Seminar @ NOTEP MINIMUM STANDARD PROTOCOL FOR PRODUCTION OF BOVINE FROZEN SEMEN





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Present Status in India GOI, Deptt. AH & Dairying

Total Cattle & buffalo	: 302.34 M (2019)
Total breedable animals (in-milk and dry)	: 125.34 M
Total semen stations	: 53 (Grade A -28; Grade B-08)
No. of bulls used for semen collection	: 4158
Total semen production	: 115.9 M
Target of semen production (2021-22)	: 140 M
AI centre	: 1,30,000
Al coverage in animals	: 30%
AI target (2021-22)	: 50%

Status of Grading of Semen Stations

Table : 1.Grading of semen stations over the years

Grades	2005	2009	2011	2013	2016	2018
А	2	12	20	30	37	28
В	12	15	17	15	14	8
С	12	7	3	-	_	-
Not Graded (NG)	33	13	7	5	2	6
Not Evaluated (NE)	-	2	2	2	5	11
Total	59	49	49	52	58	53

State-wise distribution of semen stations with the grades awarded (AR/DADF 2018-19)

Sl. No.	State	Grade A	Grade B	Not Grade d	NE	Total Stations
		80 & above	66 to 79	Below 65		
1	Andhra Pradesh			2	1	3
2	Assam	1				1
3	Bihar				1	1
4	Chhattisgarh	1				1
5	Gujarat	4	1			5
6	Haryana	3	1		1	5
7	Himachal Pradesh	1				1
8	Jammu & Kashmir				2	2
9	Karnataka	3	1		1	5
10	Kerala	2			1	3
11	Madhya Pradesh	1				1
12	Maharashtra	3	1		1	5
13	Meghalaya			1		1
14	Odisha				1	1
15	Punjab	1	2			3
16	Rajasthan	1	1			2
17	Tamil Nadu	2	1	2		5
18	Telangana			1		1
19	Uttarakhand	1				1
20	Uttar Pradesh	_ 1			2	3
21	West Bengal	3				3
	Total	28	8	6	11	53

Artificial Insemination Network (2018) Largest livestock breeding network

Institutions	Numbers
State Livestock Development Boards	28
Veterinary Hospitals/Polyclinics	12,099
Veterinary dispensaries	25,263
Veterinary aid centres	27,628
Intensive Cattle Development projects	346
Cattle breeding farms	185
Buffalo breeding farms	35
Goat breeding farms	161
Sheep breeding farms	88
Intensive sheep development projects	69
Pig breeding farms	366
Semen production centres	53
Frozen semen banks	226
Liquid nitrogen plants	92
AI Centres	1,30,000

Total Frozen Semen Production (In Million)

The future of Indian dairy industry lies with bull semen





Bulls under FSD Production

Annual growth rate: 4.4%







Central Monitoring Unit (CMU)

- Review & revise minimum standards as per <u>MSP guideline</u>
- Evaluation of sperm stations
- Submission of reports to the department
- Inspection at 2 Yrs interval



CMU Grading

A (Excellent semen station without deficiencies)	:28
B (Deficiency in one or two aspects)	:08
C (Deficiencies in many aspects)	
D (Deficiencies in most of the aspects)	



Artificial Insemination - best tool world wide for <u>genetic improvement</u> through dissemination of superior germ plasm

Achieved only if the <u>frozen semen used</u> in AI programme conforms to the <u>quality standards</u>



Bulls used in AI programme satisfy quality norms Bulls - disease free Semen - harvested & processed as per MSP

Failure to observe the guidelines

Poor quality semen Unfit for distribution to AI centres

1. Standard for Genetic Merit of Breeding Bulls

Bulls procured should follow

Minimum Standard Protocols (MSP) & Standard Operating Procedures (SOP) for Progeny Testing (PT) through Government approved PT Programmes (Breeding value estimation committee, GOI)

If such bulls are not available

based on the <u>dam's Standard lactation yield</u> {Recorded as per International Committee for Animal Recording}.

Standards for Dam's Lactation Yield (wef 1st April 2020)

Breed	Dam's lactation yield (Kgs)			
	First	Best	Fat %	
Pure HF*	7000	10000	3.5	
Pure Jersey*	5000	6000	5.0	
Sahiwal	2400	3000	4.0	
Tharparkar	2000	2500	4.0	
Hariana	1600	2000	4.0	
HF cross F2*	5000	6000	4.0	
Jersey CB-F2*	3500	4500	4.5	
Murrah	2400	3000	7.0	
Bhadawari	1300	1600	8.0	

Dam's milk yield for F-1 cross will be as indigenous dam's yield

2. Physical Examination of Bull

Before procuring

Thorough physical examination (by an accredited Official)



Scrotal circumference once in 3 months



Body weight - once a month

Thorough breeding soundness examination

3. Karyotyping & testing for genetically transmitted diseases

To rule out any chromosomal defects

Breed	Specific Tests to be carried out
Indigenous cattle & buffaloes	Factor XI deficiency syndrome Bovine Leukocyte Adhesion Deficiency (BLAD) Citrullinemia
Jersey & Jersey Crossbreds	Above tests
HF & HF crossbreds	Above tests plus Deficiency of Uridine Monophosphate Synthase (DUMPS)



4. Quarantine Quarantine period - minimum 60 days compulsory before bringing new bulls into semen station **Ouarantine activities Place** – 5 Km from semen station Manpower, equipments, feeding, watering & cleaning - Separate **Tests for major contagious diseases** - TB, JD, Brucellosis, IBR/IPB, Campylobacteriosis, Trichomoniasis & BVD (by accredited agency) Vaccination - FMD, HS, BQ, Theileriosis & Anthrax

Vaccination Schedule







6. Culling of Bulls & Semen Doses due to Specific Diseases

Diseases	Bulls	Semen doses
i. FMD	Retain	Last one month's doses to be discarded
ii. Brucellosis	Castrate & remove	Discarded since the last negative test
iii. TB	Remove	do
iv, JD	Remove	do
v. IBR/IPB	Retained provided semen sample -ve	do, if virus is isolated on culture on semen samples.
vi. Campylo- bacteriosis	Treat and retain	Discarded since the last negative test
vii. Trichomoniasis	Treat and retain	do
viii BVD	Isolate & remove	do buils culled when

- i. +ve for above diseases (ii, iii, iv, v & viii) (within 48 h)
- ii. +ve for Campylobacteriosis & Trichomoniasis isolated & treated
- iii. Completed 6 yrs of productive period or 2 lakh semen doses
- iv. Poor libido, poor semen quality, incurable lameness, etc.



8. Management of Bulls

- a) Hygienic conditions
- b) Body coat
- c) Hooves
- c) Prepucial hairs
- d) Brushing and grooming
- e) Prepuce washing

- at all times
- clean & generally short.
- regularly trimmed.
- cut to about 2 cm (too short, it may cause irritation)
- regularly, special attention to the underside abdomen.
- sterile normal saline solution,
- every 10 days (if the microbial load, within the prescribed limits -5000 CFU/ml).
- Cleaning prior to the day of collection if the m/load beyond the prescribed limit.
- f) Scientific feeding schedule followed.
- g) Quality analysis of feed and fodder routine .





At least mounting area as above or Rubber matting on hard floor On the day of collection – washing & cleaning of bulls, prepuce cleaning externally with NSS Use of sterilized bull aprons



Sexual preparation - by false mounts (2-3)

Semen collector - wash hands with 0.1% Savlon soln. or use disposable gloves

- do not touch the penis
- AV shd. not thrust on penis, instead penis shd. be guided to AV.
- Appropriate size AVs, ranging from 8-14"
- AV cone ; top quality Neoprene rubber.



Semen ejaculates

- min. two ejaculates/collection & min. two collections /bull /week

Annually target

- at least 90 collection days and 180 ejaculates from each adult bull.

Post-collection

- Cleaning & spray of odorless disinfectant solution, e.g. Colloidal iodine
 - Cleaning of AV by non-spermicidal neutral detergent.







+1 MM



+2 MM



+3 MM



+4 MM



+5 MM



After dilution





PTM in Low Magf.

PTM in High Magf.

10. Handling, processing & freezing of semen

A) Premises

- □ Sufficient trees to reduce dust.
- □ Entry in the laboratory, other than laboratory personnel strictly restricted.
- □ Airlock system or air curtain must in processing laboratory.
- □ Preferably split AC to maintain the room temperature at 20° C (Maintaining this temperature is most important to achieve the best results of semen freezing).
- **Relative humidity** 55% processing lab.
- □ UV lights appr. Number
- □ Lab. fumigation weekly for 2 hr

(with 12 ml 37% formadehyde soln. in 100ml cu. Mt. using humidifier)

- □ Work platform cleaned with 70% alcohol or Glutaril.
- □ Sink not be placed in the semen processing room.
- **Floors** preferably made up of vitrified tiles.



(B) Equipments

- Digital photometer / Computer aided Spectrophotometer shall be validated with Haemocytometer readings for sperm concentration twice a year.
- Automatic straw filling & sealing machine thoroughly cleaned, immediately after use.
- The microscope lens gently cleaned daily with a mix. of ethyl & methyl alcohol (1:1) or a mix. of 80% ethyl alcohol & 20% ether.
- Incubators cleaned & disinfected with 70% alcohol.
- Single distilled water shall be used in autoclave & thermo-controlled water bath.
- Provision for two refrigerators,
- •Separate for storing eggs, antibiotics & buffers.
- Separate for chemicals.



(C) Personnel Hygiene

Bull attendants- tested for TB every year.Other staff- tested for TB once in two years.

(D) Diluents

- Prolonged storage of purified water not recommended
- After preparation buffer sterilized again.

Antibiotics in diluents:

- **To control Mycoplasma**, Gentamycin, Tylosin, Lincospectin & Spectinomycin (GTLS) may be preferred.

- Combination of Penicillin & Streptomycin.

Eggs :

- fresh & from known source

- sterilized with 70% alcohol.

(E) Evaluation & Processing



(F) Colour Specifications:

Standard colour codes of semen straws to be followed by all semen stations:

Breed	Colour of straw
Holstein	Pink/Rose
HF Crossbred	Pistachio Green (light green)
Jersey	Yellow
Jersey Crossbred	Salmon
Indigenous cattle	Orange
Sunandini	Blue
Buffalo	Grey
Others	Transparent



(G) Printing of Straws

Printing information on straws:

- bull number, breed, name of the organization, year, batch number, ejaculate number, preferably after filling and sealing

- room temperature : 20° C.

- 1.* SS Bull Id- alphanumeric
- 2. *Breed
- 3. *Semen station Name
- 4. *Batch No. (Eja. No.)
- 5. *Dam's Lactation yield & source
- 6. Brand image
- 7. Brand code

*Essential & 6,7 - Optional





Three Character Semen Station Codes			Breed Code formation		
Sr. No.	Semen Station	State	Туре	SS Code	Printing abbreviations to be followed: Jersey – JY Holstein – HF
1. 2.	ABC, Salon AMUL	UP Gujarat	Trust Coop	ABC AMU	HF Cross – CB HF Jersey Cross – CB JY
3.	BAIF	Maha- rashtra	Trust	BAF	Sunandini – SUN Sahiwal – SAH Red Sindhi – RS
4.	CFSP&TI, Hessarghatta	Karnataka	GOI	CSF	Kankrej – KANK Gir – GIR
5.	SAG Bidaj	Gujarat	Trust	BDJ	Tharparkar – THAR
6.	Rishikesh	Uttara- khand	Govt	ULD	Hariana – KATHI Hariana – HAR Murrah Buffalo – MBF
7.	Alamadhi Semen Station, Chennai	Tamil Nadu	Trust	ALM	Surti Buffalo- SBFJaffrabadi Buffalo- JBFMehsana Buffalo- MSNB

Allotted codes till date – 54 SS

(H) Post-thaw motility

Examined - 24 h after freezing.

If any doubt, examined by two experienced persons.

(Preferably, the person involved in evaluation of neat semen, shall not check the post-thaw motility).

PTM - below 50% - discarded.







Thawing unit

(I) Quality Checks for frozen semen

A summary of quality tests, cut-off values :

Sr. **QC** Parameters **Cut- off Values Check period** No. 1 **Bacterial Load** 5000 CFUs /ml For each batch 2 Hypo-osmotic Swelling For all bulls, once in a $\geq 40\%$ **Test (HOST)** quarter 3 standard drop in motility by **Incubation / Thermo** Do-10% after every 30 minutes resistance Test 4 **Intact Acrosome (PIA)** $\geq 65 \%$ Dousing Giema stain 5 **Sperm Concentration** 20 million sperm/dose Once in 6 month by random sampling (0.25 ml Mini straw) 6 **Morphological** < 20%Doabnormalities

(J) Information System

Following record must be maintained –

- 1. Use suitable software to record data to avoid manual data entry.
- 2. Barcode system for semen stations producing more than one million doses may be introduce (ejaculates, production, storage & dispatch of semen).
- **3.** Imp. records of semen Volume, density, motility, concentration, dilution rate, total extended volume, pre-freeze motility, PTM & total number of doses produced etc.
- 4. Miscellaneous records
 - Actual reason(s) for not donating semen, undesired % of gross morphological defects, pH, presence of dirt, dust, blood, pus etc.
 - ii. Details of semen supplied to various agencies (name of agency & PTM, date).
 - iii. Conception rate of bulls, records of the progeny associated with any genetic defect, % male / female born, etc.
 - iv. Microbiological load.
 - v. Record of all quality tests for neat and frozen semen.

(K) Semen Storage

- Frozen semen doses produced at least 30 days prior to the date of dispatch.
- After checking PTM, if acceptable, kept in temporary storage for 7 days & then transferred to the bulk storage containers.
- Liquid Nitrogen replenished at regular intervals depending on the evaporation rate.





13. Cleaning and Sterilization

Laboratory and other areas: Cold fumigation Artificial Vagina (AV): autoclaved at 5 psi pressure x 20 mts or AV sterilizer **Glassware:** Non-spermicidal neutral detergent solution for 30 mts Hot air oven at 160°C for 1h or at 180°C x 30 mts. Rubber wares: autoclaved at 3-4 psi x 10 mts. (The rubber tubing for semen filling shall not be reused). Distilled Water: Fresh triple glass distilled water or Milli-Q purified water shall be autoclaved at 15 psi x 15 mts **Buffer:** autoclaved at 5 psi pressure x 20 mts Bacteriological Media: autoclaved at 15 psi pressure x15 mts. Filter Papers: Whatman No.1 -wrapped in thick cotton cloth for sterilization in an autoclaved at 5 psi pressure x 20 mts.



a) Autoclave

Summary of Sterilization

S. No.	Items	Pressure (psi)	Time (Min)
1	Artificial Vagina	5	20
2	Buffer	5	20
3	Plastic Tips	5	20
4	Filter Papers	5	20
5	Bull Apron	5	20
6	Thermo-resistant Rubber wares	3-4	10
7	Bacteriological Media	15	15
8	Distilled Water	15	15
9	Surgical Equipment	10	10

b) Hot Air Oven

Sr.No	Item	Temperature	Time (min.)
1.	Glass wares	160°C / 180° C	60/30
2.	Filling Nozzles	160°C / 180° C	60/30

c) Gas sterilizer

Using ethylene oxide gas (900 mg/cu.lit. for 6 h)





Quality Control of Consumables

Chemicals:

Analytical Reagent (AR) / Guaranteed Reagent (GR) from reputed companies. Purity > 99%

Straws:

- 1. Reputed companies.
- 2. The factory plug should not be loose.
- 3. The straws remain intact (without cracks / dents, etc.) during and after freezing.
- 4. Easily printable.
- 5. Movement of the factory plug should be free.
- 6. Straws should be routinely checked for microbial load.

Note: The semen stations should avoid purchase of consumables on lowest quotation basis. e.g.: To produce top quality semen, it is better to use AR / GR reagents manufactured by reputed companies whose products are reliable. This is true with other consumables also.

Manpower Requirement for semen production

Designation	Up to 5 lakh doses	5-10 lakh doses	10-25 lakh doses	Above 25 lakh doses
Officer Inch.	1	1	1	1
VO	1	2	2	3
QCO	1	1	1	1
Lab. Tech.	1	2	3-4	4-5
Lab. Attendant	2	3	3-5	4-6
Office Asst.	1	1	1	1
Bull Attendant	1 person per 7- 8 bulls			

For dispatch of semen – separate official **Refresher training / visit** to must be arranged compulsorily once in two to three years at reputed semen production stations.

List of lab. Equipment for frozen semen station:

Sr. No.	Items	Nos.
1	Phase contrast microscope with biotherm (Nikon/Olympus)	1
2	Slide warmer	1
3	Auto/eppendorf pipette	3
4	Water bath (IMV)	1
5	Laminar air flow	1
6	CCTV	1
7	Photometer (IMV)	1
8	Ph meter	1
9	Straw filling, sealing & printing machine	1
10	Cold handling cabinet	1
11	Biological freezer	1
12	Freezing rack (mini)	30

Sr. No.	Items	Nos.
13	Distribution ramp (mini)	1
14	Incubator	2
15	Autoclave	2
16	Hot air oven	2
17	Double glass distt. plant	2
18	Millipore water purification system	1
19	ETO sterilizer	1
20	Magnetic stirrer	1
21	Electronic weighing machine	1
22	WM bulk FS storage container	2
23	bulk FS storage container	1
24	Geyser	2

Sr. No.	Items	Nos.
25	Vacuum cleaner	1
26	Central voltage stabilizer	1
27	FS storage Cont. (12000 mini str)	5
28	LN storage cont (50 lt.)	10
29	PC	1
30	Fumigator/ humidifier	1
31	Thaw unit	2

Conclusion

- An ideal semen station should follow prescribed MSP.
- All sanitary measures from rearing of bull to collection, processing, freezing storage & transportation of semen must be followed.
- Semen should of high quality and should maintain good fertility rate.

Kindness makes you most beautiful person in the World, no matter what you look like.

> THANK YOU