Manual for the Artificial Insemination (AI) of Local breeds Cattle in Ethiopia

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Summary: Biological cells like bovine sperm are frozen by exposing them to cryoprotectants then slow cooling the samples at specific rates to allow the exodus of intracellular water molecules prior to being plunged into liquid nitrogen for long term storage. Bovine embryos are typically exposed to either glycerol or ethylene glycol for several minutes at room temperature and then ramped to a temperature of about – 35° Celsius (C) before being plunged into liquid nitrogen (– 196° C). The success of any artificial insemination program is dependent on numerous factors. Success starts with a bull that is healthy, disease-free, and produces ample quantities of high quality semen. However, equally important is the fertility potential of the female, competency of the inseminator and quality of the environment and also the prolonged postpartum interval between calving and conception and low conception rate are the major constraints limiting the success of AI for cattle development. Two of the major goals of artificial insemination of domesticated animals are to achieve continuous genetic improvement and to prevent or eliminate venereal disease. In comparison with natural service, fewer males are needed to artificially inseminate the same number of females and to produce the same number of offspring. However, there are risks associated with artificial insemination, which has the potential to disseminate genetic defects and also to spread infectious disease nationally and internationally.

[Abebe M.A. Manual for the Artificial Insemination (AI) of Local breeds Cattle in Ethiopia. *Life Sci J* 2025;22(2):29-40]. ISSN 1097-8135 (print); ISSN 2372-613X (online). http://www.lifesciencesite.com. 03. doi:10.7537/marslsj220225.03

Key words: Artificial Insemination; breeds; cattle; Ethiopia

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1. Introduction

Artificial insemination is an assisted conception method that can be used to alleviate infertility in selected couples. The rationale behind the use of artificial insemination is to increase the gamete density near the site of fertilization. The effectiveness of artificial insemination has been clearly established in specific subsets of infertile patients such as those with idiopathic infertility, infertility related to a cervical factor, or mild male factor infertility (Alborzi *et al*, 2003)

Artificial insemination (AI) has contributed enormously to the genetic improvement of beef and dairy cattle in the last 50 years. Frozen semen in 0.5 ml or 0.25 ml straws has become the universally accepted unit of storage and transfer of bovine genetics to cattle producers. This manual elaborates the steps performed for freezing bull semen in straws, beginning with semen collection through storage in liquid nitrogen tanks (Bacha, 2007).

It is theorized by low temperature biologists that frozen semen and embryos stored properly in liquid nitrogen at – 196° C, or liquid nitrogen vapor below – 130° C, will stay viable for at least one thousand years. Cellular metabolism at those temperatures essentially ceases, therefore the cells don't age.

Historically, Artificial insemination has been used in clinical medicine for more than 200 years for the treatment of infertile couples. In 1785 John Hunter, a Scottish surgeon from London, advised a man with hypospadias to collect his semen and have his wife inject it into her vagina. This was the first documented case of successful artificial insemination in a human. Today, artificial insemination is frequently used in the treatment of couples with various causes of infertility, including ovulatory dysfunction, cervical factor infertility, and unexplained infertility as well as those with infertility caused by endometriosis female and immunologic factors. Artificial insemination with donor semen has become a well-accepted method of conception (Brad, 2012).

Agriculture (mainly crop and livestock production) is the mainstay of the Ethiopian economy employing approximately 85% of the total population. Livestock production accounts for approximately 30% of the total agricultural GDP and 16% of national foreign currency earnings. The total cattle population

for the rural sedentary areas of Ethiopia is estimated at 43.12 million, of which 55.41% are females. Out of the total female cattle population, only 151,344 (0.35%) and 19,263 (0.04%) heads are hybrid and exotic breeds, respectively (Bungum *et al*, 2004).

Artificial insemination (AI) has been defined as a process by which sperm is collected from the male, processed, stored, and artificially introduced into the female reproductive tract for the purpose of conception. 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 (Cantineau *et al*, 2004).

In Ethiopia, AI was introduced in 1938 in Asmara (the current capital city of Eritrea), the then part of Ethiopia, which was interrupted due to the 2nd World War and restarted in 1952. It was again discontinued due to unaffordable expenses of importing semen, liquid nitrogen and other related inputs requirement. In 1967, an independent service was started in the then Arsi Region, Chilalo Awraja under the Swedish International Development Agency (SIDA). The technology of AI for cattle has been introduced at the farm level in the country over 35 years ago as a tool for genetic improvement. The present National Artificial Insemination Center (NAIC) was established in 1984 to coordinate the overall AI operation at national level. The efficiency of the service in the country, however, has remained at a very low level due to infrastructure, managerial and financial constraints, as well as poor heat detection, improper timing of insemination and embryonic death (Duran *et al*, 2004).

2. Importance of AI

The main objective AI is to improve production per unit of land or animal, using the available resources in a sustainable manner. Ideally animal recording schemes (milk recording for dairy animals and performance recording for beef animals) should be in place. This will allow for the selection of sires used in artificial insemination (AI) based on Estimated Breeding Values (EBVs) of the sire's parents or, in the case of beef breeds, his own EBVs for different traits. The availability of EBVs in a population depends on an internationally approved pedigree registering and recording system. An overview of sire proofs and cow indexes (Estimated Transmitting Ability or ETA), milk recording, linear classification and aspects of genetic improvement are given (Erel *et al*, 2000).

It should be stressed that fulfillment of breeding goals requires rigorous selection and culling. Contract mating using semen from the best bulls inseminated into cows that are ranked high in the population on their production provide the source of bull calves. A selection panel of people knowledgeable about cattle and the industry advises on contract matings and inspects the calves, selecting individuals on breed type, health and conformation for the progeny test team. One thousand doses of each young bull's semen are distributed randomly to farms participating in the progeny test scheme (Goverde *et al*, 2000).

2.1. Criteria for selection of bulls

The selected bull must be best in milk recording, beef recording, Reproductive efficiency and Likeability.

2.1.1. Milk recording

Individual animals should be clearly identified by ear tag or other equally effective technique (collars, freeze brands, electronic subcutaneous devices). Basic milk recording entails regular milk weights and analysis for percentage butter fat and percentage protein. Approved meters or scales and an analyzing laboratory using accurate and validated techniques should be available. A minimum number of recordings per lactation is required, as stipulated by the International Committee for Animal Recording (ICAR), which regulates and approves animal recording schemes and will provide assistance (see ICAR, 2001, International Agreement on Recording Practices, website: http://www.icar.org).

2.1.2. Beef recording

Identification of individual cattle is necessary. Approved weighing scales should be used. Calving information to assess calving interval and ease of calving should form part of such a system. Weaning weights and daily weight gains are important. Carcass evaluation and fertility parameters such as scrotal circumference in males and age at first heat or first calving in females are valuable additions.

Breed Societies should encourage members to measure performance and record it in the breed

association data file. "Breed plan International" is based in Australia. The BLUP system is used making maximum use of the pedigree information available. EBV's are calculated for birth weight, 200 day weight direct, 200 day weight maternal, 400 day weight and 600 day weight. The 200 day weight maternal is an estimate of the milking ability of the dam based on weaning weight of the calf. EBV's for scrotal size, gestation length and days to calving are included. "Days to calving" is the time between the date of entry of the bull into the herd and the calving date for each cow. Carcass characteristics are another important component. Further information is available in Hammond et al. (1992) and at the website http://breedplan.une.edu.au/bplan.html.

2.1.3. Reproductive efficiency

Bulls selected on the basis of their genetic merit should be subjected to a general clinical examination, an examination of the reproductive organs, semen examination and serving ability assessment. This will be done routinely at the time of collection of progeny test doses of semen. The fertility performance of each bull should be recorded from conception rates based on pregnancy diagnosis. Non-return rates (NRR) may be used in situations where pregnancy diagnosis is not available to farmers. NRR are only reliable as an index of bull fertility in artificial insemination where heat detection efficiency is very high. Selection of bulls with high efficiency of reproductive functions will improve the running of the AI center and ensure improvement of male reproductive efficiency in the population (Hammadeh *et al*., 2001).

2.1.4. Likeability

Where measurement of milk production in individual cows is difficult, farmers could be asked to rank cows on likeability according to the following scheme (McClintock, Genetics Australia, personal communication): when it is 5 = Excellent animal; liked in all respects, 4 = Very good animal; likable with respect to most characteristics, 3 = Average animal, 2 = Below average but acceptable and 1 = Not a good animal; not liked at all (Montanaro *et al*, 2001).

This would be a useful guide to the bulls whose daughters were best fulfilling the farmers' needs. For dairy breeds likeability would include milk production, temperament and resistance to disease. In beef breeds it would include calving ease, weight gains and fertility. Oral information from farmers' knowledge of their animals should be used for evaluating the bulls that were used to produce these cows.

3. Constraints of AI

The conception rate is influenced by cattle rearing systems (intensive vs. extensive), purpose of rearing cows (dairy vs. dairy + draught); body condition scoring (BCS) and milk production is significantly related. The degrees of vulvar swelling, nature of genital discharge, tonicity of uterus, and interval between oestrus and AI had significant effects on the conception rate.

The prolonged postpartum interval between calving and conception and low conception rate are the major constraints limiting the success of AI for cattle development. The nutritional condition of the cow at calving and thereafter, weaning age of calves, frequency of suckling, cattle rearing system, accuracy of heat detection, interval between oestrus and AI, the oestrus signs and semen quality are the important determinants of the interval to conception and conception rate (Ohl *et al*, 2001).

4. Semen collection

Bulls are ready for semen collection and freezing procedures after they have passed a complete physical examination, the required federal health tests and a breeding soundness evaluation. There are different methods that can be used to collect semen from bulls.

4.1. Collection area and facilities

The semen collection area should be as close as possible to the semen evaluation laboratory (not more than 30 m). For teaser bull restraint a stanchion made from strong metal bars or smooth treated wooden poles and timber is recommended (Fig 1). The floor of the collection site should not be slippery. It can be made of rough concrete or a dug-out filled with sand and sprinkled with water to avoid dust. Rubber mats can also be used (Osasuna *et al*, 2004).

Facilities for the restraint of bulls awaiting their turn for semen collection should be near enough to enable them to see clearly the mounting bull and serving area (Fig. 2). The collection area should be ringed with strong metal bars or timber for the safety of people and the bulls themselves. The construction should be high enough to protect the full height of an average person (1.75 m). Spaces between rails should be small enough to prevent a bull getting his head through. Escape spaces in the surrounding fences should be placed at regular intervals. The collection area should be sheltered and must have adequate ventilation and light.

Fig. Figure 1: Strong timber construction of stanchion for teaser bull restraint in South Africa. Note the non-slip floor.

Figure 2: Holding facilities for bulls in South Africa.

4.2. Preparation of bulls

Unless the semen donor bulls are housed under clean dry conditions and are clean when they arrive at the collection area, they should be washed and cleaned. The washing area should not be more than 20 m from the serving area and should be made of rough concrete with a slanting floor to facilitate drainage of water, dung and urine. Adequate clean water with reasonable pressure should be provided through a hose pipe at this area. Prior to cleaning, the preputial hair should be cut short leaving a tuft of 2 cm length all rounds. Ordinary washing soap and a mild brush

should be used to clean the bulls. During cleaning emphasis should be put on the lower abdomen and the preputial area. Disinfectants should not be used. Clean dry paper towels should be used after washing to remove excess water.

If the teaser bull or steer is dirty he should be cleaned at the back with water and soap and dried thoroughly. There is little risk of contamination of the penis or the semen if the teaser is clean and collection technique is good, allowing no or little contact of the penis with the teaser. Equipment and materials in the laboratory for the whole production process should be thoroughly cleaned, rinsed with distilled water and sterilized in an autoclave or hot air oven.

4.3. Artificial vaginas

A 45 cm long outer rubber-barrel with rough inner rubber liner that is not spermiotoxic is recommended. The inner liner should periodically be checked for possible leakages. The rubber cones should be also be non-spermiotoxic and a correctly labeled collection tube should be attached. A jacket for the cone should be provided to prevent breakage and avoid direct exposure to sunlight. Rubber bands for holding on the cones and two ends of the reflected inner lining onto the outer barrel should be strong. Sterile and non-spermiotoxic lubricant (eg. KY jelly, which has been tested and found to be non-toxic in diluted form) should be applied sparingly and just before collection (Fig. 3). The lubricant can be replaced by a small amount of diluent to moisten the entrance to the artificial vagina.

Figure 3: Preparation of the artificial vagina; lubricating with a glass rod using KY jelly.

Water for the outer jacket filling should be warmed to 60ºC. Enough of this should be poured into the inner chamber to provide the required pressure. This may range from 500-750 ml. Inner temperature after lubrication should range between 42-48ºC. Assembled AVs should be kept in incubators at 55-60ºC. If there is a delay between preparation of the AV and collection, the temperature should be checked. Just before collection excess water is poured off from the AV and enough air blown in to provide adequate internal pressure.

4.4. Electro ejaculators

Electro ejaculators should only be used when absolutely necessary. Injured or sick bulls should not be subjected to the technique. Good training and good handling procedures allow most bulls to be collected with the artificial vagina. Some Bos indicus bulls with low libido may not always respond to standard procedures and will require electro ejaculation. The prepuce should be washed and dried. The rectum should be emptied of faces and the probe inserted to lie over the seminal vesicles and ampullae. Stimuli

should be applied with great care to achieve a very slow and gradual increase in intensity.

4.5. The collector

A collector should be selected on the basis of his/her ability, enthusiasm and experience to work with livestock. Protective gear should include gum boots with steel or wooden-toed caps, apron, head cap and thin half-length plastic hand gloves.

4.6. Steps of semen collection

Bulls should be lead to the teaser in a gentle friendly manner by the handler paying attention to the temperament of the particular bull, preferably using a halter. The bull should be allowed to watch other bulls mounting before collection. He is lead around behind the teaser and may be allowed to mount other bulls. Two false mounts are given (Fig. 4). These measures constitute good sexual preparation which increases sexual excitement and the amount of semen collected. The bull is then allowed to mount for the first collection (Richthoff *et al*, 2003).

Figure 4: Sexual preparation assists in improving the bull's serving behavior at collection. It helps to obtain more spermatozoa in the collected ejaculate. The bull at the top is a slow server and is being given a false mount in a different environment to the collecting area.

At this time the collector should gently grasp the prepuce behind its opening and direct the fully erect penis into the lubricated end of the AV (Fig. 5). The handler may rest his shoulder against the bull's flank and move with the movement of the bull as he thrusts. The AV should be held so the bull withdraws as he dismounts.

Fig. 5: Collection of semen with the artificial vagina. The left hand touches only the preputial skin, not the penis itself.

The ejaculate should be taken immediately to the evaluation room. Handling of semen should be always done with great care to avoid cold shock, contamination, excessive agitation and direct sunlight.

4.7. Evaluation of semen quality

The semen should be transferred to a water bath maintained at 33-36ºC. Visual evaluation for volume, colour, consistency/density, odour and observation for presence of foreign material should be made and recorded.

Microscopic evaluation is done using a phase contrast microscope for mass activity and individual motility. Determination of concentration is done with a hemocytometer or a calibrated photometer. At this point, if required, smears can be made for morphological studies and live/dead count. Nigrosin-eosin stain is recommended. Buffered nigrosin eosin solution is mixed with a drop of semen and smeared on a glass slide for morphological examination. It should be examined under oil immersion. The smear is made by drawing the drop along to avoid mechanical damage to the spermatozoa.

For percentage alive at least two counts of one hundred spermatozoa should be made. If there is not good agreement between the two another two hundred are counted. Live cells have no pink eosin stain in them and appear uniformly white. If the spermatozoa are dead, the membrane is damaged and it is permeable to eosin. The dead spermatozoa have pink stain within them. Sometimes it is concentrated behind the acrosome, sometimes it is uniformly spread throughout the cell. Sperm concentration of the sample is determined using a hemocytometer (Saleh and Agarwal, 2002).

Morphological examination can be done on the nigrosin eosin stained smear under oil immersion. Two counts of one hundred heads should be made or more if there is not good agreement. The most common abnormalities of the head are narrow, narrow at the base, pear shaped, abaxial and “undeveloped”. This last includes severely deformed spermatozoa with the tail coiled within or around the head and microcephalic heads. A count of structural defects of the midpiece may be made but is not done routinely since these are uncommon in bulls. Similarly acrosome abnormalities can be counted but they are not common in good quality semen.

At least two counts of one hundred spermatozoa (more if not good agreement) should be made of the midpiece and tail defects. This may be done on the nigrosin eosin smear or alternatively on a wet preparation of diluted semen (saline or buffered formal saline if the sample is to be kept) with a phase contrast microscope. The common abnormalities counted are proximal cytoplasmic drops, distal

cytoplasmic droplets, tailless heads, singly bent tails, doubly bent tails and coiled tails.

The following are guides to the values of semen characteristics in the bull that indicate normal reproductive function: when it is a) Motility (moving actively forward): > 50%, b) Concentration: > 500 million /ml, c) Live sperm: > 50%, d) Abnormal sperm heads: < 20% (range for bulls with good fertility is 8–12%), e) Proximal droplets: < 4 %, f) Distal droplets: < 4%, g) Tailless: < 15%, h) singly bent tails: < 8%, i) Double bent tails: < 4% and j) Coiled tails: < 3%. Cells other than spermatozoa: none, or very few leucocytes or epithelial cells (Schrick *et al*, 2003).

Automated computerized machines for recording motility and concentration and calculating the required dilutions are now frequently used in AI centers that can afford them. They incorporate additional qualities of the motility including speed of movement and linearity. Semen used for artificial insemination should be of high quality. Characteristics will be better than the above limits indicating the "normal" range. When reproductive function is excellent, motility will be greater than 70%, head abnormalities will be below 10%. Centers should develop a system of morphological assessment and guidelines for limits beyond which semen is discarded. A routine count of normal/abnormal can be used as a screening test to ensure that semen processed and sold contains at least 70% normal spermatozoa.

The definition of motility is often ambiguous. Since the important criterion is "progressive forward motility", this should be the basis for judgement. If there are 70% or more of spermatozoa moving actively forward the semen sample is of good quality and acceptable for processing. The post freeze examination assesses both the ability of the semen to withstand freezing and thawing and the efficiency of the processing itself. If there are 40% or more of spermatozoa moving actively forward after freezing and thawing the quality is acceptable for AI. For selection/rejection purposes it does not matter very much if the others are slow, swimming backwards in circles (singly bent tails) or immotile. However, these characteristics are important for diagnostic purposes, because they help to define the disturbance of function.

Some systems of evaluation characterize motility as follows: (a) % direction motility (moving forward); (b) % local motility (wiggling around without going forward); and (c) % no movement (possibly all dead). To judge this under the microscope, the general picture is first assessed, and then the type of motility of those moving is assessed. To be acceptable more than 50% should be moving, and of these more than 70% should show progressive motility.

Twenty million spermatozoa per straw (one cow dose) has been the standard for many AI centers. Some bulls reach their potential fertility with 15 million spermatozoa per dose, provided semen processing, handling and AI technique are excellent. Regular counts of sperm numbers per straw should be made for quality control. Many centers have standardized their own ways of assessing semen quality and sperm numbers per straw; they can be recommended for use provided they serve the purpose effectively.

4.7.1. Semen traits to be evaluated include the following**:**

*4.7.1.1. Volume***:** The capacity to produce spermatozoa per gram of testicular tissue (Daily Sperm Production; DSP) is well correlated to scrotal circumference measurement in young bulls. In bulls on a regular semen collection schedule, volume and sperm concentration indicate a bulls capacity to produce sperm and also allow an AI center to monitor testis function.

*4.7.1.2. Density***:** Density may be classified as follows: **a)** Very Good (VG): creamy, grainy semen with 750 to 1 billion or more spermatozoa per ml, **b)** Good (G): milk-like semen with 400 to 750 million spermatozoa per ml**, C)** Fair (F): skim milk-like semen with 250 to 400 million spermatozoa per ml **and d)** Poor (P): translucent semen with less than 250 million spermatozoa per ml

Semen collected by AV may be more concentrated, and cleaner, than samples collected by electro ejaculation or by massage. With the latter two methods, volume and density may not be representative of a bull's normal capacity to produce sperm; however, with good technique, clean ejaculates with good concentration are often possible when the bulls are sexually rested and handled in a calm way.

*4.7.1.3. Motility***:**

A) **Gross Motility:** A 5 mm diameter drop of the semen is placed on a warm glass-slide and mass motion is observed under bright field microscopy at 40 X magnification with the field diaphragm closed. Factors that affect mass motion of the spermatozoa include concentration, percentage of progressively motile cells and the speed/vigor of sperm motion. If one or more of these factors is compromised the swirling of mass motion will be suppressed. Descriptive assessment of gross motility: a) Very Good (VG): rapid dark swirls and eddies, b) Good (Good): slower swirls and eddies, c) Fair (F): no swirls, but prominent individual cell motion and d) Poor (P): little or no individual cell motion

B) Individual Progressive Motility: a) A 5 - 7 µl volume of the semen is placed on a new warm

glass-slide creating a drop approximately 3-5 mm in diameter, which is then covered with a coverslip. The volume of semen used (5 vs. 7 µl) for evaluation will depend on the size of the coverslip preferred (18x18 mm or 22x22 mm coverslips).

b) The sample is observed under phase contrast microscopy at 200 - 400 x magnification and the percentage of sperm cells having progressive linear motion is determined. If the semen is too concentrated, the semen sample may be diluted with a buffered diluent or semen extender before cover slipping (Sori, 2004).

Visual microscopic analysis of individual progressive motility is somewhat subjective even when performed by very skilled people and becomes tedious when large numbers of samples must be analyzed. Computer Assisted Semen Analysis (CASA) systems (Fig. 6 and Fig. 7) have the potential to increase objectivity of analysis and reduce worker fatigue. In addition, CASA systems should reduce variability in analytical results between AI centers.

Figure 6. Sperm Vision CASA system. (Courtesy of Minitube International). - To view this image in full size goes to the IVIS website at www.ivis.org.

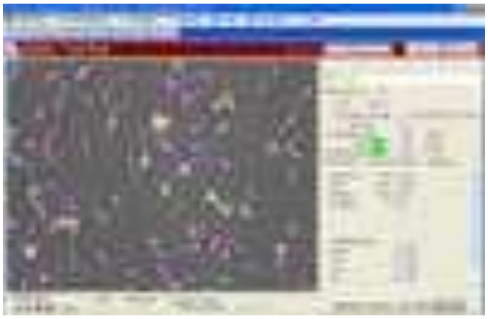

Figure 7. View of motility analysis by the Sperm Vision CASA system. (Courtesy of Minitube International). - To view this image in full size goes to the IVIS website at www.ivis.org.

4.7.1.4. Sperm Morphology Evaluation

Sperm cells are translucent when observed with bright field microscopy; therefore, phase contrast microscopy or the use of sperm stains are needed for analysis of sperm morphology. Eosin-nigrosin stain is commonly used as a "live/dead" stain because in addition to providing background-contrast for sperm cells with the nigrosin component, sperm membrane penetration by eosin, or lack thereof, is an indicator of sperm membrane integrity and thus of sperm viability.

Technique: 1) Put a glass slide on a warming plate (37°C) for 30 - 60 seconds, 2) Put a 5 - 6 mm droplet of eosin-nigrosin stain at one end of the glass slide, 3) Put a droplet of semen beside the droplet of stains (Fig. 8). The droplet's size depends on the density of the semen: a) 3 mm for VG density, b) 4 mm for G density and c) 5 mm for F or P densities, 4) Mix the stain and the semen on the slide, 5) Spread the mixture slowly on the slide from one end to the other using a wooden applicator stick or the edge of another glass slide (Fig. 9), 6) Dry the smear quickly by blowing air over it and 7) Perform the sperm morphology evaluation at 1000 x magnification using immersion oil, counting at least 100 sperm per sample (Fig. 10). If a high number of abnormalities are observed, a count of 300 or more sperm will give a more accurate differential count.

Eosin-nigrosin stain is very hypotonic and, therefore, may cause artifacts in sperm morphology. Drying stained smears quickly by using warm glass slides and blowing over them after spreading the stain will minimize formation of artifact. The percentage of individual progressive motile sperm and the percent of sperm staining alive are highly correlated when semen quality is good.

Figure 8. Glass slide on a warming plate with droplets of stain and semen before making a smear for sperm morphology evaluation. - To view this image in full size goes to the IVIS website at www.ivis.org.

Figure 9. A second glass slide may be used to spread the semen – stain mixture. - To view this image in full size goes to the IVIS website at www.ivis.org.

Figure 10. A bovine semen smear stained with eosin-nigrosin at 1000 X magnification. The white cells are live sperms (6 cells) and the red ones are dead sperms (1 cell). - To view this image in full size goes to the IVIS website at www.ivis.org.

4.7.1.5. Semen Concentration

Although a rough prediction of sperm concentration can be determined by visual evaluation of a semen sample, exact concentrations must be known to determine the number of doses of semen to be frozen. The following methods can be used to determine sperm concentration: 1. Electronic Counting: - Photometers such as the Photometer SDM5 (Fig. 11) are in common use. A sample of raw semen is diluted in a cuvette with a predetermined volume of diluent and analyzed with the photometer. This analysis takes about 30 seconds. The volume, progressive motility, number of sperm per dose and the volume per dose is entered. The number of doses to be frozen and the final extender volume will be calculated.

Figure 11. Photometer SDM5 for determination of sperm concentration, and automatic calculation of extender volume required and number of doses that can be produced from an ejaculate. (Courtesy of Minitube International). - To view this image in full size goes to the IVIS website at www.ivis.org.

2. CASA Systems: - Systems such as Sperm Vision (Fig. 6 & Fig. 7), use computer programmed digital analysis of microscope fields of moving sperm. Several characteristics of motility are quantified, and sperm concentration is also determined. These systems require appropriate dilution of semen samples before filling of one or more commercially supplied disposable counting chambers with a depth of 20 microns. A counting chamber is placed under a microscope and up to 7 microscope fields are analyzed in just seconds per field. Data can be stored and provided on customized printed forms.

3. Hemocytometer (Fig. 13):- Hemocytometer are used as the standard method for determination of cell concentrations and for calibration of electronic systems of cell counting. The method is very reliable and inexpensive, but will take about 10 minutes per sample and involves somewhat tedious visual counting of sperm. Sperm dilution prior to filling the 2 chambers of the hemocytometer can be done in a variety of ways. One method which facilitates accurate dilutions of semen is the Unopette® micro collection system (Becton-Dickinson, Rutherford, New Jersey). Unopette® micro collection systems (Fig. 12) are used in laboratories to count red or white blood cells, but also work well for counting sperm. Unopettes utilize a plastic reservoir containing an exact volume of a diluent and a capillary pipette of exact volume.

Figure 12. Components of a Unopette® micro collection system: plastic reservoir with a known volume of solution and a capillary pipette stored inside a sharp -ended cover to perforate the plastic reservoir. - To view this image in full size goes to the IVIS website at www.ivis.org.

Figure 13. Hemocytometer with the Neubauer chamber. It requires an exclusively designed cover slip that comes in the original hemocytometer packaging. (Courtesy of the Melissa Rouge). - To view this image in full size goes to the IVIS website at www.ivis.org.

Technique:-The sharp-ended cover over the capillary pipette is used to puncture the top of the fluid container. The capillary pipette is then withdrawn from the sharp-ended cover to be filled with semen. The pipette is held almost horizontally and touched to the edge of a semen sample to allow filling by capillary action. The diluent vial is squeezed slightly before the pipette is inserted through the perforated top. Releasing the squeeze draws the semen from thecapillary pipette into the vial. Several slight squeezing actions rinse the inside of the capillary tube. The collar end of the capillary tube is fitted onto the diluent vial and the capillary tube can then be used to fill both chambers of the cover slipped hemocytometer (Fig. 13). The solution present in the Unopette plastic reservoir inhibits sperm motility. The filled hemocytometer is then allowed to stand for 3 - 5 minutes to let the sperm settle to the bottom of the chambers. The full grid on each hemocytometer chamber has 9 main squares, each of which is 1 mm square (Fig. 14). Each of the main squares (1 mm^2 each) located on the four corners of the grid are divided into 16 smaller squares (Fig. 14). The main central square (1 mm^2) is divided into 25 squares and each of these large squares is divided into 16 tiny squares (Fig. 15). With a phase contrast microscope using 400 x magnifications, count the number of sperm in a minimum of 2 main squares (2 mm^2) per grid, for a total of 4 main squares (4 mm^2) per chamber, and determine the mean number of sperm per square mm (Fig. 14). Count sperm heads that are present inside or touching the left and top side of each square mm (Fig. 15).

The mean number of sperm per square mm is multiplied by factors for chamber depth and dilution of semen and then converted to sperm per cubic centimeter, for example: a) Hemocytometer depth 0.1 mm x 10, b) Dilution factor x 200, c) Sperm/mm^3 x 1000 = sperm cm^3, d) 0.25 or 0.5 ml straw x 0.25 or 0.5 ml=sperm/dose and e) Sperm/dose x % motile = ***motile sperm /dose***.

Figure 14. Left: Top view of the hemocytometer showing the location of the chamber's full grid in one of the two sides where the count is done. Right: Magnification of the hemocytometer full grid that contains nine main squares, each of which is 1 mm x 1 mm. Each corner main square has 16 small squares. (Courtesy of the Melissa Rouge). - To view this image in full size goes to the IVIS website atwww.ivis.org.

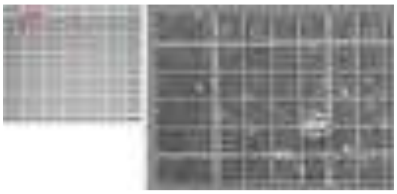

Figure 15. Left: Higher magnification (100 x) of the main central counting square area (1 mm^2) of the hemocytometer grid showing the 25 squares. Each of these squares contains 16 tiny squares. The red lines are bordering one square where sperm are counted. If red dots represent sperm, one would count 3 sperm in the top middle large square. (Courtesy of the Melissa Rouge). Right: Higher magnification (200 x of the main central counting square area showing the top- left large square with sperm cells inside. - To view this image in full size goes to the IVIS website at www.ivis.org.

4.7.2. Progressively Motile Morphologically Normal Sperm Cells

After the semen quality evaluation has been performed and a calculation of the concentration of sperm is obtained, it is important to determine the number of Progressively Motile Morphologically Normal (PMMN) sperm in the ejaculate. PMMN is used to calculate the number of doses that can be frozen. Example: 9.5 ml ejaculate with the following characteristics: a) 75% progressively motile sperm, b) 80% morphologically normal sperm and c) Concentration of 620 million spermatozoa/ml

1. Calculate the number of sperm in the ejaculate: 9.5 ml x 620 million/ml= 5.89 billion sperm
2. Calculate the number of PMMN sperm: 5.89 billion sperm x 75% motile x 80% normal = 3.534 billion PMMN sperm in the ejaculate.

4.7.2.1. Calculation of the Number of Doses and Dilution

The average number of sperm provided per dose in the last 5 decades in North America has been 10 million progressively motile sperm post-thaw. Since approximately 50% of sperm die in the freezing process, it is necessary to double the number of sperm per dose; i.e., if 10 million progressively motile sperm post-thaw were the goal, the number of sperm per dose would be 20 million progressively motile sperm. To continue with the previous example, the number of doses that can be packaged with an ejaculate that has 3.534 billion PMMN spermcells would be 176 straws after doing the following calculation. 3.534 billion PMMN/20x10 6 PMMN/straw = 176 straws

4.8. Dilution and extension

The diluent type and extension ratio depends on the type of semen produced: deep frozen semen (DFS), chilled semen (CS) or room temperature semen (RTS).

For DFS the recommended diluents are: a) Egg yolk - citrate - glycerol extenders, b) Skimmed milk - egg yolk – extenders, c) Tris-buffer based diluents (synthetic) and For CS and RTS the recommended diluents are: a) Coconut milk - egg yolk extender, b) Egg yolk - citrate extenders and c) Caprogen extender (common in New Zealand; not yet tested under African conditions).

Technical details of preparation of the extenders for DFS and CS are fairly standardized within the AI industry, and are available at all semen processing centers. RTS is a relatively novel procedure, and therefore an example of diluents is given. For RTS addition of antibiotics, antifungals and peroxidases (eg. Catalase) is necessary. Peroxidases are not added to CS.

Caprogen extender is saturated with nitrogen and contains catalase. Using this diluent it is possible to transport semen anaerobically and at ambient temperature and to use it for up to 4 days with a 60–70% non-return rate. There is a recent trend towards synthetic extenders and ones that do not contain animal products such as milk and egg yolk (Sovino *et al*, 2002).

Dilution should aim at 15 to 20 million total spermatozoa per cow dose for deep frozen semen. Concentration of the raw ejaculate, of the final dilution of the semen and the sperm content of straws should be checked periodically with a hemocytometer using duplicate dilutions and counts. These checks serve to maintain accurate calibration of instruments used for assessing concentration.

4.9. Processing and packaging

A standardized daily routine should be adopted for all types of semen processing. For example, the following routine is recommended: 1) Diluent preparation, 2) Raw semen collection and evaluation, 3) Extender A at 30ºC added to semen 1:1 and allowed to cool to room temperature (approximately 20 minutes), 4) Complete dilution with Extender A at room temperature and placed in 4-5ºC for at least 4 hours, 5) Extender B is held at 4-5ºC and added in two steps, 30% and then 70% at that temperature, 6) Fill, seal and label straws, 7) Place straws on freezing racks in liquid nitrogen vapour to -140ºC over 10 minutes (straws should be 5cm above the liquid nitrogen surface; in the absence of freezing machines this step can be done in a large semen storage tank or a big polystyrene container containing liquid nitrogen), 8) Place racks in liquid nitrogen at -196ºC, 9) Collect straws with gloved hand and store in goblets in liquid nitrogen, 10) Wash and sterilize glassware for the next day.

In this system the extender is added in two fractions. Fraction A contains no glycerol; fraction B contains 14% glycerol. The final concentration of glycerol is 7%. The 4 hour time lapse between adding fraction A and the first part of fraction B is to allow antibiotics to work before they are inhibited by glycerol.

The common types of packaging used for processed semen are: a) DFS - packaged and sealed in straws, mini (0.25 ml) or medium (0.5 ml), or pelleted (0.1 ml drops). Straws and pellets contain a minimum of 20 million spermatozoa per dose and b) RTS and CS - packaged and sealed in ampoules or vials of 1.0 ml, containing 15-20 million spermatozoa. In some cases this can be reduced to 5 million spermatozoa (e.g. the caprogen diluted semen used in New Zealand).

4.10. Preservation and storage

DFS is preserved and stored in liquid nitrogen at -196ºC. Transferring of semen between containers must be done quickly. Canisters containing

packages when raised from the tank should remain in the neck of the tank for less than 10 seconds. Liquid nitrogen is dangerous and must be handled carefully. CS is stored at 4-6°C in the refrigerator and transported in insulated containers at the same temperature. RTS is held at ambient temperatures ranging from 18-26°C. The containers of straws, ampoules and pellets should be properly labeled and records maintained on their location and contents.

4.11. Post packaging quality control

Motility of samples from processed batches of semen should be checked before dispatch. Post thaw motility should be 40% or more for DFS. All semen storage containers should be regularly checked for liquid nitrogen level and replenished as required.

5. Consequence of mishandling frozen semen

Applied reproductive technologies like AI and embryo transfer have developed into huge global industries due to the ability to cryopreserve male gametes and embryos and subsequently thaw them with predictable results. However, many of those directly involved in implementing these technologies have, at some time, experienced poor, if not disastrous, results post thaw. Based upon careful statistical analysis of fertilization rates involving embryo transfer and semen evaluation, along with observations in the field by professionals trained in handling cryopreserved and stored samples, it's evident that mistakes made during routine handling can expose cryopreserved sperm and embryos to thermal insults that can damage or even destroy them.

It's normal for professionals in the AI and embryo transfer industries to look forward to emerging biotechnologies so their clients can take advantage of them. No veterinarian or animal scientist wants to be left behind. However, it behooves practitioners in the field to train animal breeders and their staff in basic handling techniques involving frozen semen and embryos, no matter what the species. It's imperative that fundamental handling techniques be employed in the field else the technologies will fail.

It's often very difficult to re-train experienced AI technicians who have established bad handling habits when thawing frozen semen, taking inventory, or moving canes of semen from vessel to vessel. The easy excuse for them when confronted with low conception rates is to blame the bull, the bull stud, or the cow but seldom their AI skills or semen handling techniques. After all, they've been handling frozen semen for years and not had any problems with damaged semen due to mishandling, at least that's been diagnosed. Also, AI schools have not thoroughly addressed the issue of proper handling techniques.

Perhaps more research is needed in this area to specifically point out damage done to sperm and embryos during brief or extended thermal exposures. Maybe ultra-structural analysis of sperm membranes using scanning electron microscopy could pin point specific physical damage to individual sperm and help determine under what conditions sperm undergoes significant changes after an exposure. Additionally, maybe a temperature specific dye could be added to semen samples that would react if the internal temperature of a straw of frozen semen warmed to above - 130° C then was re-exposed to liquid nitrogen. If a cane or even a canister of frozen semen turned up bright purple after being thawed, the technician would instantly know the sperm has been exposed to potentially damaging temperatures.

In any case, the value of bovine genetics is ever increasing due to the utilization of existing technologies like AI and embryo transfer. With new biotechnologies looming in the forefront like genetic markers and others, the value of superior genetics will only increase in value. But even with commercial dairies where semen costs are affordable, the expense of low conception rates can be devastating to the bottom line. Proper handling techniques for frozen semen and embryos are easy to implement and should be as fundamental as heat detection or passing an AI gun through a cervix. Implementing safe handling procedures for cryopreserved reproductive cells will increase the efficiency of applied reproductive biologies.

6. Selection of AI site

6.1. Technique of AI

Several different techniques have been used for artificial insemination. The original technique used for over a century was intravaginal insemination, where an unprocessed semen sample is placed high in the vagina. In the latter half of the 20th century, the cervical cap was developed to maintain the highest concentration of semen at the external os of the cervix. It was soon discovered that placing the semen sample into the endocervix (intracervical insemination) resulted in pregnancy rates similar to that obtainable using a cervical cap and superior to those seen with high vaginal insemination.

6.1.1. Intrauterine Versus Intracervical Insemination

A major breakthrough came in the 1960s when methods were developed for extracting enriched samples of motile sperm from semen. These purified samples were free of proteins and prostaglandins, and thus could be placed within the uterus using a technique designated intrauterine insemination (IUI). This technique was found to result in pregnancy rates 2 to 3 times those of intracervical insemination.

However, intracervical insemination is still utilized in some practices (Zollner *et al*, 2003).

In an effort to further improve pregnancy rates, techniques were developed to place washed sperm samples directly into the tubes via transcerival cannulation (intratubal insemination) or into the peritoneal cavity via a needle placed through the posterior cul-de-sac (intraperitoneal insemination). Another technique developed in Europe, termed fallopian tube sperm perfusion, involves pressure injection of a large volume (4 mL) of washed sperm sample while the cervix is sealed to prevent reflux of the sample. This technique appears to have a higher pregnancy rate than IUI in couples with unexplained infertility. The remainders of these technically difficult approaches have never been shown to result in better pregnancy rates than IUI. One prospective, randomized study found that simultaneous intratubal insemination actually decreased the pregnancy rates associated with IUI. In modern clinical practice in the United States, IUI is the predominant technique used for artificial insemination.

7. Selection of inseminated cows

7.1. Heat detection

Under herd conditions farmers should be advised to observe cows for heat signs at least three times in a day (20 minutes of visual observation each time: morning, afternoon and late evening). This should be done at times other than during feeding and milking. It may be conveniently done during communal grazing. One or more of the following signs should be observed as indicators of the different stages of oestrus: 1) **Pre-heat signs**: restlessness, separates from herd, ear movements, attempts to mount others, clear mucus, reduced milk production, bellowing, 2) **Standing heat**: stands still when mounted; other signs include clear and copious mucus, vulva enlarged, rests head on back of other cows, tail head roughened (the last sign could also be seen post-heat) and 3) **Post-heat** (2-3 days after start of heat): moves away when mounted, tired and lying while others graze, clear or bloody mucus on tail or legs. Cows/heifers observed with these signs should be recorded for future management of heat/reproduction to reduce the economic loss due to missed heat. Ideally, if a cow is first seen in heat in the morning, she should be inseminated in the afternoon of the same day and if she is first seen in heat in the afternoon or evening, she should be inseminated the next morning. This well documented rule has recently been questioned. Good conception rates are being obtained with a once per day service.

Education of farmers is needed on heat detection methods, adequate feeding and observation of cows for heat signs, identification of cows truly on heat and recording the time of heat observation and if possible informing the inseminator the time of first heat detection.

Aids to heat detection such as tail paint, heat mount detectors, teasers and heat synchronization may be used under certain economically warranted situations. The measurement of progesterone by RIA in samples of milk collected on the day of insemination provides valuable retrospective information on the accuracy of heat detection.

7.2. Body condition at calving and at insemination

Body condition at calving and at the subsequent insemination influence the interval from calving to first oestrus and also conception rate, and are therefore important. Farmers should aim to have cows in a condition score between 2.5 and 3.5 (based on a scale of body condition score from 1-5) and to minimize loss of score between calving and insemination. Cows that are too fat at calving are likely to have calving difficulties and are more prone to early fetal death. Cows which are too thin, especially if they are losing condition, will have delayed oestrus and poor conception rates.

7.3. Other factors to be considered before insemination

Cows should be at least 42 days after calving before they are served again. For high yielding cows a longer period may be necessary to obtain good conception rates and to reduce embryo and early fetal losses.

The cow should be in good health. Specifically, she should be free of any evidence of infection of the reproductive tract. Particular attention should be paid to cows that have had abnormal calvings (e.g. dystocia, retained placenta and prolapse of the uterus), as they may require a longer period after calving for involution of the uterus and to return to normal fertility. The AI technician must make sure that the cow is genuinely in oestrus and that she is not pregnant. If there is any suspicion that the cow may be pregnant, the insemination should be done only half-way in to the cervix.

8. Dystocia and AI

When cows body condition score (BCS) is below 3.5 and the cow is malnutrition, it leads to difficulty in birth (dystocia).

9. Status of AI in Ethiopia

AI service in Ethiopia has been given little or no emphasis at the federal, regional or woreda levels during the last years even though it is a widely practiced animal biotechnology all over the world. The

most important constraints associated with AI service in Ethiopia include less structural linkage between AI Center and service giving units, absence of collaborations and regular communications between NAIC and stakeholders, lack of breeding policy and herd recording system, inadequate resource in terms of inputs and facilities, and absence of incentives and rewards to motivate AI technicians.

10. Fertility potential

Fertility potential is seriously depending on the bull and cow fertility, the inseminator ability and safety of environment (the bull + the cow + the inseminator + the environment).

10.1. The bull

It has long been recognized that a major benefit of A.I. is the reduced risk of disease transmission. However, A.I. can be a very efficient means to spread disease if attention is not paid to the health status of the donor sires. Major A.I. centers that are members of Certified Semen Services (CSS) go to extensive lengths to ensure each dose of semen is safe and disease free. The average bull in a major A.I. center may receive 30 or more health tests in a given year while screening for 12 or more different diseases. Although semen processed on-farm or by non- CSS custom-collection centers "may be" equally safe, there are no guarantees and the risk will almost always be much greater than with sires residing in CSS approved A.I. facilities. Bulls with sufficient health status to qualify as semen donors must also produce semen of acceptable quality to achieve normal conception potential. Bulls at major A.I. centers are critically scrutinized for semen quality on each collection day using state of the art technologies in semen evaluation. Samples of less than acceptable quality are discarded. Bulls that continually produce substandard quality ejaculates, perhaps due to illness or adverse weather conditions, may be temporarily removed from the collection schedule. Some sires are simply culled from the program. These procedures ensure that only highly fertile semen makes it to the salable inventory. As a result of these intense A.I. center quality control procedures, the variation in A.I. sire conception rate is extremely small. As estimated by both Agritech Analytics and the United States Department of Agriculture-Animal Improvement Programs Laboratory (USDA-AIPL), greater than 90 percent of "all" A.I. sires (progeny test and inactive included) have a fertility deviation that is > -3% of average fertility. However, when you consider the Active A.I. list from which semen is actually available for purchase, greater than 98 percent of sires will meet this threshold. Because of the transient nature of both semen quality and sire health status, A.I. will always have an advantage over natural service sires.

10.2. The cow

No matter how good the semen quality, semen has no therapeutic effect to "cure" fertility problems in the cow. Cows that experience any type of health problem are predisposed to reduced conception rates. Cows that experience one health problem have increased susceptibility to other health conditions, which then compounds the degree of difficulty in getting them rebred. For example: cows that get milk fever have an increased probability of experiencing ketosis, a displaced abomasum, metritis, mastitis and cystic ovaries than cows that do not get milk fever. When cows experience health and reproductive problems, we want to find a magic potion that can be applied to fix the problem "today". However, today's fertility is a reflection of the cow's environment and management during the previous two or three months. Similarly, the decisions made today can affect cow fertility for several months to come. There is no single factor that affects cow fertility more than nutrition.

10.3. The inseminator

When we discuss inseminator competence, we usually focus on issues like semen handling and A.I. technique. Can they retrieve semen from the tank without damaging semen that remains in the tank? Can they thaw semen, load an A.I. gun and arrive at the rear end of the cow with semen that is still alive and viable? And finally, can they proficiently manipulate the gun through the cervix and precisely deposit semen in the uterine body? It's true, each of these factors are important, however no attribute of inseminator competence is more important than: Can he accurately identify cows that are truly in estrus and ready for insemination? In many of today's large dairies, the breeding philosophy is: "Stick a dose of semen in her just in case." This practice is not only wasteful, but it can actually be counterproductive. Research has shown that cows that are pregnant less than 25 to 30 days have a 90 percent probability of abortion if mistakenly re-inseminated. Some herds can improve conception and pregnancy rates simply by taking a more conservative approach to estrus diagnosis. Proper timing of A.I. is also an important component of inseminator efficiency. It's not enough just to put semen in a cow that is in heat. The timing of A.I. must ensure that the fertile life of sperm and egg will overlap. Frozen-thawed sperm survive approximately 20 to 24 hours in the female reproductive tract. However, the fertile life of an unfertilized ovum is only six to eight hours after ovulation. Since ovulation occurs 25 to 32 hours after the first standing mount, ideal timing is to A.I. four to 14 hours later. However,

it is important to distinguish the difference between "first standing mount" and "first observed standing mount". In today's large dairies cows are often only locked-up at a single time each day, which diminishes the opportunity to A.I. at "the right time". A once daily A.I. program must simply appreciate that many cows will be bred too early and must be re-bred if they are still standing for an extended period of time.

Hygiene is an over-looked attribute of inseminator proficiency. Maintaining the hygienic integrity of the uterus is an important factor influencing success. Little things like clean hands, clean paper towels, clean equipment and perhaps double sheath breeding may make a meaningful difference in the long run.

10.4. The environment

Environment is the overwhelming factor influencing conception potential. Heat stress is one of the most obvious environmental culprits. Facility design and strategic use of fans and sprinklers are essential to maintain acceptable conception rates through the warm summer months. Cool, clean water must be provided in all locations, especially in holding pens.

Environmental disease exposure can dramatically impact reproductive performance. Many herds have found out the hard way that vaccination programs are insurance policies but by no means are they guarantees. Work with your veterinarian to establish a routine herd health program. Vaccination must be combined with close surveillance and disease testing as necessary. Testing is especially important for deaths and abortions.

11. Diseases related with AI

The regular testing of semen donors under official veterinary supervision has been adopted by governments world-wide as a means of avoiding the spread of pathogens and reducing excessive contamination of semen by ubiquitous bacteria (WHO, 1967).

National standards for semen production and distribution are usually based on regulatory programmes to ensure that diseases of importance are identified and appropriate tests are applied to all sires entering and residing in artificial insemination (AI) centers. Two of the major goals of artificial insemination of domesticated animals are to achieve continuous genetic improvement and to prevent or eliminate venereal disease. Some lists of venereal diseases are:

11.1. Infectious bovine rhinotracheitis/infectious pustular vulvovaginitis

Bovine herpesvirus-1 (BHV-1), also called infectious bovine rhinotracheitis/infectious pustular vulvovaginitis virus, is a member of the subfamily Alphaherpesvirinae and is one of the most common viral pathogens found in bovine semen.

Reproductive disorders caused by BHV-1 include infectious pustular vulvovaginitis, endometritis, salpingitis, shortened oestrus cycles and abortions in susceptible female cattle and balanoposthitis in susceptible bulls. In BHV-1 infection of the genital tract of the bull, the virus replicates in the mucosae of the prepuce, penis and distal part of the urethra, and semen is most likely to be contaminated during ejaculation by virus shedding from infected mucosae.

11.2. Bovine virus diarrhea

Bovine virus diarrhea (BVD) virus, a ribonucleic acid (RNA) virus, has two main types characterized by non-cytopathic (NCP) or cytopathic (CP) effects on cultured cells. They are indistinguishable serologically. The NCP biotype may infect the fetus and establish a persistent infection (PI) which continues into post-natal life. A high proportion of adult cattle world-wide have antibody to BVD virus (BVDV), although most infections in adults are subclinical.

11.3. Bovine brucellosis

Bovine brucellosis is a bacterial disease caused mainly by Brucella abortus. In cattle, the disease is characterized by abortion and is often associated with retained placenta, metritis and a subsequent period of infertility. Brucellosis affects approximately 5% of livestock world-wide and continues to increase. It is also an important zoonosis. Some authorities in Brucella-face countries consider that the serum agglutination test is sufficiently sensitive for health certification of Al bulls (69), although more sensitive tests are available (38, 93). Reports of bulls shedding B. abortus in semen while their serum agglutination titers were low or negative (6) indicate the benefits of testing semen for the presence of the organism, or testing seminal plasma for agglutinins, particularly in areas of high risk. Live strain 19 vaccine and the killed 45/20 vaccine have both played an important role in the control of brucellosis. However, strain 19 may produce permanent infections in bulls similar to those of natural disease (60). New vaccines such as RB51, which is an avirulent rough mutant lacking an O-chain, can induce a protective cell-mediated immune response without an accompanying seroconversion, but the value of these vaccines in the field remains to be tested.

11.4. Leptospirosis

Leptospirosis survives in extended unfrozen bovine semen, either with or without antibiotics (18), and also in frozen semen without antibiotics. Treatment of bulls with 25 mg of dihydrostreptomycin

(DHS) per k g - 1 body weight has been approved internationally to stabilize low antibody titers and to prevent shedding of leptospira, but the efficacy of such treatment is questionable. DHS has, furthermore, been withdrawn in some countries which mean that other antibiotic treatments will require certification. As the currently available serological tests cannot differentiate vaccine from infectious liters, vaccination of bulls in AI centers is not usually appropriate.

11.5. Bovine genital campylobacteriosis

Venereal campylobacteriosis, a widespread bacterial disease associated with both bovine infertility and abortion, is caused by Campylobacter (Vibrio) fetus, particularly the subspecies venerealis. C. fetus infection in cattle has decreased in regions where AI and vaccination are practiced, yet the disease continues to be an important pathogen causing reproductive problems in many countries.

Preputial scrapings and semen from bulls are the usual specimens taken for laboratory tests. Infected bulls produce IgA in their preputial secretions but the titers tend to be so low that they are indistinguishable from those present before infection.

11.6. Trichomonosis

Trichomonosis is a venereal disease of cattle caused by the protozoan parasite Tritrichomonas fetus. In the female, it is characterized by infertility, early abortion and pyometra but in the infected bull, a symptomless carrier state occurs with T. fetus being found on the penis and preputial membranes.

This does not interfere with spermatogenic function or the ability to copulate (31). Trichomonosis occurs world-wide, particularly among range cattle (44, 67). High herd prevalence has been reported in areas of North America where natural breeding is practiced (13, 73). Control can be achieved through a policy of testing breeding animals and the widespread use of AI (87).

12. Import and export of bovine genetics

The import and export of the cattle/ buffalo germplasm is under restricted list and is allowed against the license issued by Directorate General of Foreign Trade, Ministry of Commerce on the recommendation of this Department.

Introduction of temperate dairy breeds in the country for crossbreeding indigenous non - descript cattle has been accepted for quite some time now. In pursuance to this, the need has been felt by number of State Governments/ Organisations to import exotic germplasm to produce the quality cross-bred animals. With the extension of the breeding program and the artificial breeding network, a surge in the demand for the exotic germplasm is also expected. Accordingly, the import of the germplasm must be from the sires, which have been progeny tested and are in active use in the cattle breeding from which such germplasm are being sourced.

There is a definite demand for the germplasm of Indian breeds of cattle and buffalo in South America, South Asia and other countries. Keeping in view our responsibility towards conservation of the rich diversity, it is important to broadly categorize the germplasm of cattle and buffalo meant for breeding purposes and for the export purposes. Imposing a complete ban on the export of Indigenous germplasm because of conservation concern could be counterproductive, since such a ban may encourage the flow of germplasm through illegal trade which is not desirable. It can be used for the upgradation of the indigenous stock.

13. Pregnancy diagnosis (PD)

Pregnancy diagnosis in cow is defined as to detect the matted cow/the cow which got improved breed of sperm via Artificial insemination (AI) technique is either conceive the fetus or not. It also simply defined as the determination of pregnancy in cattle.

When the **cow is non-pregnant** the following signs are observed: **a**) both the uterine horns are symmetrical **b**) uterine horns are located intrapelvic **c**) feel of uterine horns are normal **d**) ovaries are at normal position and **e**) one of the ovaries exhibit corpus luteum.

When the **cow is pregnant** the following signs are observed.

1) 5th pregnant cow shows a) uterus hangs on the brim of pelvis b) uterus is tonic/increase uterus muscle vigour/ c) pregnant horn is further enlarged d) exhibit pregnancy corpus luteum which is differ from periodic corpus luteum is not having a neck.

2) End of 2nd month pregnant shows a) slippery feel of fetal membrane when horn is palpated between fingers b) cervix stretched/pulled forwarded c) uterine wall is thinner than normal due to increase diameter of uterine horn.

3) End of 3rd month pregnant shows a) rebound effect is detectable b) uterus sinking in the abdominal position c) thinning of uterine wall is continues d) fluctuation can be felt e) fremitus (+) can be felt and f) middle uterine artery (major blood supply) palpated.

4) 6th month pregnant shows a) uterus sinking in the abdomen b) fetus and fluctuation are felt c) from 0-5 month growth are observed and d) fremitus (++) can easily felt.

5) From 7th month to birth shows a) uterus is entirely located abdominal b) fetus sink more deep in the abdominal cavity c) fetus parts can be clearly felt or identified and d) fremitus (+++) is very strong.

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2/6/2025