TEXT

THE STANDARDS AND PROCEDURE FOR BREEDING ANIMALS, 2015

LIVESTOCK BREEDING SERVICE AUTHORITY, PUNJAB

[30th January 2015]

NOTIFICATION

In exercise of the powers conferred under section 8, 9,10,11 and 12 of chapter- III of the Punjab Livestock Breeding Act 2014 vested to the Livestock Breeding Service Authority and now the said authority is pleased to make the following standards and procedures of breeding:-

1**. Short title, extent and commencement.-**These standards may be called the Standards and Procedure for Breeding Animals, 2015 and they shall come into force at once.

2. I: **Chapter 1**

Standard Operating Procedure

for

Performance Recording and Progeny Testing

Performance recording is basic requirement for any progeny testing program. It includes economically and aesthetically important qualities. Progeny testing is utilized to select males/bulls for genetically controlled traits/qualities of economic importance (such as milk production) for which they possess genes but they do not exhibit those traits. Successes in artificial insemination and embryo transfer technologies in dairy cattle and buffaloes necessitate that bulls with better genetic potential be disseminated widely. To judge that a bull has better potential for traits which are not expressed in the males, female relatives are evaluated. When a bull calf is born, information on its mother and other ancestors (e.g., grand mother) and even sisters (if they had performance records) may be utilized for such a judgment. Such a decision making is called pedigree or family selection. The problem with such a decision is that such an information is very limited (e.g., few lactations in few herds) and therefore level of accuracy in declaring a calf good or bad may not be very high. As such a male can have a very profound influence on the population, level of accuracy needs to be much higher to use it extensively.

Information on the performance of large number of daughters can help to really estimate whether a bull has good potential for traits like milk yield and such a selection is therefore called progeny testing. Now a days, availability of computers have made it possible to utilize all available information on the relatives (pedigree, progeny and other relatives) of a bull and therefore it is not just the progeny that contributes to the evaluation of a bull. Still we may call it progeny testing instead of animal model evaluation or some other kind of evaluation, for simplicity.

Breeding Objectives

Before taking up the progeny testing program, breeding objectives i.e main purpose of keeping that particular breed should be clear. It may be combination of few or many traits. For Sahiwal cattle or Nili-Ravi buffaloes, for example, progeny testing is being done for milk yield while traits like fat yield, type/beauty etc are only rarely considered. For Beetals it may be meat with milk yield as secondary trait. The procedures outlined are therefore focused on progeny testing for milk production.

Steps required

1. Survey of the area for identification of potential farmers

Identification of potential farmers to be involved in progeny testing program. Bigger farmers (>10 breeding females of the breed) that are willing to cooperate, in terms of actual pedigree and performance recording and use of 100% A.I is needed. Agreement should delineate the services provided to the farmer and information to be collected from him. It should for example, specify that he will not keep any bull and that about half of the females will be inseminated with young bulls and other half with proven bulls.

2. Training of AI technicians and recorders

Milk recorders and artificial insemination technicians recruited for the project need to be trained /made available. Milk recorder should be a veterinary assistant level person with good social skills.

3. Tagging and registration of cows/buffaloes

Animals should be tagged or freeze branded to have a unique identification. A digital photo is now a days quite cheap. A closer photo of head to capture side face and horns, eyes and face should supplement the tag/brand. About 200 pregnancies per bull are intended (and assuming a calving interval of 1.5 years and two inseminations per conception) it would .mean that 600 cows/buffaloes per bull need to be identified for getting preliminary evaluation of a bull. To test 100 bulls therefore 50-60 thousand females are needed to be registered. All the animals of the breed with the registered farmer/herd need to be tagged and recorded. No minimum milk production level is required. As all the animals served with natural service bulls and through Artificial Insemination also need to be tagged. Ear tag should be unique and should have district code and animal number. For example a Nili Ravi buffalo in Lahore district may have NI00001LHE. This will be the same number as registration number with the Authority. AI technicians will also be using the same number even when animal is not under performance recording program.

4. Performance recording

Milk recording of all the cows/buffaloes in any registered farm is needed as per international norms, not just good/elite females. For milk yield, morning and evening recording at approximately monthly interval is an acceptable frequency. When cows/buffaloes are milked in the presence of calf, milk consumed by the calf (e.g, one teat) may be appropriately accounted for the recorded production or calf may be fed separately and cow/buffalo recorded completely. Lactation yields should be projected using Test Interval Method (www.icar.org). Multiple lactation records should be transformed into an index compensating for repeatability of the trait.

Other than milk and fertility records, health status also needs to be recorded. Beauty may be an important trait and prospective bull mothers should be above average in beauty standards, especially the udder and feet-and-legs. Supervision of performance recorders is needed and accurate workers should be recorded. Planned mating is mandatory for progeny testing programmes.

5. Breeding control of registered cows/buffaloes

All the cows/buffaloes may be inseminated artificially with semen from young bulls and technical experts should make such a choice to ensure randomness in usage of young bulls to be tested. Record of every insemination, bull used, pregnancy and birth will be kept on a specific recording sheet.

6. Processing of recorded information

The recorded data on farmers cows/buffaloes may be edited monthly after they are received from the field, may be edited for consistency and for generation of reports for feed back to the farmers and for editing to check if it conforms to the already available information of the animal and the farmer. Although such processing of information may be done routinely, generation of reports for feed back may be done quarterly (every three months) and genetic evaluations done biannually if not at a more frequent interval. Lactation yields need to be predicted from monthly records and decision for any cow/buffalo to be elite or not elite needs to be made and communicated to the relevant field staff for planned breeding if needed. Choice of lactation length may be 10 months (both for cattle and buffaloes) to conform to international standard. Lactations ending normally may not be projected to any standard lactation.

7. Identification of elite cows/buffaloes

Elite cows and buffaloes are routinely declared and there is no minimum level of milk production for such a declaration. Generally these are the best animals within a management group. Their number may depend on the number of bulls to be tested every year. For example if 500 male calves are intended to be produced to get 200 candidate bulls, 800 births are expected which would require at least 2000 elite cows/buffaloes and if they are top 20% of the recorded population, recorded population will be ten thousand cows/buffaloes. For better selection intensity, recorded population may be even higher. It may be made clear that under good management, elite may a cow/buffalo producing more than 3500 liters a lactation while under ordinary management, elite may be a cow/buffalo producing more than 2000 liters a lactation (adjusted for age/parity, season of calving and other differences). Farms may thus be categorized as good management farms (concentrate feeding possible along with good health care) and mediocre management farms (concentrate feeding rare and grazing mainly offered along with other management issues).

8. Feedback mechanism to the farmers

Under small and medium sized farming system, farmers generally know how their individual animals perform or at least they can easily rank their animals from best to worse. The central idea of feedback is to inform them how their animals perform compared to the animals of other farmers in the area and even across various production systems. This helps them keep involved in the system because selection and culling decisions can be more judicious. Monthly lactation summary reports ranking farmers for average production of their cows/buffaloes and periodic rewarding of those who excel may help farmers pay attention to recording and improving their management. Culling decisions may also be helped by the experts.

9. Selection and raising of bull calves

The calves born to the elite cows/buffaloes may be monitored carefully based on the breed characters and physical conditions of the calf.

10. Selection of candidate bulls

The bull calves that mature to a breeding age (at the calf raising centers) and donate semen for preservation and later use in the registered herds are called candidate bulls. Their selection is made from among the calves bought to CRCs on the basis of breed characters, libido and semen quality. Sometimes indices are used to rank them. Well-fed young bulls should donate semen at 2 to 2.5 years of age. Age is slightly more in buffaloes than in indigenous cattle. A performance sheet may be used to record performance of bull calves and bulls.

11. Semen collection and storage

The semen production units generally have adequate expertise to train bulls for semen collection and then actual collection of semen, its evaluation and storage after freezing. At least 2 thousand doses should be used to get elite dams (bull mothers) pregnant for getting progeny records and another 8-10 thousands for future use when daughter information becomes available. This means that a bull needs to spend about 2-3 years at the SPU. This time can be reduced tremendously by better management at the SPU. After collection of desired doses of semen such bulls can be denoted to the farmers for natural mating especially for farms where AI facilities are difficult to reach.

12. Artificial breeding in the registered herds

The registered herds may be provided with AI facility as and when they need it. Preferred method would be a central calling facility where all the AI technicians are hooked and nearest one may reach the farm as and when such a request is received (like a taxi service). Periodic monitoring of the system may also be needed to improve efficiency.

13. Genetic evaluation of bulls

The bulls to be ultimately used in AI are tested at many stages but final selection is through genetic evaluation for the milk yield (and other traits) i.e estimated breeding values. These evaluations should be done sooner than on yearly basis. Software to be utilized for genetic evaluation is also important. Programs such as ASREML may be bought or free software such as DFREML or JAA can also do the job. Minimum criteria for any bull to qualify for getting PTAs published may be reliability which may be 50% in the beginning and can be improved gradually to say, 70% and more. Number of daughters to publish any bull's EBVs should not be less than 10.

14. Disposal of semen from below average bulls

Bulls having negative (below average breeding values for the traits like milk yield (or index having other traits) need to be disposed off even when reliabilities on the estimated breeding values are not very high. The bulls if alive may also be disposed off accordingly. At least bulls ranked at the bottom (below 25%) should be culled and semen discarded.

15. Contractual matings of elites with elites

The top most bulls (say top 5%) need to mated with top cows/buffaloes to get 200 inseminations for each bull to get bull calves again. The choice of the elites will be from step 13 where genetic evaluation of cows/buffaloes and the bulls is done simultaneously.

16. Continuity of the progeny testing program

System should be developed in such as way that if a person or two leave, the system does not collapse. So more than one persons be trained at every level. One should have capacity to adjust to new technologies and innovations in the field of biotechnology and computational breeding. It may start from simple and feasible data collection protocols and should lead towards an ideal situation of automation with the passage of time.

17. Practicability and authenticity

Starting any progeny testing program is difficult and maintaining it is even more difficult as at administrative and political level quick fixes have more attraction than any long term endeavor. Recording less may be acceptable but recording false is not. Simplicity helps at least in the beginning and socioeconomic factors may be very important to any tailor-made effort. Sustainability of the progeny testing program is extremely important and requires dedication.

2.II : Chapter 2

Standard Operating Procedure

for

Selection of Breeding Males for Artificial Insemination

Bull is the most important component of any breeding program as everything revolves around it. Bull used for widespread use especially through A.I must be better than others not just in its abilities to transfer desired characters, it should be free form genetic and other diseases. Best option is that it is progeny tested i.e information on daughters performance for traits like milk yield was available and estimated breed value(s) calculated. But when/until such bulls are not available, at least it should have performance recorded pedigree i.e it should come from a dam which is better than others in a similar production environment. In progeny testing programs such dams are recorded for traits such as milk yield (morning-evening approximately at monthly interval) for a standard duration of 10 months. Following minimums are to be considered for various breeds and bulls without a recorded pedigree should not be used for semen collection and freezing. Dams are expected to be born and perform under agro-climatic production setup of Punjab. Bulls of indigenous breeds are expected to have been registered with relevant registered associations/societies and pedigrees authenticated. Yet, for breeds where a registered association does not exist, Livestock Breeding Services Authority will issue the breed standards and register such bulls directly for semen production businesses (template for registration of bulls is attached as Annex-1). Unique number will be issued to each bull. They may retain their ear tag numbers and branded numbers.

Minimum criteria for dam's 1st and best lactation yields of bulls brought to semen production facilities.

Breed	Dam's 1st lactation milk yield*(litres)	Dam's best lactation milk yield (litres)	Fat %
Sahiwal	2500	3000	4.5
Cholistani	2000	2500	4.5
Red Sindhi	2000	2500	4.5
Nili-Ravi	2500	3000	6.5
Holstein (purebred)	5000	6500	3.5

Jersey (purebred)	3500	4500	5.0
Holstein crossbred	4000	5000	4.0
Jersey crossbred	3000	4000	4.5

*in a standard lactation of 305-days

a. Physical Examination

Introducing any new bulls into a semen production facility requires quarantine measures and a thorough physical examination which will be conducted by a designated/accredited Official / Veterinarian to ensure that the bulls do not display clinical symptom(s) of any infection or any contagious diseases and are structurally correct. Structural anomalies such as cryptorchidism, penile frenulum and (moderate to extreme forms of) leg defects (e.g., hocked-in) or (moderate to extreme forms of) testicular shapes (e.g., asymmetrical testis) are unacceptable even when pedigrees are popular. In bucks and rams obvious mouth defects (under or overshot jaws) should also be avoided. Testicular size may vary with the age of the male yet, very narrow scrotal circumference is neither economical nor desirable. Breed associations may also fix certain minimums for weight, height or other attributes for breeding males so that semen collection facilities can adhere to these parameters. Gait defects may be temporary or permanent and breeding soundness examination (Appendix ??) should help avoid undesirable males.

b. Genetic abnormalities

Bulls will be required to have normal karyotype certificate to rule out chromosomal defects. Specific tests may also be conducted for genetically transmitted diseases like Bovine Leukocyte Adhesion Deficiency (BLAD), Citrullinemia and Deficiency of Uridine Monophosphate Synthase (DUMPS) for exotic breeds and their crossbreds. Beta casein variants (A1 and A2) are also becoming important and in future exotic bulls (Holstein and Jersey) and their crossbreds might be screened to only allow A2 bulls for semen production. Our local cattle and buffaloes naturally possess A2 variant, the desired genotype.

c. Quarantine tests

A minimum quarantine period of 60 days is compulsory before bringing new bulls in a semen station (in case of bulls are brought from known sources, 30 days quarantine is sufficient). This period shall be divided in two periods of 30 days during which a series of compulsory examinations and tests are carried out. Only after favorable results from the health control point, the bull shall be admitted to the semen station.

a). In the quarantine station new bull/s shall be housed for minimum of 60 days in a place which is effectively separated from the facilities occupied by resident bulls and all equipment used in handling, feeding, watering and cleaning the new bulls shall not be shared with the resident herd(s). Quarantine station and the semen production facility can not share the same boundary wall. Both facilities should not share labor as well.

b) Each new bull in quarantine station will be tested against major contagious diseases before its entry to resident herd e.g. TB, Brucellosis, Campylobacteriosis and Trichomoniasis. All tests shall be done by an accredited institute or disease diagnostic laboratory.

c) If any bull is found positive for TB, or Brucellosis, it shall be removed immediately.

d) During quarantine period, the bulls shall be vaccinated against FMD, HS, BQ, Theileriosis and Anthrax. However, vaccinations against bacterial diseases shall

be done only if there is an outbreak or prevalence of a particular disease. Level of comfort (shade, feeding, watering etc) will be similar to the donating bulls.

Once the quarantine period is over, all bulls shall be introduced to the resident herd. A written consent / certification of designated official / veterinarian will be required before semen collection can be initiated.

d. Testing of Bulls

The resident bull herd would also be required to go through periodical testing and vaccinations. Testing protocols for bulls against Brucellosis, Campylobacteriosis Trichomoniasis, Leptospirosis, Tuberculosis, and IBR are given in Annex III to VII. For international trade OIE guidelines (http://www.oie.int/fileadmin/Home/eng/Health_standards /tahm/A_index.htm) will need to follow.

e. Vaccination Schedule

The bulls shall be vaccinated against FMD, HS, BQ and Anthrax. However, vaccinations against bacterial diseases shall be done only if there is an outbreak or prevalence of a particular disease. To reduce lay off time, the bulls shall be vaccinated on the rest day or the day after completing semen collection. Sexual tests may not be required unless otherwise febrile condition is noticed.

The semen station in collaboration with local governments shall arrange for carrying out ring vaccinations for all animals against FMD, HS and BQ within a radius of 5 km around the semen station. Vaccinations against HS and BQ shall be carried out in the areas having incidence of these diseases.

Culling of Bulls and Semen Doses due to Specific Diseases

Diseases	Bulls	Fate of semen doses
Foot and Mouth Disease (FMD)	Retain	Last one month doses to be discarded, refer Annex-XX
Brucellosis	Castrate & remove	To be discarded since the last negative
Tuberculosis (TB)	Remove	To be discarded since the last negative
Campylobacteriosis	Treat and retain	To be discarded since the last negative
Trichomoniasis	Treat and retain	To be discarded since the last negative

The semen station must remove bulls (within 48 hours) which are positive for above diseases, poor libido, poor semen quality, incurable lameness, etc. Besides, the semen station shall also cull those bulls which have completed six years of productive period or 2 lakh semen doses, whichever is achieved earlier. These conditions will be relaxed for progeny tested (positive) bulls.

f. Parentage Confirmation

Parentage confirmation through molecular evaluation for all the candidates bull entering the semen production unit would be compulsory.

2.III : Chapter 3

Standard Operating Procedure for Selection of Breeding Males for Natural Service

Males used for providing natural service should have all the basic norms laid down for those used for collection of semen for Artificial Insemination except that their progeny information may not be available. Individual's own performance (for traits like growth) and pedigree performance therefore could be the main information sources. This is especially true for rams and bucks where males have recorded phenotype for traits like growth rate and breeding objectives include growth as selection criteria. Males used for breeding commercially will be registered under the authority and following guidelines are expected to expected be helpful for provide a level playing field as well as in the improvement of genetic potential of our breeds.

1. Registration with breed association/society
2. Basic phenotypic qualities
3. Breeding soundness examination
4. Genetic and venereal diseases
5. Maintenance of breeding records

Registration with breed association/society

Registration with the Livestock Authority is through the breed associations for those breeds and species for which such farmers/breeders groups exist. For other breeds and species such registration will be by a cell in the Authority headquarters and minimum criteria will be published/declared by the authority.

Basic phenotypic attributes

For the main local breeds such as Nili-Ravi and Sahiwal (or even for Beetals for which semen is available) associations should come up with minimum criteria for beauty traits apart from the authentication of pedigrees in the form of pedigree certificates.

Nili-Ravi bulls are required to have black color and curled horns (level of curl may vary but tighter is better) along with wall eyes (white eye balls, partial or complete) and white switch of tail (length of white part may vary but less than 1/3rd is preferred). White patch on forehead is liked but albinism (of head, partial or complete is not). Length of body and height are preferred physical attributes. Size of testicles may vary and both single and double pouched scrotums are acceptable yet, symmetry is crucial. Similarly, being hocked-in (rear leg rear view) is not a preferred attribute. Strength of rear legs is actually quite important breeding males of all species as they have to bear all the weight during mounting. Hooves should also be compact.

Sahiwal bulls are required to have reddish dun color (a variant of red color). Variation of brown shade may vary from greenish red to darker red yet very dark chocolate or very light brown shades are not preferred. Hump and dewlap are characteristically large. Folds are preferred in the dewlap while hump must not be erect and not broken. Longer tails are preferred with black switch. While body spots are not acceptable yet, small (less visible) spots in the underline may be acceptable. Symmetry of testicles is mandatory and hocked-in bulls are less acceptable. Hooves should not be worn out especially at a younger age.

Beetals strains have characteristic colors that may easily distinguish one from the other. Nuqri for example, has to be while, Faisalabadi, black and white spotted (or even

red and white with varying share of the two colors). Makhi Cheeni has to have brown splashing on light background (with light brown color, Phikki Cheeni, preferred over darker shades, Ratti Cheeni). Single and double pouching of testicles exist yet, symmetry is the most crucial attribute. Under and overshot jaws are not acceptable while extremely hocked-in bucks are not recommended. Horns should be small and close to body although polledness is preferred in Nuqri strain. Body length is the most important single body trait in goats and taller and longer animals are preferred.

In all the breeds, males should have known and performance recorded pedigrees. Performance of dams is crucial and therefore instead of opinion, physical records are preferred. For goats, seeing the dam (if alive) can further help decided selecting of bucks from dams with good body length and uniform large udders (not large teats). Same may be true for Sahiwal and Nili-Ravi where udder is the single most important trait to look at. Yet, when performance recorded dams are available, following minimum performance (similar to those of AI males/bulls) of dams is desired. These naturally servicing males will have the same pattern of registration as with AI males.

Minimum criteria for dam's 1st and best lactation yields of bulls brought to semen production facilities.

Breed	Dam's 1st lactation milk yield*(litres)	Dam's best lactation milk yield (litres)	Fat %
Sahiwal	2500	3000	4.5
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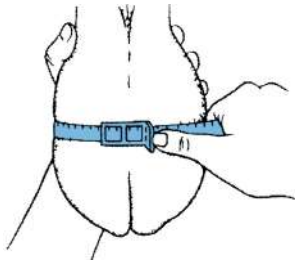
*in a standard lactation of 305-days

Breeding soundness examination

The objective of BS exam is to identify infertile and subfertile males and promote fertile bulls/bucks. There are many components to a bull breeding soundness examination that can be simplified as follows.

1. **Health status:** This may involve reviewing the health status of the herd of origin and reviewing the results of previous health records, collecting samples and commissioning more laboratory tests, reviewing vaccination and other treatment history and reviewing previous breeding performance (if available).

2. **Physical condition:** Assessing body size relative to age, body condition, conformation, locomotion and noting signs of any infectious or contagious disease. A more detailed examination may then be required particularly to assess the eyes, the teeth, the legs and feet and, of course, the reproductive apparatus. The prepuce will be examined for any evidence of constriction or discharge. The penis will be palpated to ensure that it is freely moveable within the prepuce and that there is no evidence of any abnormal swellings or growths. The testes will be measured for scrotal circumference and palpated to ensure



Measuring scrotal

uniform size and consistency. They should be freely moveable within the scrotum. Any defect observed (orchitis, excessive fat around testes, hernia, cryptorchidism, hypoplasia, etc.) need to be carefully diagnosed. Trans-scrotal ultrasonography and testicular fine needle aspiration cytology have also been used under advanced setups to diagnose subfertile males. An internal examination will also usually be carried out to palpate the accessory reproductive apparatus and ensure normality.

3. **Semen examination:** Electro-ejaculator is commonly used for getting semen sample yet, teasing the bull with a cow that is in oestrus and then using an artificial vagina may be equally good. An assessment can be made of ejaculate volume and density before the sample is viewed using a microscope to assess sperm motility and the number of abnormal or damaged sperm.

4. **Sexual behavior assessment:** Keenness to serve (libido) and ability to serve are important for any breeding male. It is particularly important during this part of the examination to watch carefully for any penile deviations which may prevent intromission (the penis entering the vagina) and for evidence of an ejaculatory thrust.

Genetic and Venereal diseases

Periodic physical examination of any candidate or serving bull / buck is needed to ensure that the bulls do not display clinical symptom(s) of any infection or any contagious disease. Testing for TB and Brucellosis should be mandatory every six months. Ideally, bulls should be karyotyped to rule out chromosomal defects. For exotic and crossbred bulls, specific tests may also be conducted for ruling out genetically transmitted diseases like Bovine Leukocyte Adhesion Deficiency (BLAD), Citrullinemia and Deficiency of Uridine Monophosphate Synthase (DUMPS) and Complex Vertebral Malformation (CVM). Vaccination for known viral/bacterial diseases should be compulsory and vaccination records should also be maintained.

Maintenance of breeding records

Service records will be required to be maintained by the bull service provider in the form of register to be periodically verified by designated vetnarian from the Authority and these records must be communicated electronically with the Authority for maintenance of a database through the breed associations as frequently as desired. Moreover, bulls should not be allowed to remain in an area for more than 3 years and in case of rams and bucks, for 1 year, to avoid inbreeding in the population.

2.IV : Chapter 4

Standard Operating Procedure

For

Physical Infrastructure for Semen Production Facilities

Physical infrastructure and general management conditions at the semen production and freezing facility can easily affect the quality of semen and consequently the whole livestock value chain if minimum standards are not ensured. Any semen production facility will be required to be registered with the Authority. These will be required to follow minimum standards laid down here.

1. Premises

Premises for semen production facilities should be away from public areas with minimum traffic and flow of people. Sufficient trees shall be planted and lawns prepared around the semen station to reduce dust and heat in the summer.

2. Housing

There should be provision of animal housing for individual bulls, a semen collection yard, a semen processing room and a semen storage room. For equipment cleaning / disinfection / sterilization there should be a separate room. Bull sheds shall have spacious individual pens with adequate loafing area, manger and water trough with access to drinking water all time. Adequate shade around the bull shed shall be provided. The roof shall be made of asbestos or some other suitable material. During summer, cooling system with sprinklers and fans may be required particularly for the buffaloes and exotic bulls.

At one corner of the farm, there shall be an isolation shed for separating ailing / sick bull(s) for treatment. Bull(s) once diagnosed suffering from infectious diseases shall be removed immediately from semen station for safety of other bulls. There should be separate staff and separate bio-security arrangements for semen station and quarantine facilities where fresh entrants will stay for 1-2 months. Entry of unauthorized persons must be prohibited.

3. Management of Bulls

A satisfactory state of cleanliness of barns and bulls should be ensured. For proper management of bulls, the following points shall be considered:

a) The bulls shall be kept under hygienic conditions at all times.

b) The coat of the bulls shall be kept clean and generally short. The hooves shall be regularly trimmed.

c) The length of the tuft of hairs at the preputial orifice, which is invariably soiled, shall be cut to about 2cm. The hair would not be removed altogether, because of its protective role. If cut too short, it may cause irritation of the preputial mucosa.

d) Bulls shall be brushed and groomed regularly, and where necessary, special attention shall be given to the underside of the abdomen, a day prior to semen collection.

e) Cleaning of the prepuce with sterile normal saline solution may be done every ten days if the microbial load is within the prescribed limits. Cleaning prior to the day of collection can be practiced if the microbial load in frozen semen is beyond the prescribed limit.

f) In the event of obvious soiling, careful cleaning of the preputial orifice and the adjoining areas with soap or a detergent is recommended; followed by thorough rinsing and drying.

g) Scientific feeding schedule shall be followed for the bulls. Semen station shall carryout routine quality analysis of feed and fodder for arriving at a balanced ration.

h) Vaccination schedule should also be strictly followed.

4. Conditions for Semen laboratory

a) The premises and inside of the laboratory should always be neat and clean. The ceiling and walls of the laboratory shall be made up of non-porous materials. All cracks and crevices shall be sealed to control pests and insects.

b) Entry of persons to the laboratory, other than laboratory personnel, shall be strictly restricted. Airlock system or anti-room shall be provided to avoid direct entry to the semen-processing laboratory.

c) Laboratory windows shall preferably be made of double sheet glass with fixed aluminium frame. The glass panes shall be plastered with sun control films to avoid direct sunlight. The doors shall be kept closed, especially during dilutor preparation and semen processing.

d) Preferably cassette type or, split type air conditioners fitted with air purifying system with remote temperature control mechanism should be installed to maintain the room temperature at 20°C - 22°C. The number of ACs to be fixed to sustain this temperature shall depend on the size of the processing room. Maintaining this temperature is most important to achieve the best results when single step dilution method is followed for freezing semen. The flow of air from AC must not be towards the front side of the Laminar Air Flow Unit. Adequate number of thermometers shall be kept in a few places in the laboratory to check the room temperature. Alternatively, central cooling with 10 to 15 air exchanges should be fixed, especially for the semen processing laboratory. This helps to control the bacterial load in the semen-processing laboratory and in removing obnoxious odour. The processing laboratory should ideally maintain around 55% relative humidity.

e) Sink drains shall be decontaminated routinely with a disinfectant. Sink shall not be placed in the semen processing room.

f) The floors shall be preferably made up of vitrified tiles. Floors and horizontal surfaces shall be cleaned and mopped with a disinfectant solution, as dirt and dust, which settle on these surfaces, are the main sources of contamination.

g) Unwanted furniture, equipment and materials shall not be kept in the laboratory as they only provide additional area for dust and spores to collect.

h) Appropriate number of germicidal UV lights with respect to area of laboratory, laminar airflow unit, apron and laboratory footwear cabinet may be fixed with a common operating switch outside the laboratory. These lights shall be switched ‘on’ at least 8 hours prior to commencement of work in the laboratory and shall be switched ‘off’ before beginning work. The date of installation of the UV lights shall be noted to facilitate replacement as the life of UV tube is limited. A logbook should be maintained for timely replacement of UV lights.

i) The laboratory shall be fumigated twice a week with a suitable, using humidifier.

j) Fumigation should be supported by monitoring laboratory environment by bacterial load test. The bacterial load shall be measured every week to monitor pollution of the laboratory atmosphere.

k) The work platform, the parts of equipment and other items to be handled during processing of semen, shall be cleaned with suitable disinfectant. It is advisable to repeat cleaning schedule after completing processing of semen.

l) Clean laboratory footwear, apron, hand gloves, mask and caps shall be compulsorily put on while working in the laboratory.

m) Eating, drinking, smoking, etc. shall be prohibited in the laboratory and unnecessary conversation should be discouraged. Besides, entry of persons shall be strictly restricted.

n) Long exposure of semen to ultraviolet rays, visible light in direct sunlight and white florescent light causes chromosomal damage and hence, direct exposure to such sources of light shall be avoided. Hence, there shall be provision for indirect or diffused lighting inside the semen processing room.

5. Personnel Hygiene

Clothing, skin and hair of laboratory personnel are the sources of contamination. Hence, all should wear laboratory aprons and footwear all the time while they are in the laboratory. Hands shall be washed with soap and water and rinsed with 70% alcohol, before commencing work in the laboratory. The bull attendants must undergo test for TB every year**.** Other staff working on the station should be tested for TB once in two years. Restricted entry inside the semen processing room and freezing room shall be strictly adhered to.

6. Equipments

a) The exteriors of all equipment and furniture shall be cleaned weekly. The equipment shall be kept covered by plastic covers when not in use.

b) The pre-filter of Laminar Airflow unit shall be cleaned weekly. Routine servicing and testing twice a year will ensure efficiency of air filters. Alternatively, culture plate test shall be carried out at frequent interval to assess bacterial load of the air passing through the filters.

c) Digital photometer / Computer aided Spectrophotometer shall be validated with Haemocytometer readings for sperm concentration twice a year separately for cattle and buffalo (20 samples each).

d) The automatic semen straw filling and sealing machine shall be thoroughly cleaned, immediately after use.

e) The microscope lens shall be gently cleaned daily with a piece of cotton soaked in a mixture of ethyl and methyl alcohol (1:1) or a mixture of 80% ethyl alcohol and 20% ether)

f) The bio-freezer shall be defrosted and thoroughly cleaned and dried, immediately after use.

g) Incubators to maintain artificial vagina shall be cleaned and disinfected with 70% alcohol.

h) Single distilled water shall be used in autoclave and thermo-controlled water bath. The water bath shall be cleaned and filled with single distilled water on a regular basis.

i) The thermometer kept immersed in water bath shall be cleaned daily to have precise temperature reading or water bath fitted with digital display temperature indicator should be used.

j) The Liquid Nitrogen containers returned / received from foreign countries and contagious disease prone areas shall be disinfected thoroughly.

k) The refrigerator meant for storing eggs, antibiotics and buffer shall not be used for storing vaccines and other materials. All such materials shall be stored at a place away from semen laboratory. The refrigerator used for storing eggs, etc. shall be sterilized every week using alcohol swab.

l) All equipment used in semen processing should be covered under annual maintenance contracts.

List of equipments

1	Standard Thermometer	11	Refrigerator(s)
2	Water Bath	12	Laminar Air Flow Units
3	Weighing Balance	13	Biological Freezer
4	Incubator	14	Microjet Ink Printer
5	Autoclave Hot Air Oven	15	Straw Filling & Sealing Machine
6	Microscope	16	Water distillation unit
7	Slide Warmer	17	Semen tank (large and small)
8	Micropipettes	18	Computer, etc
9	pH Meter		
10	Photometer		

2.V: Chapter 5

Standard Operating Procedure

For

Semen Collection and Preservation Standards

Semen Collection

Semen collection is generally done in the morning hours. Ideally, the floor of the collection yard shall be made of concrete layer at a depth of one foot from the ground level. It should be comforting for proper footing of the bulls. Good quality rubber mat (with interlocking arrangement) or coir mat may be put on the mounting area for adequate cushioning effect. After collection, the area must be thoroughly cleaned and disinfected. A dusty floor shall be avoided to prevent dust falling on the AV / semen samples. On the day of collection, before collecting semen, the bulls shall be properly washed and cleaned. After that, the prepuce shall be cleaned externally with normal saline and a sterilized paper napkin or sterilized cloth napkin soaked in normal saline to remove any sand or dust particles. For each bull a separate napkin shall be used.

The person responsible to carry out preputial wash must use disposable gloves and separate sterilized nozzle for each bull to avoid transmission of infection from one bull to another. Semen collection should be individualized based on the bull. Sexual preparation (number of false mounts and restraint) of the bulls may be done considering the individual behavior of the bulls and not generalized. For this purpose, the sexual behavior of the individual bulls shall be studied and documented. As a general rule, bulls shall be sexually prepared by giving two / three false mounts followed by restraint. The gap between two ejaculates shall be half an hour to one hour depending on the bull. Second ejaculate shall be taken with proper preparation of bulls. Sterilized bull aprons shall be used to avoid penis touching hindquarter of the dummy. Before every collection, the semen collector shall either wash his hands with a disinfecting solution or use disposable gloves or do both. The semen collector shall not touch the penis.

Preferably veterinarians shall perform semen collection. If semen is collected by staff, a veterinarian shall remain present to supervise the collection process. While taking collection, it shall be ensured that AV is not thrust on penis of bull, instead penis should be guided to AV. Immediately after collection, the AVs shall be thoroughly cleaned by non-spermicidal neutral detergent. Separate AVs shall be used for each ejaculation. The AV shall be changed even if the bull has inserted its penis without successful ejaculation. The same AV shall not be used twice. The AVs shall always be kept inverted and the collection tube shall be covered with felt / water jacket (plastic bottle filled with warm water at 37^{o}C) to avoid cold shock. The open end of sterilized AVs shall be covered with aluminum foil, which would be removed at the time when bull is ready for giving semen.

Appropriate size AVs, ranging from 8-14", shall be used for cattle and buffaloes to ensure semen is ejaculated in cone. The cone shall be of top quality Neoprene rubber. Use of lubricant shall be avoided. If it is extremely essential to use lubricant, separate sterilized glass rods shall be used for smearing the Jelly on each AV. The AV shall not to be shaken after ejaculation; otherwise lubricant and debris may mix with the semen samples. As soon as the first ejaculate is taken, the bull apron should be removed and dipped in the plastic tub filled with detergent lotion. For second ejaculate, a fresh apron should be tied to the bull.

The entry of visitors and staff / labourers (other than those not involved in semen collection) shall be strictly prohibited in the collection arena at the time of semen collection. Protective clothing (barn coat) and gumboots shall be used by the veterinarians and personnel during semen collection. Gumboots and barn coat should be washed immediately after completion of semen collection work. Semen stations may follow the norm of minimum two ejaculates per collection and minimum two collections per bull per week for taking 180 ejaculates annually from each adult bull. However, a maximum number of collections per bull would depend on the individual capacity of the bull.

Semen Evaluation

Semen evaluation, freezing and packing are critical quality control segments. As soon as the neat semen is received from the collection area, it shall be kept in the laboratory in a thermo-controlled water bath at 37^{o}C under aseptic conditions (such as Laminar Air Flow Unit) and semen volume should be recorded. After examination of sperm concentration and initial motility, semen samples shall be primarily diluted with dilutor maintained at 37°C. After initial dilution of semen in the ratio of 1:1, the semen should be extended further after 7 minutes of cooling at 20°C with dilutor maintained at the lab temperature. The semen samples should not get accumulated for long time in water bath, which may reduce their viability. Sperm concentration shall be checked preferably by a digital photometer with auto dilutor, manufactured by a reputed company. The photometer shall be calibrated separately taking 20 readings each for cattle and buffalo semen, at least once in six months, with haemocytometer readings. Semen samples showing less than 500 million / ml sperm concentration shall be discarded. The volume of straws should be determined as it may vary from batch to batch. While determining the dilution rate as per the photometric reading, the actual volume of mini straw should be fed to the photometer. Straw volume of randomly drawn straws from a day's production should be checked as part of quality assurance and documented. Appropriate antibiotic must be added to the extender.

Semen samples selected for freezing should have minimum 70% initial progressive motility. Final dilution of semen, keeping a minimum of 20 million spermatozoa per dose, shall be done in appropriate flasks with the dilutor maintained at 37^{o}C. Filling and sealing of semen shall be done under aseptic conditions (such as Laminar Air Flow

Unit) using sterile straws, filling nozzles and fresh rubber tubings. French Mini straws should be used.

Unused straws shall be repacked (air-tight) under Laminar Air Flow Unit before storage. Immediately after use, all the glass ware, rubber ware, plastic tips and other re-usables shall be immersed in neutral detergent solution (to be kept in a plastic tub near the Laminar Air Flow Unit). The freezing should be carried out as per the recommended protocols for freezing cattle and buffalo semen. After freezing gets over, the straws should be collected from the racks using scoop tongs. The operator should wear woolen gloves with leather gloves over it to avoid frost injury.

Colour specifications for different species and breeds:

All semen stations shall follow the following colour codes for filling of semen in straws:

Printing of Straws

After filling and sealing, information pertaining to supplier (towards left side), bull name, registration number, date of collection, batch number and breed (towards right side) should be printed on straws. Bull registration numbers will follow international norm (www.icar.org) and following abbreviations will be used for various breeds. For example for a HiFi Lahore semen station (registered with the Authority as 007), semen packed on 28th January 2015 for a Sahiwal bull having popular name as Jumbola registered as 00786 with the Authority (bull code), the straw should read as:

HiFi Lahore Jumbola PK007SW00786 28Jan2015 Sahiwal

Colour codes for filling of semen in straws

Breed	**Color**
Sahiwal cattle	Red
Cholistani	Light blue
Dhanni	Transparent
Holstein cattle	Pink
Holstein Crossbred cattle	Light pink
Jersey cattle	Yellow
Jersey Crossbred cattle	Light yellow
Nili-Ravi Buffalo	Light grey
Goat	Light green
Sheep	Orange

Breed codes for printing on semen straws

Breed/species	**Abbreviation**
Holstein cattle	HO
Holstein Crossbred cattle	XHO
Jersey cattle	JE
Jersey Crossbred cattle	XJE
Sahiwal cattle	SW

Cholistani	CH
Dhanni	DN
Nilli Ravi Buffalo	NI
Beetal Makhi Cheeni goat	GMC
Beetal Nuqri goat	GNQ
Beetal Faisalabadi goat	GFS
Kajli sheep	SKJ
Lohi sheep	SLO

Straws manufactured by reputed companies are safer to use for production of quality semen. While buying straws, package volume and microbial load in straws shall be checked randomly from the consignment. In addition, some empty straws should be placed in filling and sealing machine and the machine should be run to see the sealing quality of the straws. In case of any foul smell, it should be presumed that the straws are manufactured from poor plastic which could be toxic to the spermatozoa and can even result in reduced motility on long storage.

Post thaw motility

After freezing, the semen straws shall be stored in a separate container. Post-thaw motility of semen should be examined at 24 hours (after freezing). Differences in observations shall be updated and recorded for the purpose of accepting a particular batch of semen doses. Whenever there is any doubt, post-thaw motility shall be examined by two experienced persons. Preferably, the person involved in evaluation of neat semen, shall not check the post thaw motility. For a minimum concentration of 20 million per dose, minimum acceptable post thaw motility shall be 40%. Semen doses below 50% progressive motility shall be discarded.

Quality Checks for frozen semen

Quarterly testing of random samples from each batch for bacterial load using standard plate count (the standards for acceptable colony forming units (CFUs) in processed semen is 5000 per ml as per OIE norm. If the bacterial load exceeds the OIE limit, the semen doses are to be discarded.) The frozen semen samples should not have uncountable CFUs as they may have pathogenic organisms. Therefore, semen showing crowded CFUs should be subjected to testing for pathogenic organisms by an outside laboratory. Other tests to check the quality of semen may also be beneficial. Validation of photometer shall be done once in 6 months by checking at least 20 samples each for cattle and buffalo. Neat semen shall be examined at an interval of every six months for morphological abnormalities, particularly for crossbred bulls. Morphological examination of sperms of young bulls must be carried out (at least six samples at weekly intervals) before introducing them in the herd. Semen should not be used if the sample contains a total abnormality of more than 20% and head and mid-piece abnormality (alone) of 7%. Quality checking of semen straws, drawn randomly from the long storage containers once in three months, should be done as a part of quality assurance.

Information System

In order to facilitate the information system, all the bulls maintained by the semen station must be identified by ear tags/ cold branding. The semen stations shall use suitable software to record data pertaining to various activities and also should have

online facility for the same. Barcode system can also be used to facilitate traceability. Volume of semen, density, motility, sperm concentration, dilution rate, total extended volume, post-thaw motility (24 hrs after freezing), and total number of doses produced, etc. shall be maintained. Pre-freeze and post-thaw motility shall be checked for new and problematic bulls.

Miscellaneous information regarding actual reason(s) for not donating semen, undesired percentage of gross morphological defects, semen pH, presence of dirt, dust, blood, pus, etc. in semen samples shall be noted and recorded. Details of semen supplied to various agencies, including post-thaw motility at the time of dispatch, shall be recorded. Fertility data of bulls, conception rate, records of the progeny associated with any genetic defect, percent male / female born, etc. shall be noted and recorded. Report on microbiological examination of semen samples shall also be maintained alongwith record of all quality tests for neat and frozen semen samples shall be maintained.

Semen Storage

To avoid accidental spread of diseases, the semen station shall follow the procedure of preserving semen doses for at least 30 days after production. Frozen semen doses produced at least 30 days prior to the date of dispatch should only be supplied for AI. Semen is preserved in liquid nitrogen at -196°C. Transferring of semen must be done quickly. After checking post-thaw motility, if found acceptable, frozen semen doses shall be kept in temporary storage for 7 days. After temporary storage, the semen goblets shall be transferred to the bulk storage containers with proper recording of position in the canisters. After each dispatch, records redefining the position of remaining doses shall be updated.

Frozen semen should be packed in mini goblet (10-15mm) and stored in medium goblet (35mm) and large goblet (65mm). Liquid Nitrogen shall be replenished at regular intervals depending on the liquid nitrogen evaporation rate as per season of the year. The level of liquid nitrogen in the storage tank should not be allowed to go below l/3rd the depth of the tank. In shipping and handling, the exposure time of frozen semen to air must not be more than 3 seconds. All transfers should be made in mini goblets within 3 seconds.

Two reference samples of the doses dispatched to be drawn and retained for six months or a screen shot of randomly selected sample should be stored and a soft copy of which should be given to the customer. The goblets containing the semen should be well identified and precaution should be taken to see that each goblet has sufficient space for liquid nitrogen. Mini straws need special care and should not be exposed above liquid nitrogen even for a short time (10 seconds) as they get warm faster and any exposure causes irreversible damage to sperm viability.

2.VI: Chapter 6

Guidelines and Standards

for

Import of Exotic Semen

Import of exotic semen must conform to breeding policy which requires development of indigenous breeds of various species to meet the indigenous and export demand of various livestock products and services. Progeny tested cattle semen is however, allowed for crossing with non-descript cattle both for dairy and beef purposes. Import of

semen for species other than cattle for experiment (or other purposes) will require special permission.

1. Dairy Cattle

Breeds: Holstein and Jersey

Form of semen: Frozen in liquid nitrogen (in plastic straws)

Bull's attributes:

1. Origin should preferably be (but not restricting to) United State of America, Canada, Germany and Australia. Other countries only when average performance of daughters meet the laid down criteria.
2. For Holsteins, average lactation milk yield of daughters in a standard lactation of 305 days should be at least 10500 litres for North American, 8500 litres for European and 6500 litres for Australian setups. Daughters under multiple production systems should have average milk yield equivalent to European production setups. Average lactation fat% of 3.7% or better and average protein 3.0% or better.
3. For Jerseys, average lactation milk yield of daughters in a standard lactation of 305 days should be at least 7500 litres with average fat% of 4.8% or better and protein, 3.6% or better. These averages may vary and will be kept close to yearly averages reported by International Committee for Animal Recording (www.icar.org) for various countries.
4. Should have at least 50 daughters performance recorded in 20 or more herds with better than 80% accuracy of production traits. If genomic information is added to recorded performance, accuracy will increase.
5. Estimated Breeding values or Predicted Transmitting Abilities (Gnomic or otherwise) or for milk, fat and protein yields should be positive in the most recent genetic evaluation. Semen from negatively ranked bulls for production traits is not allowed.
6. Bull should be improver for overall Type (positive PTA for overall Type) alongwith positive PTAs for Udder and Feet & Legs conformation.
7. Bulls should not cause more dystocia than average bulls in the recent most genetic evaluation nor should their daughters prone to mastitis than the average bulls.
8. Bulls should preferably be A2A2 genotype for Beta casein.
9. Bull should be free from genetic disease such as Bovine Leukocyte Adhesion Disease, (BLAD) Deficiency of Uridine Monophosphate Synthetase (DUMPS), Complex Vertebral malformation (CVM), Citrulinemia, Factor XI etc and should have normal karyotype.
10. Should be free from scheduled reproductive problems transmittable venereal diseases duly certified by the concerned department of the country. Health certificate from competent authority of the country of origin will have to be supplied.
11. The motility percentage of sperm in frozen semen should not be less than 50% after thawing of semen.
12. Each semen straw (0.25/0.5 ml capacity) should carry at least 20 million sperms for non-sexed and 2 million for sexed semen. Accuracy of female births should be 90% or better with sexed semen.

13. Should be registered with and traceable from Breed Association / Semen Supplier Website

2. Beef cattle

Breeds: Angus, Hereford, Charolais, Brahman (for export of beef from crossbreds)

Form of semen: Frozen in liquid nitrogen in plastic straws

Bull's attributes:

1. Origin should preferably be (but not restricting to) United State of America, Canada, Germany, Australia and Brazil.
2. Must be registered with relevant Breed Association
3. Expected Progeny Difference (or breeding value) should be positive (above average) for weaning weight, yearling weight, calving ease and dressing percentage.
4. The accuracy value for various traits should be greater than or equal to 70%.
5. The sperm concentration per frozen dose of semen should be at least 20 million in 0.5/0.25ml straws The motility of sperms in after thawing of frozen semen should be better than 50%.
6. The donor bull should be certified not to have any genetic and venereal diseases.

2.VII: Chapter 7

Standard Operating Procedure

for

Artificial Insemination Training and Training Institutions

Artificial Insemination Training Institutes operating in Punjab will have to register with the Authority and get accredited for issuing AI training certificates. This also applies to any NGO or Government institution involved in the business. Quality of AI training varies across the organizations due to absence of a uniform training module, standard protocol and a mechanism to ensure its effective implementation by the training institutes. The standards are basic minimums of training for undertaking artificial insemination services successfully.

A. Duration, qualification and Curriculum

1. Duration and qualification

1.6-months (out of which at least 3 months should be extensive practical training in the filed)

2. Matriculation (with minimum of 18 years of age)

2. Curriculum

a. Theory

1) Different breeds of cows and buffaloes and their production and reproduction parameters

2) Introduction to AI, and its importance, role of AI in genetic upgradation

3) Natural Service (NS) vs AI, advantages and limitations.

4) External and internal body parts of a dairy animal and their functions

5) Male reproductive organs & their functions

6) Semen, its collection, evaluation, processing, preservation

7) Reading a semen straw

8) Female reproductive organs & their functions

9) Important reproductive hormones and their role in reproductive cycle

10) Normal reproductive cycle

11) Oestrus cycle and signs of estrous:

12) Puberty, Maturity, Breeding, Fertilization, Implantation, Gestation and Calving

13) Service period, calving interval, dry period and gestation period

14) Synchronization of breeding females

15) Process of insemination using liquid and frozen semen

16) Importance of fixed time AI

17) Care of animals during & after insemination

18) AI equipment and accessories & their care

19) Liquid nitrogen handling

20) Significance of early pregnancy diagnosis

21) Ultrasonography

22) Methods of calculating conception rates and factors affecting conception rates

23) Common reproductive disorders/ diseases, repeat breeding, causes of abortion, etc.

24) Measures to obtain maximum fertility

25) Ear tagging, importance of record keeping, recording formats and submission of records using data transfer devices and web services

26) Method of non-surgical castration

27) Care and management of new born calf and heifers till it becomes pregnant.

28) Care and management of animals before and after calving, precautions at the time of calving and use of naval kit for disinfection of naval cord

29) Importance of bio-security measures to be adopted during AI

30) Basic aspects of nutrition

31) Importance of proper nutrition including feeding of vitamins and mineral mixtures and deworming in fertility management

32) Body condition (scoring) and its relationship with fertility

33) Conservation and development of indigenous breeds through selective breeding

34) Benefits of crossbreeding and genetic improvement of dairy animals

35) The existing Breeding Policy and its enforcement

36) Various government schemes in the livestock sector

b. Practical

1) Identification of different female reproductive organs on morbid genitalia
2) Palpation of female genitalia in a Phantom box and passing of AI gun
3) Structure of LN container:
    - different models
    - handling & care
    - filling and checking LN level
4) AI equipment & accessories:
    - handling & care including sterilization
    - reading and recording of semen straw information
5) Handling of animals under different situations
6) Palpation of female genitalia in live animal
7) Passing of AI gun in live animals
8) Reproductive examination of cows/buffaloes in estrus
9) Demonstration and extensive practice of:
    - proper method for withdrawal of straw from containers
    - proper thawing procedure
    - proper preparation of gun
    - correct site of semen deposition
10) Demonstration of semen motility as affected by long time exposure and thawing process
11) Introduction of metritis and its basic treatment
12) Pregnancy diagnosis at 40 days & beyond
13) Assessing the stage of fetal life
14) Ear tagging
15) Record keeping and communication
16) Study visits should be arranged to AI Centre and Semen Production and Processing facility with a visit of any modern Dairy Farm / Feed factory/ fodder farm

3. Tests and examination:

- Two quarterly written tests on topics covered (10% each
- Final written test at the end of 6-months (30%)
- Final practical test to evaluate the skills learnt (50%)

4. Pass marks:

- Minimum 50% each in theory and practical

5. Attending of in-service short training course of 1-2 days (every year)

B. Physical facilities

1. Class room facilities:

For a batch of 25 trainees, a class room having minimum of 400 square feet area will be required. If there are more than 25 trainees, there should be an additional class of 400 square feet area.

A laboratory having minimum 500 square feet area for practical classes will also be required. This laboratory should have facility to store reproductive organs and keep different models of animals and reproductive organs and space to keep semen and liquid nitrogen storage containers. Disposal of wastes should also be appropriate to keep the environment neat and tidy.

There should be a library/reading room having books and journals on cattle, breeding, indigenous breeds and dairy apart from rooms for teachers and administrators.

2. Teaching aids

a. The class rooms must have the following:

- Adequate chairs and tables for trainees
- White board
- LCD Projector
- Computer
- Charts and Models
- The centre must have the required quantity of semen doses and LN storage containers, AI guns and other AI accessories.
- Reproductive organs must be obtained from a nearby slaughter house for palpation and passing AI guns.
- Ear tags and ear tag applicators
- Measuring tapes and rulers for estimation of heights and body weight
- Other communication technology aids (notebooks, android phone, printers etc).

b. Animals and housing facilities for practical training

- For practicals, the centre should have at least one animal for every 3-4 trainees. Alternatively, centre may have contractual arrangement with some culled animal facility, slaughter house or any other farm for practical training. Every trainee must pass AI gun in at least 20 live animals during entire period of class room training before going for field training during later half of the course. Animals used for training should have normal genital tract. Pregnant animals at various stages f
- pregnancy should also be arranged for demonstration.
- If the centre has its own animals, there should be kept in a comfortable shed and fed, watered and vaccinated properly. Restraining facilities for individual animals should also be appropriate. These experimental animals should be replaced every six months. Disposable gloves, dungarees and gumboots are expected to be born by working with animals including the trainees.

3. Lodging and boarding facilities for trainees

- The centre should have proper residential facilities for trainees including kitchen and minimum recreational facilities. However, if this not feasible, boarding and lodging facilities from other agencies can also be hired. Copy of the formal agreements with such agencies should be available for record for requirement at the time of Accreditation process.

4. Field training

- The Centre should have some formal arrangement with AI service providing organizations for its trainees to receive apprenticeship training for at least 90 days. During this period, each trainee should do minimum 75-100 AIs and the same numbers of pregnancy tests.

5. Documentation of AI training activities

Trainees' records of registration, daily attendance, list of passing out trainees, feedback files, training materials and annual progress reports should be available with the centre.

C. Faculty profile and requirement (for a batch size of 25 trainees)

1. Veterinary Officers:

Minimum two Veterinarians/Teachers registered with PVMC having 3 years of work experience in AI, breeding, health and management of dairy animals along with experience in providing on the job practical training and delivery of lectures.

2. Support Staff:

Minimum one support staff is required with graduation in any discipline

3. Labour for cleaning and other services

Number should be sufficient to keep the facilities neat and clean and well guarded as well

D. Accreditation of Training Institutes

Each accredited AI Training Institution needs to:

a) Follow the standard curriculum and duration for class room and practical training as described in above

b) Have arrangements for class rooms with teaching aids and hands on training in palpating morbid reproductive organs in the laboratory and in live animals and for carrying out artificial insemination as mentioned above

c) Have arrangements with AI Service providing organizations to provide practical training to its trainees for the duration approved

d) Have a registered veterinary practitioner under whose supervision the institute imparts training

e) Evaluate the trainees and award a certificate after successful completion of the course. Furthermore accredited institutes will send the names of the successful trainees to the Authority for issue of a license bearing a photograph and a registration number of the trainee. On successful training the training institute shall equip each trainee the AI kit as well

The process of accreditation will start by a written request of any institution intending to award AI training. Submission of application should accompany the

required documents which will be verified by the Authority against prescribed Minimum Standards detailed above. Following documents are needed:

- Mission & Objective of AI training Institute
- Memorandum of Article / Bylaws of the Organization/AI training Institute, as applicable
- Details of Infrastructure (Office, Classroom, Board & Lodging, etc.)
- Learning resources /teaching aids
- Facilities available for practical and on the job field training on animals
- Details of training programmes, duration, course curriculum, training schedule, admission norms and internal evaluation process
- Details of training faculty
- Copies of records maintained by the AITI regarding previously trained persons (names, addresses and contacts)
- Progress for the last two years (except for the 1st time)

In case application is accepted, the application is further processed for a visit by a team and upon verification of required minimums, Accreditation certificate will be awarded to the applicant for a period of two years. For renewal of accreditation, the AITI has to reapply and same procedure will be followed. The AITI must respond to any queries of the Authority as and when asked. Once, accredited, annual progress reports have to be submitted before the end of July every year.

All the AI technicians working in the field before these laws have to go through a refresher course (to be arranged by accreted institutions) to get practicing license from the Authority.

2. VIII: Chapter 8

Guidelines and Standards

for

Use of Exotic Semen

Exotic semen of cattle is imported to upgrade non-descript local cattle for dairy and beef purposes. Semen of following breeds is allowed for such crossbreeding projects/schemes.

Dairy cattle:

1. Holstein Friesian
2. Jersey

Beef cattle

1. Angus
2. Hereford
3. Charolais
4. Brahman

For dairy cattle, restricting semen to few dairy cattle breeds is basically to manage the availability of semen from crossbred bulls of these breeds. This is in conformity to the breeding policy where two internationally recommended breeds Holstein and Jersey have been allowed. Even with just two breeds, four types of semen will be needed to keep the desired level of exotic inheritance between 50 and 75% i.e two purebreds and two 50% crossbred bulls. Semen from Brown Swiss has also been imported previously but experience from other countries with similar production system as that of Punjab, Holstein and Jersey should be sufficient. It may also be mentioned that Friesian being a large sized breed is suggested for crossing non-descripts when cow being inseminated has a larger size and production system is not subsistence low input type. Jersey semen should be preferred for breeding smaller sized cattle. Yet, in both cases production system should be at least medium input system.

For beef cattle, 3 out of 4 allowed breeds (Angus, Hereford and Charolais) have previously been tested for producing crossbred beef animals in Punjab. Addition of Brahman is for production of beef from crossbred cattle for export purpose (beef qualities almost similar to our local cattle). Cows selected for crossing with thee exotic breeds should be of bigger size because genetic potential for birth weight of all these cattle is almost double than our local non-descript cattle. The precise breeding guidelines will be as follows:

Dairy cattle

1. Purebred Sahiwal and Sahiwal like cows – should be bred only to Sahiwal semen
2. Purebred Cholistani and Cholistani cows – should be bred only to Cholsitani semen
3. Crossbred cattle having 75% or higher exotic inheritance – should be bred with 50% exotic bulls
4. Crossbred cattle having less than equal to 50% exotic inheritance – may be bred with purbred exotic semen for high input systems and with 50% crossbred bulls for low to medium input systems.
5. Non-descript cattle (smaller size) – should be bred with Jersey semen but under low-medium input system, Sahiwal or Cholistani may be used as well
6. Non-descript cattle (bigger size) – may be bred with Holstein or Jersey semen (depending upon the input system, high input production system may prefer Holsteins)

Crossbreeding local cows with exotic bull/semen for dairy purpose

	Bull/Semen					
Cow	SW	CH	HO100	HO50	JE100	JE50
Sahiwal (SW)						
Cholistani (CH)						
Crossbred ≥75% exotic						
Crossbred 50-75% exotic						
Crossbred <50% exotic						
Non-Descript1 (ND1)						

Non-Descript2 (ND2)						

ND1 means smaller sized non-descript cattle, upgraded with Sahiwal or Cholistani

ND2 means bigger sized non-descript cattle

HO means exotic Holstein (50, 100 is proportion of exotic level)

JE means exotic Jersey

Males produced from all these crosses should be culled and only top class males be retained (by planned matings) from top class cows for production of future sires of various exotic level.

Beef cattle

Crossbreeding for beef cattle is not generally recommended for day to day beef production. This is mainly due to high cost of inputs especially when millions of surplus males are available in the province for fattening. However, this may be economical for export (both veal and beef). Another use may be producing of sacrificial animals for big city markets. Following mating plan is suggested assuming that crossbreds (both males and females) will be consumed at a suitable age for sacrificial purpose or for export.

Crossbreeding local cows with exotic bull/semen for beef purpose

Exotic semen	Scope	Local cows
Angus	Good scope for sacrificial local markets	Nondescript and low yielding crossbred cows
Hereford	Good scope for sacrificial local markets	Nondescript and low yielding crossbred cows
Charolaise	For leaner beef export markets	Nondescript and low yielding crossbred cows
Brahman	For gulf markets requiring zebu beef	Non-descript local cows

2.IX: Chapter 9

Standard Operating Procedure

for

Artificial Insemination Services

Genetic potential of AI bulls is expected to be better than those used for natural service. Risk of transmission of venereal and genetic diseases is also lower yet, conception rate is generally low which can be greatly affected by AI technicians and AI service providers.

The first and foremost issue is clarity regarding the breeding policy of the province. While crossbreeding of cattle is allowed but for non-descript and crossbreds cattle only. Only two breeds are allowed, Holstein and Jersey and desired level of exotic inheritance should be within 50 and 75% range. Holstein Friesian is not preferred for

short sized cattle not it is for low input system / poor farmers. Purebreds cattle such as Sahiwal and Cholistani (and cows which as almost similar to them) should be bred with Sahiwal and Cholistani cattle semen only. Nili-Ravi buffaloes should be bred with Nili-Ravi buffalo semen. Similarly, Nuqri, Faisalabadi and Makhi Cheeni or Rahim Yar Khan strains should be bred with respective bucks or semen from them. Same shall be true for sheep. The general slogan is: purebreds for purebreds and exotics and crossbreds for non-descript and crossbreds.

The second issue is availability of semen for Authority registered semen production facilities only. Top graded semen production facilities should be preferred.

Semen storage

- Store frozen semen doses in a well-ventilated, all weather safe storage area.
- Ensure a proper and foolproof identification system for each semen container, canister, and goblet so that a bull's semen can be traced with ease.
- While transferring semen doses, goblets should be well identified and precaution should be taken to see that each goblet has sufficient space for liquid nitrogen. Frozen semen should not be exposed above liquid nitrogen as it may cause irreversible damage to sperm viability.
- All transfers of semen straws into goblets should take place under liquid nitrogen, in a polystyrene / thermopol box.
- Liquid Nitrogen should be replenished in both storage and distribution containers at regular intervals to ensure proper level of liquid nitrogen.
- Details of semen doses supplied to various AI technicians at the time of dispatch should be recorded. After each dispatch, records redefining the position of remaining doses should be updated.

Liquid Nitrogen

Service provider shall have a bulk Liquid Nitrogen (LN) sourcing, storage and delivery facility. A schedule of LN replenishment to all AI centres on fortnightly / monthly / quarterly basis, whichever is convenient depending on the field containers shall be worked out by each service provider and shall be adhered to in the interest of maintaining quality of semen. A log book shall be maintained for all such schedules at different locations/ starting points of supply routes. The AI centre should have a bigger container for LN and semen straw storage, preferably of 35 litre capacity, and a small portable LN container, preferably 2-3 litre capacity, to carry the semen to the place where the AI is carried out. The LN containers in the AI centre shall be protected sufficiently to avoid damage to container. AI centre should have a dip stick with critical level marks and a ready-reckoner for assessing the LN levels and quantity of LN in litres. Supply of LN should be either through portable LN tankers of 500 to 2000 litre capacity with gravitational flow or through the LN delivery pump and not by pouring LN from one container to another container.

AI guns and sheaths

Stainless steel AI guns from an agency whose AI guns are tested and approved by Authority shall be used. Same shall be true for AI. AI accessories like forceps and scissors shall be made of good quality stainless steel. Thermos flask and thermometer shall be of good quality.

AI Technicians

AI Service providers should ensure that they engage only those AI technicians who have undergone a training course in AI from a government recognized AI training institute and collect a copy of their training certificate at the time of appointment.

Animal Identification

Every animal receiving AI shall be identified with an Ear Tag with a unique number and (preferably) a barcode. These numbers shall enable generation of reports concerned to the individual animal and the associated information through an information system. Only polyurethane laser printed ear tags having a 12 digit number and a bar code shall be used. The numbering system followed shall be unique with the last digit of the number being a “check digit” to ensure that no two animals are tagged with the same number. Only numbers supplied by an agency identified by the Authority shall be used for unique identification of animals. An example may be for a buffalo tagged in Lahore district (digit code 16) and registered as 000001 may have ear tag as 16NR000001

Supervision, Review of Activities and Communication

There shall be a hierarchy of supervisory mechanism. Every 20 AI technicians should have an AI supervisor. Every 60-65 AI centres should have a veterinarian to provide advisory services. A team of 200 AI technicians, 10 supervisors and 3 veterinarians shall form a region controlled by a Regional Officer. An effective communication network shall be in place for communication among the team members in a given area. AI technicians, supervisors and veterinary officers of an area shall meet once in a month for a review of technical programme, business transactions as well as for scheduling the extension programmes. There shall be a monthly review meeting at regional level involving Regional Officer, veterinary officers and AI supervisors. Effective supervision is reflected in the accuracy of reporting, fixing the problems faced efficiently and effectively, acceptance of progress records by the system, promptness in business transactions and minimum backlogs.

Annex-I

CLINICAL EXAMINATION OF BULL

UNIT:		
NAME/IDENTIFICATION NUMBER OF BULL:		
BREED		
DATE OF BIRTH:		
REGISTRATION NUMBER:		
1	CLINICAL EXAMINATION (On first day of quarantine) (General health, testis, penis, accessory glands and presence of hereditary defects)	
	Date of examination _____ / _____ / _____ yr mo day	
	Finding:	
	Remarks:	
2	ROUTINE TEST (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: Agglutination test.	

3	CERTICATION
	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 Signature Date Name in Capital letters PVMC registration number
5	ENDORSEMENT (Bull Approval Committee)
	We hereby endorse the certification as done above and recommend/do not recommend the use of above described bull for semen collection for Artificial Insemination purposes.

	Signature Name in Capital letters	Date Official Stamp

Annex-II

BREEDING SOUNDNESS OF A BULL

Date of Examination …….

Owner:

Address:

(A**) Identification** Name - (Ear tattoo, Ear tag, Brand No.)

Breed ---------- Age----------

(B) **Record**

i) Pedigree covering information upto grand grand maternal and paternal sides

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ii) Progeny: No. of herds/No. of daughters - PD - Progeny Testing based on BLUP

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iii) Proven /Test:

---

iv) A. I. /Natural:

(C) **General Clinical Examination**

i) Weight: ..... Condition Score: .....

ii) General Health: ……

iii) Teeth and Jaws: ……. Eyes: ……. …. Thorax: …….Abdomen:…….

iv) Feet: ……..Legs:……Joints ……...Gait:……

(D) **Physical Examination of the Reproductive Organs**

i) Scrotum: …… Testes: ……. Scrotal Circumference……. (cm)

ii) Normalcy/ Consistency.....

iii) Epididymides.....Spermatic Chord: .....

iv) Accessory organs:

Seminal vesicles.....

Ampullae:.....

Prostate: .....

Bulbourethral: .....

v) Penis and

Prepuce: .....

(E) **Semen Examination**

i) Collection Method: ……

ii) Volume (ml): …….. Density (0-6)…….....

Wave Motion (0-6)……..... Motility (%)……..

iii) Examination of Serving Behavior:

Serving Ability:

Libido..... Erection (Stiffness ..... Protrusion …… Deviation ….....)

Seeking.....Ejaculatory Thrust..... Protrusion on thrusting.....

Body Position..... Serving Capacity: …… acceptance to vagina ……

Mating & semen ejaculation: ……..

F) **Vaccination Record**

Foot & Mouth: ……

Black Quarter: ……

HS: ……

(G) Tests for Infectious Disease:-

Brucellosis: ……

Leptospirosis: ……

Trichomoniasis: ……

Vibriosis: ……..

Tuberculosis: ……

IBR: ……

BVD: ……

(H) **Semen Results Sheet**

Concentration (million per ml): ……

% spermatozoa alive (Nigrosin eosin): ……

% abnormal sperm heads: ……

% spermatozoa with:

Proximal cytoplasmic droplets ----------

Distal cytoplasmic droplets ----------

Tailless heads ----------

Bent tails ----------

Coiled tails ----------

Acrosome defects ----------
Structural abnormalities ----------

Cells other than spermatozoa ----------

Comments:

Annex – III

BRUCELLOSIS TESTING

I	Screening Test Details	Name	ELISA, RBPT, CFT, SAT
		Sample	Serum
		Testing at	VRI, Lahore, UDL, UVAS or any other recognized lab
II	Eligible animals		• All above one year • In females 14 days after calving or abortion
III	Frequency of testing	Positive herd	30 to 60 days after culling of last positive animal
		Negative herd	Exactly one year (± 1 week) after last whole herd negative testing
		Negative herd (optional	Where the disease has been maintaining a very low profile (less than l % positive) quarterly or six monthly sample could be collected to minimize losses
IV	Action on finding a positive bull	Animal	Immediate isolation, castration and culling
		Semen	Destroy semen doses since last negative test
V	Brucellosis free herd (OIE)		Herd found negative on two consecutive annual tests
VI	Quarantine	Duration of quarantine	Minimum 30 days
		Test schedule	Two tests, Serum ELISA, interval of 30 days between tests. Only negative animals to be allowed to mix with the rest of the herd
VII	Additional testing at sexual maturity		Serum ELISA before bulls are used for semen collection and distribution for Al

UDL- University Diagnostic Lab
UVAS-University of Veterinary & Animal Sciences, Lahore
VRI- Veterinary Research Institute, Lahore

Annex – IV

CAMPYLOBACTERIOSIS (VIBRIOSIS) TESTING

I	Screening test details	Name	Bacterial isolation & identification
		Sample	Perpetual washing, semen
		Testing at	VRI, Lahore, UDL, UVAS or any other recognized lab
II	Eligible animal		All male animals
III	Prevention		Annual sheath washing
IV	Frequency of testing	Positive herd	30 days after culling of positive animal
		Negative herd	Exactly one year (± 1 week) after last whole herd negative testing
V	Action on finding a positive bull	Animal	Treat the animal
		Semen	Destroy semen doses since last negative test
VI	Quarantine	Duration of quarantine	Minimum 30 days
		Test schedule	One test if age is less than 6 months, others 3 consecutive tests at weekly interval

Annex – V

TRICHOMONIASIS TESTING

I	Screening Test details	Name	Agent isolation & identification
		Sample	Perpetual washing
		Testing at	VRI, Lahore, UDL, UVAS or any other recognized lab
II	Eligible animals		All male animals
III	Prevention		Annual sheath washing
IV	Frequency of testing		Annual
V	Action on Finding a Positive animal	Animal	Treat the animals
		Semen	Destroy the collections since last negative test
VI	Quarantine	Duration of quarantine	Minimum 30 days
		Test Schedule	One test if age is less than 6 months, others 3 consecutive tests at weekly intervals
VII	Additional Testing at sexual Maturity		Protozoa isolation before bulls are used for semen distribution for A.I.

Annex – VI

LEPTOSPIROSIS TESTING

I	Screening Test details	Name	Agglutination test, Immuno-fluorescence, Immuno-histochemistry, PCR
		Sample	
		Testing at	VRI, Lahore, UDL, UVAS or any other recognized lab
II	Eligible animals		All animals
III	Prevention		Screening and isolation of animals Prevention from rodents
IV	Frequency of testing		Annual testing
V	Action on Finding a Positive animal	Animal	Treat the animal
		Semen	Destroy the collection since last negative test
VI	Quarantine	Duration of quarantine	Minimum 30 days
		Test Schedule	Annually
VII	Additional Testing at sexual Maturity		ELISA, annually

Annex – VII

TUBERCULOSIS

I	Screening Test details	Name	Delayed Hypersensitivity – Single Intradermal test
		Reagent	Bovine tuberculin PPD
		Testing at	VRI, Lahore, UDL, UVAS or any other recognized lab
		Positive result criteria	As per OIE norms *Negative*: Increase in skin thickness less than 2 mm & without clinical signs viz; exudation, necrosis, pain, inflammation of the lymphatic duct of that region or the lymph node, 72 hours post-inoculation. *Inconclusive*: Increase in skin thickness more than 2 mm & less than 4 mm, absence of above clinical signs, 72 hours post-inoculation. *Positive:* Increase in skin thickness 4 mm or more, or presence of clinical signs viz; exudation, necrosis, pain and inflammation of the lymphatic duct of that region or the lymph node, 72 hours post-inoculation.
II	Eligible Animals		All animals above 6 weeks of age
III	Frequency of testing	Positive herd	Minimum 60 days after culling of last positive animal.
		Negative herd	Annual test is minimum. Six months (±1 week) after last whole herd negative testing, desirable.
IV	Action on finding a positive bull	Animal	Immediate isolation and culling
		Semen	Destroy semen doses since last negative test
V	Tuberculosis free herd (OIE)		Herd found negative on two consecutive tuberculin tests at an interval of 6 months, the first being performed 6 months after the slaughter of last affected animal
VI	Quarantine	Duration of quarantine	Minimum 90 days
		Test schedule	Two tuberculin tests, minimum interval of 60 days between tests.

PPD- Purified Protein Derivative

Annex – VIII

FOOT AND MOUTH DISEASE TESTING

I	Action during FMD outbreak	Animal	Isolate diseased animals till recovery, do not cull
		Semen	Semen from FMD infected bulls : Destroy semen collected during one month before onset of outbreak. Do not collect semen from bulls during the outbreak and three months after the last case of FMD recovered in the farm Infected animals must be given 90 days rest Semen from healthy bulls maintained in FMD infected farm : Destroy semen collected during one month before onset of outbreak. Do not collect semen from bulls during the outbreak and one month after the last case of FMD recovered in the farm. Semen could be used other than the above mentioned periods, if there is no new case of FMD develops during three months period after last FMD case recovered in the farm
II	Quarantine		Vaccination - as per manufacturer's recommendations
III	Vaccination	Oil vaccine	Annual in farm and 5 to 10 km around the farm Test for seroconversion, by collecting serum on the day of vaccination and 21 days later.

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