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NATIONAL DAIRY RESEARCH AND DEVELOPMENT CENTRE
DEPARTMENT OF LIVESTOCK
MINISTRY OF AGRICULTURE & FORESTS
YUSIPANG, THIMPHU

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STANDARD OPERATING PROCEDURES FOR BOVINE ARTIFICIAL INSEMINATION IN BHUTAN

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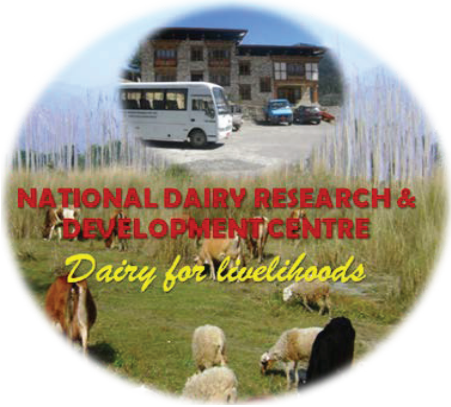

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STANDARD OPERATING PROCEDURES FOR BOVINE ARTIFICIAL INSEMINATION IN BHUTAN

Purpose

The Standard Operating Procedures (SOP) is required to guide field AI technicians (AITs) while performing Artificial Insemination (AI) in bovine. This SOP provides sequential steps that need to be strictly followed to improve conception rate in the field. Artificial Insemination Technicians and Community Artificial Insemination Technician (CAITs) should be familiar with the SOP as outlined below.

Artificial Insemination Centre (AIC) should have basic facilities and equipment to render efficient professional AI service to the beneficiaries. Basic facilities includes a separate room for storage of Liquid Nitrogen (LN2) containers, Semen bank, AI register and all essential AI equipment / items required to carry out AI service professionally.

Failure to observe the SOP will lead to compromised results.

Safety & Environmental Considerations

Safety procedures should be followed while performing Artificial Insemination (proper restraining of animals, proper usage of safety gears, proper handling of Liquid Nitrogen and Frozen Semen) and roper disposal of AI consumables post insemination.

1. General procedures

1.1 Keep AI Centre (AIC), AI equipment, and premises clean and tidy.

1.2 Personnel should be present at the AIC to receive LN_2 and other AI inputs on the specified schedule.

1.3 Monitor Liquid Nitrogen (LN_2) level in Semen bank and regularly refill with LN_2 from storage container at weekly

interval to ensure adequate quantity of LN_2 **(minimum of 20 cm level)** in semen bank.

1.4 Frozen semen stock in Semen bank should be identified with clear tagged canister/goblet with complete description of semen details.

1.5 Maintain accurate semen inventory and updated AI records at the AIC.

2. Taking the history of animal

It is imperative to obtain detailed information of the animal in heat from the owner. Identify the animal in estrus and check past breeding records and history. The person presenting the cow should be asked questions as outlined below to get a clear picture about the animal in heat.

2.1 When was the animal observed in heat?

(Optimal conception rates are achieved when AI is performed within 8 -12 h after onset of heat symptoms).

2.2 Date of previous calving?

(Optimum time for breeding after calving in normal healthy cow is 60 days post parturition).

2.3 What is the breed of the animal?

(Crossing of more than two breeds (tri hybridization) shall not be allowed as per the Livestock Breeding Guidelines of Bhutan. For details refer to Guidelines for use of pedigree selected and imported bovine frozen semen.

2.4 In case of cow, what is the lactation number? In case of heifers, what is the age of heifer and estimated body weight?

Breeding is recommended in heifers that attains about 70% of mature body weight for the particular breed.

2.6 What are the heat signs observed by the owner and has any abnormal discharge or abnormal behaviors been noticed?
Observe body condition score (BCS) of the cow and should be in the range of 2.5 – 3.5 for higher conception rate.

3. External Examination

3.1 Examine the animal externally and ascertain whether that animal is in heat.

3.2 Carefully check tail, vulva & adjacent parts for presence of mucus or discharge.
(**The best sign of heat is clear, transparent, viscous and ropy vaginal discharge**).

3.3 Check animal for signs of standing heat on the rump or the tail.

3.4 Open vulva lips and check clitoris & mucus membrane.
(**In case of abnormal discharges, flecks of pus are detected on clitoris**).

3.5 Animals that show abnormalities in external examination should not be inseminated & necessary treatment carried out.

4. Rectal Palpation of cow

4.1 Ensure animal inside the AI crate or chute is properly restraint for safety of animal & inseminator.

4.2 Lubricate gloved hand with non-irritant lubricant.

4.3 Carefully insert gloved hand into rectum with gentle movements.

4.4 Remove dung from rectum (if any) by gently back racking to prevent ballooning.

4.5 Carefully locate and fix cervix through rectal wall.

4.6 Carefully palpate cervix & two uterine horns.

4.7 If cow is in heat, Cervix is slightly soft and pliable & uterine horns are turgid (tonic).

4.8 If Cervix is hard / rigid & uterine horns are soft / flappy, Cow is unlikely in heat.

4.9 Uterine horn should be carefully checked for pregnancy, pyometra or other abnormalities.

4.10 Cows with abnormalities or without obvious signs of heat should never be inseminated.

4.11 Avoid touching ovaries during rectal palpation. **(Palpating ovaries of cows in heat can rupture the follicle and the cow will not conceive).**

5. Thawing of Frozen semen

5.1 Proceed with thawing of semen and preparation of AI gun only after completing the above procedures and ascertaining that the animal is in standing heat.

5.2 Ensure that all items required for thawing & performing AI are assembled and kept ready before thawing of semen. The following items are required: hot water, cold water, Thawing Tray (Kidney tray / thawing container), Scissors, Thermometer, Forceps, Clean towel / tissue paper, AI gun, AI sheath and AI gloves.

5.3 Adjust thawing temperature by adding cold or hot water to 37-39°C in the thawing tray.

5.4 Place the thawing device near semen bank.

5.5 Remove neck plug / cap from the semen bank.

5.6 Pre cool the tips of forceps in LN2 vapor for few seconds (inside the neck of semen tank).

5.7 Raise / Pull out the Canister containing semen just high enough for the semen straws to be visible and well below the frost line in the container neck.

5.8 Keep all unused straws well below frost line in neck of semen bank.

5.9 With the pre cooled forceps, identify the semen from the

desired bull. Remove one semen straw using tweezers / forceps. Do not pull out and thaw more than one semen straw at any time.

5.10 Lower the canister and arrange it properly inside the semen bank.

5.11 Ensure removal of excess LN2 retained in cotton plug by gently shaking the semen straw as soon as it is taken out of the semen bank to avoid bursting of semen straw.

5.12 Never allow semen to refreeze after removal from semen bank.

5.13 Quickly plunge straw into thaw unit and ensure semen straw is fully immersed in thawing water.

5.14 Replace neck plug of the semen bank.

5.15 Thaw straw at 37°C for 30 seconds.

5.16 During thawing period, pre warm AI gun by rubbing it with towel/ tissue paper/ cotton, but do not over heat the gun. Ensure that AI gun and sheath are also maintained at temperature of around 37°C.

5.17 After thawing period, remove straw from thawing water and dry it completely in tissue paper or towel or using cotton. (**Remember Water is lethal to spermatozoa**).

5.18 Do not touch the portion of the straw which contains semen while handing the semen.

5.19 Always ascertain that air space / air bubble if any in the semen straw is at the laboratory seal end.

(Grasp the semen straw at laboratory seal end and shake it once or twice. This will force the air bubble to move to the laboratory sealed end of the semen straw. The location of the air bubble at the laboratory seal end is important so that when the semen straw is cut no semen is lost or there is no discontinuity to the flow of semen during AI).

5.20 Check sealed ends of semen straw for any leakages / breakage before loading into the gun.

5.21 Carefully load the semen straw in the pre warmed AI Gun.

6 Loading of AI Gun

6.1 Assemble parts of the AI Gun.

6.2 (Universal AI gun that accommodates both straw types and specific AI guns that accommodate either 0.5 ml or 0.25 ml straws can be used).

6.3 While using universal AI gun, ensure that specific bore diameter of AI gun is used for the specific straw type.

6.4 Carefully pullout one AI sheath about 3 cm from the sterile AI sheath package.

6.5 Pull plunger and lock of gun, 10cm out of the AI gun.

6.6 Put manufacturer's end (double wad end) of semen straw into pre warmed AI gun as far as it goes.
(This should lead to the semen straw protruding a few cm out of the AI gun).

6.7 Hold loaded AI gun vertically at eye-level and with clean sharp scissors cut straw horizontally (right angle) just below laboratory seal.

6.8 Take out one AI sheath by holding bottom of the sheath from protective cover & place it over AI gun.

6.9 (**The AI sheath prevents contamination of AI gun and holds straw in place during expulsion of semen**).

6.9.1 Place and secure the sheath firmly over AI gun to lock the AI gun.

6.9.2 The AI gun is ready to use.

(Please note that after AI gun is loaded, insemination should be performed as early as possible).

7 Insemination Technique

7.1 Wear shoulder length AI glove, preferably on left hand and hold the gun with right hand. Ensure to keep loaded AI gun warm and hygienic during transit from thaw water to the cow. Repeat steps from rectal palpation of cow clause no. 4.1 to 4.5.

7.2 Clean vulva thoroughly with water and wipe dry with a dry towel / tissue paper prior to insertion of gun.

7.3 Ask owner to help spread / open the vulva.

7.4 Never allow AI gun tip to touch external coat, anus or vulva of the animal.

7.5 Insert insemination gun at 30° to 45° angle to avoid entry of gun into urethral opening.

7.6 Hold cervix firmly through rectum and slightly stretch it forward to unfold the vaginal folds.

7.7 Gently and smoothly pass the gun through the vagina to the opening of the cervical canal.

7.8 Hold the external os of cervix ahead of the gun's tip and negotiate vaginal folds and cervical rings to pass the gun through the cervix till the gun's tip reaches internal os.

7.9 Be firm, yet gentle, do not overdo it nor be too aggressive and take your time.

7.10 Remember AI is a two step process: first get AI gun into cervix, then place cervix over AI gun.

7.11 Feel the tip of AI gun at internal os with tip of index finger to ensure that the gun is in correct place (just at the internal os / uterine body).

7.12 Be certain gun tip is not caught in thin area between cervical rings or vaginal folds or fornix.

7.13 Slowly deposit semen in internal os / uterus body by push the gun piston with your thumb.

7.14 After depositing semen, gently withdraw / remove AI gun and gently massage cervix and vulva few times.

7.15 Check AI gun and semen straw for any leakage of semen between straw and AI sheath and to ensure complete semen deposit in internal os or body of uterus.

8 Post Insemination

8.1 After insemination, recheck semen ID and other details on the straw.

8.2 Properly dispose used AI sheath, semen straw, AI gloves, tissue paper and other items.

8.3 Place any contaminated AI gun for thorough cleaning before next use.

8.4 Wash hands particularly the non gloved hand.

8.5 Record all required information in the specified AI register provided.

8.6 Ask animal owner to release the animal and let her calm down.

8.7 AI is performed in relatively unhygienic surrounding so keep AI kit clean, organized & tidy at all times and wash it thoroughly at regular intervals

8.8 AI Gun should be washed and rinsed with 70% alcohol at least once a week

9 Post Insemination advice to animal owner

9.1 Ask farmer to keep the animal under observation for next 12-24 hrs.

9.2 Advice the owner not to let inseminated animals mix with bulls during the remaining part of present heat.

9.3 Advice the farmer to observe for heat symptoms after 18-21 days.

9.4 If animal does not repeat heat at 18-21 days intervals for two consecutive times, call for pregnancy diagnosis after 3 months from the date of insemination.

10 Post insemination follow up

10.1 Follow each and every animal inseminated after 18 - 21 days to find out whether it has repeated and for pregnancy diagnosis after 3 months.

10.2 Follow each and every pregnant animal and record calving detail of the animals inseminated in the AI register provided.

10.3 Send AI monthly progress report of AIC to the Dzongkhag Livestock Sector and concerned RLDC in the format provided by NDRDC, Yusipang.

10.4 Always maintain all records related to AI, pregnancy diagnosis, calving and other details in the AI register provided.

10.5 Advise animal owners on proper heat detection, timing of AI, feeding, management and healthcare of animals from time to time.

11 Care and handling of LN_2 and Frozen semen

All AITs and CAITs should follow the **Guidelines on handling of Liquid Nitrogen (LN_2) and Frozen Bovine Semen (**circulated separately) for proper care, handling and management of LN_2, Frozen semen and AI equipment.

བ་ཨ་བའི་རྟགས་མཚན་དང་ཐ་སྙགས་ནི་གི་དུས་ཚོད།
HEAT DETECTION & INSEMINATION

བ་ཨ་བའི་རྟགས་མཚན་དང་ཐ་སྙགས་ནི་གི་དུས་ཚོད། HEAT SYMPTOMS & TIME OF INSEMINATION

ཨ་ཡང་ནི་སྐོར་ཤྱིན་འཕྲོད།

རྟགས་མཚན། སྐང་ཆུབ་ནི། ནོར་གཞན་ལྟ་དྲིམ་ཉམ་ནི། ནོར་གཞན་གུར་བཞོན་ནི། མོ་མཚན་ཆོན་མཁྲོ་ཞིན་མ་ལས་དམར་དོ་སྨུ་སྨོ་ནི། ཇོང་ས་ཅིག་ལྟ་གསང་མི་ཆགས་ནི། ཚོམ་མར་འབབ་འཕྲོམ་ནི།

ཐ་སྙགས་ནི་དང་ཐ་སོན་བསྟུན་ནི་གི་དུས་ཚོད་མ་རན་ཚེན།

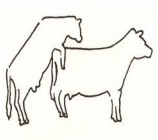
COMING INTO HEAT (8 hours)

Signs: Bellows, Sniffs other cows, rides other cows, Red and swollen vulva, Restless, decrease milk production

Insemination : Early

ཨ་ཡང་སྐྱེ་རྒྱ་ཚོད་ ༤ ་དང་ ༡༢ ་གི་བར་ན།

རྟགས་མཚན། གཞན་ལྟ་བཞོན་པ་བཏུག་ནི། ལོག་ལོག་ཅིག་སྐད་ཆུབ་ནི། ཇོང་ས་ཙང་མ་ཆགས། མོ་མཚན་རྒྱན་ཉམ་ནི། ཞིན་མར་དམར་དོ་སྨུ་སྨོ་ནི། མོ་མཚན་ནང་ལས་ སྨུབས་ལྟ་བཟུམ་ ཅིག་འཐོན་མ་ཤེན།



STANDING HEAT (8 to 12 hours)

Signs: Stands to be ridden, frequent bellowing, restlessness, Red and swollen vulva , Clear mucus discharge from vulva

Insemination: Right time and high likelihood of resulting in pregnancy

རྒྱ་ཚོད་ཀཱ་ཀྱི་རྒྱལ་ཨ་ཡང་ནི་ བསཉན་ནི།

རྟགས་མཚན། ནོར་གཞན་ལྟི་བཞོན་མ་བཏུག་ནི་། ནོར་གཞན་ལྟ་དྲིམ་ཉམ་ནི། མོ་མཚན་ནང་ལས་སྨུབས་ལྟང་བཟུམ་ འཐོན་མ་ཤེན།

ཐ་སྙགས་ནི་དང་ཐ་སོན་བསྟུན་ནི་གི་དུས་ཚོད་འཕྲི་ནི།

CESSATION OF HEAT (After 18 hours)

Signs: Will not stand to be ridden, smells other cows, clear mucus discharge

Insemination: Too Late & less likely to result in pregnancy

ཐ་སྙགས་ནི་དང་ཐ་སོན་བསྟུན་ནི་གི་དུས་ཚོད། TIME OF INSEMINATION

ཨ་ནི་གི་དུས་ Time of heat	ཐ་སྙགས་ནི་གི་དུས་ Time of Insemination	དུས་ཚོད་འཕྲི་ནི། Too late
ཇོ་ག Morning	ཕྱི་ཐུ་ཡས་ར། Evening	དེ་གི་ནངས་པ་ཡས། Next morning
ཉིམ། Afternoon	དེ་གི་ཕྱི་ཐུ་ར། Late evening / early morning	ཟླུས་མ་གི་ཉིམ་ཡས། Next day afternoon
ཕྱི་ཐུ། Evening	དེ་གི་ནངས་པ་ཇོ་ག Next Morning	ཟླུས་མ་གི་ཕྱི་ནོ། Next evening

བ་ཡུ་ཐ་སོན་བསྟུན་ཐབ། INSEMINATION TECHNIQUE

ལག་པ་གཡོན་དང་ རུབས་གསང་ཆུས་ཡིར་ དོར་ནང་བསྟུན་ཞིན་ཡས་ ཨངས་ཚང་སྐོ་ (Cervix) འདི་ལེགས་ཐོན་སྐེ་བཟུང་ ཞིན་ཡས་ ལག་གཡས་དེ་ཁིས་ ཐ་སོན་ ཁས་འདི་བསྟུན་དགོ།

ཕྱི་ བསྟུན།

ཕྱིག་ཏྲི།

རྒྱལ་ཡོངས་ཨོ་ཚས་ཞིན་འཚོལ་དང་གོང་འཕེལ་སྐྱེ་བ།
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