

# ***Improving artificial breeding of cattle in Africa***

## ***Guidelines and recommendations***

*A manual prepared under the framework of an  
IAEA Technical Cooperation Regional AFRA Project on  
Increasing and Improving Milk and Meat Production, with  
technical support from the Joint FAO/IAEA Division of  
Nuclear Techniques in Food and Agriculture*



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International Atomic Energy Agency

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## FOREWORD

The International Atomic Energy Agency (IAEA) and the African Co-operative Agreement for Research, Development and Training Related to Nuclear Science and Technology (AFRA), with technical support from the Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture, implemented a technical cooperation project entitled Improving and Increasing Milk and Meat Production. The objectives of this project were to be achieved by (a) assessing the performance of existing artificial insemination (AI) programmes for small-scale dairy farmers and identifying constraints; (b) formulating and assisting in the implementation of remedial measures including appropriate strategies; (c) establishing sustainable routine non-pregnancy diagnosis (N-PD) and related services to farmers; and (d) harmonizing managerial and field practices and sharing of expertise within the region. The radioimmunoassay (RIA) for measurement of progesterone in milk and blood of cattle and use of the computer database AIDA (Artificial Insemination Database Application) are important components of an integrated approach to these activities.

The project commenced in 1999 and, in addition to other national and regional activities, two meetings were held specifically to address objective (d) above:

- Task Force Meeting on Training of Artificial Insemination (AI) Technicians, Field Assessment of Fertility and Database Management, November 1999 in Pretoria, South Africa.
- Task Force Meeting to Harmonize Procedures for Selection and Management of AI Bulls and Use of Semen Technology in African Countries, May 2001 in Arusha, Tanzania.

The meeting in Tanzania provided a comprehensive overview of the current practices being adopted for selection and management of AI bulls and use of semen technology in five African countries, and compared these with international practices. It also provided an opportunity for participants to discuss technical issues related to provision of improved breeding services to cattle farmers and to arrive at a consensus on what can be considered 'best practice' in the countries represented.

It was concluded that: (a) methods of keeping records, their analysis and use for evaluating genetic merit and fertility of AI bulls require improvement in most AFRA Member States; (b) the technical aspects of providing N-PD and related services to farmers based on progesterone assay have been clarified in many Member States; (c) the modalities and logistics of practical application under the different conditions in countries need to be further developed; and (d) there is a clear need for guidelines that can be used for improvement and harmonization of currently used technical procedures for AI in the Member States.

Participants of the meeting developed a framework for the guidelines, formulated the first draft of the manual and identified the additional work necessary for its completion. The manual was subsequently compiled and the second draft was circulated to all AFRA national project coordinators for comments and suggestions for improvement. It was finally discussed at a Project Review, Coordination and Planning Meeting held in Addis Ababa in March 2002 and further material incorporated as required.

This manual combines the outputs from the three meetings referred to above, and is the result of collaboration between the contributors listed at the end of the publication.

The manual is aimed at all levels of administrative and technical personnel involved in the provision of AI services to cattle farmers in Africa. This includes livestock specialists in Ministries of Agriculture/Livestock, Departments of Livestock and Veterinary Services, AI centres, semen distribution centres and field level AI Service points. It is also expected to be a useful resource for teachers and students, in faculties of veterinary and animal sciences, institutions for livestock breeding research and those involved in the training of AI technicians.

Research over the last 50 years has produced a considerable body of knowledge about artificial breeding. Much of this is shared by people involved in the industry. The challenge is to apply what is known in an efficient, cost effective and sustainable way in different cattle farming systems under varying socio-economic environments. It is hoped that this manual will provide guidelines that will assist in that process in Africa.

The IAEA officer responsible for this publication was O. Perera of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture.

### *EDITORIAL NOTE*

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## 1. SELECTION, HEALTH AND MANAGEMENT OF BULLS

### 1.1. Selection of bulls

Methods of selection should be based on clear breeding goals, aimed at increased milk and/or beef, and improved productivity. The main objective 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 in **Annex 1**. In the absence of these schemes a "likeability" scheme should be established (see 1.1.3).

#### 1.1.1. 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 analysing 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>)

#### 1.1.2. 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. "Breedplan 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>.



### 1.1.3. 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):

- 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
- 1 = Not a good animal; not liked at all

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.

### 1.1.4. 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 centre and ensure improvement of male reproductive efficiency in the population. An example of suitable forms for records of reproductive characteristics of bulls is given in **Annex 2**.

## 1.2. Genetic improvement

It should be stressed that fulfillment of breeding goals requires rigorous selection and culling. Contract matings 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 is distributed randomly to farms participating in the progeny test scheme. This must result in at least 25 herd recorded daughters in 15 herds for minimum reliability of the EBV's derived from the production of those daughters.

The basis for selection and ranking of bulls with respect to their genetic value for different attributes is the EBV. The EBV for a characteristic such as milk production of daughters is Heritability x Phenotypic Superiority. The latter is the difference between the value for a bull and the mean value for the population in a country or specified area (Hammond *et al.*, 1992).

Heritabilities for different traits are given in Table 1. Recently somatic cell counts, udder characteristics (depth, milking speed) and reproductive efficiency (56 day non-return

rate of daughters) are being included in the criteria for selection of dairy bulls. Generally, traits such as conception rate have low heritability due to the relatively large influence of factors related to environment and management. However, certain specific reproductive disorders such as cystic ovarian disease are likely to have higher heritability. It should be mentioned that AI focuses on intensive genetic improvement using sires, whereas embryo transfer can exploit the merits of the dam as well.

Table 1. Heritabilities of some economically important traits in beef and dairy cattle (Hammond *et al.*, 1992)

Type of cattle and traits	Heritability (%)
<b>Beef Cattle</b>	
<i>Highly heritable traits</i>	
Birth weight	35
Milk production	40
Feedlot gain	40
12 month carcass weight	45
Carcass characteristics	40
Age at puberty	40
Scrotal circumference	50
Mature weight	50
<i>Traits of medium heritability</i>	
Weaning weight	25
Carcass yield grade	30
<i>Traits of low heritability</i>	
Calving interval	10
Longevity	0
<b>Dairy Cattle</b>	
<i>Highly heritable traits</i>	
Birth weight	50
Mature weight	35
<i>Traits of medium heritability</i>	
Milk production	25
Fat production	25
Protein	25
Solids not fat	25
Excitability	25
<i>Traits of low heritability</i>	
Teat placement	20
Services per conception	5
Mastitis susceptibility	10

### 1.2.1. Cornerstones for genetic improvement

*Objective measurements of performance:* in areas with small-holder farms some deliberate selection of the better farms for contract matings is probably necessary to obtain accurate and regular results. Records on AI dates and calving dates are essential. Some form of approved milk recording or the use of a likeability score is necessary. An incentive scheme is usual for farmers participating in progeny test programmes. Herds accepting semen from bulls to be progeny tested may have the cost of that semen discounted.

*Normal conformation and functionality:* selection of bulls, semen or cows for genetic improvement under African conditions should be based on fertility and production performance within African environments. Animals should be able to reproduce and produce efficiently. The first requirement is that the AI sires should be born without assistance. Their daughters should have regular and normal calvings. Cows for contract matings should have a record of regular and normal calving. The progeny of a cow tell how good she really is. The cow has to produce a daughter that is better than she is (measured by Kg of milk, likeability or other measure). Function in the herd is first based on production and freedom from disease, then on conformation and other traits. Inspection of animals and records of their reproduction and production is generally done by a panel including a veterinarian and a geneticist.

*Adaptability:* ability to sustain production under adverse conditions. Records of progeny of AI bulls and contract mated cows will provide a measure of reproductive and productive adaptability. African environments are harsher than the European and North American environment. Selecting for sustained high production under different African conditions should be a priority. Large frame size, very high milk production, high feed intake and ability to sustain production under housed conditions may not be the best genetic base for many African conditions. Ability to produce and reproduce under poor nutritional conditions together with tolerance to heat, ticks and tick borne diseases are important attributes often found in indigenous breeds (Fig. 1). The length of time a cow remains in the herd and reasons for culling may form a useful basis for measuring sustainability. These cornerstones are the same for the improvement of indigenous breeds, exotic breeds, cross breeding and the formation of synthetic breeds.



*Fig. 1. An indigenous bull in the tropics at an AI station. His genetic worth for the local small-holder farmer needs to be defined. Note cool housing.*

### 1.2.2. Cross breeding

Cross breeding is the mating of animals of different breeds. The incentive for cross breeding is the exploitation of hybrid vigour or heterosis as a result of which the performance of cross-breeds exceed the average of the parental breeds. This is due to the fact that parental animals differ in gene composition and that dominant genes carry more favourable traits than recessive genes.

Several systems of cross breeding are applied (Maree and Casey, 1993):

- *Single cross* – This is the crossing of any two breeds selected on the basis of their performance traits to produce cross-bred offspring with considerable hybrid vigour. Heterosis is fully expressed.
- *Back cross* – A cross-bred female (F<sub>1</sub> cross) from a single cross is mated in alternate generations to unrelated pure-bred males belonging to the original parental breeds (some heterosis may be lost in later generations). Heterosis expression is half that of the single cross between breeds.
- *Rotational crossing* – A third or fourth breed is systematically introduced into a back-cross programme to maintain heterosis. Pure-bred males are used on cross-bred females.
- *Three-breed terminal cross* – The F<sub>1</sub> cross-bred females are mated to males of a selected third breed and all offspring (F<sub>2</sub>) slaughtered for meat production. More heterosis can be achieved with this method than with a three-breed rotational cross.

The Friesland and Boran crosses have done very well under African dairy ranching conditions. The Bonsmara breed is a synthetic breed developed for Africa providing a method of gene diffusion into African beef animals. The first generation often do not do well due to tick borne diseases and one has to aim for less than 25% Friesland to allow for the desired advantages to survive.

In tropical Latin American countries the F<sub>1</sub> cross between *Bos indicus* and *Bos taurus* is the best compromise in dairy cattle between productive characteristics and adaptability to the environment. But it is not possible to sustain this degree of heterosis. Synthetic breeds have been developed in Australia (Australian Milking Zebu and Australian Friesian Sahiwal) but they have not become popular. There have been no dairy synthetic breeds developed in Africa.

Many milking cows in African countries are likely to be cross-bred and based on Friesian genes for the *Bos taurus* component. Most semen from purebred bulls is not going to be put into pure bred cows. Guidelines are important for managing the genetic resources available in a way that is sustainable and which provides the best promise for increased milk and meat production with heat tolerance and resistance to tick and tick-borne diseases. Recording milk production and grading up valuable locally adapted *Bos indicus* breeds by selection should be part of this livestock development agenda. The 7<sup>th</sup> World Congress on Genetics Applied to Livestock Production in August 2002 highlighted strategies for animal breeding in developing countries. Further information is available at the website: <http://wcalp.toulouse.inra.fr>.

### 1.3. Statutory requirements for disease testing and quarantine

These should be set out and monitored for compliance by a government or other statutory authority separate from the AI organization. They include:

- Registration of premises

- Approval of animals as donors of genetic material
- Keeping and care of animals
  - maintenance of quarantine and Veterinary surveillance of the management, nutrition, health and welfare of the animals
- Technical activities at centres
  - proper identification of genetic material, cleanliness and sterilization
- The records to be kept at centres, and
- Disease testing.

Suitable regulations for the above have been developed for South African conditions, and are given in **Annex 3**. Some of these may be universally applicable, but others need to be modified and/or adapted for the needs of other countries which have different conditions.

A specimen form to apply for approval of a bull for use in AI is given in **Annex 4**.

The Office International des Epizooties (OIE) has, under its International Animal Health Code (2001), detailed recommendations for collection and processing of semen (see website: [http://www.oie.int/eng/normes/mcode/A\\_summry.htm](http://www.oie.int/eng/normes/mcode/A_summry.htm); Section 3.2). The specific diseases against which semen donor bulls and teaser animals should be tested are given in the OIE Code under Article 3.2.1.5 and are reproduced in **Annex 5**.

## 1.4. Management of bulls

### 1.4.1. Housing

Housing may be closed, semi-open or open. Bulls in tropical and subtropical conditions require protection from heat and adequate ventilation. Shade trees, shade cloth and thatch are effective. Fine water sprays with fans can be used to cool *Bos Taurus* bulls under hot conditions. Bulls should be housed securely so there is no chance of escape and interaction with other bulls, staff and the general public.

### 1.4.2. Feeding

A balanced ration should be fed (see **Annex 6**). This could be home grown or bought in or both. Care should be taken not to over-feed bulls as fat deposition in the inguinal canal negatively affects fertility. Condition score is an important guide to nutritional requirements. Working bulls should have a score of 3 to 3.5 on a scale of 1-5 (see **Annex 7**). Feeding of mineral licks and clean water should be *ad libitum*.

### 1.4.3. Handling

The establishment of a firm relationship between the handler and the bull is essential and cannot be overemphasized. The bull should be at ease when he is handled and the handler should not feel threatened. The proper application of a bull nose ring is required as soon as the bull arrives at the centre. The bull should be handled by both a halter and the bull ring. Care should be taken to use the ring only when the bull becomes unruly and difficult to handle by the halter alone. Bulls should be led by the halter and not by their nose (Fig. 2). Bulls should always be handled in such a manner that semen production is optimized. This includes taking note of all aspects of the physiology of male sexual behaviour. Negative stimuli should be avoided in the collection area as far as possible. This includes pain delivered via the nose ring, which could lead to low libido (e.g. many of the difficult, slow, low libido *Bos indicus* bulls may have been made that way by poor training and handling techniques).



*Fig. 2. Young bulls should be taught to lead with the halter. The nose ring is used sparingly for restraint and not at all when the bull is immediately behind the teaser.*

#### 1.4.4. Health

Once the bulls have passed all the quarantine tests for disease control, normal routine preventive medicine should be practiced in the AI Centre. Care should be taken that bulls remain in excellent health for continuous semen production. The necessary vaccinations, regular deworming and control of ectoparasites should be implemented to meet national and regional requirements. Continuous monitoring of diseases should be undertaken whether statutory or not. It is in the best interest of the AI Centre to be able to certify at all times that all animals are fit to produce semen for sale and distribution.

#### 1.4.5. Records

A complete history of every animal should be kept from the time of arrival until the day of departure from the centre. All incidents, ailments and medications should be recorded.

## **2. SEMEN TECHNOLOGY AND FIELD PRACTICES**

### **2.1. Semen technology**

#### 2.1.1. 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. 3). 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.

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. 4). 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. 3. Strong timber construction of stanchion for teaser bull restraint in South Africa. Note the non-slip floor.*

#### 2.1.2. 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 round. 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.



*Fig. 4. Holding facilities for bulls in South Africa.*

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 sterilised in an autoclave or hot air oven.

### 2.1.3. 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 labelled 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 (e.g. KY jelly, which has been tested and found to be non-toxic in diluted form) should be applied sparingly and just before collection (Fig. 5). The lubricant can be replaced by a small amount of diluent to moisten the entrance to the artificial vagina.





*Fig. 5. Preparation of the artificial vagina; lubricating with a glass rod using KY jelly.*

Water for the outer jacket filling should be warmed to 60°C. 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 42-48°C. Assembled AVs should be kept in incubators at 55-60°C. 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.

#### 2.1.4. Electroejaculators

Electroejaculators 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 electroejaculation. The prepuce should be washed and dried. The rectum should be emptied of faeces 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.

#### 2.1.5. 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.

#### 2.1.6. Collection procedure

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. 6). These measures constitute good sexual preparation which increase sexual excitement and the amount of semen collected. The bull is then allowed to mount for the first collection.



*Fig. 6. Sexual preparation assists in improving the bull's serving behaviour 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 from 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. 7). 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.

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.



*Fig. 7. Collection of semen with the artificial vagina. The left hand touches only the preputial skin, not the penis itself.*

#### 2.1.7. Evaluation of semen

The semen should be transferred to a water bath maintained at 33-36°C. Visual evaluation for volume, colour, consistency/density, odour and observation for presence of foreign material should be made and recorded (see Semen Result Sheet **Annex 2**).

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 (**Annex 8**). 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 haemocytometer and is calculated as described in **Annex 2**.

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.

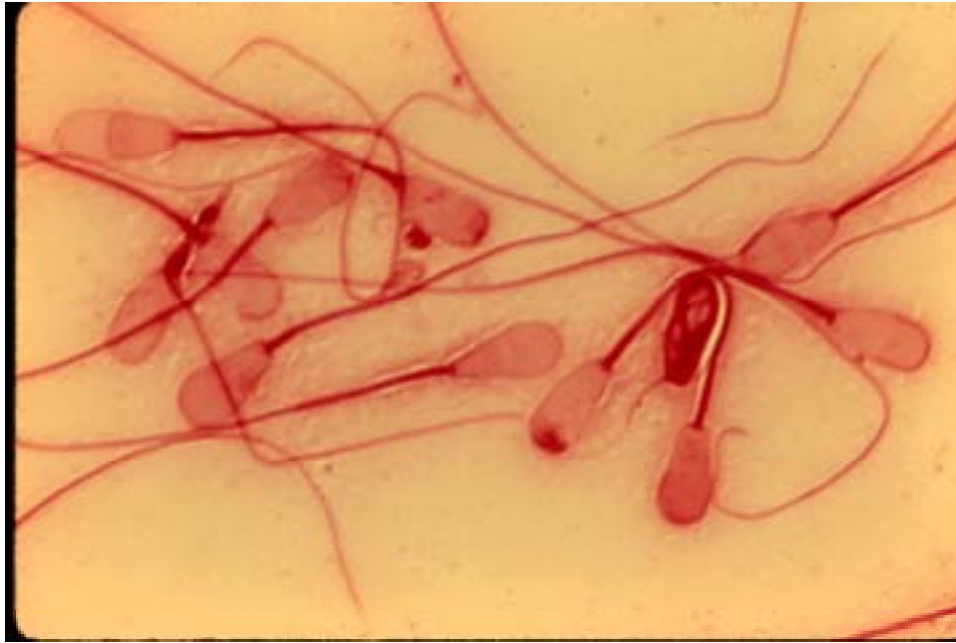
The following are guides to the values of semen characteristics in the bull that indicate normal reproductive function:

- Motility (moving actively forward): > 50%
- Concentration: > 500 million /ml
- Live sperm: > 50%
- Abnormal sperm heads: < 20% (range for bulls with good fertility is 8–12%)
- Proximal droplets: < 4%; Distal droplets: < 4%
- Tailless: < 15%; Singly bent tails: < 8%; Double bent tails: < 4%; Coiled tails: < 3%
- Cells other than spermatozoa: none, or very few leucocytes or epithelial cells.

Automated computerized machines for recording motility and concentration and calculating the required dilutions are now frequently used in AI centres 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%. Centres 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.

Detailed morphological examination (Fig. 8) is generally reserved for borderline samples. The assessment is also valuable in helping to reach a diagnosis when a bull fails to produce semen of processable quality as assessed by concentration and motility. Morphology is also useful in monitoring the semen of bulls with disturbances of reproductive function (e.g. testicular degeneration) to establish a prognosis for future production of semen suitable for processing.



*Fig. 8. Semen smear stained with carbol fuchsin eosin. Narrow at base, pear shaped and undeveloped heads are visible. One spermatozoa has an acrosome defect. The bull consistently gave semen of poor freezability.*

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 centres. 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 centres 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.

### 2.1.8. 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:

- Egg yolk - citrate - glycerol extenders
- Skimmed milk - egg yolk - extenders
- Tris-buffer based diluents (synthetic)

For CS and RTS the recommended diluents are:

- Coconut milk - egg yolk extender (**Annex 9**)
- Egg yolk - citrate extenders
- 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 centres. RTS is a relatively novel procedure, and therefore an example of diluents are given in **Annex 9**. 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 four days with a 60–70% non-return rate (Shannon, 1965 & 1968).

There is a recent trend towards synthetic extenders and ones that do not contain animal products such as milk and egg yolk (**Annex 9**).

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 haemocytometer using duplicate dilutions and counts. These checks serve to maintain accurate calibration of instruments used for assessing concentration.

### 2.1.9. Processing and packaging

A standardized daily routine should be adopted for all types of semen processing. For example, the following routine is recommended:

- Diluent preparation,
- Raw semen collection and evaluation,
- Extender A at 30°C added to semen 1:1 and allowed to cool to room temperature (approximately 20 minutes),
- Complete dilution with Extender A at room temperature and placed in 4-5°C for at least four hours,
- Extender B is held at 4-5°C and added in two steps, 30% and then 70% at that temperature,
- Fill, seal and label straws,
- Place straws on freezing racks in liquid nitrogen vapour to -140°C 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),

- Place racks in liquid nitrogen at  $-196^{\circ}\text{C}$ ,
- Collect straws with gloved hand and store in goblets in liquid nitrogen,
- Wash and sterilise 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 four 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:

- 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.
- 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).

#### 2.1.10. Preservation and storage

DFS is preserved and stored in liquid nitrogen at  $-196^{\circ}\text{C}$ . 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. Safety precautions are given in **Annex 10**.

CS is stored at  $4-6^{\circ}\text{C}$  in the refrigerator and transported in insulated containers at the same temperature. RTS is held at ambient temperatures ranging from  $18-26^{\circ}\text{C}$ .

The containers of straws, ampoules and pellets should be properly labelled and records maintained on their location and contents.

#### 2.1.11. Post packaging quality control

Motility of samples from processed batches of semen should be checked before despatch. 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.

## 2.2. Field practices

### 2.2.1. Heat detection

Farmers should be encouraged to keep proper fertility records of individual cows in their herds for efficient reproductive management. Artificial insemination technicians (AITs) play a key role in encouraging this to be done or doing it themselves for some clients. The essential information includes identity of cow, dates of observed oestrus, dates of mating or insemination, pregnancy/non-pregnancy tests (e.g. progesterone assay and/or manual pregnancy diagnosis), date and result, date of calving and milk production.

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:

- Pre-heat signs: restlessness, separates from herd, ear movements, attempts to mount others, clear mucus, reduced milk production, bellowing.
- Standing heat: stands still when mounted (Fig. 9); 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).
- 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.



*Fig. 9. The cow in oestrus stands to be mounted.*

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. The Australian In Calf programme has demonstrated an average first service conception rate of 48% in 69 herds with once per day insemination. In 99 herds inseminating twice daily the average first service conception rate was 50%. Where the inseminator visits a particular location only once a day, the cow should be inseminated at the first visit after the farmer has observed standing heat. Theoretically, the best time to inseminate is between 6 to 18 hours after detection of heat.



Education of farmers is needed on heat detection methods, adequate feeding, 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.

#### 2.2.2. 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 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 foetal death. Cows which are too thin, especially if they are losing condition, will have delayed oestrus and poor conception rates.

#### 2.2.3. 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 foetal 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.

#### 2.2.4. Semen handling and insemination technique

The cow to be inseminated should be properly restrained. A crush is recommended. Where a crush is not available the cow should be tethered and body movements restricted by a person standing alongside. Excitement and stress should be avoided since adrenaline release disturbs sperm transport.

When DF semen is used, insemination kits should contain a small liquid nitrogen container, a vacuum flask for hot water, a thawing flask and thermometer, gloves, tweezers, insemination guns, plastic sheaths, scissors, paper towels, soap and record books. Inseminators require protective clothing and a watch suitable for controlling thawing time.

Care and proper handling of the liquid nitrogen containers, semen and other AI equipment is important. Mechanical damage to containers through rough handling will reduce their efficiency. There must be always adequate liquid nitrogen in the tank. Preferably the tank should be kept full.

Transferring of semen from the tank to the thawing water must be done quickly. Canisters should remain in the neck of the tank (Fig. 10) less than 10 seconds and preferably no longer than five seconds. Frequent opening of semen containers is to be discouraged (not more than 10 seconds in any 10 minute period).



*Fig. 10. When transferring frozen straws the canister should not be brought beyond the neck of the container. It should not remain there for more than 10 seconds.*

Recommendations for thawing DF semen vary considerably in the literature. Generally, thawing should be in warm water at 35°C for a minimum of 20-30 seconds. The straw should be wiped dry, cut at right angles and properly loaded into the insemination gun (pistolette). Prior to loading, the gun should be briskly rubbed with a piece of paper towel to warm it. This helps to prevent sudden changes in temperature which are detrimental to the semen. Under warm temperature conditions the gun may be held in the mouth while the cow is being prepared and the hand is being inserted in the rectum. In cooler conditions the gun may be placed down the back between the clothing and the body to avoid changes in temperature. Faeces should be removed from the rectum. The uterus should be examined for size and consistency. Insemination must not be carried out if the uterus is enlarged.

The vulva lips should be wiped clean with a dry paper towel. The vulva lips are parted and the gun is introduced gently through the cervix under control of the hand in the rectum. The whole semen dose is deposited just in front of the internal opening of the cervix into the body of uterus. After withdrawal of the gun the vulva may be massaged to assist sperm transport.

Thorough cleaning of the person (inseminator) and equipment in between farms is mandatory. Inseminators should be aware of diseases likely to be spread between farms and measures to prevent this.

### 2.2.5. Follow-up advice to farmer

The nutritional plane should be maintained after AI. Shade should be available since heat is detrimental to embryo survival. Between 18-24 days after AI the cow should be watched carefully for signs of a return to oestrus. A milk sample for progesterone assay as a test of non pregnancy is best taken on days 21-23.

## **3. DELIVERY OF IMPROVED GENETICS AND BREEDING SERVICES TO FARMERS**

### **3.1. Organization**

#### 3.1.1. Artificial insemination services

Provision of AI services requires active participation of, and cooperation between, the stakeholders in dairy production. This includes farmers, inseminators, AI centres and organizations involved in milk recording, collection and marketing.

Genetic improvement depends on the accurate measurement of milk production in identified cows and the utilization of this data for bull selection. Getting cows in calf requires good semen, good heat detection and good insemination technique. An adequate infrastructure needs to be in place and maintained. Telephone services or transport systems for messages from the farmer must be reliable. Inseminators should have reliable and fast means of transport. Motor vehicles or light motor bikes are recommended. Contingency plans are needed to continue to provide services when vehicles require repairs or when the inseminator is on holiday or is sick.

In each country, the policies and practices for delivery of improved genetics and related services to farmers should be formulated in relation to: country situation (animal population, production milk and meat, etc.); environmental conditions and availability of resources for livestock production; and social and economic situation of farmers and people. Governments should formulate appropriate breeding policies and provide guidelines to AI services and farmers on the choice of suitable breeds and, if importation of semen is done, on its genetic value.

#### 3.1.2. Co-operatives and farmer organizations

In many countries these organizations provide the best structure for the development of AI services. Co-operatives assist the farmers in a number of ways including reduction in the cost of AI and drugs and in the collection and marketing of the products (milk and meat). They should also provide information services and education programmes for members.

#### 3.1.3. Linkages, information, education and extension

Good communication and cooperation are needed between AI Centres, herd recording organizations, farmers' organizations, breed societies, research organizations and government or other authorities involved in programmes and services outlined in 3.3.6. The AI organization should work with the cooperatives in providing good information to farmers on: the AI service itself; genetic improvement; sanitation and hygiene; farm economics; fertility; the bulls; breeds and quality of semen available; and the progeny test scheme. All these organizations should be involved in an integrated programme of education of farmers.

Cultural aspects need to be taken into account when AI is being advocated to traditional farmers for whom breeding cattle and the ownership of bulls forms an important part of their way of life.

#### 3.1.4. Development of services and increasing numbers of animals inseminated

Development of services includes the improvement of infrastructure, ways of making Government and private AI Centres cost effective, the question of privatization (if and how it should be done), and economic management of the service. Co-operative ownership of centres is one approach.

Fertility results need to be good to help convince farmers of the advantages of AI. Field days (e.g. “Progeny days” in South Africa, with farm visits to give farmers the opportunity of seeing calves born as a result of AI), brochures and other publicity materials are valuable extension tools.

### **3.2. Genetics, product quality and marketing**

#### 3.2.1. Milk

Governments (and other organizations concerned with agricultural development) should encourage the consumption of milk and promote its marketing and distribution. This will provide a stimulus for more efficient production of milk and meat. They should encourage the formation and development of farmer organizations. There needs to be a well monitored system of marketing and distribution of milk, with milk collection centres and small processing units.

Simplified herd milk recording is needed in areas with small holder farms. Quantity control and quality control of milk production should be under the care of Government or its assigned authorities. Local consumption of milk should be encouraged as in school milk programmes. Encouraging milk production on the small farms is a way of empowering the disadvantaged, especially women.

#### 3.2.2. Meat

Meat quality control should be in the hands of the proper national authority. Local consumption should be encouraged. Marketing and distribution of meat and other products (skins, etc.) is often best done by co-operatives in collaboration with health authorities. Ways of achieving better revenue for the farmers should be explored.

#### 3.2.3. Semen

Semen must be of good quality with the required standards for motility and numbers of spermatozoa depending on the type of processing and packaging (see sections 2.1.7 to 2.1.11). Selling semen is selling genetics and the semen should as far as possible be from progeny-tested bulls. The advantages of using particular bulls in particular areas and for specific purposes need to be well known to farmers so they can make an informed decision when they purchase semen from the AI service.

### 3.3. Farmer services, records and economics

#### 3.3.1. Standard of management and heat detection

Before AI services are introduced to a farm there needs to be a reasonable standard of management and hygiene and an understanding of the importance of heat detection and pregnancy diagnosis (including early non-pregnancy diagnosis using RIA for progesterone as a management tool where this service is provided, and clinical pregnancy diagnosis at 45-90 days after service).

Heat detection is covered in section (2.2.1) and farmer services using RIA of progesterone are described in section (3.3.3).

#### 3.3.2. The inseminator, technique and remuneration



*Fig. 11. An inseminator performing artificial insemination on a cow.*

The inseminator (Fig. 11) is a key person in the industry. There are two main categories of AI service providers in African countries. One category is AI Technicians (AITs), who provide services to a farming community, require certification and/or registration, and may also be responsible for other livestock services. The other category is Do-It-Yourself inseminators (DIYs), who are farmers or are employed by a farmer, for AI services on their own or their employer's farms only.

AITs are currently employed in a variety of ways, e.g. government, co-operatives, AI organizations, non-governmental and self-employed (including veterinarians and DIYs). The majority of countries use frozen semen, packaged in 0.25 and 0.5 ml straws. The sources of semen are mainly national or sub-national AI centres and importation, but also include custom frozen semen.

The current entry characteristics for training of AITs ranges from the basic ability to read and write (for DIYs) through secondary to tertiary educational levels (for AITs), depending on intended functions and duties. Training is conducted through residential courses of one to six weeks duration, containing theoretical and practical components, followed by evaluation.

Sources of financial support for courses vary, and include employers (e.g. government, co-operatives or AI associations) and fees charged from the trainees. Refresher courses and continuing education activities are held when necessary, but are sometimes constrained by lack of funds.

Guidelines for training AITs, based on a previous Task Force meeting held under the AFRA project, are given in **Annex 11**.

### 3.3.3. Diagnosis of pregnancy and non-pregnancy

If a cow has not returned to heat after AI, manual diagnosis of pregnancy should be done by rectal palpation, preferably around 60 days after the last service. Earlier diagnosis from about 45 days is possible but, unless the operator is highly skilled, can result in damage to the conceptus. Also, spontaneous foetal losses can occur up to 60 days after conception and can invalidate an earlier positive diagnosis.

The use of progesterone measurement for non-pregnancy diagnosis (N-PD) at an earlier stage can serve as a useful monitoring tool to assist in improving reproductive management by farmers as well as to increase the effectiveness of AI programmes. This is based on a single sample of milk or blood collected at 21-23 days after AI and is likely to be most effective where heat detection by farmers is good and the fertility resulting from AI is high. In situations where a high proportion of cows submitted to AI are in anoestrus, the use of progesterone measurement will be a waste of resources to diagnose animals which in any case could not have got pregnant. Similarly, if many cows are submitted during the luteal phase, a high proportion of false positive diagnoses will result.

Based on results obtained under the Joint FAO/IAEA Programme Coordinated Research Projects and IAEA Technical Cooperation Projects, the following procedures are recommended for N-PD using milk samples:

- Collect a sample of milk between 21-23 days after AI, in accordance with the procedure described below, label the tube and send to the RIA laboratory together with a copy of the completed record form, *within one week*.
- The methods for collecting and returning these samples to the laboratory will vary depending on local conditions. One possibility may be for farmers to collect the sample, using pre-labelled vials with the cow number and appropriate date which are provided by the AIT at time of AI, and to return these to the laboratory through existing milk collection systems or co-operatives.
- Provide the results of progesterone RIA to farmers, together with appropriate interpretation and advice, *within 7-10 days* of receipt. This should normally be through the veterinarian and/or AIT, but direct information to farmers may also be feasible in some situations.
- The veterinarian (together with the AIT) must follow-up on the outcome of recommendations, examine non-returning cows for pregnancy at 45-60 days after AI,

diagnose any infertility problems that persist, provide appropriate treatment and inform the laboratory of the responses.

- Other related services which could be incorporated in such a programme include diagnosis and treatment or control of other disorders, such as mastitis, foot problems, calf diseases and sub-clinical conditions affecting productivity.



*Fig. 12. Milk samples from a herd in Tunisia, preserved with sodium azide, being sent on ice to the laboratory for RIA of progesterone.*

Sampling and sample processing can have a major influence on progesterone concentrations in milk. In order to achieve reliable results it is recommended that the following procedures be followed:

- Use milk samples from the same milking time (either morning or afternoon milking) and milking stage (preferably composite milk or strippings). Add one tablet of sodium azide (100 mg) as a preservative per 10-20 ml of milk and mix well.
- Transport in ice to the assay laboratory as soon as possible (maximum storage times should be one week if kept at room temperature and 4-5 weeks if kept at 4°C). Whole milk should not be frozen.
- To remove the fat, centrifuge samples at 2000 x g for 15 minutes at the same temperature each time (preferably 4°C, if refrigerated centrifuge is available).
- If milk was centrifuged at room temperature, place in refrigerator for 15 minutes to harden fat layer (not necessary if centrifuged at 4°C). Use a glass rod to pierce fat layer and transfer entire skim milk to a storage vial (5-10 ml) using a pasteur pipette.

- Skim milk samples with preservative can be stored as follows: (a) at room temperature or 37°C for 1-2 weeks; (b) at 4°C for at least three months; and (c) at -20°C indefinitely.
- Materials required for collection and transport of milk samples by AITs include: plastic sample vials (10-20 ml), sodium azide tablets, adhesive labels, marker pens and a cool box with ice.

Where milk sampling is not possible, as in the case of heifers or certain beef production systems, blood can be collected and processed to obtain plasma or serum. The assay procedure for measuring progesterone in plasma and serum is basically similar to that for milk, but requires progesterone standards made up in bovine plasma and serum.

The following procedure is recommended for obtaining plasma:

- Take blood from the jugular or tail vein, using evacuated tubes or syringes containing heparin or EDTA as anticoagulants;
- Place immediately in a cool box or ice bath at 4°C;
- Centrifuge for separation of plasma as soon as possible (within 2-4 hours), at 770 x g (approx. 2000 rpm) for 20 minutes at the same temperature each time (preferably 4°C, if refrigerated centrifuge is available);
- Draw off the plasma using a pasteur pipette and transfer to a storage vial;
- Label and store at -20°C.

The following procedure is recommended for obtaining serum:

- Take blood from the jugular or tail vein, using evacuated tubes or syringes without any anticoagulant.
- Place immediately in a cool box or ice bath at 4°C and transfer to a refrigerator as soon as possible.
- After a firm clot has formed ring the clot to promote retraction and keep for a further 2-4 hours at 4°C.
- Centrifuge for separation of serum at 770 x g (approx. 2000 rpm) for 20 minutes at the same temperature each time (preferably 4°C, if refrigerated centrifuge is available).
- Draw off the serum using a pasteur pipette and transfer to a storage vial.
- Label and store at -20°C.

#### 3.3.4. Records and their use

The Joint FAO/IAEA Division has developed a computer database named the Artificial Insemination Database Application (AIDA), through a Co-ordinated Research Project that was undertaken by 14 countries, to be used for recording, managing and reporting information from the field and the laboratory. This application was subsequently modified and is now being adapted for routine use in AI services in Asia and Africa (as AIDA Asia and AIDA Africa, respectively). For the purpose of the AFRA project, a set of essential or core data that needs to be recorded has been identified. This is termed the Minimum Data-Set (MDS) and can be recorded on one sheet of paper for each cow (**Annex 12**). Additional information may be recorded as necessary, based on other fields available in the AIDA computer program and its associated data record forms.

The MDS includes the following:

- Farmer, farm and address



- Identification of cow (number or name)
- Breed of cow and breed of its sire and dam
- Last calving date
- Whether milking only; if not, the type of suckling (once, twice or *ad libitum*)
- Date of AI
- Interval from heat to AI (hr)
- Time of AI (AM/PM)
- Site of semen deposition (uterus/cervix/vagina)
- Semen used (bull, breed and batch)
- Milk sampling dates
- Progesterone values in milk samples (to be entered by RIA laboratory)
- Date of PD and result
- Remarks.

The record form should be completed on-farm at time of AI and sent to the RIA laboratory together with the milk samples. Copies of forms may be kept with the farmer and/or AIT depending on requirements. On receipt at the RIA laboratory, record information on the samples received and file the record forms. Enter this data regularly into the AIDA computer database. Refer to the AIDA User's Manual for the sequence of data entry and other operational aspects. The forms for Farm Information, Inseminator, and Semen Batch must be entered before a record for an inseminated cow is entered.

Assay milk samples for progesterone in batches, preferably of 65 samples each (an assay of 150 tubes, containing standards, internal quality controls and samples in duplicate). Full details of procedures for progesterone RIA are contained in the User Manual provided to all counterpart laboratories in countries participating in FAO/IAEA projects.

Enter the assay data in AIDA. During the survey phase these should be summarized using the reports feature and, together with interpretations and recommendations, sent periodically to the veterinarian and/or AIT. For the N-PD service individual results on each cow must be sent to the farmer, either directly or through the veterinarian and/or IAT, together with interpretation and recommendations, within 7-10 days of receipt of the sample. The record forms sent back from the field could also be used for this purpose if appropriate.

### 3.3.5. Value of services

A system should be set up to determine the costs and benefits of AI in a region. The basis for this is milk recording. Cows with AI sires should be producing more milk and have better calves than cows from natural service sires. The advantage needs to be sufficient to more than cover the costs of AI. A quantified economic benefit can be used in promotion of AI services.

### 3.3.6. Herd Health Services in relation to AI

Artificial insemination services should be integrated with other programmes and services that influence its efficiency and the efficiency of animal production. These include disease diagnosis and control programmes, especially for infectious reproductive disorders (Brucellosis, Leptospirosis and Tuberculosis), mastitis diagnosis and control, calf rearing, heifer rearing, nutrition improvement programmes and other veterinary and animal husbandry education services.

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## **Annex 1**

### **SIRE PROOFS AND COW INDEXES**

Genetic evaluation should be published in terms of Estimated Transmitting Ability (ETA) which is EBV/z or estimated progeny differences (EPD). A bull's ETA is referred to as a sire proof while a cow's ETA is known as the cow index. Sire proofs and cow indexes are calculated for production and type using BLUP methodology with an individual animal model.

Systematic methods of recording should be established and a central body should be set up to receive records and be responsible for publication of sire proofs.

Production records and sire ratings for production traits should be expressed as Breed Class Average (BCA) points. For example 1 BCA point for a mature Holstein cow is equivalent to 53 kg of milk, 1.96 kg of fat and 1.68 kg of protein. Therefore the average daughter of a Holstein sire with a +1 rating for milk would produce 53 kg of milk more per 305 days lactation than the average genetic merit of all cows in milk and recorded with complete records in the most recent year. This is known as a moving or rolling cow base as opposed to a fixed cow base.

Depending on abundance of the breed there should be a minimum number of daughters in a number of herds with a repeatability of a given percentage. For Canadian proofs the minimum should be 12 daughters in 10 herds with 60% repeatability (a measure of Accuracy). All sire proofs should be updated regularly (annually) and percentile rankings published for milk, fat and protein yield as an indication of the relative placing of a bull for a specific trait in comparison to all bulls evaluated. A bull in the 90<sup>th</sup> percentile indicates that he is in the top 10% of the breed in Canada.

#### **Milk recording**

Milk recording determines individual cow production. This information is used by the dairy farmer to make management decision relating to feeding, breeding, selection and marketing. Production records are used to calculate AI Sire proofs for genetic evaluation.

A centralized milk recording system needs to be put in place and governments should determine official standards for milk recording and supervision of the recording exercise. Central laboratories to test the milk samples should be established to determine fat, protein content and somatic cell counts. Farmers should pay for the laboratory costs. It should be encouraged that these tests add value to the milk and should fetch slightly more than untested milk.

Breed class average (BCA) is an index used to compare 305 day production of dairy cows and it is the production of a cow expressed as a percentage of the standard.

#### **Linear classification**

Type classification is an evaluation of body conformation as defined by the Breed Associations. Each breed should develop its true type cow and bull models, which are standards that ensure uniformity and accuracy in type classification.

There is a high positive correlation between dairy character and milk yield. Important characters are feet and legs, the mammary system and general appearance. The dairy farmer uses type classification as a management tool in breeding and selection decisions. Typing may be used to develop sire proofs and cow indexes.

Milk yield is an important predictor of herd life while sound functional type plays a significant role in lifetime production. Longevity depends on acceptable levels of production in combination with good functional type characteristics, particularly mammary system feet and legs. However, there is need to harmonize type classification for all breeds.

## **Genetic improvement**

Methods to establish long-term selection goals require knowledge on genetic and phenotypic parameters of traits that may contribute either directly or indirectly to improved profitability. The parameters are estimated from data derived from parents, progeny and other close relatives. These include heritabilities, phenotypic and genetic correlation of identified traits. These parameters are estimated from phenotypic and genotypic variances and covariances of important economic traits.

Livestock populations have been improved genetically for important quantitative traits that are affected by a large number of genes located at many loci as opposed to qualitative traits that are of less economic importance and are effected by few genes and include traits like skin and coat colour, absence of horns etc. Examples of quantitative traits are milk yield, growth rates, carcass yield etc.

The raw material for livestock improvement is genetic variation due to genes that are additive in their effects, and the non-additive gene effects (i.e. epistasis and dominance). Additive gene effect is that which is passed on from one generation to the other.

Observed phenotypic variation can be attributed to genetic and environmental attributes. The genetic attributes can be additive or non-additive while the environmental attributes can be permanent or temporary. Dominance variation is created when two genes (alleles) at a particular locus on a chromosome interact, and one gene completely overrides the effects of another (e.g. the dominance of the polled trait in cattle over horned recessive gene).

Epistasis is gene action where genes at one locus interact with genes at another locus to cause variation, e.g. the gene that restricts colour in the Charolais cross calves out of Angus dams. The calves are dun in colour because the gene for restriction of colour from the Charolais sire is epistatic to the gene for black colour from the Angus dam.

Within herd or flock ranking of animals leads to accurate genetic evaluation, i.e. comparing animals that are herd mates or contemporaries. This process helps to remove sources of environmental variance (non-genetic) that impacts on the phenotype.

Physiological effects cause systematic non-genetic variations and adjustment for these reduces the non-genetic component of variance. So measurements of performance may be adjusted for a variety of physiology effects such as age, parity, stage of lactation and sex which may influence non-genetic variations.

Some herds may have higher genetic merit than others due to the breeding programme in place. Progeny testing of sires in herds of different genetic merit leads to mis-rankings, the sires of progeny tested against herdmates of higher genetic merit being underrated and vice versa. Handerson (1984) discovered a method of incorporating the pedigree or record of ancestry into genetic evaluation systems and solving the large number of linear equations simultaneously to rank males and females for their genetic merit, taking into consideration the genetic merit of animals performing in the same herd-year season subclass. The Best Linear Unbiased Prediction (BLUP) model evaluation system of genetic evaluation is accepted as the most accurate system available for genetic ranking of males and females to day and its application to large populations of livestock is occurring rapidly. This method removes a very high percentage of environmental variance.

The BLUP Animal Model Methods is the most appropriate to rank animals because of the properties of the predictor and its accuracy. BLUP simultaneously ranks males and females, thus adjusting for non-random mating. This method also identifies all animals by pedigree, thus tying the various herds together and ensuring more accurate across herd rankings.

Heritability broadly defines the percentage of total variations that is due to genetic effects. The higher the non-additive genetic variations within a line or breed, the greater the heterosis in the cross. Usually the breeder chooses the animals to be parents of the next generations, while disposing of others. The selection process may consider a number of suitable traits simultaneously. The progress resulting from selection of superior parents for a given trait depends on heritability, selection differential and generation interval in years.

$$\text{Predicted genetic response due to selection} = \frac{h^2(O_s - O_A)}{L}$$

$O_s$  is the mean performance of selected individuals.

$O_A$  is the mean performance of individuals in population.

$L$  is the generations length in years (average age of parents when their progeny are born), and

$(O_s - O_A)$  is the selection differential (SD).

Rapid genetic progress is expected per year when  $h^2$  is high, selection is intense (large SD) and the generation interval is kept short.

Genetic variation decreases as selection progresses and inbreeding may set in if selection goes on for long because the selected population becomes very closely related.







## Semen Results Sheet

### Laboratory:

#### Initial examination of semen in the field:-

	Sample A	Sample B
Volume (ml)	-----	-----
Density (0-6)	-----	-----
Wave motion (0-6)	-----	-----
Motility (%)	-----	-----

#### Laboratory results :-

Concentration (millions per ml)	-----	-----
% spermatozoa alive (Nigrosin eosin)	-----	-----
% abnormal sperm heads	-----	-----
% spermatozoa with		
proximal cytoplasmic droplets	-----	-----
distal cytoplasmic droplets	-----	-----
tailless heads	-----	-----
singly bent tails	-----	-----
doubly bent tails	-----	-----
coiled tails	-----	-----
acrosome defects	-----	-----
structural abnormalities of the midpiece	-----	-----
Cells other than spermatozoa	-----	-----

#### Comments

## Calculation of Concentration of spermatozoa in French straw

$$T = (d/80) n$$

where

- T is the number of sperm expressed in million per milliliter,
- d is the dilution rate used to prepare sperm-water suspension,
- n is the total number of sperm present within 20 counting squares in a Neubauer haemocytometer.

Example:

- Assume you added 3.9ml of water to 0.1ml of semen resulting in a (0.1ml/3.9ml) 40 times dilution rate (d = 40).
- Further assume you counted 263 sperm within 20 squares (n = 263).

$$T = (d/80) n$$
$$T = (40/80) 263 = 131.5$$

- Thus, the straw contained 131.5 million sperm per milliliter.
- A mini straw contains on average 0.22 ml semen. Therefore the total number of sperm per straw is approximately  $0.22 \times 131.5$  million sperm (28.93 million sperm per mini straw).

The method described above may also be used to determine the concentration of sperm in a freshly collected ejaculate. Due to the high sperm concentration in the ejaculate one should however dilute 1:80 or 1:160 prior to filling the counting chamber.



**Annex 3**  
**STATUTORY REQUIREMENTS FOR DISEASE TESTING AND QUARANTINE**  
(Source: South Africa)

Government Veterinary Health Departments require specific conditions under which semen is collected to comply with standards laid down to prevent the spread of disease within the borders of the artificial insemination centre and also when semen is distributed. Statutory requirements for registration of premises as centres, approval of animals as donors of genetic material, keeping and care of animals at centres, technical activities at centres as well as records to be kept at centres are thus laid down.

**A. Registration of premises as centres**

- (1) First time application for the registration of premises as a centre shall:
  - (a) be made on a form that is obtainable from the registrar for this purpose;
  - (b) be made before genetic material destined for sale is collected on the premises concerned;
  - (c) be accompanied by:
    - (i) the application fee specified, and
    - (ii) two copies of a site plan of the premises concerned and of detailed ground plans.
- (2) An application as referred to in subregulation (1) shall lapse within two years after the date of such an application if the premises concerned do not comply with the requirements for registration as set out in this regulation.
- (3) A site plan referred to in subregulation (1)(c)(ii) shall indicate the location of the facilities specified below in relation to other buildings on the same premises and surrounding properties and building complexes and places, if any, where other animals are kept:
  - (a) office and laboratory complexes;
  - (b) stables, pens, collecting stocks and crushes in which animals will be kept and handled in quarantine with a view to their approval to be admitted to the centre.
  - (c) stables, pens, crushes, kraals and if applicable, collecting stocks, as well as any other places where approved as well as other animals, shall be kept and handled at the centre.
  - (d) public roads and thoroughfares on and around the premises and the public entrances to the premises.
- (4) A detailed ground plan referred to in subregulation (1)(c)(ii) shall indicate the measurements and description of:
  - (a) every room that will be used as offices and laboratories including:
    - (i) the location of rooms for the evaluation, processing, packing, labelling or storage of genetic material,
    - (ii) the location of rooms for cleansing and sterilisation of equipment,
    - (iii) the location of cloakrooms and toilets, and
  - (b) stables, pens, collecting stocks, crushes and places referred to in subregulation (3), and
  - (c) kraals and barns.
- (5) Premises shall be registered as a centre if it complies with the following requirements:
  - (a) It shall be fenced in such a manner that animals that are kept there shall not have physical contact with any other animals.
  - (b) The premises shall be large enough to provide for the exercising of animals therein.

- (c) The quarantine area shall:
  - (i) be designed and fenced in such a manner that the animals concerned shall not be able to make physical contact with each other nor with any other animal;
  - (ii) be equipped with the necessary stables, pens, collecting stocks and crushes for keeping, examination and testing of the animals therein; and
  - (iii) be so situated or screened off that the effluent cannot flow from one quarantine stable or pen to another or from that area over to any other portion of the premises.
- (d) In the case of a centre for pigs. Persons working in the area referred to in paragraph (c), shall have no contact with other workers on the premises of that centre.
- (e) Excess water shall drain rapidly and efficiently from camps, crushes and other places where animals are to be kept on the premises.
- (f) Separate rooms for the following shall be provided for at a centre:
  - (i) administrative activities;
  - (ii) apparatus required for the evaluation, processing, packing, labelling and storage of genetic material as the case may be; and
  - (iii) the cleaning, disinfection or sterilisation and preparation of the equipment used for the collection of genetic material, and the activities referred to in subparagraph (ii).
- (g) The rooms for the different activities referred to in paragraph (f) shall be effectively screened off from each other if they are in the same building.
- (h) The place at a centre where genetic material is sold, or from which they are dispatched, shall be so situated that the persons being served there shall have no access to the rooms referred to in paragraph (f)(ii) and (iii).
- (i) Floors, walls and ceilings of rooms where genetic material is handled at a centre, shall be finished off in such a manner, and the workbenches therein shall be of such a standard that they can be cleaned and disinfected effectively.
- (j) Floors and walls of stables, pens and collecting stocks at a centre shall be impenetrable and shall be finished off in such a manner that:
  - (i) they can be cleaned and disinfected effectively; and
  - (ii) the animals kept therein, will not be injured thereby.
- (k) All stables, pens, kraals, camps and other places where animals are kept at a centre shall provide adequate space, ventilation, light and protection for shelter from heat, cold or inclement weather for the animals kept therein.
- (l) Measures shall be taken at a centre to control flies, animal parasites, other insects and rodents.
- (m) The facilities at a centre that are used for the collection, evaluation, processing, packaging, labelling and storage of genetic material shall be maintained in such a condition that the genetic material handled therewith or therein shall not be contaminated or the quality thereof be detrimentally affected in any way.
- (6) The registration of premises as a centre shall be subject to the following conditions:
  - (a) The person in charge of the centre shall notify the registrar in writing of:
    - (i) any proposed structural alteration in respect of the building complexes or other construction on the premises of the centre concerned, as indicated on the site plan and detailed ground plan submitted in terms of subregulation (1);
    - (ii) any proposed change in the maximum number and kinds of animals kept at the centre concerned;

- (iii) any change in respect of the person to whom the certificate of registration has been issued;
  - (iv) the termination of services at the centre concerned; and
  - (v) the date on which an animal approved for the collection of semen is removed from that centre, and the reason for such removal.
- (b) A notice referred to in paragraph (a) shall be submitted to the registrar by certified post within 14 days after the change took place, services have been terminated or an animal has been removed from the centre.
  - (c) The animals at the centre shall be kept and cared for in accordance with the requirements set out.
  - (d) The technical activities at the centre in respect of collection, evaluation, processing, labelling and storage of genetic material shall be carried out in terms of the requirements set out.
  - (e) Records shall be kept and preserved at the centre in accordance with the requirements set out.

## **B. Approval of animals as donors of genetic material**

- (1) An application for the approval of an animal for the collection of genetic material shall:
  - (a) be made on a form that is obtainable from the registrar for this purpose; and
  - (b) be accompanied by:
    - (i) the application fee specified;
    - (ii) an extended two generation pedigree of the animal concerned;
    - (iii) a blood typing of DNA certificate as required by the animal breeders' society concerned confirming parentage and/or individual identification.
    - (iv) a certificate based on the pedigree of the animal concerned, as issued by the relevant registering authority; and
    - (v) the performance or breeding values data of the animal, certified by the organization contracted by the Department to operate the integrated registration and genetic information system or by an independent registering authority operating an approved performance testing scheme for the breed and the animal concerned.
- (2) After the documentation in subregulation 1(b) has been furnished to the registrar, the registrar shall:
  - (a) verify the information supplied with the animal breeders' society concerned;
  - (b) notify the applicant to arrange for the examination of the animal concerned by a veterinarian, with a view to the furnishing of a certificate required;
  - (c) such an examination referred to in paragraph (b) shall be conducted under the conditions set out in the certificate obtainable from the registrar.
- (3) An animal of a kind referred to in column 1 of Table 2 of the Annexure (not included in the manual) that is intended for the collection of genetic material, shall only be approved for this purpose in the absence of hereditary defects referred to in column 2 of the said table.
- (4) Where known chromosomal abnormalities occur in a specific breed, a karyotyping certificate of clearance shall be submitted.
- (5) An animal of a breed referred to in column 1 of Table 3 of the Annexure (not included in the manual) shall have proven performance data with reference to at least the required performance parameters referred to in column 2 of the said table opposite thereto, in order to be considered for approval for the collection of genetic material.

- (6) The Act shall be applicable to all breeds of animals specified in Table 6 of the Annexure.

### **C. Keeping and care of animals at centres**

- (1) Subject to the provisions of sub-regulation (2):
- (a) only animals that are approved for the collection of genetic material may be admitted to or kept in a centre other than the quarantine centre thereof; and
  - (b) and animal shall be removed from a centre within 14 days of the date of a written notice by the registrar that:
    - (i) an application for the renewal of the approval of such an animal has been refused;
    - (ii) the approval of such an animal has been withdrawn; or
    - (iii) the registrar has withdrawn an approval granted in terms of subregulation (2).
- (2) The registrar may on application approve in writing that an animal other than one referred to in subregulation (1)(a), may be kept at a centre for the purpose specified in such approval.
- (3) An application referred to in subregulation (2) shall:
- (a) be made on a form that is obtainable from the registrar for that purpose; and
  - (b) be accompanied by:
    - (i) the application fee
    - (ii) a certificate issued by a veterinarian who is an officer, setting out the general state of health of the animal concerned and confirming that the animal is free of any disease.

### **D. Technical activities at centres**

- (1) The technical activities at a centre shall:
- (a) in so far as they apply to the state of health of the animals kept therein, be under the control of a veterinarian; Provided that if a full time veterinarian is not in the full time employment of a centre, the centre shall be visited on a regular basis by a veterinarian for the said purpose; and
  - (b) in so far as they apply to the collection, evaluation, processing, packing, labelling and storage of genetic material, be under the control of a veterinarian or a registered semen collector or an embryo collector; as the case may be.
- (2) The equipment at the centre for the collection of genetic material shall be cleaned, sterilised prepared prior to their use and the apparatus to be used for the evaluation, processing, packaging and labelling thereof, shall be clean and sterile.
- (3) Equipment and apparatus shall be used in such a manner that genetic material of different animals shall not become mixed, and that such genetic material shall not be contaminated or damaged.
- (4) The diluent for semen and the medium in which an embryo is prepared or preserved for transfer, shall not contain any micro-organisms of substance injurious or be detrimental to such semen, embryo or animal that is inseminated or to which an embryo is transferred.
- (5) Each dose of semen, excluding semen packed in pelleted form, and each embryo/ovum or batch of embryos/ova shall be packed in separate container that shall be sealed in such a manner that the semen or embryo/ovum shall not spill or become contaminated.
- (6) When the semen is packed in pelleted form, the semen of each animal from which it is collected shall be packed separately in the manner explained in subregulation (5)

- (7) Each container in which a dose of genetic material is packed shall be marked or labelled either in codified form or otherwise, with the following particulars:
  - (i) The name or code number of the centre where such genetic material has been collected.
  - (ii) The identification of the animal from which it has been collected.
  - (iii) The date on which such genetic material has been collected, or the batch number of the genetic material from which such dose of genetic material has been obtained.
  - (iv) In the case of an embryo, the identification of both the donor of the semen and the ovum used in the fertilisation and nidation thereof.
- (8) The particulars referred to in subregulation (7), shall be marked or labelled in a manner that is clear and legible and that shall not be effaced during storage, conveyance or handling.
- (9) Each dose of semen from an animal of a kind specified in column 1 of Table 4 in the Annexure shall contain at least the number of unfrozen spermatozoa specified in column 2 of the said table.

#### **E. Records to be kept at centres**

- (1) The holder of a registration certificate in respect of a centre shall keep the following records in respect of an animal from which genetic material is collected and of such genetic material:
  - (a) The identification of the animal from which the semen or ova are collected and, in the case of an embryo, the identification of the animal from which the semen has been used for the fertilisation of the ovum concerned as well as the identification of the donor animal of the ovum concerned.
  - (b) The dates on which genetic material has been collected from each such animal, and if applicable, the batch number allocated to such genetic material: Provided that if a batch of genetic material is unfit for use, the date on which it is destroyed shall be recorded.
  - (c) The number of doses of genetic material packed from each such batch.
  - (d) The name and address of each person to whom genetic material from each animal has been sold, the date of such sale and the number of doses of genetic material thus sold.
- (2) The records referred to in subregulation (1) shall be kept on the premises of the centre concerned for at least two years after the date on which the last genetic material of the animal concerned has been sold or destroyed.

#### **F. Disease testing**

As previously stated a specific veterinary certificate is required with the application for approval for a bull for artificial insemination. This certifies the following:

- (1) Donor bull identification;
- (2) Clinical examination and findings (entry);
- (3) Routine tests for disease;
- (4) Semen evaluation;
- (5) Clinical examination and findings (exit).

This is certified by the veterinary surgeon in control of the AI centre and pertains to examinations done whilst the bull is in pre-quarantine. Once the endorsement by an official veterinarian, the bull may enter the quarantine area of the AI centre.





**Annex 4**  
**APPLICATION FOR APPROVAL OF A BULL FOR USE IN**  
**ARTIFICIAL INSEMINATION**  
 (Source: South Africa)

**Veterinary Certificate Issued in Terms of the Requirements of Article 9(3)(A) of the**  
**Livestock Improvement Act, 1977 (No25/77) and Animal Disease Act, 1984**

CENTRE:
NAME/ IDENTIFICATION/ MICROCHIP NUMBER OF BULL:
BREED:
DATE OF BIRTH:
REGISTRATION NUMBER:
AI CODE:

1	<b>Clinical examination</b> (ON FIRST DAY OF QUARANTINE) (General health, testis, penis, accessory glands and presence of hereditary deficiencies)
	Date of examination: ____/____/____ yy      mm      dd
	Findings:
	Remarks:
2	<b>TEST ROUTINE</b> (Done within a month of semen collection) <span style="float: right;"><b>RESULTS</b></span>
	<u>TUBERCULOSIS:</u> Date of intradermal injection: _____ Date of reading: _____
	<u>BRUCELLOSIS:</u> CFT or ELISA
	<b><i>TRICHOMONIASIS: (Three sheath washes at one week interval)</i></b> Date of first washing: _____ Date of second washing: _____ Date of third washing: _____
	<b><i>CAMPYLOBACTEROSIS: (Three sheath washes at one week interval)</i></b> Date of first washing: _____ Date of second washing: _____ Date of third washing: _____
	<u>LEPTOSPIROSIS:</u> MAT

3	<b>Semen evaluation</b>	
	<i>MACROSCOPIC</i>	
	Amount:	
	Density:	
	Colour:	
Date:		
	<i>MICROSCOPIC</i>	
	Mobility:	
	Neutrophiles:	
	% abnormalities:	
	Date:	
4	<b>CLINICAL EXAMINATION</b> (on last day of quarantine) (General health, testis, penis, accessory glands and presence of hereditary deficiencies)	
	Date of examination: ____ / ____ / ____ yy mm dd	
	Findings:	
	Remarks:	
5	<b>CERTIFICATION</b> (by private veterinarian)	
	I hereby certify that the above information is to the best of my knowledge true and correct. A clinical examination was performed by me on _____ and _____ and the above described bull was found healthy and free from any infectious disease to which cattle are susceptible.	
	_____	_____
	Signature	Date
	_____	_____
	Name in capital letters	SAVC registration number

6	<b>ENDORSEMENT</b> (by an official veterinarian)				
<p>I, a veterinarian authorised by the South African Veterinary Administration hereby endorse the certification done above by the qualified veterinarian and <b><u>recommend/do not recommend</u></b> the use of above described bull for semen collection for artificial insemination purposes.</p> <table border="0" style="width: 100%;"> <tr> <td style="width: 50%; border-top: 1px solid black; vertical-align: bottom;">Signature</td> <td style="width: 50%; border-top: 1px solid black; vertical-align: bottom;">Date</td> </tr> <tr> <td style="border-top: 1px solid black; vertical-align: bottom;">Name in capital letters</td> <td style="border-top: 1px solid black; vertical-align: bottom;">SV area</td> </tr> </table> <div style="text-align: center; border: 1px solid black; width: fit-content; margin: 0 auto; padding: 10px;"> Official Stamp </div>		Signature	Date	Name in capital letters	SV area
Signature	Date				
Name in capital letters	SV area				



**Annex 5**  
**RECOMMENDATIONS OF OIE ON DISEASE TESTING**  
**FOR BULLS AND TEASERS**

**The Office International des Epizooties (OIE)**  
**International Animal Health Code (2001), Section 3.2**

(Website: [http://www.oie.int/eng/normes/mcode/A\\_summry.htm](http://www.oie.int/eng/normes/mcode/A_summry.htm))  
Article 3.2.1.5.

**Conditions applicable to testing of bulls and teaser animals**

Bulls and teaser animals can enter an *artificial insemination centre* only if they fulfil the requirements laid down by the *Veterinary Administration*.

1. *Pre-quarantine testing*

Bovines must appear healthy and normal and must comply with the following requirements prior to entry into isolation at the *quarantine station* prior to entering the semen collection facilities.

a) Bovine brucellosis

The animals should comply with the provisions referred to in Article 2.3.1.5. of the *Code*.

b) Bovine tuberculosis

The animals should comply with the provisions referred to in Article 2.3.3.4. of the *Code*.

c) Bovine viral diarrhoea-mucosal disease (BVD-MD)

A virus isolation test or a test for virus antigen (immunoperoxidase, PCR or ELISA) should be carried out, with negative results.

d) Infectious bovine rhinotracheitis-infectious pustular vulvovaginitis (IBR-IPV)

The animals should comply with the provisions referred to in Article 2.3.5.4. of the *Code*.

2. *Testing in the quarantine station prior to entering the semen collection facilities*

Prior to entering the semen collection facilities of the *artificial insemination centre*, bovines must be kept in a *quarantine station* for at least 28 days. The animals should be subjected to diagnostic tests as described below a minimum of 21 days after entering the *quarantine station*, except for *Campylobacter fetus* and *Trichomonas fetus*, for which testing may commence after at least 7 days in quarantine, and the results should be negative except in the case of BVD-MD antibody serological testing (see point 2c)i below).

- a) Bovine brucellosis  
The animals should comply with the provisions referred to in Article 2.3.1.5. of the *Code*.
- b) Bovine tuberculosis  
The animals should comply with the provisions referred to in Article 2.3.3.4. of the *Code*.
- c) BVD-MD
  - i) All animals should be subjected to a serological test to determine the presence or absence of BVD-MD antibodies.
  - ii) All animals should be tested for viraemia as described in point 1c) above.
  - iii) Only if all the animals in quarantine test negative for viraemia may the animals enter the semen collection facilities upon completion of the 28-day quarantine period.
  - iv) If any animals test positive for viraemia, all these and the other animals of the same group should remain in quarantine and be retested not less than 21 days after the positive test. Animals that are positive to this second test for viraemia should be considered persistently infected with BVD-MD virus and should not be allowed entry into the semen collection facilities. Animals that are negative to this second test should be considered not persistently infected with BVD-MD virus and may enter the semen collection facilities.
- d) *Campylobacter fetus* subsp. *venerealis*
  - i) Animals less than 6 months old or kept since that age only in a single sex group prior to quarantine should be tested once by culturing a preputial specimen, with a negative result.
  - ii) Animals aged 6 months or older that could have had contact with females prior to quarantine should be tested three times at weekly intervals by culturing a preputial specimen, with a negative result in each case.
- e) *Trichomonas fetus*
  - i) Animals less than 6 months old or kept since that age only in a single sex group prior to quarantine should be tested once by culturing a preputial specimen, with a negative result.
  - ii) Animals aged 6 months or older that could have had contact with females prior to quarantine should be tested three times at weekly intervals by culturing a preputial specimen, with a negative result in each case.
- f) IBR-IPV  
The animals should comply with the provisions referred to in Article 2.3.5.4 of the *Code*.

3. *Testing for BVD-MD prior to the initial dispatch of semen from each serologically positive bull*

Prior to the initial dispatch of semen from BVD-MD serologically positive bulls, a semen sample from each animal should be subjected to a virus isolation or virus antigen ELISA test for BVD-MD. In the event of a positive result, the bull should be removed from the centre and all of its semen destroyed.

4. *Testing programme for bovines resident in the semen collection facilities*

All bovines resident in the semen collection facilities should be tested at least annually for the following diseases, with negative results:

a) Bovine brucellosis

The animals should comply with the provisions referred to in Article 2.3.1.5. of the *Code*.

b) Bovine tuberculosis

The animals should comply with the provisions referred to in Article 2.3.3.4. of the *Code*.

c) BVD-MD

Animals negative to previous serological tests should be retested to confirm absence of antibodies.

d) *Campylobacter fetus* subsp. *Venerealis*

i) A preputial specimen should be cultured.

ii) Only bulls on semen production or having contact with bulls on semen production need to be tested. Bulls returning to collection after a lay off of more than 6 months should be tested not more than 30 days prior to resuming production.

e) *Trichomonas fetus*

i) A preputial swab should be cultured.

ii) Only bulls on semen production or having contact with bulls on semen production need to be tested. Bulls returning to collection after a lay off of more than 6 months should be tested not more than 30 days prior to resuming production.

f) IBR-IPV

The animals should comply with the provisions referred to in Article 2.3.5.4. of the *Code*.





## Annex 6 NUTRITION OF BULLS

Rations for bulls in South Africa are made up as follows:

The raw materials included in the bull concentrate are:

- Yellow Maize meal
- Wheat middling
- Soya oil cake
- Sunflower oil cake
- Molasses
- Limestone powder
- MCP
- Urea
- Vitamin/ Mineral pre-mix

The following table is used as guide for feeding the bulls. The feeding is to suit a blend of the 17% crude protein concentrate and hay.

BODY WEIGHT (Kg)	FEED (KG DM)	PROTEIN (g) REQUIRED (Dairy)	TDN (Kg) REQUIRED	CONC. (Kg) REQUIRED	HAY (Kg) REQUIRED
150	4.5	540	2.75	3.0	1.5
200	6.0	750	3.9	3.5	2.5
280	8.0	900	4.55	3.8	4.0
350	8.8	950	5.6	4.0	4.8
400	9.5	1000	6.2	4.2	5.3
450	10.0	1050	6.4	4.3	5.7
500	10.2	1055	6.6	4.5	5.9
Greater	-	-	-	4.5	<i>Ad Lib</i>

Important points:

- a) The range of concentrates is between 3 and 5 Kg per day.
- b) Depending on condition, slightly more or less concentrates could be fed, e.g., 1 to 5 Kg.
- c) The above table does not allow for wastage.
- d) The hay is obviously fed to maximum that the bull can eat.



## **Annex 7**

### **BODY CONDITION SCORING SCHEME**

#### *Condition Score 1:*

The cow is emaciated. The ends of the short ribs are sharp to the touch and together they give a prominent shelf like appearance to the loin. The individual vertebrae (spinous processes) of the backbone are prominent. The hook and pin bones are sharply defined. The thurl region and thighs are sunken and incurving. The anal area has receded and the vulva appears prominent.

#### *Condition score 2:*

This cow is thin. The ends of the short ribs can be felt but they and the individual vertebrae are less visibly prominent. The short ribs do not form as obvious an overhang or shelf effect. The hook and pin bones are prominent. But the depression of the thurl region between them is less severe. The area around the anus is less sunken and the vulva less prominent.

#### *Condition Score 3:*

A cow in average body condition. The short ribs can be felt by applying slight pressure. The overhanging shelflike appearance of these bones is gone. The backbone is a rounded ridge and hook and pin bones are round and smoothed over. The anal area is filled out but there is no evidence of fat deposit.

#### *Condition score 4:*

A cow in heavy condition. The individual short ribs can be felt only when firm pressure is applied. Together they are rounded over with no shelf effect. The ridge of backbone is flattening over the loin and rump areas and rounded over the chine. The hook bones are smoothed over and the span between the hook bones over the backbone is flat. The area around the pin bones is beginning to show patches of fat deposit.

#### *Condition score 5:*

A fat cow. The bone structure of the topline, hook and pin bones and the short ribs is not visible. Fat deposits around the tailbone and over the ribs are obvious. The thighs curve out, the brisket and flanks are heavy and the chine very round.

These descriptions are taken from the Ontario Ministry of Agriculture and Food. Illustrations can be found at the website:

<http://www.gov.on.ca/OMAFRA/english/livestock/dairy/facts/92-122.htm#Score>.

The Australian system for condition scoring beef cattle is similar. See website:

[http://www.nre.vic.gov.au/web/root/domino/cm\\_da/nrenfa.nsf/frameset/NRE+Farming+and+Agriculture?OpenDocument](http://www.nre.vic.gov.au/web/root/domino/cm_da/nrenfa.nsf/frameset/NRE+Farming+and+Agriculture?OpenDocument)



**Annex 8**  
**SEMEN EVALUATION USING NIGROSIN-EOSIN STAIN**  
**AND BUFFERED FORMOL-SALINE SOLUTION**

A buffered nigrosin-eosin solution is used to prepare the semen for a count of live spermatozoa. It is most convenient if this solution is readily available in small vials for field use. Semen and stain should be at the same temperature, preferably 35-37°C. Semen is mixed with the stain. Immediately afterwards or after standard incubation time (e.g. 3 minutes at 35-37°C) a thin smear is made from the mixture and allowed to dry. Examination is preferably done using the oil-immersion lens of a light microscope.

Where nigrosin-eosin has diffused into the cell, it is pink and is counted as dead. Live sperm have no pink colour present and remain white. At least 200 sperm should be counted and scored as live or dead, in two sets of 100, so that good agreement can be seen between the two sets. If there is not good agreement another 200 spermatozoa should be counted. A mean percentage of live sperm calculated.

Another 200 sperm heads can be counted in a similar way to record head shape. They are classed as normal head shape or abnormal (narrow, narrow at base, pear shaped, abaxial, small, large, undeveloped, other).

A further 200 spermatozoa can be counted in a similar way to record findings on the mid-piece and tail. They are classed as normal mid-piece and tail or abnormal (proximal cytoplasmic droplet, distal cytoplasmic droplet, tailless head, singly bent tail, doubly bent tail, coiled tail, other). For this count a wet fixed preparation of the semen in buffered formol saline can be used with phase contrast or differential interference contrast microscopy.

### **Nigrosin-Eosin Stain**

#### Nigrosin solution

Dissolve 10 g nigrosin (G.T. Gurr) in 100 ml of distilled water. Boiling and adding small amounts of nigrosin at a time will help in the dissolving process.

#### Stock buffer solution

- (A) Disodium hydrogen phosphate ( $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ ): 21.682 g in distilled water to make a volume of 500 ml.
- (B) Potassium dihydrogen phosphate ( $\text{KH}_2\text{PO}_4$ ): 22.254 g in distilled water to make a volume of 500 ml.

*Stock Buffer Solution* consists of 200 ml of Solution A and 80 ml of Solution B.

#### Stock glucose solution

48.3 g Glucose ( $\text{C}_6\text{H}_{12}\text{O}_6$ ) in distilled water to make a volume of 500 ml.

*Composition of Nigrosin-Eosin Stain solution (Used in Eppendorf Vials)*

Nigrosin solution	150 ml
Eosin yellowish	5 g
Stock buffer solution	30 ml
Stock glucose solution	30 ml
Water to make up a volume of	300 ml

**Buffered Formol-Saline Solution**

A buffered formol-saline solution is used to prepare the semen for a count of abnormalities associated with the mid-piece and tail under phase contrast at 400x. This is included since it is useful for making a permanent fixed preparation of a bull's spermatozoa. When phase contrast is not available the same assessment can be made on the nigrosin-eosin stained smear.

Stock saline solution:

9.01 g NaCl in distilled water to make up a volume of 500 ml.

Composition of Buffered Formol-Saline Solution:

Stock buffer solution (see above)	100 ml
Stock saline solution	150 ml
Formalin solution 40% w/v	62.5 ml
Water to make a volume of	500 ml

**Annex 9**  
**SEMEN DILUENTS AND EXTENDERS**

**A. Preparation of Room Temperature Diluent for Semen (from Central Artificial Insemination Station, Kabete, Kenya)**

Dissolve 300 mg of Sulphanilamide in 25 ml of distilled water by warming to allow it to dissolve.

Add the following to a separate flask

Sodium Citrate	2200 mg
Penicillin	60 mg
Dihydrosterptomycin	135 mg
Polymixin B sulphate	10 mg
Dissolved in	50 ml distilled water

Add

Coconut water	17 ml
Egg yolk	7 ml
Mycostatin solution	1 ml
Catalase	0.5 ml (few drops)
Sulphanilamide	300 mg

Fill up to 100 ml using distilled water

Adjust pH to 7.4 with a 10% NaOH solution (10 g NaOH + 100 ml distilled water)

Mycostatin solution: 10 mg Mycostatin and 50 ml distilled water (use only for two weeks after preparation). At present Mycostatin is not available. Instead, Lincomycin is used (dissolve 375 mg Lincomycin HCl in 50 ml water and use 6 ml in the diluent)

For coconuts, use a ripening stage (called madafu). This is before the soft kernel becomes hard. Boil for 15 minutes, cool and filter.

**B. Caprogen (MacMillan and Shanon, 1982)**

The stock buffer is made up as follows.

20 g trisodium citrate
10 g glycine
3 g glucose
12.5 ml glycerol
25 mg sodium sulphacetamide
1.25 x 10 <sup>6</sup> units crystalline benzyl penicillin
1.25 x 10 <sup>6</sup> units streptomycin sulphate



For 20% egg yolk Caprogen use:

- 77 parts of the stock buffer
- 1 part 2.5% solution of n- hexanoic acid
- 1 part 0.05% Chloramphenicol
- 1 part of a solution of 0.45 mg beef liver catalase per ml
- 20 parts fresh egg yolk.

The pH should be 6.8.

The diluent is prepared in a 1 litre cylinder and then chilled to 2°C. Nitrogen is then bubbled through the mixture for 30 minutes to saturate it and stored overnight at 2°C. It is warmed to room temperature before use.

**C. TRILADYL<sup>®</sup> (Minitube) is a synthetic diluent made up as follows:**

- 1 x Triladyl concentrate
- 1 x egg yolk
- 3 x distilled water

TRILADYL<sup>®</sup> contains lactose as a non-permeating and glycerol as a permeating cryoprotectant. These two agents bind and compete with water and reduce the freezing point of the liquid.

Egg yolk contains lipoproteins and phospholipids that bind to cell membranes and protects them against cold- and osmotic shock helping them through the critical temperature range.

TRILADYL<sup>®</sup> also contains TRIS and citric acid as pH buffers and 4 different antibiotics (Tylosin, spectinomycin, gentamycin and lincomycin).

**D. BIOXCELL (IMV Technologies)** is another recently released synthetic diluent for freezing semen which is made up with just the addition of ultrapure water. It does not contain animal products. The makers claim high fertility with low concentrations of spermatozoa.

**E. ANDROMED (Minitube)** also contains no egg yolk but synthetic agents.

**Annex 10**  
**LIQUID NITROGEN SAFETY PRECAUTIONS**

Liquid nitrogen has a temperature of  $-196^{\circ}\text{C}$  and is extremely hazardous to handle. If the skin comes in contact with the material serious burns can occur. Nitrogen vapour, if inhaled, can cause hypoxia leading to respiratory distress.

The following recommendations must be followed:

- Avoid all skin contact with liquid nitrogen.
- Ensure adequate ventilation of the room when working with it.
- Secure tanks well during transport.
- Avoid transporting tanks in the passenger compartment of a vehicle.

Spillage through dislodgement or a traffic accident can result in burns and dangerous levels of nitrogen gas in the vehicle.

If a skin burn occurs flood the area with cold water and apply a cold compress. Seek immediate medical attention if eyes have been affected or the skin is blistered.

If dizziness or loss of consciousness occurs due to lack of oxygen move the person to a well ventilated area. If breathing has stopped apply artificial respiration and call an ambulance.

Check liquid nitrogen tanks regularly for evidence of frost on the outside and for excessive loss of liquid. Either state indicates a breakdown of insulation. Check the level of the liquid nitrogen using a solid dipstick (caution: hollow dipsticks can cause the liquid to spray upwards).



## Annex 11 GUIDELINES FOR TRAINING AI TECHNICIANS

The following core competencies are essential for an AI technician (AIT) who requires certification or registration. Other components or modules may be added as required by individual countries.

- (i) The theoretical component of the course should be designed so that the trainees will acquire a *comprehensive knowledge* of:
  - Anatomy and physiology of bovine male and female reproductive systems
  - Heat detection methods and importance of correct timing of AI
  - All steps involved in the AI technique and the hygienic requirements
  - Hygienic and safe handling of semen
  - Types of AI equipment, their use and cleanliness
  - Semen production procedures at AI centres
  - Factors influencing AI results, errors and inefficiencies
  - Herd fertility and its economic importance
  - Nutrition and its effects on fertility
  - Maintaining good working relationships with farmers and other service providers
  - Legislation relating to livestock breeding in his/her country
- (ii) It should also provide them with an *understanding* of:
  - Selection of breeding stock, interpretation of indices and progeny testing
  - Good record keeping and reporting
- (iii) The practical component of the course should include:
  - Examination and handling of specimens of reproductive organs of the cow, both directly and using a simulated cow where available
  - Palpation *per rectum* of the reproductive organs in live cows to assess their reproductive status
  - Handling and manipulating AI equipment
  - Handling semen correctly and performing all steps in transferring semen from the transport container to the cow
  - Restraining and handling cows
  - Passing the insemination pistol/gun through the cervix of live cows easily and safely, and correctly placing the semen
  - Accurately filling in the records required

For “Do-it-Yourself” technicians (DIYs) who do not require registration, all the above should included, *except* the following theoretical components:

- Semen production procedures at AI centres
- Herd fertility and economic importance
- Nutrition and effects on fertility
- Maintaining good working relationships with farmers and other service providers
- Legislation relating to livestock breeding in his/her country
- Selection of breeding stock, interpretation of indices and progeny testing

For AITs who will be involved in farmer services based on milk progesterone assay, the following components should also be included:

- Hormonal changes during the oestrous cycle of the cow
- Basis of the progesterone measurement for assessing reproductive status
- Collection, transport, processing and storage of milk samples and factors influencing progesterone concentration
- Records necessary at the time of insemination
- Interpretation of progesterone levels in milk samples
- Advice to be given to the farmer based on progesterone results

Evaluation of the trainee's knowledge and competencies on the above course components should include both theoretical and practical examinations.

Where possible, trainees completing the course should obtain field experience by inseminating a minimum of 30 cows under appropriate supervision of an experienced AIT before commencing independent work.

Refresher courses and continuing education are encouraged, and should be designed according to the above objectives and guidelines.

**Annex 12**  
**INDIVIDUAL COW AI RECORD**  
*IAEA/AFRA Project on Improving Milk and Meat Production*

Farmer: \_\_\_\_\_ Farm: \_\_\_\_\_  
 Address: \_\_\_\_\_  
 Breed: \_\_\_\_\_  
 Breed of Sire: \_\_\_\_\_ Breed of Dam: \_\_\_\_\_ Milking only: Yes/No  
 Birth Date: \_\_\_\_\_ Lactation No.: \_\_\_\_\_ Suckling: for let down only/once per day/twice per day/  
 Last Calving date: \_\_\_\_\_ Remarks: \_\_\_\_\_ *Ad libitum/other* .....

AI No	Date	Heat to AI (hr)	AI Time (am/pm)	Site of AI (U/C/V*)	Bull & Breed	Semen Batch	Date of Milk Sample			Result of Milk Progesterone (nmol/l)			PD date & Result	Remarks
							1	2	3	1	2	3		

(\* U = uterus; C = cervix; V = vagina)

Name of Inseminator: \_\_\_\_\_ Signature: \_\_\_\_\_ Date: \_\_\_\_\_

Laboratory Interpretation and Recommendations:

Name: \_\_\_\_\_ Signature: \_\_\_\_\_ Date: \_\_\_\_\_



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