IAEA-TECDOC-1480

Improving artificial breeding of cattle and buffalo in Asia Guidelines and recommendations

A manual prepared under the framework of an IAEA Technical Cooperation Regional RCA Project on "Improving Animal Productivity and Reproductive Efficiency", with technical support of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture

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The originating Section of this publication in the IAEA was:

Animal Production and Health Section
International Atomic Energy Agency
Wagramer Strasse 5
P.O. Box 100
A-1400 Vienna, Austria

IMPROVING ARTIFICIAL BREEDING OF CATTLE AND BUFFALO IN ASIA
GUIDELINES AND RECOMMENDATIONS
IAEA, VIENNA, 2005
IAEA-TECDOC-1480
ISBN 92–0–112005–2
ISSN 1011–4289

Printed by the IAEA in Austria
November 2005

FOREWORD

The International Atomic Energy Agency (IAEA) and the Regional Cooperative Agreement for Asia and the Pacific Region (RCA), with technical support of the Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture, implemented a Technical Cooperation (TC) project entitled Improving Animal Productivity and Reproductive Efficiency. The dual objectives of this project are (a) strengthening and extending the field applications of Urea Molasses Multinutrient Blocks (UMMB) and other feed supplementation strategies, and (b) monitoring and improving the reproductive management and fertility of smallholder dairy cattle subjected to Artificial Insemination (AI). The radioimmunoassay (RIA) for measurement of progesterone in milk and use of the computer database AIDA (Artificial Insemination Database Application) play important roles in the success of the latter objective. The first meeting to plan project activities was held in January 1999 in Yangon, Myanmar and the second meeting to review progress and develop further work plans was held in February 2000 in Kuala Lumpur, Malaysia. The latter meeting concluded that the procedures currently used by different Asian countries for evaluation of breeding bulls should be standardized and unified protocols developed for ensuring quality control of semen during processing, storage and field use. It was recommended that this should be accomplished through a regional workshop of national consultants.

A workshop of national consultants from 10 RCA Member States was therefore held in April 2002 in Faisalabad, Pakistan, to consider and discuss the following aspects and arrive at a consensus on the best procedures and practices to be adopted to suit conditions and needs in developing countries of Asia:

- Selection, management and health control of AI bulls
- Semen technologies from collection through processing to storage
- Delivery and follow-up of field AI services to farmers

The IAEA has also supported a similar project in Africa under the AFRA programme, to monitor and improve AI services. A recent Task Force meeting held under this project to "Harmonize Procedures for Selection and Management of AI Bulls and Use of Semen Technology in African Countries" resulted in the compilation of a manual of guidelines similar to that envisaged for Asian countries. That manual was therefore used as a basic framework and necessary modifications and additions were done to meet the needs of RCA Member States and, in particular, to include aspects relevant to buffaloes.

The main conclusions and recommendations from the RCA workshop were that the major challenges in the Asian region were:

- Lack of a sound system for evaluation of breeding bulls;
- Improper semen handling;
- Inadequate and expensive supply of liquid nitrogen;
- Inconsistent heat detection, incorrect timing of AI and poor hygiene;
- Inadequate AI recording, follow-up and reporting;
- Lack of clear breeding policies for cross-breeding;
- Inadequate logistic support to field services; and
- Lack of incentive schemes and accountability.

It was clear that no universal remedy was possible for the identified problems, but there were common technical and procedural aspects that can be improved and standardized

for application in the Asian region. The workshop therefore considered and reached consensus on the most appropriate techniques and procedures for obtaining optimum results under the prevailing conditions in Asia.

The draft document prepared during the workshop was edited and the second draft was circulated to all national project coordinators for comments and suggestions for improvement. It was finally discussed at a Project Review and Planning Meeting held in Hangzhou, China in November 2002 and further material was incorporated as required.

This manual therefore builds upon the experience gained in compiling a similar manual for use in African countries and is the result of collaboration between the national project coordinators of the RCA project, several experts in AI in the participating Member States, IAEA experts who assisted with the project and the technical officer from the Joint FAO/IAEA Programme.

This manual is aimed at all levels of administrative and technical personnel involved in the provision of AI services to cattle and buffalo farmers in Asia, including Ministries of Agriculture/Livestock, Directorates of Livestock and Veterinary Services, AI Centres, Semen Distribution Centres, local authorities responsible for livestock development services, Faculties of Veterinary and Animal Sciences, and Institutions for breeding research and training of AI technicians.

It is hoped that the manual will assist livestock personnel in Asia to apply the knowledge that already exists in an efficient, cost effective and sustainable way in different cattle and buffalo farming systems under varying socio–economic environments.

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

CONTENTS

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 over view of sire proofs and cow indexes (Estimated Transmitting Ability or ETA), milk recording, linear classification and aspects of genetic improvement are given in Annex 2. In the absence of these schemes, a "likeability" system should be established (see Section1.1.3).

The major breeds of cattle and buffaloes of importance for milk, meat and work in the Asian countries represented at the RCA Workshop of National Consultants that developed this manual are given in Annex 3.

1.1.1. Milk recording

Individual animals should be clearly identified by ear tags or another equally effective technique (ear notching, collars, freeze brands, electronic devices). Basic milk recording entails regular milk weights/volume and analysis for percentage of butterfat and protein. 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 best linear unbiased prediction (BLUP) system is used, which makes maximum use of the pedigree information available. The EBVs are calculated for birth weight, 200-day weight (direct and 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. The EBVs 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; likeable with respect to most characteristics
3 = Average animal
2 = Below average but acceptable
1 = Not a good animal; not liked at all

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

1.1.4. Reproductive efficiency

Bulls selected on the basis of their genetic merit should also be subjected to a general clinical examination, an examination of the reproductive organs, a semen examination and freezability test and an assessment of serving ability. These tests 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 and the first service conception rate should be at least 50 %.

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 record keeping, from the University of Melbourne, Australia, is given in Annex 4.

1.2. Genetic improvement

It should be stressed that fulfilment 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 breeding livestock and the industry advises on contract matings and later inspects the calves, selecting individuals on breed type, health and conformation for entry into the progeny test programme. A minimum of 500 doses of each young bull's semen should be used in 20 to 30 herds in the progeny test scheme. This distribution process should result in at least 20 daughters from each bull. However, under small-holder farming systems, obtaining this number of daughters is often not possible and alternative procedures need to be developed and documented.

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, traits such as 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. 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 moderate 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

Type of cattle and traits	Heritability (%)
Beef Cattle	
Highly heritable traits	
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
Traits of medium heritability	
Weaning Weight	25
Carcass yield grade	30
Traits of low heritability	
Calving interval	10
Longevity	0
Dairy Cattle	
Highly heritable traits	
Birth weight	50
Butter fat percentage	40
Mature weight	35
Traits of medium heritability	
Milk production	25
Fat production	25
Protein production	25
Excitability	25
Traits of low heritability	
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. Insemination 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 schemes.

Normal conformation and functionality: selection of bulls, semen or cows for genetic improvement under Asian conditions should be based on fertility and production performance within Asian 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. Asian environments are harsher than the European and North American environment. Selecting for sustained high production under different Asian 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 Asian 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).

Fig. 1. Indigenous bulls in the tropics at an AI station. Their genetic worth for the local small-holder farmer needs to be defined. Note cool housing.

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.

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-breds exceeds the average of the parental breeds. Heterosis occurs because the parental animals differ in gene composition and that dominant genes carry more favourable effects on traits than do 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 female from a single cross (F1 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 maximum heterosis. Pure-bred males are used on cross-bred females.
- *Three-breed terminal cross* – The F1 cross-bred females are mated to males of a selected third breed and all offspring (F2) slaughtered for meat production. More heterosis can be achieved with this method than with a three-breed rotational cross.

Under conditions in Asia, cross breeding of native cattle with several exotic dairy breeds like Holstein-Friesian and Jersey has been practiced on a large scale. The F1 progeny thus produced have given very promising results, particularly in terms of increased milk production, in almost all involved countries. However, in the absence of clear-cut breeding plans and programmes, further breeding of F1 progeny has resulted in subsequent generations of F2 and beyond. In these later generations the advantages observed in the F1 generation have markedly deteriorated, causing great frustration and disillusionment among cattle raisers regarding the value of cross breeding. There is a need for each country to examine the issue critically, to clearly define the breeding goals and to implement strict selection programmes.

The improvement of indigenous breeds through selective breeding, where relevant, should also be given serious consideration.

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 organisation. 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 sterilisation
- 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 5. Some of these may be universally applicable, but others need to be modified and or adapted for the needs of the Asian countries, which have different conditions.

A specimen form to apply for approval of a bull for use in AI is given in Annex 6.

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 ; 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 7.

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. 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. Breeding bulls should have a score of 3 on a scale of 1-5. Bulls should have access to mineral licks and clean water *ad libitum*.

1.4.3. Handling

The establishment of a firm relationship between the handler and the bull is essential and can not 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 optimised. This includes taking note of all aspects of the physiology of male sexual behaviour. Negative stimuli should be avoided in the collection area. 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. Adequate exercise and regular hoof care should be provided. Continuous monitoring of diseases should be undertaken whether statutory or not. All quarantine requirements, including restrictions in the movement of animals and personnel, must be strictly observed. 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.

Fig. 3. Strong timber construction of stanchion for teaser bull restraint. Note the non-slip floor.

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. 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.

2.1.2. Preparation of bulls

The semen donor bulls must be housed under clean dry conditions and should be washed and cleaned before they arrive at the collection area. 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 soft brush should be used to clean the bulls. During cleaning, emphasis should be put on the lower abdomen and the preputial area. If necessary, washing of the preputial sheath with normal saline solution can be done once every week or fortnight to reduce bacterial contamination of semen. Disinfectants should not be used. Clean, dry paper towels should be used after washing to remove excess water.

If the teaser bull or steer is dirty, its back should be cleaned with water and soap and dried thoroughly. An apron may be used if necessary. 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.

2.1.3. Preparation and sterilization of equipment and materials

All equipment used for the collection, evaluation and processing of semen must be clean and sterilized. The following procedures are recommended:

a) Glassware

- Wash with detergent containing 2% Na_2Co_3. If glassware has become cloudy through repeated use, leave it submerged in potassium dichromate solution ($K_2Cr_2O_7$, 8 g; H_2SO_4, 12 ml; distilled water, 100 ml) for 24 hr.
- Wash with tap water
- Rinse with distilled water
- Dry and cover in clean paper or aluminium foil
- Place in hot air oven (160°C) for 30 minutes
- Transfer to a closed, dust free incubator (37°C)

b) Rubber materials

- Wash in detergent
- Wash repeatedly in tap water
- Boil for 10-15 minutes
- Swab dry
- Store in a dust-free chamber fitted with ultraviolet radiation.

c) Buffers

- After preparation, autoclave at 120°C and 15 lb pressure for 20 minutes
- Fresh eggs of Grade A should be collected from pathogen free flocks and the shell washed and swabbed with 70% alcohol

2.1.4. Artificial vaginas

An outer rubber barrel (usually 45cm long) with rough inner rubber liner that is non-spermiotoxic is recommended. The inner liner should periodically be checked for possible leakage. The rubber cones should be also non-spermiotoxic and a correctly labelled collection tube should be attached. A jacket should be provided for the cone to prevent breakage and avoid direct exposure to sunlight. Rubber bands for holding on the cones and the two ends of the reflected inner lining onto the outer barrel should be strong.

A lubricant that is sterile, non-spermiotoxic, non irritant to the penis and easily washable (eg. KY jelly or white Vaseline that has been sterilized by boiling) should be applied sparingly and just before collection at the entrance of the artificial vagina (AV, Fig. 4). The lubricant can be replaced by a small amount of diluent to moisten the entrance to the artificial vagina.

Fig. 4. Preparation of the artificial vagina; lubricating with a glass rod using KY jelly.

Water for the outer jacket filling should be warmed to 60^{o}C. Enough of this should be poured into the inner chamber to provide the required pressure. This quantity may range from 500-750 ml. Inner temperature after lubrication should range between 40-45^{o}C. Assembled AVs should be kept in incubators at 55-60^{o}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.

2.1.5. Electroejaculators

Electroejaculators should only be used when absolutely necessary. Only lame or injured bulls should be subjected to the technique. Good training and good handling procedures allow most bulls to be collected with the artificial vagina. 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.

2.1.6. 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.

2.1.7. Collection procedure

It is advised to collect the semen early in the morning. Bulls should be led, preferably using a halter, to the teaser in a gentle friendly manner by the handler, who should pay attention to the temperament of the particular bull. The bull should be allowed to watch other bulls mounting before collection. He is led around behind the teaser and may be allowed to mount other bulls. Two false mounts are usually given (Fig. 5). These measures promote good sexual excitement, which improves the quality of semen by cleansing the urethral passage and increasing the amount of semen collected. The bull is then allowed to mount for the first collection.

Fig. 5. Sexual preparation assists in improving the bull's serving behaviour at collection. It helps to obtain more spermatozoa in the collected ejaculate.

At this time the collector shall gently grasp the prepuce behind its opening and direct the fully erected penis into the lubricated end of the AV (Fig. 6). The penis should not be touched. 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 that the bull withdraws as he dismounts, and should not be pulled away from the penis.

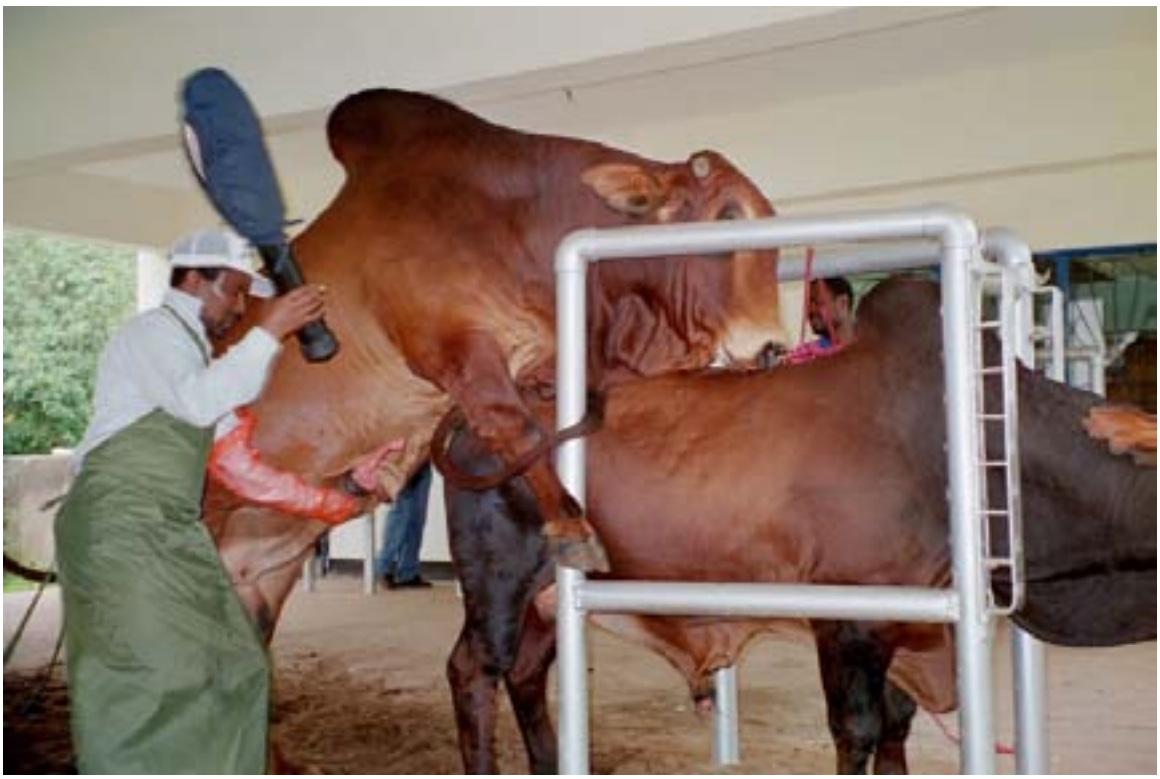

Fig. 6. Collection of semen with the artificial vagina. The left hand touches only the preputial skin, not the penis itself.

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

2.1.8. Evaluation of semen

a) Macroscopic examination

The semen should be transferred to a water bath maintained at 35±1°C. Visual evaluation for volume, colour, consistency/density, odour and observation for presence of foreign material (blood, pus cells, dung, hair, etc.) shall be made and recorded (see Semen Result Sheet of Annex 4). If dung or hair is found in the semen, filtration with a special semen filter is advised.

b) Microscopic examination

Microscopic evaluation is done using a simple or phase contrast microscope for mass activity (wave motion) 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 dried and examined under oil immersion.

Automated computerised machines for recording motility and concentration and calculating the required extensions are now frequently used in AI centres that can afford them.

Semen used for artificial insemination should be of high quality. The following are guides to the values of semen characteristics in the bull that indicate good reproductive function:

- Motility (moving actively forward): > 60%
- Concentration: > 500 million /ml
- Live sperm: > 70%
- Abnormal sperm: < 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.

A further technique that can be used to evaluate the semen is the hypo-osmotic swelling test (HOST), which indicates the functional integrity of sperm membranes. The procedure for conducting the test is given in Annex 9.

Centres should develop a system of morphological assessment and guidelines for limits beyond which semen is discarded. Morphological examination is generally reserved for borderline samples (Fig. 7). The assessment is also valuable in helping to reach a diagnosis when a bull begins to fail to produce semen of processable quality as assessed by concentration and motility.

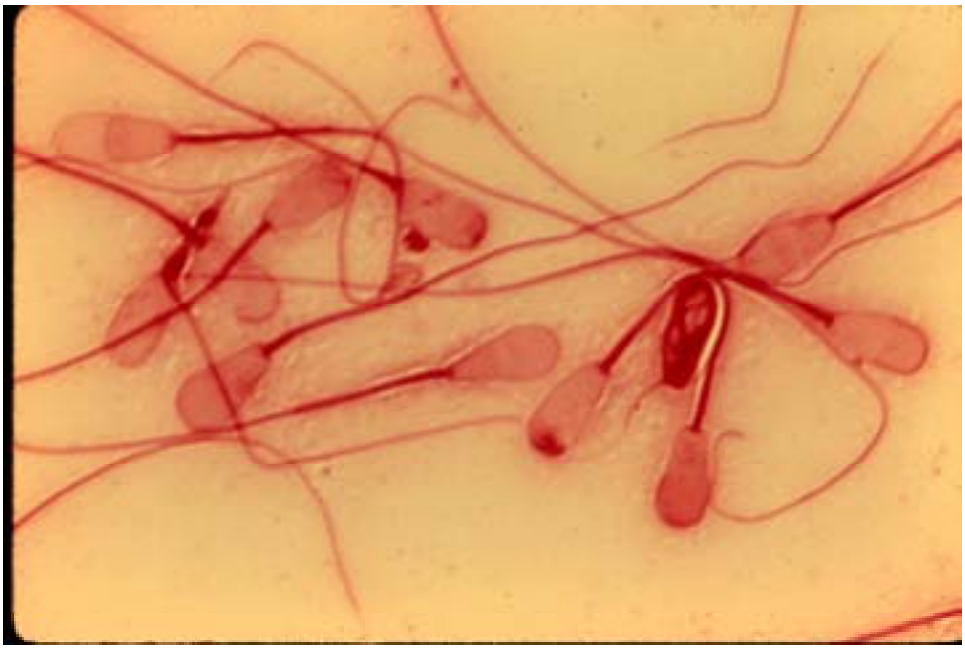

Fig. 7. Semen smear stained with carbol fuchsin eosin. Sperm heads that are narrow at base, pear shaped and undeveloped are visible. One spermatozoan has an acrosome defect. The bull consistently gave semen of poor freezability.

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. 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.

Many artificial breeding centres have standardised their own way of assessing semen quality and, provided they serve the purpose effectively, can be recommended for use.

2.1.9. Extension

The Extender 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 - egg yolk - glycerol extenders

For CS and RTS the recommended extenders are:

- Coconut milk - egg yolk extender
- Egg yolk - citrate extenders (cattle)
- Tris - egg yolk extenders (buffalo).
- Caprogen extender (common in New Zealand; not yet tested under Asian conditions)

Examples of some semen extenders are given in Annex 10.

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. For RTS, addition of antibiotics, antifungals and peroxidases (eg. Catalase) is necessary. Peroxidases are not added to CS.

Caprogen extender is saturated with nitrogen and contains catalase. Using this diluent, it is possible to transport semen anaerobically and at ambient temperature and to use it for up to 4 days with a 60–70% non-return rate (Shannon, 1965 & 1968).

Dilution should aim at obtaining 20 to 25 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.

2.1.10. Processing and packaging

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

- Extender preparation;
- Semen collection and evaluation;
- Extender A at 35°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 4 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 at 4–5°C;
- Place straws on freezing racks in liquid nitrogen vapour to -140°C over 8–10 minutes (straws should be 5 cm 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°C;
- Collect straws with a 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 4 hour time lapse between adding fraction A and the first part of fraction B is to allow antibiotics to work before they are inhibited by glycerol.

The common types of packaging used for processed semen are:

- DFS - packaged and sealed in straws, mini (0.25 ml) or medium (0.5 ml), or as pellets. Straws and pellets contain a minimum of 20–30 million spermatozoa per dose.
- RTS and CS - packaged and sealed in ampoules or airtight 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). Vials containing chilled semen are transferred to a beaker with water at 35°C and the beaker is transferred to the refrigerator at 4–6°C.

2.1.11. Preservation and storage

DFS is preserved in liquid nitrogen at –196°C. Transferring of semen 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 11.

CS is refrigerated at 4–5°C. RTS is held at ambient temperature (18–26°C).

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

2.1.12. Post packaging quality control

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

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; 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.

In buffaloes the heat signs are not as pronounced as in the cow. In addition to the above, swelling of vulval lips resulting in effacement of the folds at the edge of the vulva can be used as an indicator of heat. The duration of heat is also short, may be no longer than 6 hours, and tends to occur mostly at night.

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. In situations 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 start of heat, *i.e.* before ovulation. In cattle and buffalo ovulation takes place 10–12 hr. after the end of heat.

Education of farmers is needed on heat detection methods, adequate feeding, observation of cows for heat signs, identification of cows truly in 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 synchronisation may be used under certain economically warranted situations. The measurement of progesterone by radioimmunoassay (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 minimise 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 that 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 45–50 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 preferably not be done, or if the farmer insists, 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 up to 2–3 cm above the canister level. Transferring of semen from the tank to the thawing water must be done quickly (2–5 seconds). Canisters should remain in the neck of the tank (Fig. 8) less than 10 seconds and preferably no longer than 5 seconds.

Frequent opening of semen containers is to be discouraged (not more than 10 seconds in any 10 minute period).

Fig. 8. When transferring frozen straws the canister should not be brought beyond the neck of the container. It should not remain there for more than ten seconds.

Thawing of semen 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 farmers should be advised on the following aspects:

- Records to be kept on reproductive events of each animal;
- Observing for heat daily from 16th to 24th day after AI (at least 2 times per day, morning and evening, for 15–30 minutes);
- Checking animals not seen in heat by day 21–23 after AI for non-pregnancy by progesterone estimation in milk or plasma;
- Requesting manual pregnancy diagnosis on animals not seen in heat by day 55-60 after AI;
- Observing for any abnormality in vaginal discharges;
- Appropriate feeding regime during pregnancy;
- Drying off pregnant animals 2 months before expected calving; and
- Having any animal that has an abnormal discharge, or that does not show heat within 60 days after calving, examined by a veterinarian.

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, milk collection and dairy product marketing. Governments need to be proactive in supporting and organising the administration and infrastructure for AI.

Genetic improvement depends on the accurate measurement of milk production in identified cows and the utilisation 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 the distribution of cattle population, types of production systems, environmental conditions, availability of resources for livestock production, and the 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, service committee and farmer organizations

In many countries these organisations 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.

Where co-operatives are not functioning, the formation of other organizations such as "Service Committees" should to be encouraged. These must include all stakeholders. Their tasks would be to determine how best the farmers can be served and to assist with the resolution of problems related to AI. In time, these committees should be gradually replaced by farmers' organizations.

3.1.3. Linkages, information, education and extension

Good communication and cooperation are needed between AI Centres, herd recording organisations, farmers' organisations, breed societies, research organisations and Government or other authorities involved in AI programmes and services. The AI organisation should work with these stakeholders 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 organisations 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 privatisation (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. Consideration should be given to provide adequate incentives to the inseminator to increase the coverage of AI as well as its effectiveness. Such incentives could be on the basis of the number of inseminations and the number of calves resulting from them.

Field days (e.g. "Progeny days" as 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.

In some countries the relationship between the production cost of milk, the farm-gate price and the selling price of processed milk are distorted and do not provide the farmers with a reasonable return for their investment and inputs. In such situations there is a need for clear policies and pricing structures to encourage the development of the dairy sector, and for Governments to assist with the organization of milk collection, processing and marketing.

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 increased 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 be from progeny tested bulls, if possible. The advantages of using particular bulls in particular areas and for specific purposes need to be well known to the farmer so that he can make an informed decision when he purchases 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 60-90 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

The AIT or inseminator is a key person in the industry. They are currently employed in a variety of ways, e.g. government, co-operatives, AI organizations, non-governmental and self-employed.

Training of AITs should be conducted through residential courses, containing theoretical and practical components, followed by evaluation. In most countries newly qualified AITs must initially work under the supervision of a senior inseminator for a period

of time, and are then registered. Refresher courses and continuing education activities are important for maintaining a high level of performance, and should be instituted in all countries.

A Task Force meeting held in South Africa under the IAEA AFRA III-2 project (RAF/5/046) has developed guidelines for the training of AITs. These are given in Annex 12, and could be modified as necessary by individual countries in Asia to meet their requirements.

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 is not recommended, as it can cause 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 anoestrous, the use of progesterone measurement will be a waste of resources to diagnose animals that in any case could not have got pregnant. Similarly, if many cows are submitted for AI during the luteal phase, a high proportion of false positive diagnoses will result.

The procedures recommended for N-PD by progesterone measurement using milk or blood samples are described in Annex 13.

3.3.4. Records and their use

The Joint FAO/IAEA Division has developed a computer database named 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 RCA 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 14**)**. 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 pregnancy diagnosis (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 (Fig. 9). 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.

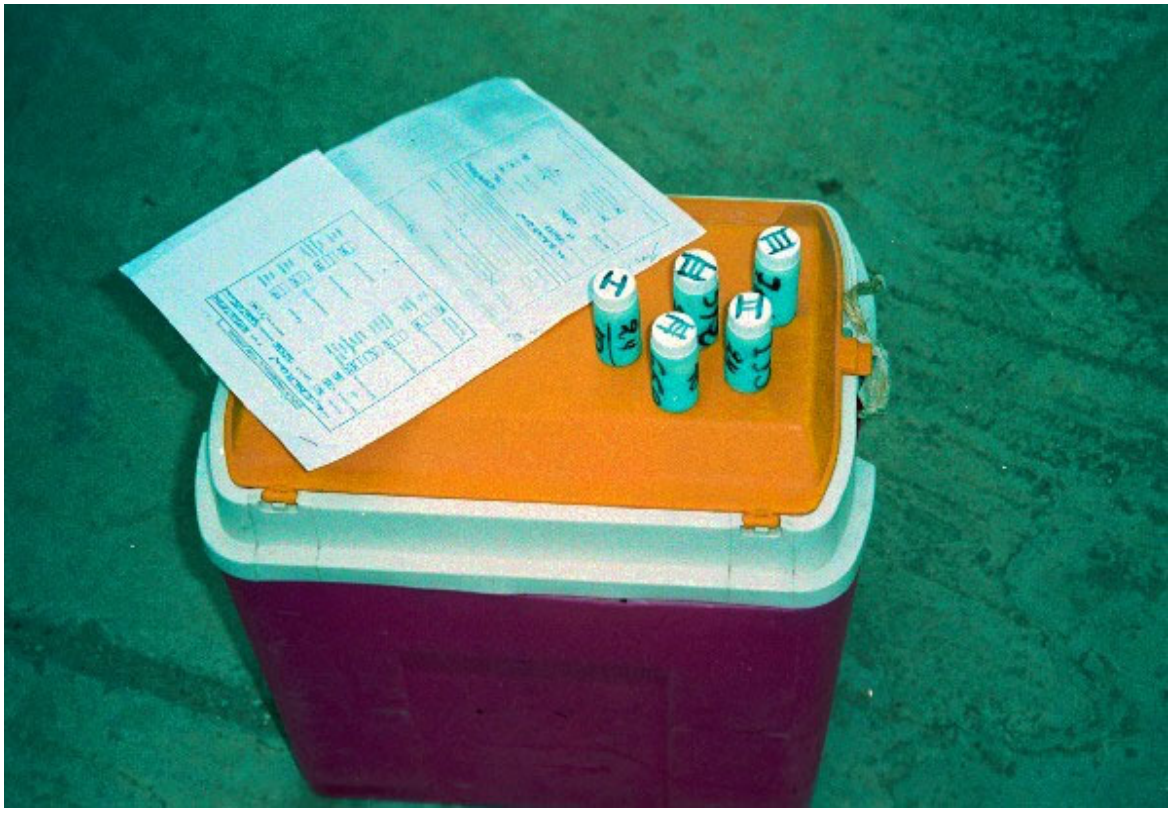

Fig. 9. Milk samples from a herd in Tunisia, preserved with sodium azide, being sent on ice to the laboratory for RIA of progesterone.

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 AIT, 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. 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, Tuberculosis, Campylobacteriosis and Trichomoniasis), mastitis diagnosis and control, calf rearing, heifer rearing, nutrition improvement programmes and other veterinary and animal husbandry education services. The visits by AITs and veterinarian to farms should be used as opportunities to provide additional services to farmers.

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ANNEXES

Note: These annexes contain materials developed or recommended for African countries under the IAEA AFRA project "Increasing and Improving Milk and Meat Production", as well as subsequent modifications and additions made for Asian countries under the IAEA RCA project "Improving Animal Productivity and Reproductive Efficiency". They should be modified and adapted according to the needs of each country.

ANNEX 1

SIRE PROOFS AND COW INDEXES

Genetic evaluation should be published in terms of Estimated Transmitting Ability (ETA), which is Estimated Breeding Value (EBV)/2 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.

A systematic method of recording should be established and a body to receive those centralized records set up to be responsible for publication of sire proofs.

Production records and sire ratings for production traits should be expressed as Breed Class Average (BCA) points according to the Canadian system. 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 is 12 daughters in 10 herds with 60% repeatability (a measure of Accuracy). All sire proofs should be updated regularly (at least 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 percentile of 90% indicates the bull is in the top 10% of his breed.

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.

The 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 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. Other important characters are feet and legs, udder conformation and general appearance. The dairy farmer uses type classification as a management tool in breeding and selection decisions. Records on type traits may be used to develop sire proofs and cow indexes. However, there is a need to harmonize type classification for all breeds.

Milk yield is an important predictor of herd life, while sound functional type plays a significant role in lifetime production. Among the type traits with the strongest influence on longevity are the shape and size of the udder, and soundness of feet and legs. Lowly heritable traits like fertility, somatic cell count levels are important, but can only be evaluated accurately in progeny tested bulls.

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 correlations 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 those 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 systematic physiological effects such as age, parity, stage of lactation and sex.

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 today 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 Method 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 variation that is due to genetic effects. The higher the non-additive genetic variation 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.

Predicted genetic response due to selection = $\frac{h^2 (0_s - 0_A)}{L}$

0_s Mean performance of selected individuals.

0_A Mean performance of individuals in population.

L Generations length in years (average age of parents when their progeny are born).

$(0_s - 0_A)$ 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.

ANNEX 2

MAJOR BREEDS OF CATTLE AND BUFFALOES IN SOME ASIAN COUNTRIES

Country	Cattle	Buffalo
Bangladesh	Red Chittagong, North Bengal Grey, Pabna, Native/Friesian, Sahiwal/Friesian	Murrah, Nili-Ravi
China	Holstein, Simental, Mongolian, Qinchun, Nanjang, Luxi Yellow, Jinnan, Yanbian, Xinjiang Brown, Angus, Limousin	Binhu, Dechang, Dehong, Diandongnan, Dongliu, Enshi, Fuan, Fuling, Fuzhong, Guizhou, Haizi, Jianghan, Shanghai, Wenzhou, Xilin, Xinglong, Xinyang, Yanjin
India	Holstein-Friesian, Sahiwal, Jersey, Jersey/Sahiwal, Brown Swiss, Brown Swiss/Sahiwal, Red Dane	Murrah, Nali-Ravi, Surti
Indonesia	Bali, Madura, Brahman, Ongole, Holstein	Swamp buffalo
Malaysia	Friesian/Sahiwal, Brahman cross, Charolais cross, Simental cross, Kedah-Kelantan	Swamp buffalo
Myanmar	Friesian/Sindhi, Friesian/Thari	Murrah, Swamp buffalo
Pakistan	Sahiwal, Red Sindhi, Cholistani, Thari, Dhanni, Dajal, Rojhan, Baghnari, Lohani	Nili-Ravi, Kundi
Philippines	Jersey, Holstein/Sahiwal, Brahman, Native	Nili-Ravi, Murrah, Bulgarian, Swamp buffalo
Thailand	Friesian, Brahman/Native, Brahman/Charolais, Charolais/Native	Swamp buffalo
Sri Lanka	Sahiwal, Friesian, Jersey, Native and crosses, AFS, AMZ	Nili Ravi, Murrah, Surti, Lanka and crosses

ANNEX 3

RECORD OF EXAMINATION OF BULLS FOR BREEDING SOUNDNESS

(Adapted from University of Melbourne, Veterinary Clinical Centre,
Animal Reproduction Section)

Date of Examination:.....…….

Owner: Veterinarian:
Address: Address:

Identification (Ear tattoo, Ear tag, Brand, Name):.....
Breed..... Age .....

History
Has the animal suffered from any condition likely to impair his fertility?

Breeding History

General clinical examination
Weight:..... Condition Score:.....
General Health:
Teeth and Jaws: Eyes: Thorax: Abdomen:
Feet: Legs: Joints Gait

Physical examination of the reproductive organs
Scrotum.....Testicles: Scrotal Circumference (cm).....Consistency.....
Epididymides:..... Spermatic Chord:.....
Seminal Vesicles:..... Ampullae:..... Prostate:.....Other:.....
Penis and Prepuce:.....

Semen examination
Collection Method:
Volume(ml):..... Density(0-6).....Wave Motion(0-6).....Motility(%).....

Examination of serving behaviour:
Serving Ability:-Libido.....Erection (Stiffness....Protrusion....Deviation....) Seeking.....
Ejaculatory Thrust....Protrusion on thrusting....Body Position.....
Serving Capacity:-.....

Tests for infectious disease:-

Tests for inherited disease:-

Diagnosis:
Prognosis:
Comments:

Semen Results Sheet

Laboratory:

Initial examination of semen in the field:-

	Sample A	Sample B
Volume (ml)	----------	----------
Density (0-6)	----------	----------
Foreign material	----------	----------
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

ANNEX 4

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 organisation 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 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 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 subregulation (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) 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 me 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:-

(a) The name or code number of the centre where such genetic material has been collected.

(b) The identification of the animal from which is has been collected.

(c) The date on which such genetic material has been collected, or the batch number of the genetic material from which such dose genetic material has been obtained.

(d) 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 recorder.

(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 have 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 5

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	**Clinical examination** (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	**TEST ROUTINE** (Done within a month of semen collection)	**RESULTS**
	TUBERCULOSIS: Date of intradermal injection: _____ Date of reading: _____	
	BRUCELLOSIS: CFT or ELISA	
	TRICHOMONIASIS: (Three sheath washes at one week interval) Date of first washing: _____ Date of second washing: _____ Date of third washing:_____	
	CAMPYLOBACTEROSIS: (Three sheath washes at one week interval) Date of first washing: _____ Date of second washing: _____ Date of third washing:_____	
	LEPTOSPIROSIS: MAT	

3	**Semen evaluation**
	MACROSCOPIC
	Amount:
	Density:
	Colour:
	Date:
	MICROSCOPIC
	Mobility:
	Neutrophiles:
	% abnormalities:
	Date:
4	**CLINICAL EXAMINATION** (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	**CERTIFICATION** (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	**ENDORSEMENT** (by an official veterinarian)

I, a veterinarian authorised by the South African Veterinary Administration hereby endorse the certification done above by the qualified veterinarian and **recommend/do not recommend*** the use of above described bull for semen collection for artificial insemination purposes.

_____	_____
Signature	Date
_____	_____
Name in capital letters	SV area

Official Stamp

* Delete as per recommendation

ANNEX 6

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).

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 diarrhea-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 7

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^{o}C. Semen is mixed with the stain. Immediately afterwards or after standard incubation time (eg. 3 minutes at 35-37^{o}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 ($Na_2HPO_4.2H_2O$): 21.682 g in distilled water to make a volume of 500 ml.
(B) Potassium dihydrogen phosphate (KH_2PO_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 ($C_6H_{12}O_6$) 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 8

HYPO-OSMOTIC SWELLING TEST (HOST)

The hypo-osmotic swelling test is performed according to the methods described by Correa and Zavos (1994). Hypo-osmotic solutions of different osmolarity (75, 100 and 150 mOsmol/L) are prepared as follows:

Solution A (150 mOsmol/L) is prepared by dissolving the following reagents in the given concentrations:

i.	Sodium citrate	7.35 gm
ii.	Fructose	13.51 gm
iii.	Double distilled water to	1000 ml

Solution B (100 mOsmol/L): 1 ml of Solution A + 0.5 ml double-distilled water (DDW)

Solution C (75 mOsmol/L): 1 ml of Solution A + 1 ml DDW

Procedure

One ml of each hypo-osmotic solution (A, B and C) is mixed with 0.1 ml of semen and incubated at 37ºC for one hr. A drop of well-mixed solution is taken on a clean dry glass slide and covered with a cover-slip. Sperm tail curling is recorded as an effect of swelling due to influx of water. A total of 200 spermatozoa are counted in different fields at 400x magnification under phase contrast microscope. Total number of spermatozoa with curled tails are calculated. Similarly, 0.1 ml of semen is incubated in normal saline and the number of spermatozoa with curled tails is calculated. This number in normal saline is deducted from the number in a hypo-osmotic solution. The resultant figure is taken as the sperm tail curling as an effect of HOST.

Reference:

CORREA, J.R., ZAVOS, P.M. (1994) The hypoosmotic swelling: Its employment as an assay to evaluate the functional integrity of the frozen thawed bovine sperm membrane. *Theriogenology* **42**: 351–360.

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.

Add the following to a separate flask:

Sodium Citrate	2,200 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 a10% 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 Lincomycion 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. DILUENTS FOR CHILLED SEMEN

B 1. Citrate Egg yolk

Citrate buffer*	80%
Egg yolk	20%
Penicillin	1,000 iu/ml
Streptomycin	0.5-1 mg/ml
Or Gentamycin	1 mg/ml

* Citrate buffer contains 2.94 g trisodium citrate dihydrate in 100 ml double distilled water.

B 2. Tris Egg Yolk

Tris buffer †	80%
Egg yolk	20%
Penicillin	1,000 iu/ml
Streptomycin	0.5-1 mg/ml
Or Gentamycin	1 mg/ml

† Tris buffer contains 3.785 g Tris, 2.113 g citric acid and 1.6 g fructose in 100 ml double distilled water.

C. Diluents for Frozen Semen

C 1. Citrate Egg Yolk Glycerol

Same as B 1 above, with addition of glycerol at:
7% for bull semen
5-6% for buffalo semen

C 2. Skim Milk Egg Yolk Glycerol

Extender A:

Skim milk buffer ‡	95%
Egg yolk	5%
Penicillin	1,000 iu/ml
Streptomycin	0.5-1 mg/ml

Extender B:

Skim milk buffer	78%
Egg yolk	4%
Glycerol	16%
Glucose	20 g/l

‡ Skim milk buffer contains 100 g skim milk powder in 960 ml double distilled water.

The semen and both fractions of extender are kept in a water bath at 35-37° C. Fraction A is added to the semen in 2 to 3 split portions at intervals of 10 to 15 minutes. The partially extended semen and Fraction B are moved to the cold handling cabinet and allowed to reach a temperature of 4 to 6°C. Fraction B is added to the partially extended semen in 2 to 3 split portions at intervals of 10 to 15 minutes.

C 3. Commercial diluents

Many commercial diluents are now available. Examples are:
- Triladyl
- Boiciphos
- Bio X-cell

ANNEX 10

LIQUID NITROGEN SAFETY PRECAUTIONS.

Liquid nitrogen has a temperature of -196^{o}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

Note: These guidelines were developed for African countries, under the IAEA AFRA III-2 project (RAF/5/046). They could be modified as necessary by individual countries in Asia to meet their specific requirements.

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 Member States (MSs).

(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 pistolet/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 be 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

RECOMMENDED PROCEDURES FOR NON-PREGNANCY DIAGNOSIS (N-PD) USING PROGESTERONE MEASUREMENT IN MILK OR BLOOD SAMPLES

Note: These generic guidelines were developed by the Joint FAO/IAEA Division, based on results from its Co-ordinated Research Projects and IAEA Technical Co-operation Projects undertaken in Asia, Africa and Latin America. They could be modified as necessary by individual countries to meet the needs of specific farming systems and local conditions.

The provision of N-PD services to farmers requires careful planning and organization of the activities. The time scales for sampling, assay and return of results to farmers is critical in order to be able to take remedial actions and derive economic benefits. The following routine is proposed:

1. 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.
2. 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 AI technician (AIT) at time of AI, and to return these to the laboratory through existing milk collection systems or co-operatives.
3. 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.
4. 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.
5. Other related services that 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.

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 1 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 2,000 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 temp or 37°C for 1–2 weeks; (b) at 4°C for at least 3 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 pasture pipette and transfer to a storage vial.
- Label and store at –20°C.

ANNEX 13

INDIVIDUAL COW AI RECORD

Farmer: _____ Farm: _____

Address: _____

_____

Cow ID: _____ 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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