ejaculates with a wave motion score of at least 4 and a percentage of abnormal sperm cells not higher than 15% are used for AI. The total number of spermatozoa differ between the two species and is 400 x 10^6 spermatozoa deposited with 0.25 ml mini-straw for sheep and from 150 x 10^6 to 400 x 10^6 spermatozoa deposited also with 0.25 ml mini-straw for goats.

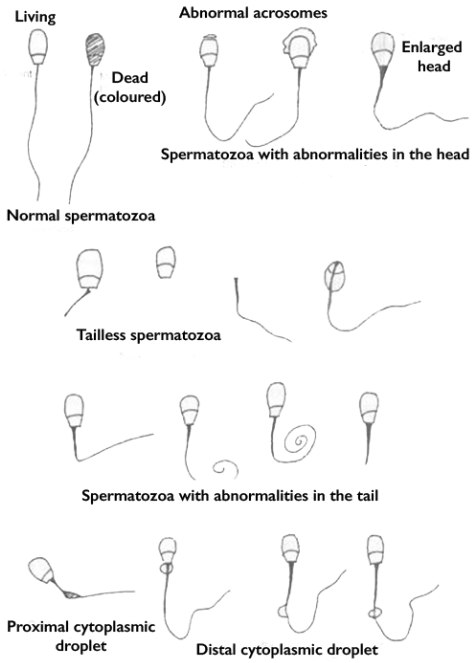

Figure 13 Classification of different sperm morphological abnormalities

Table 3 Preparation and conservation of the stain eosin-nigrosin

Eosin (water soluble)	1g
Nigrosin (water soluble)	2g
Tri Na-citrate, 5.5 H_20	3.57g
Double-distilled water	100ml
pH	6.7 – 6.8
Osmotic pressure	310 milliosmoles
Conservation	4°C (pH checked monthly)

8 Fixed time cervical artificial insemination after oestrus synchronization

Fixed-time artificial insemination works only when females are synchronized. The time of IA is fixed according to the synchronization protocol. Cervical insemination is relatively the easiest method of insemination and it is preferred option in small ruminants. After the semen collection and the straw preparation, the semen should be deposited at the entry of the cervix (ewes and the majority of does).

For that, an assistant should lift the back of the female and immobilize the animal in the right position. The vulva should be cleaned, and the operator introduces slowly the speculum with his right hand by carefully parting the vulva with fingers of the left hand. The speculum is opened when it penetrates about 8-10 cm. At the base of the vagina, the operator should identify the entry of the cervix and then introduce the syringe carefully under the small tongue of the cervix (Figure 14). In the ewe and most cases of the does it is impossible to penetrate more than 1-2 cm into the cervix. Therefore, the syringe is withdrawn some millimetres and the crosshead is pushed slowly in order to evacuate the semen. In minority of cases in the doe it is possible to penetrate through the cervix and the semen is directly deposit into the uterine corpus.

Figure 14 Cervical insemination (Photo credit: ICARDA)

9 Summary steps of semen handling, processing and deposition

The working environment targeted in these guidelines refers to extensive production systems where central laboratories for semen production do not exist or are very distant from the villages and the communities where the inseminations are to be carried out. For these reasons, we have developed mobile, low-infrastructure labs relying on the use of generators to provide electricity and using fresh non-cooled semen from the top ranked rams. Ultimately, the different labs will produce fresh-cooled semen at 15 ˚C and this will extend to 4-6 h the time lag between semen collection and insemination giving more opportunities to reach out far-off communities and villages.
Semen collection and the insemination acts include the following steps:

- Semen collection using an artificial vagina in the presence of a female induced in estrus;
- Measurement of the ejaculate volume and appreciation of the color and the consistency of the ejaculate. Volumes less than 0.5 ml are generally not used and watery ejaculates (low concentration) or with a distinct yellow color (suspicion of inflammation) are also discarded;

- Quick assessment of mass motility under a microscope. Ejaculates with mass motility scores less than 3 should be discarded;
- Measurement of the sperm concentration using a portable spectrophotometer pre-calibrated for ram semen (ovine-caprine *accuread photometer*; IMV®, France). Ejaculates with a concentration less than 3 10^9 sperm ml^{-1} are discarded;
- While being processed, ejaculates are placed in a thermos flask containing water at 35-37 ˚C;
- Ejaculates are then diluted to a final number of 400 10^6 sperm in each straw (straw volume 0.25 ml) using a commercial extender for sheep semen (Ovixcell; IMV®, France) kept at 35-37 ˚C. Final number of sperm can be further reduced to 300 or even 250 10^6 sperm if the initial quality of the ejaculate is high. For goats, final number of sperm can be further reduced to 150 10^6 sperm;
- Diluted ejaculates are then checked for individual motility under a microscope. Ejaculates with a low proportion of spermatozoa moving rapidly on a straight line (less than 40%) are not used;
- Straws are filled, then sealed with inert packing powder and immediately immersed in a thermos flask filled with water at 35-37 ˚C;
- Inseminations should be carried out immediately after packing and sealing. On average, time lag between semen collection and insemination should not exceed 15-20 minutes.

10 Post AI management and assessment of conception to AI

When inseminated, females should not be exposed to stress (fights with other animals, nutritional stress….). Any abrupt changes in the diets prior to, during and after the inseminations (up to 15-20 days) should be avoided and females should not be exposed to any management stress (vaccinations, walking or grazing over long distances, transhumance...) in the first few days following AI's.

To keep satisfactory levels of fertility at the flock level, post-mating with rams at the return oestrus is indispensable. To distinguish between females conceiving to AI and those conceiving at the return oestrus, entire rams should be reintroduced in the inseminated females between 7 and 10 days after inseminations.

Under routine field conditions, non-return oestrous after AI is not a reliable criterion to estimate conception to AI. The most reliable methods are:

- Transabdominal pregnancy diagnosis at 40-45 days after the date of insemination
- Females are considered to have been conceived at AI if lambing date $\in$ [145, 155 days after AI].

Suggested reading

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