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## Rappels sur l'insémination artificielle chez les bovins

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## **DEDICATIONS**

I dedicate this humble work:

To my mother, my father, my sisters and my brothers; may Allah grant them a  
long and happy life.

To my great teacher and my supervisor, Mrs. MIMOUNE Nora, to whom  
belongs the merit of her help for the achievement of this work.

To all the teachers who have worked hard to accomplish their noble mission  
and to fulfill their great task wholly.

## ACRONYMS

CNIAAG	National Centre for Artificial Insemination and Genetic Improvement
IBR	Infectious Bovine Rhinotracheitis
IVT	Illinois Variable Temperature
ATB	Antibiotic
CO <sub>2</sub>	Carbon dioxide
pH	Hydrogen potential
N <sup>o</sup>	Number
-	Minus
%	Percent
°C	Celsius degree
CASA	Computer-Assisted Semen Analysis
cm	Centimeter
mm	Millimeter
ng	Nanogram
ml	Milliliter
min	Minute
h	Hour
TRIS	Trihydroxymethylaminoethane
BVD	Bovine viral diarrhea
AD	Anno Domini

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# INTRODUCTION

## INTRODUCTION

Reproduction is considered one of the most important concerns of the breeder and veterinarian, prompting the search for and use of new technologies aimed at achieving multiple improvements on several levels: economic, genetic, health and technological.

These new biotechnologies that are invading the world of breeding have a number of more or less specialized techniques, the oldest being artificial insemination. AI is the instrumental introduction of sperm into the sexual organs of the female, thereby achieving the approximation of sperm and ova without sexual intercourse. It has multiple implications for livestock breeding and superiority over natural mating (**Hanzen, 2011**).

In the beef industry, insemination is an integral part of breeding practices. The bulls at the insemination centers are reproducers that have already undergone a selection process, and subjects with poor semiotic parameters have been eliminated. Moreover, the zootechnical aspect is to be taken into account, because for an elite bull, the number of straws produced is important to ensure a wide diffusion of its genetic potential. Therefore, bovine insemination is a technique that requires specific equipment and a thorough training in anatomy, physiology and surgical gesture. The success of the first insemination remains the objective of the breeders and inseminators who must take into account different factors to choose the right moment of reproduction, among which the detection of heat and the condition of the animal. This is why many methods have been developed to try to evaluate the fertility of a sire by in vitro semen analysis (**Dumont, 1997**).

Algeria has resorted to the introduction of artificial insemination, which aims to intensify milk production while minimizing the risks of transmission of sexual diseases and thus offering a better planned reproduction management through control and early diagnosis of infertility problems following an individual and permanent monitoring of inseminated cows.

## **I. Artificial insemination**

### **I.1. Definition**

Artificial insemination is a reproductive technique that consists of depositing the male's semen in the most suitable part of the female's genital tract and at the most appropriate moment, using an appropriate tool and without a sexual act taking place. The semen is obtained using variable devices on the male that has previously received a zootechnical and sanitary agreement. Artificial insemination has become one of the most important techniques ever devised for the genetic improvement of farm animals. It has been widely used for breeding dairy cattle as the most valuable management practice available to the cattle producer and has made bulls of high genetic merit available to all (Webb, 2003; Bearden et al., 2004).

### **I.2. General history**

The history of AI begins with the Arabs according to some Arabic documents dating from about 1322 AD. In fact, Spallanzani has been recognized as the inventor of AI. His scientific reports of 1780 have indicated successful use of AI in dogs. In 1899, Ivanoff of Russia pioneered AI research in birds, horses, cattle and sheep, and was apparently the first to successfully inseminate cattle artificially. Denmark was the first European country to establish an AI cooperative association in 1936. E.J. Perry of New Jersey visited the AI facilities in Denmark and established the first United States AI cooperative in 1938 at the New Jersey State College of Agriculture (Webb, 2003).

### **I.3. Artificial insemination in Algeria**

In Algeria, artificial insemination was introduced during the colonial period. Although very old, its use in our farms is very limited despite the efforts and the control of the technology by the CNIAAG (National Centre for Artificial Insemination and Genetic Improvement) from the harvesting of the semen to its placing in ready-to-use straws, everything is done according to international standards. The problem does not arise at the level of laboratories, but elsewhere, more precisely in the farms scattered throughout the country. However, in the opinion of all, this technique, which has been successful in other countries, could be very successful here, if we put the means.

## **I.4. The advantages and disadvantages of artificial insemination**

### **I.4.1. The advantage of Artificial insemination**

There are several benefits (**Rukundo, 2009**);

- **Genetic interest**

The genetic superiority of bulls selected in this way is widely spread thanks to AI. Compared to natural breeding, AI allows to increase the number of offspring per male and to dissociate, in time and in space, the places of production and semen placement. Indeed, one ejaculate allows to cover about 300 cows and can be kept for a long time (about 10 years).

- **Sanitary interest**

Artificial insemination is a tool to prevent the spread of contagious and/or venereal diseases due to the lack of direct physical contact between the female and the sire. However, there are some infectious agents that can be transmitted through the semen during AI. This is the case of foot-and-mouth disease virus, bovine pest virus, IBR virus, Brucella abortus, campylobacter.

However, the control of diseases, thanks to the strict sanitary norms required at the level of the seed producing centers, has made it possible to considerably reduce the risk of transmission of these agents by the "male" route.

Through artificial insemination, it is possible to avoid the occurrence of genetic diseases related to the prolonged use of a single sire in the same farm. Artificial insemination also makes it possible to exploit high performance breeding stock suffering from impotence as a result of accidents or fattening, through the application of collection methods with an electro-ejaculator.

- **Economic interest**

AI exempts the breeder from maintaining a bull in favor of a selected bull semen.

The breeder will no longer have to worry about feeding a bull (which is sometimes dangerous).

Thanks to AI, it is possible to crossbreed and thus benefit from a heterosis phenomenon. However, in the tropical context, its use remains linked to that of techniques of heat grouping (synchronization and/or induction of heat).

Indeed, if it is judiciously combined with heat grouping techniques, artificial insemination can contribute to a better management of the breeding through:

- Reduction of the interval between births;

- Grouping of births according to seasons.

Artificial insemination contributes to the improvement of the herd's productivity (milk-meat) which translates into an improvement of the farmer's income. This aspect is particularly noticeable in crossbred animals (obtained by artificial insemination of local cows) whose production improves by 100% compared to the local type.

Finally, AI contributes to food security through the improvement of the national production of milk and meat.

- **Technical and practical interest**

Beyond a certain number of animals, it becomes essential to run the herd in groups, for a better organization and profitability. AI allows a more rigorous organization of the productions by a planification, an organization of work and a permanent follow-up.

AI offers a great possibility to the breeder to choose the characteristics of the bull he wants to use according to the type of his breeding and the option of animal production to develop.

#### **I.4.2. The disadvantages of Artificial insemination**

The disadvantages of artificial insemination include the dangers of a poor choice of sire, a possible loss of genes (this is the case of the selection of the character of high milk production which was obtained at the expense of hardiness, longevity, fertility and inbreeding (**Rukundo, 2009**)).

## **II. Semen collection**

The semen quality of artificial insemination sires has an influence on AI success and therefore on fertility. However, the value of a bull for this male component of fertility is usually known late after a large number of AIs have been performed. The reproductive activity of the bull is a function of internal (genetic-physiological) and external (social environment, stimulation conditions) factors, explaining important inter-individual variations in sexual efficiency and sperm production (**Basso, 2005**).

### **II.1. Recruitment of semen producing bulls**

The functional value of a bull implies two conditions:

- A satisfactory sexual behaviour : mounting and ejaculation (libido appreciation).
- An optimal spermatogenesis and fertilizing value of semen.

The evaluation of the bull's reproductive capacity is based on 5 examinations:

#### **II.1.1. General examination**

It is the responsibility of the veterinarian to proceed to a general examination of the animal to specify in particular the body condition, the presence of the secondary sexual characters, the nature of the fecal matter (**Hanzen, 2009**).

- The musculoskeletal system (when moving and immobile) : postures, stance and joints.
- Good visual quality is an important parameter (**INTERVET, 1997**).
- Identity control and age.

#### **II.1.2. Genital tract examination**

The ram's penis can be easily exteriorized to see any bleeding or crusty lesions. In bulls, loosening of the penis or swelling may occur if the animal has been tranquilized during transport (for this reason, administration of tranquilizers is highly discouraged). The absence of inguinal hernia can also be checked by palpating the testicles. In both cases, the testicles are palpated by grasping the skin with both hands, on each side, close enough to the abdomen, then palpating while descending and comparing the size of the testicles, their consistency, the presence of nodules, pain, inflammation. The epididymis, which is an organ lateral to the testicle, is also palpated (**Arthur et al., 1982**).

### **II.1.3. Rectal examination**

It is of great importance and indispensable, it includes palpation of the urethra, the prostate, the vesicular glands, the ampulla, the seminal vesicles and the internal inguinal rings (INTERVET, 1997).

### **II.1.4. Sexual behaviour review**

It is reasonable to estimate that one in five bulls has a sexual instinct that is incompatible with normal fertility. Four aspects are to be distinguished: libido, copulation, penile erection and ejaculation (Hansen, 2009).

- **Libido**

Libido is defined as sexual desire, while serving capacity is the ability to complete the act of mating (Hanzen, 2006). Libido or sexual desire has been found to be affected by age, heredity, environment, and disease. Although puberty occurs, for example, in bulls at nine to ten months, in all species poor feeding retards its onset. Full libido may be achieved before normal spermatogenesis and therefore, as a rule animals are not put to stud until a few months after puberty. Bulls retain normal sexual desire until five or six years of age, but beyond this point libido very gradually wanes (Arthur et al., 1983).

- **Copulation**

Inability to perform service despite normal sexual desire is a frequent cause of bull infertility. Copulation comprises several distinct conditions, some of which are not understood. Inability to copulate has been reported to be due to skeletal or visceral pain, in others to lesions of the genital organs, inability to protrude and penile deviations, while in many cases in which no lesions can be found the nervous control of copulation is believed to be defective. The prognosis for the virgin bull has been suggested to be favorable, but grave for adult bull (Arthur et al., 1983).

- **Penile erection and ejaculates**

The penis has two-fold function: the expulsion of urine and the deposition of semen in the genital tract of the female (Roberts, 1985). Before the latter process can occur, the penis must become erect, which is accomplished by dilation of the internal and external pudendal arteries to the penis. The cavernous blood sinuses dilate with blood, the out flow of which is retarded by the increased venous pressure caused by contractions of the smooth muscles of the corpora cavernosa and the extrinsic, ischiocavernosus muscle at the base of the penis. The process of

ejaculation probably starts in the epididymis and travels along the ducts deferens at the same time the walls of the accessory glands contract and force their contents into the urethra (**Hafez, 1993**).

Semen or sperm is the entire seminal discharge of the male during normal ejaculation. It has been known that semen consists of cellular elements, the spermatozoa produced in the somniferous tubules, seminal plasma, or the liquid portion of the semen produced by secretions of the somniferous tubules, epididymis, ducti differentia, and ampulae, seminal vesicles, prostate, and bulbo-urethral glands. Failure of penile erection can, therefore, affect semen collection and it can be caused due to various reasons previously described including lack of libido, which in turn can be affected by age, heredity, environment, and disease (**Arthur et al., 1982**).

- **Other considerations**

According to various studies (**Laing, 1970; Morrow et al., 1985; Roberts, 1985**), failure to mount, failure to achieve intromission, and failure to thrust and ejaculate are other important factors that need to be considered during investigation of bull fertility.

Scrotal circumference provides a good indication of a bull's ability to produce sperm and is related to his own age at puberty (**Hanzen, 2006**). As demonstrated in Table 1, the measurement should be taken at the largest diameter of the scrotum. Both testicles should be positioned next to each other and a flexible measuring tape should be placed snugly around the scrotum. Testicles need to be descended into the scrotum, and should be of the same size and shape. Any irregular shape or swelling may indicate abnormal structure, illness, or injury.

**Table 1:** Recommended scrotal circumference for Bos taurus bulls (**Hanzen, 2006**).

Age	Very Good	Good	Poor
12-14 months	>34 cm	30-34 cm	<30 cm
15-20 months	>36 cm	31-36 cm	<31 cm
21-30 months	>38 cm	32-38 cm	<32 cm
Over 30 months	>39 cm	33-39 cm	<33 cm

### **II.1.5. Sanitary examination**

Disease prevention in bulls has been considered as essential as in breeding females, and new bulls need to be screened by a qualified veterinarian for infectious agents prior to entering a new herd. Bulls have been recommended to be purchased only from reputable seed stock producers with adequate herd health plans; including vaccination against infectious diseases, e.g. leptospirosis and campylobacteriosis. New animals should be quarantined (30 days). Bulls are also recommended to be tested annually for brucellosis, but not be vaccinated for brucellosis. In some instances, bulls need to be vaccinated for bovine viral diarrhoea 1 (BVD), infectious bovine rhinotracheitis (IBR), and trichomoniasis (**Hansen, 2006**).

### **II.2. Semen collection**

Semen collection has been considered like harvesting any other farm crop since effective harvest of semen involves obtaining the maximum number of sperm of highest possible quality in each ejaculate to make maximum use of sires. This involves proper semen collection procedures used on males that are sexually stimulated and prepared. The initial quality of semen has been determined by the male and cannot be improved even with superior handling and processing methods. However, semen quality can be lowered by improper collection and the processing techniques (**Bearden et al., 2004**).

Realization of the maximum benefits of AI depends upon the collection of maximal numbers of viable sperm cells at frequent intervals from genetically superior males (**Cole and Cupps, 1977**). The success of AI depends on the collection of a relatively large numbers of potentially fertile spermatozoa from genetically superior sires (**Garner, 1991**).

#### **II.2.1. Facilities needed for semen collection**

The routine collection of semen for AI in dairy and beef bulls is by using artificial vagina (**Faulkner and Pineda, 1980**). Several essential features have been considered in designing facilities for collecting semen, of which the safety of the handler and the collector have been found to be the most important in bulls in dairy farm. Safety fences, usually constructed of 7.6 cm. steel pipe with spaces large enough for a person to step through at 2.44 meters' intervals, should be provided. The collection area must provide good footing to prevent slipping and injury to the male being collected. An earthen floor in the immediate collection area best provides this. Means to restrain the teaser animals to minimize lateral as well as forward movement must be provided. At the same time, easy access for semen collection must be maintained (**Morrow et al., 1985; Roberts, 1985; Bearden et al., 2004**).

Appropriate and specialized facilities, equipments, and procedures have been used during collection of semen to prevent injury to the bulls and their handlers, to maximize the physiological responsiveness of the bulls in producing semen and to enhance the quantity and the quality of the semen that can be collected (**Garner, 1991**). The area for semen collection has been preferred clean, relatively quiet, free of distractions and any other stressful procedures. There has been a report of increase in spermatozoa motility by 50% through proper sexual stimulation of the bulls (**Salisbury et al., 1978**).

### **II.2.2. Procedure for collection of semen from the bull**

Standard semen collection procedures normally include sexual stimulation, sexual preparation, and collection of the semen (**Herman et al., 1994**).

- **Sexual stimulation**

Providing a stimulus situation that elicits mounting behavior in the bull is termed “Sexual Stimulation” (**Herman et al., 1994**). The stimulation process has been best practiced by exposing the bull to a mount animal in a collection environment and allowing to move briefly around female/teaser for a couple of minutes (**Morrow et al., 1985**).

- **Sexual preparation**

This has been found to determine the intentional prolongation of sexual stimulation. It is achieved through a series of false mounts (allowing the bull to mount but not ejaculate) and restraint and ultimately results in an increase in the quantity and quality of sperm ejaculated.

In dairy bulls, one false mount plus two minutes of restraint plus two additional false mounts before each ejaculation will help obtain the maximum amount of good quality semen (**Herman et al., 1994**).

### **II.2.3. Methods of semen collection**

Semen has been collected in a number of ways, and the methods of collection are governed by the intended purpose for future use. A sample for evaluation may need to be only a very small volume and not as clean a sample as one for use in artificial insemination (**Sorensen, 1979**). The following various methods have been used in collection of semen.

- **Recovery**

It follows normal copulation and can be applied in different ways. A pipette such as an inseminating catheter with an attached suction bulb may be inserted into the vagina following

ejaculation and the semen is, then, siphoned into it. This semen is contaminated with the fluids of the female tract but is satisfactory for evaluation. It may also be used for artificial insemination when trying to overcome some obstruction in the cervix or satisfy breeding restrictions of some pure bred societies. This method can be applied using different mechanisms and includes spooning, using a sponge, using a cup, and blotting (**Sorensen, 1979**).

- **Massage**

Semen has been collected from the bull, in most instances, by massage. The bull is restrained and the gloved arm and hand are lubricated before inserting through the anus into the rectum. The area of the ampulae, vesicular glands, and prostate is located under the rectum. The fingertips then are used to exert a downward pressure milking this area caudally. This stimulates and mechanically causes the sperm to be passed through the urethra by gravity to drip from the prepuce (**Sorensen, 1979; Roberts, 1985**).

- **Vaginal insert**

It consists of a tapered insert with a flange on the end that may be placed in the vagina prior to copulation (**Sorensen, 1979**). Urethral fistula: the male urethra may be cannulated with a tube just under the anus with a T type cannula allowing passage of urine through the urethra proper or collection of sperm under the anus at the time of copulation (**Sorensen, 1979; Roberts 1985**). This is only useful experimentally since rather exacting surgery is involved (**Morrow et al., 1985**).

- **Artificial vagina**

A simple and practical device, the artificial vagina has two parts. An external cylinder made of rigid material, most often hard and thick rubber (thermal insulation) or plastic with an opening closed by a plug. Its length is about 34 cm and its external diameter is between 6 and 8 cm. The inner liner made of latex or artificial rubber is inserted into the outer cylinder and its ends are folded over and held in place by an elastic band. The cavity thus formed by the outer cylinder and the inner liner is filled with water at 41- 42°C in sufficient quantity to obtain a pressure equivalent to that of the female's vagina. One end of the artificial vagina is lubricated; it will be used to introduce the penis; on the other end is fixed a rubber cone at the end of which is adapted a glass or better plastic tube graduated to collect the sperm. Some artificial vaginas are equipped with a thermometer (**Hanzen, 2009**).

Despite the apparent ease of this method, some bulls refuse the artificial vagina or are unable to mount due to arthritis or pain in the hindquarters. In these conditions, the collection can be done by electro-ejaculation (**Kabera, 2008**).

- **Electro ejaculation**

Electro-ejaculation consists in provoking the emission of sperm by the electrical excitation of the erector and ejaculatory nerves. It is performed on a standing or lying animal. After the animal is restrained, the lubricated electrode is introduced into its previously emptied rectum. Then, a series of repeated stimulations is applied to the rectum, gradually increasing the intensity according to the manufacturer's instructions until the animal is fully erect and ejaculates. Semen is collected by a collection device. The ejaculate collected by electro-ejaculation is generally of greater volume and lower sperm concentration than that collected by the artificial vagina (**Salisbury et al., 1961**).

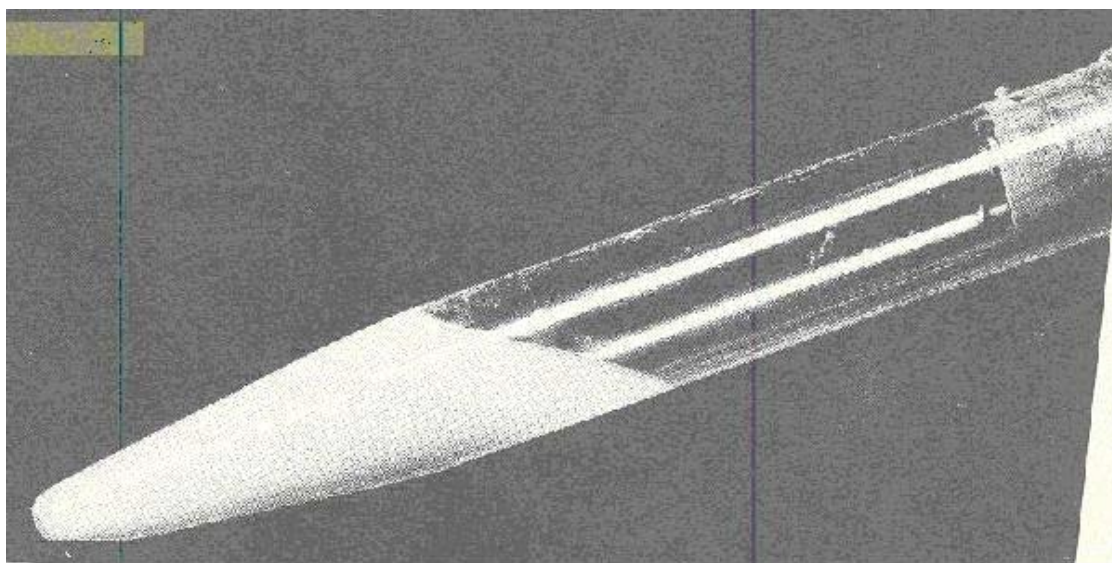
However, total sperm count, fertilizing power and freezing ability do not seem to be affected. The apparatus consists of an ogival-shaped rectal probe equipped with bipolar electrodes through which a current of medium intensity but low voltage flows, supplied by battery or by the mains by means of a transformer, the latter allowing a constant voltage. The rheostat allows the characteristics of the current to be varied in order to obtain the cycle necessary to obtain the flow of sperm. The use of electro ejaculation even during a long period of time (more than one year) has no harmful effect on the health and fertility of the animal (**Haskouri, 2001**).

### III. Semen analysis

The ejaculate is characterized by different seminological parameters which constitute the seminogram. The seminological examination of the ejaculate includes a macroscopic and a microscopic examination. It is used to assess whether the semen collected is of sufficient quality to be preserved (**Herman et al., 1994**).

#### III.1. Macroscopic analysis

Immediately after collection, the semen in the collection tube is visually examined to assess the volume, color and consistency of the ejaculate.



**Figure 1:** A Freshly collected ejaculate of good quality bull semen (**Herman et al., 1994**).

- **Volume**

The volume of the ejaculate is readily measured by collecting the sample directly into a graduated vial (**Garner, 1991**). Alternatively, it can be done by weighing the tubes after semen collection on top-loading balance, and later converting the reading into milliliter by using a computer program. The latter has been known to reduce error associated with visual reading of the tube specially when small volume or bubbles are found by 10% (**Bearden and Fuqary, 2000**). The volume has been reported to decline when young bulls are used or when there is frequent ejaculation or incomplete or failure of ejaculation and in bilateral seminal vesiculities (**Garner, 1991; Hafez, 1993**). Furthermore, those authors have described in summary that a number of factors like season of the year, method of collection, and the sexual preparation of the bull have been known to affect semen volume.

The volume of bull's semen varies between ejaculates, individual bulls, breed, and age. However, a bull with less than 2ml of semen per ejaculate is not acceptable (**Zewdie et al., 2005**).

Differences between reports on semen volume could be attributed to differences in age, breed, nutritional status geographic locations, seasons of year of study, method of semen collection and handling of bulls during collection, procedure and frequency of collection (**Caroll et al., 1963; Igboeli and Raka, 1971; Salisbury et al., 1978; Tegegn et al., 1992a; Hafez, 1993; Blezinger, 1999; Andrabie et al., 2002**).

- **Color**

The color is analyzed by simple observation of the ejaculate in the collection tube; a normal semen is whitish to yellowish-white in color. However, this color can be altered for physiological and especially pathological reasons. The yellow color of semen is due to a high content of carotene from the seminal vesicles. It can also be caused by the presence of pus or urine in the semen. A pinkish or reddish color indicates the presence of blood in the semen. Brownish discoloration indicates the presence of altered blood or degenerated blood elements in the semen or inflection. Blue color of the ejaculate is due to low sperm concentration or administration of methylene blue (**Konfe, 2014**). Any sample with abnormal staining will be discarded and exploration should be considered to characterize the origin of this abnormality (**Parez and Duplan, 1987**).

- **Viscosity**

Semen viscosity is strongly dependent on sperm concentration. The ejaculate is more viscous the higher the sperm count. Compared to distilled water, normal bull semen has a viscosity of 3.7 according to **Parez and Duplan (1987)** cited by **Konfe (2014)**. The presence of lumps in the sample or the formation of a glairy filament at the tip of the pipette indicates a pathology (**Parez and Duplan, 1987**). The opacity of the semen can also be assessed, which is related to the sperm concentration in the ejaculate.

- **PH**

PH of semen is measured with a pH meter or with the indicator paper. This is a measurement that is done immediately after collection. Sperm acidifies rapidly due to the formation of lactic acid. The pH of normal semen is between 6.2 and 6.8 in bulls according to **Hanzen (2009)** cited by **Konfe (2014)**.

### III.2. Microscopic examinations

- **Mass Motility**

Mass motility is performed from pure semen, within 10 minutes of collection, the equipment required consists of a slide previously heated to 37°C and a hot stage microscope. The operator places a drop of semen (6µL, 5 mm diameter) on the surface of a slide. At magnification 100x, the thickness of the ring formed by the spermatozoa at the periphery of a drop is also assessed. Mass motility is scored from 0 to 5 as follows in Table 2.

**Table 2:** Mass motility scoring (**Dumont, 1997**).

Score 0 :	No sperm movement
Score 1 :	Slight perceptible movement, no wave
Score 2 :	Few waves (43)
Score 3 :	Numerous waves
Score 4 :	Fast and intense waves
Score 5 :	Very fast swirls

It is possible to convert this score into an approximate percentage of motile sperm (score 3 corresponds to approximately 70% motile sperm) (**Dumont, 1997**).

A sperm whose mass motility is less than or equal to 3 is generally eliminated.

- **Individual motility**

Motility of spermatozoa has been defined as the percentage of sperm cells that are motile under their own power and progressive motility of spermatozoa has been defined as those spermatozoa that are moving or progressing from one point to another in a more or less straight line (**Bearden and Fuquary, 2000**). Spermatozoa are driven by a propulsive apparatus, the flagellum, which is equipped with contractile proteins strategically arranged in longitudinal organelles, the coarse fibers, and with associated sub filaments, and micro tubes, which provide the propulsive force necessary to overcome internal structural resistance and external viscous drag of extra cellular fluids (**Hafez, 1993**). Motility of spermatozoa at time of collection has been used commonly as a measure of the fertilizing ability of the sperm (**Roberts, 1985; Bhosrekar, 1990**). However, spermatozoa have been found to lose their fertilizing capacity before they lose motility, which puts motility estimation to be not necessary indicative of fertilizing capacity of the sperm (**Hafez, 1993**). Nevertheless, a definite correlation has been found between concentration, morphology, and motility of

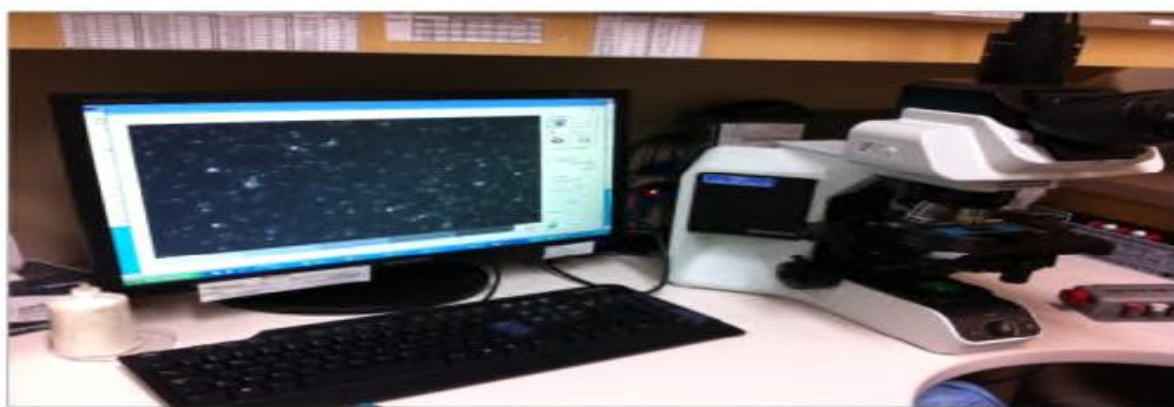
spermatozoa and the proportion of the total number of actively motile normal spermatozoa in the ejaculate (**Roberts, 1985**).

The individual sperm motility is evaluated by taking small drops of semen onto a slide with cover slip under high magnification (200X). Sperm cells moving in a straight-line forward direction are considered in the motility measure. In order to be acceptable, bull semen should have at least 70% and 40% motility respectively at the time of collection and after freezing (**Zewdie et al., 2005**).

### **Objective measurement of sperm motility by computerized semen analyzer (CASA)**

#### **a. Concept of CASA (Computer Assisted Semen Analysis)**

CASA is the acronym for "Computer-Assisted-Semen-Analysis". It is a tool that is increasingly integrated in medical laboratories, allowing the realization of seminograms. This optional device is especially useful in centers performing a large volume of analyses or specialized fertility centers. CASA systems consist of a light microscope, camera, and processor (computer). The latest devices allow the measurement and calculation of many seminogram parameters: motility, sperm concentration, morpho-metric characteristics (such as length, width, head perimeter and area, flagellum length) and morphological abnormalities (coiled flagellum, broken flagellum, vacuoles) (**OPTMQ, 2016**).

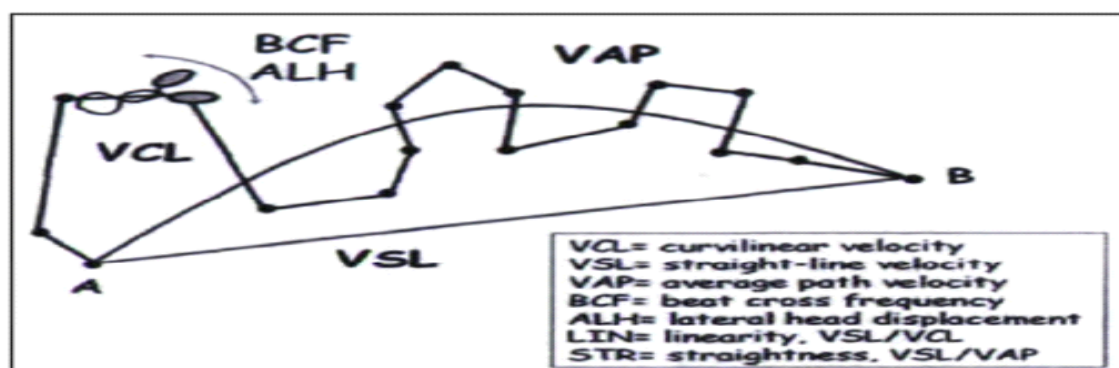


**Figure 2:** CASA system attached to a microscope (**OPTMQ, 2016**).

#### **b. Mobility assessment using CASA**

The CASA methods have made it possible to standardize total and progressive motility tests in the same laboratory and to characterize semen by several parameters. CASA analysis requires using a low concentration, this analysis is validated if the machine uses between 700 and 900 cells, which corresponds to a concentration between 20 and 30 x 10<sup>6</sup> spz/ml for most analysers (**Ponthier, 2012**). The system detects sperm movement, and tracks each sperm

separately in time and space. In practice, a sample is placed on a cell that should not be too deep so as not to interfere with the focus of the microscope (12  $\mu\text{m}$ ). A camera records all movements and analyses them according to various parameters as presented in Figure 1.



**Figure 3:** Sperm motility parameters (Hebert, 2011).

- **Sperm concentration**

The concentration expresses the number of sperm per  $\text{mm}^3$  (or per ml) of an ejaculate. It can be determined directly by counting sperm with a hematimetric cell or indirectly by visual comparison of semen to standard solutions by electronic counting or by nephelometry. The evaluation of the sperm concentration by the direct method provides a more objective result (Dumont, 1997).

- **Percentage of live spermatozoa**

The percentage of live spermatozoa is estimated approximately, under the light microscope, this value is strongly correlated with the quality of the movement. This estimation is subjective and depends strongly on the experience of the operator. The examination is more easily performed on a smear stained with eosin-nigrosin, if the rate of live spermatozoa is less than 60%, the semen is not preserved. This test is not routinely performed because the most relevant quality criterion for use in bovine AI is the percentage of live sperm after thawing (Dumont, 1997).

- **Sperm Morphology**

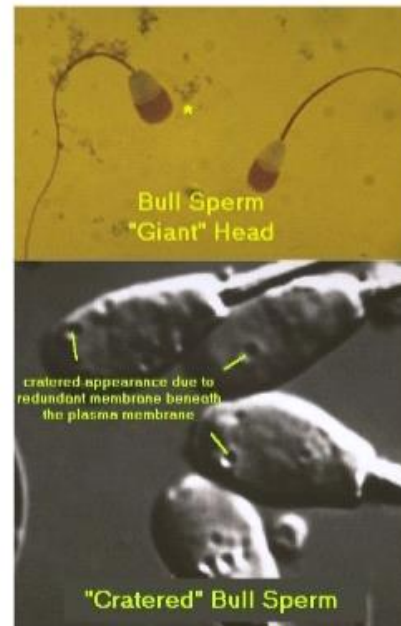
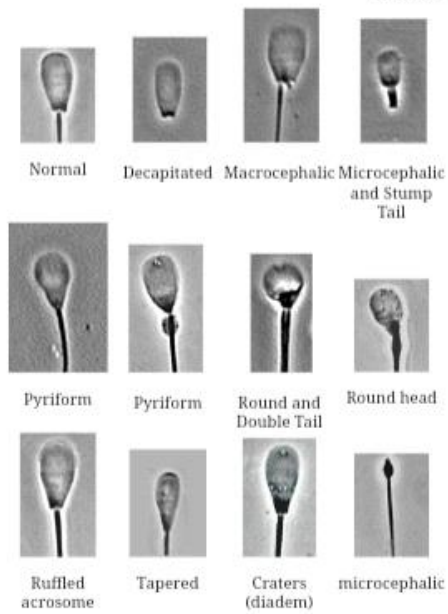
These examinations can be performed on bulls considered "doubtful" to assist the operator in making a decision to reject or retain the semen.

Previous studies have shown that the normal morphology of spermatozoa is composed of a head and a tail that is divided into a mid-piece, main-piece, and end-piece (Bearden et al., 2004). Films for microscopic examination under the oil immersion lens are made immediately

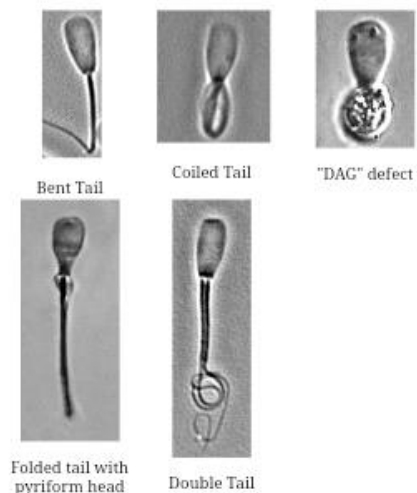
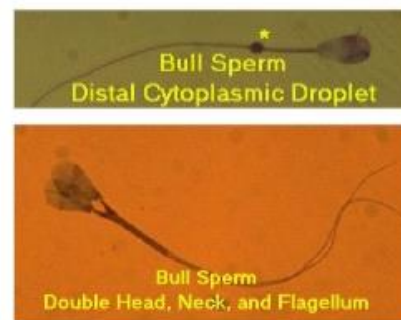
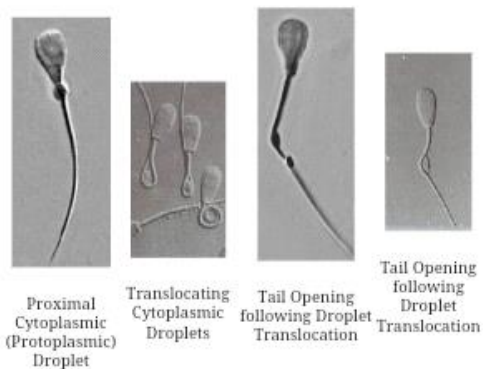
after the motility estimation, but the examination can be made, subsequently, in the laboratory (**Arthur, 1979**). To obviate temperature shock and the assumption of spurious morphological defects, a drop of semen is mixed with two drops of Indian ink previously raised to body temperature on a warm slide. The drops are mixed and spread like a blood film. Between 200 and 300 sperms are examined and classified according to their shape and appearance. Fertile bulls show about 90 percent of the morphologically normal sperms. According to previous studies (**Arthur, 1979; Bearden et al., 2004**), the following morphological abnormalities can be investigated. These include: tailless sperms and sperms with looped tail, the commonest sperm abnormalities which are detachment of the sperm head and bending of the middle piece and tail around and over the sperm head (looped tails), sperms with coiled tails (this abnormality is of two types: the coil involves the extremity of the tail, or the coil, which includes the whole of the tail & sometimes the middle piece), immature or unripe sperms (these are characterized by the presence of a droplet of protoplasm at the junction of the sperm head with the middle piece at the so-called neck), abnormalities of the sperm head and cytogenic disturbances, and other defective sperms.

Figure 4 shows some of the different abnormalities (primary and secondary) of bull spermatozoa.

### Morphology of Bull Spermatozoa Primary Abnormalities



### Secondary Abnormalities



**Figure 4:** Primary and secondary abnormalities of bull spermatozoa (Website 1).

- **Freezeability Test**

The temperature changes imposed during freezing and thawing damage the cytoplasmic membranes, the cytoskeleton, the organelles involved in motility and the acrosome. The functional capacities of the spermatozoon are therefore altered. This is why it is important, in the context of artificial insemination, to evaluate the quality of the semen after freezing. Thus, for each ejaculate stored at -196°C in liquid nitrogen, the mass (and/or individual) motility is evaluated after thawing of two to three straws as well as the percentage of dead and live spermatozoa, using the same methods as those used for the examination of fresh semen. In general, the correlation between freezeability and fresh semen quality is relatively high.

However, sometimes semen considered to be of good quality when fresh (mass motility score higher than 3 and more than 60% of live spermatozoa) turns out to be particularly poor after thawing. This is why this test is essential for any bull whose semen is intended for artificial insemination with frozen semen.

The criteria for the preservation of straws are based on the quantity of arrowing sperm. For an ejaculate to be preserved, it must have more than 25% of darting sperm and more than 8 million darting sperm per straw, after thawing (**Dumont, 1997**).

- **Interpretation of the examinations performed**

For bulls used in artificial insemination, motility must be greater than 60%, i.e., a score greater than 3 assigned in mass motility. The concentration of ejaculate must be greater than 0.5 billion per ml. The threshold for morphological abnormalities is less than 30% abnormal sperm, less than 20% major abnormalities and less than 10% for each major abnormality category. However, these criteria are less stringent for bulls used in natural breeding as the ejaculate will not be split, diluted or frozen (**Dumont, 1997**). The decision criteria for ejaculate conservation are summarized in Table 3. Table 4 presents some characteristics of ejaculation and semen in various species of domestic animals and man.

**Table 3:** Decision criteria for ejaculate preservation (**Dumont, 1997**).

Criteria	Threshold for use in artificial insemination	Threshold for use in natural breeding
<b>Macroscopic appearance</b>	"Creamy" to "Milky" appearance	"Creamy" to "Milky" appearance
<b>Volume</b>	0.5 to 14ml	1 to 10ml
<b>Mass motility</b>	Score $\geq 3$	Score $\geq 2$
<b>Individual mobility</b>	$\geq 60\%$	$\geq 30\%$
<b>Percentage of total abnormal spzs</b>	$\leq 30\%$	$\leq 40\%$
<b>Percentage of spzs with major abnormalities</b>	$\leq 20\%$	$\leq 30\%$
<b>Percentage of spzs in each major abnormality</b>	$\leq 10\%$	$\leq 20\%$
<b>Concentration</b>	$\geq 0.5 \cdot 10^9$ SPZs /ml	$\geq 0.3 \cdot 10^9$ SPZs / ml

**Table 4:** Ejaculation and semen characteristics in various species of domestic animals and man (**Sorensen, 1979**).

No.	Parameters	Bull	Ram	Boar	Stallion	Man
1	Time lapse for ejaculation	1 Sec	1 Sec	5 – 25 min	30 – 60 Sec	
2	Point of semen deposition	os cervix	os cervix	Cervix	os cervix	os cervix
3	Ejaculate volume (ml)	5-15	.8-1.2	150-200	40-100	2-6
4	Composition of ejaculate	single fraction	single fraction	fractionated sperm-free	fractionated sperm-free	coagulated single

				sperm-rich coagulum	sperm-rich mucus	fraction
5	Concentration: sperm/ml x 10 <sup>6</sup>	800- 1200	2000- 3000	200-300	200-500	50-150
6	Total sperm/ejaculate x 10 <sup>9</sup>	4-18	1.6-3.6	30-60	8-50	.1-.9
7	% motile	75	95	70	70	65
8	% normal	95	95	90	90	60

## **IV. Semen conservation**

### **IV.1. Dilutions**

Semen packaging requires some precautions such as using sterile containers, chemically pure products (they provide the nutrients and protective elements to the sperm for their survival after freezing), distilled water, no thermal shocks, and keeping the semen away from air and light. Knowing that the preliminary steps aimed at separating the spermatid fraction itself from the fraction constituted by the secretions of the adnexal glands, is not essential given that the semen is essentially constituted by testicular secretions (**Hanzen, 2016**). The dilution phase has a dual role:

- Provide protective and preservative substances.
- Fractionate the ejaculate.

#### **IV.1.1. Dilution media:**

The purpose of sperm dilution is to increase the total volume of sperm mass, to provide a favorable environment for sperm survival in vitro, and to achieve from a single ejaculate the insemination of a large number of the females (**Hanzen, 2016**).

##### **IV.1.1.1. The quality of the dilution medium**

A number of conditions must be present for a good dilution medium:

- **Osmotic pressure**
  - Isotonic with the sperm for the species involved;
  - Able to maintain it during the storage period;
  - Contain colloidal substances (egg yolk, lipoproteins, lecithins) that may protect the spermatozoa.
- **Buffer substances**
  - Maintain the pH favorable to spermatozoa (6.2 to 6.8) (**Craplet and Thibier, 1973**).
- **Nutrients**
  - Favor the metabolism, vitality, longevity of spermatozoa.

The good dilution medium must be devoid of the infectious agent because they are detrimental:

- To the survival of the spermatozoa;
- To fertilization;
- To the development of the embryo.

The correct dilution medium provides the functions prior to fertilization:

- Energy-producing metabolic activity.
- Mobility to progress through the female genital tract.
- Protective enzymes on the acrosome to facilitate its entry into the oocyte.
- Presence of proteins on the plasma membrane to ensure their optimal survival in the female reproductive tract and attachment to the pellucida of the oocyte.

#### **IV.1.1.2. The nature of the dilution medium**

There is a great variety of dilutors for the animal species, they differ by the nature, even the concentration of use of their components (**Hanzen, 2016**).

The composition of dilutor in general is:

- Egg yolk with or without milk: lecithins (and caseins) have a protective role against heat shock and buffer against the variation of pH and osmotic pressure.
- Glycerol: Cryo-protector (ability to lower the temperature of the beginning of crystallization of the dilution medium, i.e. to modify the crystallization process by avoiding the formation of large crystals responsible for mechanical alterations.
- ATB or sulfonamides against bacterial contamination.
- Buffer substances can also be used such as sodium citrate or bicarbonate; the diluent "TRIS": trihydroxymethylaminoethane + citric acid.
- Sugars: fructose, rare sugars (mannoses).

The different dilution media based on:

- Phosphated egg yolk: Lardy and Philips medium;
- Citrate: Salisbury medium;
- Sugars: glucose, fructose: Kampschmidt, Chominat, Dimitropoulos, Foote media;
- Glycol and glycerol base: Roy's medium;

- CO<sub>2</sub>: Van Demark's medium or IVT: Illinois Variable Temperature;
- Milk: Laiciphos IMT, the most traditional now, some of which are commercialized.

The sperm dilutor is maintained at 4°C for one hour after mixing to refrigerate the semen; 3 additional hours of equilibrium are necessary to allow exchanges between the dilutor and the spermatozoa.

#### **IV.1.2. The dilution rate**

For the bull, its calculation is based on obtaining insemination doses containing a concentration of spermatozoa zootechnically acceptable, and to have a certain safety margin, we retain a number of 20 million total spermatozoa per dose (straw of 0.25 ml and 2 mm in diameter), which provides an average of 10 to 12 million live and normal spermatozoa (which should allow a "fertility" success rate to be obtained); estimating 40% of losses due to the freezing and thawing processes. This value can be revised downward or upward depending on the quality of the harvested sperm (**Hanzen, 2016**).

### **IV.2. Conservation**

#### **IV.2.1. Short term semen conservation (fresh semen)**

Semen diluted at room temperature (e.g. TRIS dilutor +20% egg yolk), and stored at a temperature close to 5°C (use a medium cooling rate to avoid thermal shock; 0.5°C per minute between 37°C and 22°C and 1°C per minute between 22°C and 5°C; for half an hour). Properly diluted and stored, the semen is used within 2 to 3 days after its production, with an acceptable loss of fertility with time (the maximum time for the conservation of its fertilizing power). This type of conservation of limited duration has largely given way to the conservation of frozen semen.

#### **IV.2.2. Long term conservation of semen (freezing in straws)**

Two techniques have been widely used in the world: "straw" freezing and "pellet" freezing. Today, straw freezing dominates the market, despite its high cost, for reasons of safety and identification. French method known as "French straw". This method owes its name to the 13.3 cm long polyvinyl chloride tubes that are used to package the seed. Freezing requires the use of cryoprotective agents (**Hanzen, 2016**).

Classically, glycerol is used to freeze semen. It is worth noting that given the potential deleterious effects of cryoprotective agents on the sperm, they must be used at optimal dilution. For example, at the 4% concentration, glycerol provides the greatest mass mobility of bull

sperm, but the least amount of damage to their acrosomes occurs after freezing in a 1% solution (**Hanzen, 2010**). Two diluent solutions (10% Laiciphos, 10% egg yolk, distilled water) are required. They differ in that the second contains glycerol at a concentration of 14%. Diluent A is kept at 32°C and diluent B at 4°C (**Hanzen, 2016**).0

This technique is performed in four phases:

- Dilution phase (to have 20 million spzs/stack) ;
- Cooling phase ;
- Conditioning phase ;
- Freezing phase.

#### **IV.2.2.1. Cooling**

Sperm is added to fraction A in two steps. In the first step, an equal amount of sperm and diluent A is mixed. This mixture is added to the rest of the diluent A after 2 to 3 minutes. This pre-diluted medium is then gradually brought to a temperature of 4°C. Once this temperature is reached, diluent B is added to diluent A in 4 steps of 15 minutes (**Hanzen, 2010**).

It is important to give the glycerol time to penetrate the spermatozoa, as this process takes longer when carried out at low temperature. Equilibration therefore takes about two hours and the final dilution of glycerol will be 7% (**Hanzen, 2016**).

#### **IV.2.2.2. Packaging**

Once cooled, the semen is usually packaged in straws, glass or plastic ampoules or pellets. Three types of "polyvinyl chloride" straws are usually used. They all have a length of 133 mm, the large straw has a diameter between 3.8 and 4.2 mm and a volume of 1.2 ml. The medium straw has a diameter between 2.5 and 2.8 mm and a volume of 0.5 ml. The thin straw (most commonly used) has a diameter between 1.7 and 2.2 mm and a useful volume of 0.25 ml (**Hanzen, 2016**). Each straw is identified to the bull and the production lot/day. The straw color corresponds to a national code established for each breed. The straw is individually identified: the name or code number of the bull and the references of the ejaculate (harvest date, order No.).

For filling, about 20 straws are attached to a comb connected to a suction pump. Once filled, a slight agitation of the straws will leave room for the plug and the air bubble necessary to allow dilution of the sperm during freezing. The plugging is done manually or is more often

currently automated. It is done by means of polyvinyl alcohol powder which, once wet, turns into a gel or by crimping. Once the sperm is packaged, the straws are immersed in water at 4°C to allow the action of the glycerol (glycerolization phase) and the other constituents of the diluent. This phase also contributes to the hermetic sealing of the straw (**Hanzen, 2009**).

#### **IV.2.2.3. Freezing**

The straws are then placed on a cooling ramp for freezing. They are first placed in the nitrogen vapors a few centimeters above the liquid nitrogen level of the tank (**Hanzen, 2010**).

The cooling is obtained according to a classical curve, i.e. between 4°C and -10°C a cooling of 4°C per minute and between -10°C and -130°C a cooling of 40°C per minute. Biologically, the critical phase is between -10°C and -50°C. It is between these temperatures indeed that the phenomena of extracellular then intracellular crystallization and the movements of ions which result from it occur. The semen packaged in straws is frozen in liquid nitrogen vapors at -196°C in programmable freezing chambers (tank has a temperature descent program) after 7 to 9 min. The straws are stored in visotubes, hexagonal cylinders of variable color to facilitate their identification, themselves placed in larger cups called "canisters" stored in "tanks" that can contain several hundred liters. The straws are transported in cryogenic containers or nitrogen tanks. A regular check of the nitrogen level in these tanks is necessary (minimum level 5 cm) and the temperature (always <-120) (**Dumont, 1997**).

## V. Application of Artificial Insemination

### V.1. The modalities of detection and induction of heat

#### V.1.1. Heat

Heat is the particular behavior of a female corresponding to the estrus period, during which this female accepts mating with a male and can be fertilized. In order to determine the most favorable period for insemination, it is important to know the signs of heat and especially to recognize the three stages of heat development, which are pre-heat or pro-estrus, heat or estrus and post-heat. In addition, a fourth stage completes the cycle, the period between heats or di-estrus. The pregnancy rate varies according to the technicality of the inseminator and the regularity of his activity (**Anzar et al.** cited by **Amou'ou, 2005**).

#### V.1.2. Heat detection

Good heat detection is essential for AI and allows for predicting calving dates and detecting abnormalities in breeding stock. Missed detection results in a loss of 21 days of the cow's productive life, increasing the time to pregnancy, and indirectly the cost of AI (**Hanzen, 2005**).

##### V.1.2.1. Frequency of observation

The number and timing of heat observations greatly influence the percentage of females detected in estrus. In addition, for the same number of observations per day, the time spent on heat detection also affects this percentage as shown in Table 5.

**Table 5:** The influence of the frequency of observations on heat detection (**Haskouri, 2001**)

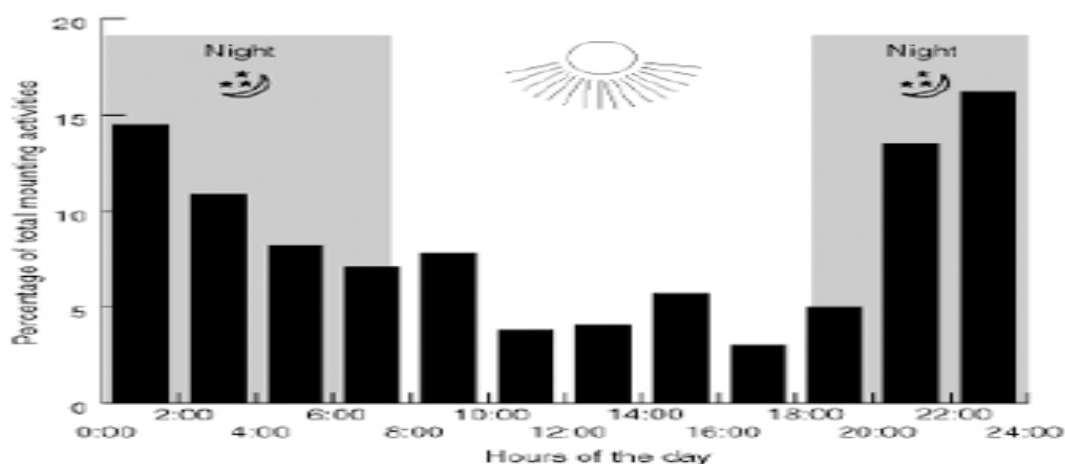
Number of observations per day	Observation period	
	30 minutes	60 minutes
1 time/day.	26 %.	30 %.
2 times/day.	48 %.	57 %.
3 times/day.	57 %.	65 %.

4 times/day.	70 %.	78 %.
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#### V.1.2.2. Observation of estrus

A poor estrus detection can be objectified by a low efficiency (proportion of possible estrus actually detected) and a poor accuracy (proportion of observed estrus correctly diagnosed) of this detection (**Saumande, 2001**). In order to detect overlap, it is necessary to spend time around the animals (periods when females are calm and free to move). For example, only 22% of heats are observed between 6:00 a.m. and 1:00 p.m.; 10% between 1:00 p.m. and 6:00 p.m.; 25% between 6:00 p.m. and midnight; and up to 43% between midnight and 6:00 a.m.. Thus, there is a maximum chance of detecting signs of heat between midnight and the morning; hence, heats should be observed for about 30 minutes at two times each day, very early in the morning between 6:00 a.m. and 7:30 a.m. and in the evening between 6:00 p.m. and 7:30 p.m.; in addition to occasional observations during the course of the day. For cows in short heat (less than seven hours), three or four periods of observation per day are necessary to observe the mounting, which lasts only a few seconds, or the secondary signs, which can also be easily missed.

It is clear, moreover, that a good heat detection is the key to the efficiency of the reproduction and that it is necessary to identify as many successive heats as possible in order to know the real individual signs and to make an evaluation allowing to increase the efficiency of detection (**Tamboura et al., 2004**). Figure 5 shows the percentage of total mounting activity during a full day.



**Figure 5:** The percentage of total mounting activity during 24 hours (**Website 2**).

### **V.1.2.3. Signs of heat recognition**

In addition to physiological changes, heat is manifested by behavioral changes that seem to be good indicators. Some of these signs are presented in Figure 6 and Figure 7 as follows.

#### **V.1.2.3.1. The primary or major signs**

The heat itself is characterized by the acceptance of overlapping which is repeated at regular intervals (about 1/4°), and lasts only a few seconds (**Thibier, 1976**). The immobilization of the female and her acceptance to be mounted by other animals (bull of the herd or a female in the pen) is the surest sign that a cow is in heat; either it is the female in heat that tries to ride her congeners (**Tamboura et al., 2004**).

The duration of heat, thus objectively defined, is on average 18 hours. (**Gilles, 2007**).

#### **V.1.2.3.2. Secondary or minor signs**

Preceding and accompanying the heat itself, irregular and not very precise warning signs have been reported (**Mamboue, 1987; Meyer and Yesso, 1987; Djabakou et al., 1992; Meyer and Yesso, 1992**). These signs are essentially the following:

- Swelling or congestion of the vulva ;
- Discharge of clear, stringy fluid or mucus between the vulvar lips ;
- Female stands more frequently and seeks the presence of other animals ;
- Alternating restlessness and resting in a recumbent position, with increased general activity and aggressive behavior toward congeners ;
- Decreased appetite and milk production, frequent emission of small streams of urine, tail deviation, attraction of other cow ;
- Frequent bellowing, frequent licking of the body, and frequent flaring or sniffing of the vulvar region of other females ;
- Aggression even toward females "higher" in the herd hierarchy, sketching to fight and seeking proximity to males.



i) Sniffing of vulva or urine of other cows



v) Scuffed tail head, dirty flanks and sweating



ii) Resting chin on other cow - both cows may be coming into oestrus



vi) Bunting



iii) Soliciting



vii) Bellowing and restless



iv) Licking - both cows may be in oestrus



viii) Mounting head to head

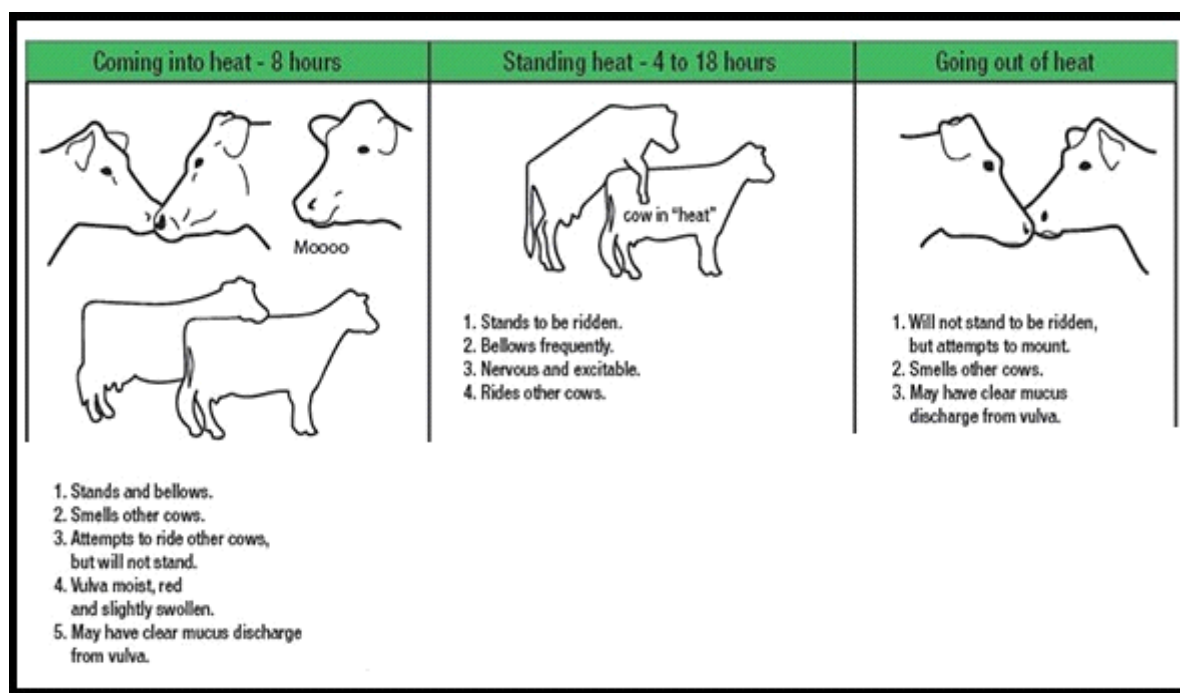


ix) Standing to be mounted

**The positive sign of oestrus is standing to be mounted**

The cow in oestrus (shaded cow) stands to be mounted and does not move away.

**Figure 6:** Some behavioural signs indicative of a cow coming into oestrus or already in oestrus (H.M.S.O., 1984).



**Figure 7:** Behavioral signs related to oestrus during the three stages of heat development (Website 3).

### V.1.3. Methods of heat detection

#### V.1.3.1. Detection of heat by the breeder (visual methods)

To be effective, observation of sexual behavior requires several conditions:

- Identification of the individual in the herd.
- The breeder must have a barn schedule on which to record the dates of events.

Manifestations that may indicate that a cow is in estrus are: Agitation, bellowing, decreased appetite, licking the vulva of other females, attempted overlapping (Parez and Duplan, 1997).

#### V.1.3.2. Tools for heat detection

- **"Kamar" and "Oestruflash" Heatmount detectors**

These instruments leave red ink marks after several seconds of sustained pressure. They perform well on cows in normal heat, but this sometimes leads to a problem of false positives. The cow in heat (or believed to be in heat) must be removed from the herd, which does not help to sexually activate the other cows (Lacrete, 2003).



**Figure 8:** Kamar and Oestrufash heatmount detectors (Pulvery, 2017).

- **Detector animals (with mount detectors)**

The animals used are an androgenic bull or cow or a bull with penile deviation. One animal per 30 cows is required. The detection rate would be between 70 and 90% with one observation period per day (Parez and Duplan, 1997).

- **Markers**

This is a technique that involves marking the top of the tail of the cow to be detected in heat with pencil, chalk or paint. When the cow is mounted, the marker is erased (Lacrete, 2003).

- **Progesterone dosage (milk or serum)**

By comparing the progesterone level on the day of insemination with that on day 22-24 after insemination, one can tell with 95% accuracy if the animal is in heat. The progesterone level is then low. If the cow is not "coming on" heat, she may have been in silent heat. Be wary if the progesterone level is high, as this does not necessarily mean the cow is pregnant; it is only presumed to be pregnant. The fastest test takes about 10 minutes.

- **Detection systems integrated to the milking system**

Many milking equipment companies offer options for heat detection.

- **Pedometer (wristband) or neck motion sensor**

The pedometer measures the activity of the cow and transmits a signal. The effectiveness of the pedometer to detect cows in heat is around 83% and its accuracy (reporting cows really in heat) is around 85%.

- **Measurement of the electrical conductivity of milk**

At each milking, the milking system measures the conductivity of the milk. A variation in this level indicates a probable heat of the animal in question.

- **Milk quantity**

It has long been known that milk production can be affected during heat. Many milking systems, both robotic and conventional, measure the amount of milk produced at each milking, so variations can be easily observed. In general, the main factors that are responsible for a lack of efficiency in detecting heat are:

- Inadequate and poorly distributed time allocated daily to heat observation ;
- Most mounting activity occurs at night, 70% between 6 :00 p.m. and 6 :00 a.m. ;
- Heats are often short. According to some studies, 65% of cows let themselves be mounted for 16 hours or less, 25% for less than 7 hours.
- The fewer cows in heat, the lower the level of activity and heat expression in the herd as a whole. This becomes a problem especially in smaller herds.
- Mating lasts 10 seconds or less, and breeders too often combine observation periods with other activities.
- Heat externalization is often reduced by foot and limb problems, slippery floors, summer heat, winter cold, and other environmental factors such as lack of exercise that promote slower basal or intrinsic metabolism of the genitals.

In order to maximize the effectiveness of heat detection, a heat detection program must be developed that limits the negative effects caused by " humans" and " animals" (**Lacrete, 2003**).

#### **V.1.4. Timing of insemination**

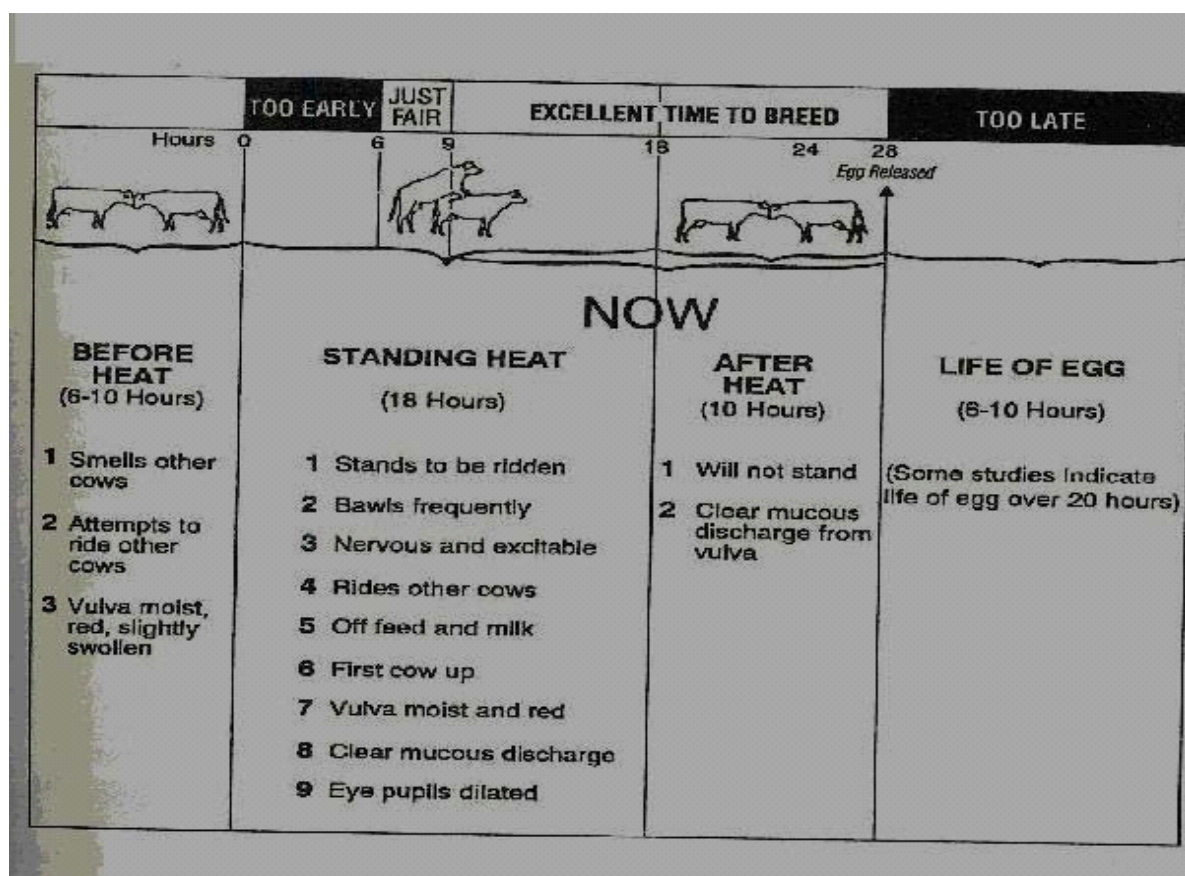
The control of sexual cycles is a set of techniques aiming at grouping heats (to trigger estrus at the same period in a number of females) in order to plan, control and program all the stages of reproduction at convenient times for the breeder (**Derivaux and Ectors, 1989**).

In the cow, maximum fertility has been achieved if inseminated from mid estrus to the end of estrus (**Gomes, 1977**). Fertilization of the ovum has been reported to occur in the oviduct at the junction of the isthmus and ampulla (**Daris, 1998**). The life span of the ovum is around 12 – 18 hours and its viability decreases with time. About 8 hours after service sufficient spermatozoa have reached the isthmus of the oviduct. For fertilization to take place,

capacitation of the spermatozoa is required. Capacitated sperm cells show a hyper motility and have undergone the acrosome reaction. The life span of spermatozoa is limited. If insemination takes place too early, the sperm cells will die before fertilization of the ovum can occur. Conversely, when insemination is over delayed, the ovum has lost its capacity to be fertilized (Daris, 1998). Table 6 and Figure 7 display timing of insemination in relation to estrus.

**Table 6:** Optimum time of insemination in relation to estrus (Daris, 1998).

Fertility	Poor	Fair	Good/Excellent				Fair	Poor
Hours	0	5	10	15	20	25	30	
Standing estrus								



**Figure 9:** Timing guide for inseminating the average cow (Herman et al., 1994).

### **V.1.5. Control of estrus**

#### **V.1.5.1. Reason for control of estrus**

The estrus cycle can be regulated pharmacologically to induce or control the time of estrus and ovulation. The main reasons for estrus control are: induction of estrus in lactating dairy cows that are not observed in estrus by 45 days post-partum, synchronization of groups of heifers for insemination with semen of easy calving bulls, reduction of the time necessary for estrus detection, to facilitate the use of AI under extensive conditions, synchronization of donor and recipient cattle for embryo transfer and induction of ovarian activity in beef cows with lactation anoestrus (**Morrow et al., 1985; Daris, 1998; Bekana et al., 2005**).

#### **V.1.5.2. Methods of controlling estrus**

In cattle with active ovaries, the estrous cycle can be manipulated by administration of prostaglandin to induce early regression of the corpus luteum (**Davis et al., 1987; Daris, 1998; Bekana et al., 2005**) and by the use of progestagens that act as an artificial corpus luteum (**Daris, 1998**). The detection of estrus by regular surveillance or by the use of vasectomized teaser males may not be warranted or possible and, in any event, neither of these approaches in it enables insemination to be performed at the optimum time with regard to fertility (**Hunter, 1980**). Detection of estrus is getting more and more difficult according to the extension of the numbers of cows reared, the improvement of milking cows with a high milk production and the changes of the circumstances of feeding. Other factors include management of cows, implying the dependence on the techniques for estrus and ovulation synchronization in the reproductive management being very high (**Sugawara, 2004**). More logical and satisfactory way of estrus detection by far would be a situation in which the estrous cycles of animals to be bred could be controlled by a pharmacological or pseudo-physiological treatment such that the time of onset of estrus could be predicted in the great majority of animals receiving the treatment (**Hunter, 1980**). Furthermore, the author showed that the precise time of ovulation has been predetermined, and thus the animals can be inseminated at a fixed time without reference to the state of behavioral estrus, thereby avoiding the penalties associated with incorrectly timed insemination and the aging of gametes.

In essence, therefore, a system of estrous cycle control would attempt to detect the desired timetable of breeding rather than permitting females to impose their own reproductive rhythms on the farming system. Synchronization of estrus has been known to have many advantages including the reduction of time needed for detection of estrus (**Hunter, 1980; Hailu, 2007**).

Synchronization of estrus and ovulation can be conducted by the use of either, PGF2 $\alpha$  or GnRH or the combination of the two where the former is injected 7 days before the latter to induce a new follicular wave (**Sugawara, 2004; Hailu, 2007**).

## **V.2. Techniques of artificial insemination**

### **V.2.1. Precautions**

In practicing AI, the following precautions should be taken:

- The equipment must be in good condition so as not to cause injury to the female's reproductive system ;
- The equipment must be sterile ;
- The procedure must be done gently because the uterus is fragile.

The inseminator is an itinerant person who has an on-board laboratory in the back of his utility vehicle. Very organized, he checks every morning, before leaving on his rounds, the operating condition and availability of all his equipment (**Niang, 2012**).

### **V.2.2. Site of semen deposition**

The genital tract of the cow has four distinct segments as shown in Figure 10.

#### **V.2.2.1. Oviducts or uterine tubes or fallopian tubes**

They are two sinuous ducts intended to convey the ovum towards the uterus 20 to 30 cm long, located close to the ovaries and terminated by a pavilion which has the shape of a funnel where the matured ovules fall (**Craplet et Thibier, 1973**). The duct itself consists of 3 parts:

- Ampulla, where fertilization takes place, the meeting or fusion of the egg and sperm ;
- Isthmus, of reduced caliber ;
- Utero-tubal junction area where the oviduct and the corresponding uterine horn join.

Thus, the oviducts provide a triple role:

- Oviductal capture at the time of ovulation.
- Transport of the egg or ovum to the uterus.
- Modification of sperm (capacitation) to be suitable for fertilization.

#### **V.2.2.2. Uterus or womb**

The uterus is a pouch that extends from the sublumbar region to the entrance of the pelvic cavity (**Craplet and Thibier, 1973**), and divided into three parts (**Derivaux et al., 1980**):

- **Horns**

The horns are long and curved downwards, they are tapered at their anterior end and welded over a hundred extended at their posterior part where they are joined, in the angle of bifurcation by two superimposed serous muscular folds between which it is easy to introduce the finger

- **Body of uterus or uterine cavity**

It is short, the mucosa presents a series of rounded, convex outgrowths, 70-150 in number, they are the cotyledons.

- **Cervix**

The cervix is long (10cm), narrow, thick-walled and hard and the mucosa, radially pleated forms two, three and even four successively arranged and even concentric blossoms, cut into unequal lobes with an almost cartilaginous consistency the irregularity of the blossoms makes the lumen of the duct realize more of a broken line than a straight one, which makes catheterization of the cervix difficult in the heifer.

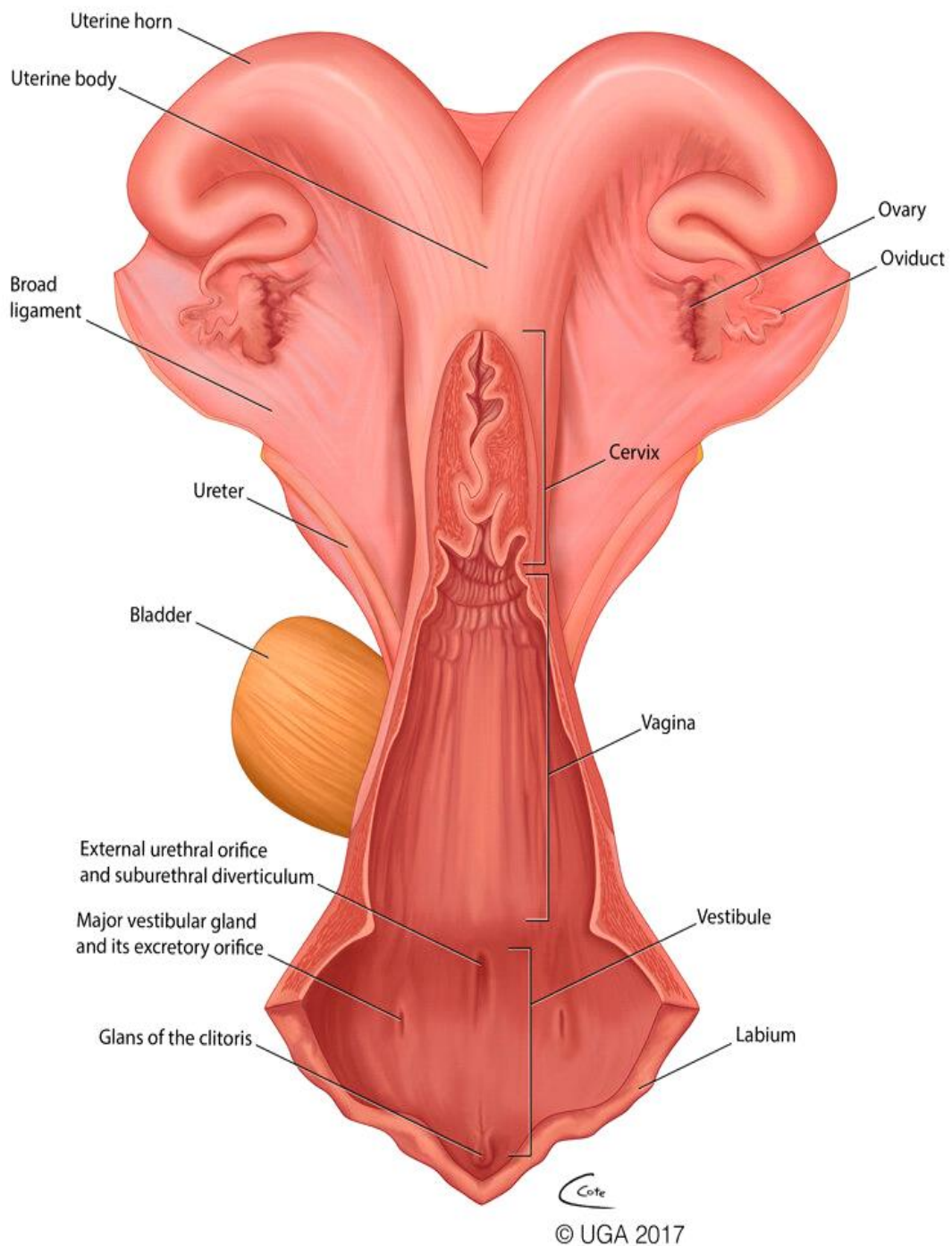
#### **V.2.2.3. Vagina**

Uneven and median duct of a 30 cm length and whose internal surface pleated. The vagina is entirely lodged in the pelvic cavity, its anterior end is inserted around the cervix, it is the organ of mating, the vagina also allows the passage of the fetus at parturition (**Craplet and Thibier, 1973**).

#### **V.2.2.4. Vulva and urogenital sinus**

The vulval cavity constitutes the vestibule common to the genital and urinary tracts, it is delimited from the vaginal cavity at the level of the floor of the vagina, the urinary meatus is located at 10 or 20 cm from the lower commissure of the vulva (**Ghoribi, 2004**).

Dorsal view of the different components of the cow reproductive tract



**Figure 10:** Dorsal view of the different components of the cows reproductive tract (**Website4**).

### **V.2.3. Supplies and Equipments**

Supplies and equipment needed or useful for AI programs include these (**Nurlign, 2020**):

- Bull semen ;
- Semen storage tank ;
- Liquid nitrogen ;
- Insemination gun
- Electronic thawing device or insulated water bath ;
- Plastic obstetrical sleeves ;
- Thermometer ;
- Obstetrical lubricant ;
- Timer ;
- Paper towels ;
- Straw-cutting device ;
- Record-keeping supplies ;
- Future trends in AI.

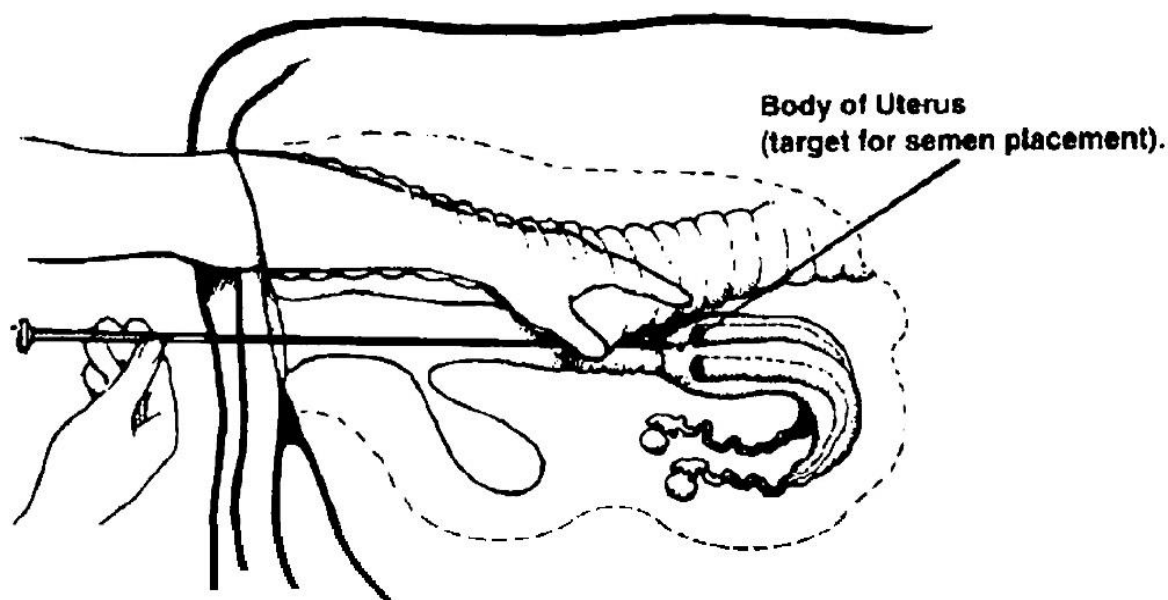
### **V.2.4. Artificial insemination procedures**

The technique of inseminating a cow is a skill requiring adequate knowledge, experience and patience. Improper AI techniques can negate all other efforts to obtain conception, Semen must be deposited within the tract of the cow at the best location and at the best time to get acceptable conception rates. Early methods of AI involved deposition of the semen in the vagina, as would occur in natural mating. Those methods are not satisfactory. Fertility is low and greater numbers of sperm are required. Another method which gained popularity was the "speculum" method. This method is easily learned, but proper cleaning and sterilizing of the equipment is necessary, making it more impractical to inseminate than with the rectovaginal technique which is the most widely used AI method today (**Baracaldo, 2007**).

In the rectovaginal technique a sterile, disposable catheter containing the thawed semen is inserted into the vagina and then guided into the cervix by means of a gloved hand in the rectum. The inseminating catheter is passed through the spiral folds of the cow's cervix into the uterus as illustrated in Figure 11. Part of the semen is deposited just inside the uterus and the remainder in the cervix as the catheter is withdrawn. Expulsion of the semen should be accomplished slowly and deliberately to avoid excessive sperm losses in the catheter. The body of the uterus is short; therefore, care should be taken not to penetrate deeply which

might cause physical injury. In animals previously inseminated, the catheter should not be forced through the cervix since pregnancy is a possibility.

Since research data show little variation in conception rates when semen is placed in the cervix, uterine body or uterine horns, some people recommend incomplete penetration of the cervical canal and deposition of semen in the cervix. The rectovaginal technique is more difficult to learn and practice is essential for acceptable proficiency but the advantages make this method of insemination more desirable than other known methods. With practice, the skillful technician soon learns to thread the cervix over the catheter with ease. If disposable catheters are used and proper sanitation measures are followed, there is little chance of infection being carried from one cow to another (Road, 2007).



**Figure 11:** Proper placement of insemination gun to deposit semen in the body of the uterus (Website 5).

#### **V.2.5. Factors affecting success of artificial insemination**

The site of semen deposition has been an important factor in the success of AI in cattle. In addition, the deposition of semen in the uterine body resulted in a 10% higher non-return rate than did cervical deposition (Macpherson, 1968). An increase in the conception rate has been reported when semen was deposited in the uterine horns rather than the uterine body (Senger et al., 1988). In contrast, no difference was found in the fertilization rate, conception rate or nonreturn rate, respectively, between uterine body and uterine horn inseminations (Hawk and Tanabe, 1986; Williams et al., 1988; and McKenna et al., 1990). In super

ovulated cows, **Hawk et al. (1988)** used a modified insemination device requiring two technicians to deposit semen near the uterotubal junction and as compared to uterine body deposition; he found no effect upon the fertilization rate.

The major factors that determine AI efficiency are heat detection skills, fertility level of the herd, semen quality, and efficiency of inseminators (**Barrett, 1974**). Similarly, a successful insemination requires the acquisition of quality semen from a bull, the detection of estrus in the female, and the ability to properly place the semen in the reproductive tract of the female (**Damron, 2000**). Detection of estrus has been known to be one of the most difficult tasks for successful AI activities, which in turn is affected by diseases of testis, epididymis, and accessory glands in the male (**Sori, 2004**), and diseases of the female reproductive tract (**Roberts, 1985**). The success of AI depends upon various factors such as the efficiency, capacity and commitment of AI centers in procedurally and ethically producing, processing, handling and distributing semen; the commitments and efficiencies of AITs; presence of appropriate breeding policy along with proper control of indiscriminate crossbreeding; proper heat detections by farmers and other factors (**GebreMedhin, 2005**).

## CONCLUSION

AI is a great tool to improve genetic potential and consequently to increase animal production. It allows especially by the multiplication of an ejaculate thanks to the techniques of dilution and conservation, to increase in the framework of genetic improvement, the progeny of males recognized as improvers. However, its success requires the breeder and the inseminator to apply technical and herd management expertise. This technology can then be used for the betterment of the livestock.

The study that we have carried out consists in showing the technique of artificial insemination by the method of semen collection, namely the tools used and the way of recovering the ejaculate (electro ejaculation and artificial vagina), then it is necessary to evaluate if the collected semen will be of good quality to be preserved at an adequate temperature and in appropriate media, this analysis includes a macroscopic and microscopic examination with the help of the CASA systems that allow the realization of seminograms.

AI requires not only a good quality semen but also the preparation of the reproductive females which starts with the detection of heat of the cow by the inseminator using different methods, it is the first step in artificial insemination and represents a fundamental and essential element of yield of the herd. It also needs a high level of knowledge of this technique on the one hand and a great mastery of the technique itself on the other hand. This allows AI to be of great use and offer genetic, sanitary and economic interests.

In Algeria, artificial insemination was introduced during the colonial period. Although very old, its use in our farms is very limited despite the efforts and the mastery of the technology by the CNIAAG, it remains neglected because of the lack of instruction of the breeders.

## **RECOMMENDATIONS**

Based on the above conclusions the following are recommended:

- Selection of bulls for AI should strictly follow the standard guidelines and procedures set for the purpose and also the national livestock development policies of the country.
- One national body responsible to coordinate and monitor AI service, herd recording and also livestock breeding programs needs to be established and be very well organized in human and material resources.
- The AI service provision should be restructured in such a way that it responds well to the breed improvement programs of the country.
- To increase the chances of success in the first AI, it is strongly recommended:
  - To note all heat,
  - To inseminate on a reference heat,
  - To inseminate between 6 and 24 hours after the beginning of heat,
  - It is important that the inseminators are well trained and that the farms are well managed.

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(05/31/2021 at 11pm)
5. **Website 5:** [www.extension.okstate.edu](http://www.extension.okstate.edu)  
(06/01/2021 at 9pm)

## Abstract

Artificial insemination (AI) is the most widely used reproductive biotechnology in the world. In order to increase the reproductive capacity of the herd and to ensure a good management, the AI is done on the one hand after the preparation of the semen which is shown in our work. The collection of semen on selected males must pass first by the good recollection of the ejaculate, then this last one undergoes macroscopic examinations and another microscopic examination with the help of the CASA systems which allows the realization of seminograms. The collected semen is diluted and then preserved in the short term or in the long term. The preservation is done with the help of straws which are frozen in nitrogen at a temperature of  $-196^{\circ}\text{C}$  to be thawed during their use. On the other hand, the success of the AI requires the preparation of the producers in heat induction by the different protocols of synchronization and the good detection of the heat in order to determine the most favorable period for the insemination. AI offers many genetic, sanitary, economic and practical interests but it also has disadvantages. In Algeria, although AI has been practiced since the colonial era, its improvement remains very limited. Therefore, it is necessary to rebuild the inseminator through training courses that aim at good practice and knowledge of the technique before its realization.

Key words: artificial insemination, cattle, semen, preparation of reproducers, improvement.

## Résumé

L'insémination artificielle est la biotechnologie de reproduction la plus utilisée dans le monde. Afin d'augmenter les capacités de reproduction du cheptel et d'assurer une bonne gestion, l'IA se fait d'une part après la préparation de la semence ce qui est montré dans notre travail. Le prélèvement de la semence sur des males sélectionnés doit passer d'abord par la bonne récolte de l'éjaculat, ensuite cette dernière subit des examens macroscopique et un autre examen microscopique à l'aide des systèmes CASA qui permet la réalisation de spermogrammes. La semence prélevée est diluée puis conservée à court terme ou à long terme cette dernière est réalisée à l'aide des paillettes qui seront congelée dans l'azote à une température de  $-196^{\circ}\text{C}$  pour être décongelé lors de leur utilisation. D'autre part la réussite de l'IA exige la préparation des productrices en induction des chaleurs par les différents protocoles de synchronisation et la bonne détection des chaleurs afin de déterminer la période la plus propice à l'insémination. L'IA offre beaucoup d'intérêt d'ordre génétique, sanitaire, économique et pratique mais elle a aussi des inconvénients. En Algérie bien que l'IA est pratiquée depuis l'époque coloniale, son amélioration reste très limitée donc il faut bien reconstruire l'inséminateur par des formations qui ont pour but la bonne pratique et la connaissance de la technique avant sa réalisation.

Mot clés : insémination artificielle, bovins, la semence, préparation des reproductrices, amélioration.

## المخلص

التلقيح الاصطناعي هو التكنولوجيا الحيوية الإنجابية الأكثر استخداما في العالم. من أجل زيادة القدرة الإنجابية للقطيع وضمان إدارة جيدة، يتم التلقيح الاصطناعي من جهة بعد إعداد السائل المنوي الذي يظهر في عملنا. يجب أن يمر جمع السائل المنوي على الذكور المختارين أولا بالتذكر الجيد للقطيع، ثم يخضع هذا الأخير لفحوصات مجهريّة وفحص مجهري آخر بمساعدة أنظمة تحليل السائل المنوي بمساعدة الكمبيوتر. يتم تخفيف السائل المنوي الذي تم جمعه ثم الحفاظ عليه على المدى القصير أو على المدى الطويل. يتم الحفاظ بمساعدة القش الذي يتم تجميده في النيتروجين عند درجة حرارة  $-196^{\circ}\text{C}$  درجة مئوية ليتم إذابته أثناء استخدامها. من ناحية أخرى، يتطلب نجاح التلقيح الاصطناعي إعداد المنتجين في الحث الحراري بواسطة بروتوكولات التزامن المختلفة والكشف الجيد للحرارة من أجل تحديد الفترة الأكثر ملاءمة للتلقيح. يوفر التلقيح الاصطناعي العديد من المصالح الوراثية والصحية والاقتصادية والعملية ولكن له أيضا عيوب. في الجزائر، على الرغم من ممارسة التلقيح الاصطناعي منذ الحقبة الاستعمارية، إلا أن تحسينه لا يزال محدودا للغاية. لذلك، من الضروري إعادة بناء الملقح من خلال الدورات التدريبية التي تهدف إلى الممارسة الجيدة والمعرفة بهذه التقنية قبل تحقيقها.

الكلمات المفتاحية: التلقيح الاصطناعي، الماشية، البذور، تربية إعداد وتربية.