Introduction

Today India stands on the threshold of a new era with pragmatic programme of reformation, liberalization and globalization, which destined to spur economic growth. In this direction, agriculture in general and livestock in particular is in a position to be a major driving force for fast and sustainable growth of national economy.
Present scenario

• The population available for breeding is 105.5 millions which includes 57 millions of indigenous cattle, 8.5 millions of crossbred and 40 millions of buffalo. The inseminations carried out are 19.5 millions covering 18.8 percent of the population.
• The pregnancy rate on inseminated population is only 23.0 percent
• This is because either the frozen semen produced is of poor quality or the handling of the frozen semen in the field post freezing is poor along with poor insemination technique and improper heat detection
A wide variation in milk productivity per animal between developed and developing countries is due to various reasons, but in India the low productivity can be attributed to the following factors.

Despite having large number of bovine population, average lactation yield per animal is 795 kg, but in developed countries like US, UK and Israel average production per animal varies from 9000 to 14000 kg per lactation. (Export prospects for agro-based industries world trade Center, Mumbai).

• i). Poor genetic potential and severe genetic deterioration over a period of decades,

• ii). Inadequate feeding, poor health and poor management,

• iii). Poor AI coverage
The present low level of productivity cannot be expected to change dramatically in the near future with the existing scenario of breeding practices. Still there is a tremendous scope for enhancement of animal productivity in India by adopting suitable genetic improvement programmes.

Today, reproductive biotechnologies allow us to make rapid changes in the genetics of milk producing animals. Maximum utilization of elite sires for artificial insemination has been proved as one of the most successful unique reproductive biotechnologies in cattle breeding with its emergence as the cheapest and fastest mode of genetic improvement.

It has had an enormous impact worldwide in many species, particularly in dairy cattle. Natural service would limit the use of one bull to less than 100 mating per year. In addition exposure of sires to infectious genital diseases and its subsequent spread to livestock population through natural service is also prevented by use of AI.

Time required for establishing reliable proof on young bull is reduced through AI.
• Systematic selection of superior bulls for AI is very important and the progress made in India in this regard is negligible.

• Semen of bulls of high breeding value and of desired genetic trait shall be adopted for mating to the bull mothers.

• To gain maximum and rapid genetic progress, proven superior sires should be employed for breeding.
To support the genetic improvement programme in the country there is a need to produce quality semen from genetically superior bulls and adoption of large scale AI programme.

The success of AI depends on how best the various steps right from the selection of bulls to insemination in the field are followed.

However, to meet basic objective of increasing animal productivity, there should be rigorous selection of young bulls of desired traits and adoption of improved methods to produce quality semen and its usage in the field.

One of the important factors that determine the degree of success of AI is quality of frozen semen used.
• Artificial Insemination is a double edged sword. It helps in improving the cattle population faster at the same time, if genetic merit, health and hygienic protocols are not followed for bulls and processing of semen respectively, this biotechnique can also affect adversely the animal population at large.

• It is therefore very essential to adopt certain minimum standards for bulls, semen processing and quality
Selection of bulls

- **Genetic Merit** - This objective can be achieved by procuring the bulls which will satisfy the pedigree norms adopted in the minimum standard protocol.

- **Physical Soundness** - The bulls should be physically sound. The joints, hooves and gait should be normal.

- The bulls while mounting put the weight of their body on hind legs, the hind legs should not have any abnormality like sickle shaped, bow or straight hocks.

- The neck, eyes and shoulders should be normal.
Selection of bulls

• Reproductive soundness
• The bulls should be examined for the size of the testis (length and breadth), scrotal circumference etc as the semen production capacity depends on these parameters.
• The feel of the testis is also very important. Normally the testis should be firm and not soft, the soft feel indicates degeneration of the tissue, testis should not be hard also, hard testis indicate fibrosis. There should not be any injury or swelling on the scrotum.
• The scrotum should have proper neck and the testis should be suspended in vertical plane in it. There should not be any rotation of the testis, however, rotation of 90 degree or lesser is tolerable and is not going to affect the semen quality
examining the scrotum

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placement of hand to measure scrotal circumference

RIGHT!

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• EXAMINE THE TESTIS FOR
• SOFTNESS/HARDNESS
• FREE MOVEMENT IN THE SCROTUM
• TORSION OF TESTIS
• EPIDIDYMIS(CAUDA,CORPUS AND CAPUT)
• LENGTH AND BREADTH OF TESTIS
• BIFID CHARACTER
Selection of bulls (continued)

- The bulls should be examined per rectum for accessory sex gland and their soundness.

- After the andrological soundness examination, the semen of the bulls should be collected and examined for ejaculate volume, mass activity, sperm concentration and initial motility.

- The smears of semen should be taken for sperm morphology acrosome integrity and live count.

- The semen collection will also throw light on the sexual behavior of the bulls, penile movements seeking ability and type of thrust etc.
Health protocol

- Once the bulls are selected, they should be quarantined in quarantine shed away from main herd (at least 2 to 5 kms.)
- During this period they should be tested for TB, JD, Brucellosis, IBR-IPV, Trichomoniasis and Campylobacteriosis and declared free
- The bulls should be vaccinated for foot & mouth, HS and BQ. Ring vaccination, covering a radius of 5 kilometers shall be done for foot and mouth in all cloven footed animals.
- If the bulls are purchased from known source or from own farm, the quarantine of 30 days is quite sufficient. If the bulls are purchased from unknown source but of good pedigree as per MSP, they should be quarantined for at least 60 days. First 30 days they should be kept under observation, and all tests should be carried out in 2nd 30 day period and if found negative should be added to the main herd of bulls.
Health protocol (continued)

Not only the bulls should be screened against the common diseases like TB, JD, Brucellosis, but also for genetic carrier diseases like BLAD; Citrulanemia, DUMPS etc.

Bulls should be karyotyped and studied for any chromosomal aberrations.

The bulls should be regularly dewormed by broad spectrum dewormer like Fenbendazol, Albendazol or injectable ecto-endo parasitacide (Dectomax or Ivermectin).
Bulls are housed in scientifically designed sheds
Bull housing

• The bulls should be provided with comfortable housing. Single bullpens having loafing area is suitable for breeding bulls. The bulls should be kept free. The bullpens should have feeding manger and water trough. In summer there should be cooling arrangement for the exotic and buffalo bulls. The bullpens should be kept clean.

• The bullpens should be surrounded by good shady trees and the construction of pens should be such that the bulls are not exposed to direct sun.

• Strong smelling disinfectants like phenyl or formalin should not be used in bull shed instead compound like gluteraldehyde, colloidal iodine or chlorine compounds like hypo chlorate be used.

• Weekly spraying of 4% soda be practiced. The floors should be scrubbed and burnt at least once in a year by blow lamp.

• There should be isolation shed for sick animals.
Loafing area with soft bed
Buffalo enjoying shower
Preputial Washing
Preputial Washing
Managing breeding bulls

• Proper care and comfort of breeding bulls should be taken to get maximum production of semen
• The bulls should be kept under hygienic conditions all the time
• The hooves should be regularly trimmed
• Regular exercise should be given to the breeding bulls to keep them trimmed. This also helps reducing their reaction time
• The preputial hair should be trimmed to 2 cm. length, long preputial hair cause adhering of the dung to it, spoiling the ejaculate. If closely trimmed it will cause irritation leading to frequent masturbation by the bulls
• Bulls shall be washed and groomed regularly, while washing special attention shall be given for washing underside of abdomen
• In case of obvious soiling of prepuce careful cleaning of preputial orifice and the adjoining areas with soap and detergent, followed by thorough washing and drying should be done
Feeding of bulls
Feeding of the breeding bulls

- Daily nutrient requirements of mature and young growing bulls has to be worked out from available fodder and feed resources. A ready table of requirement according to bodyweight of bulls is given at annexure ‘ix’ of MSP.
- Roughly 2.5kg of dry matter per 100 kg bodyweight should be given to adult bulls. This also includes the feed concentrate.
- The dry matter should come from 60% of green roughages and 40% dry fodder.
- The concentrate feed should form 0.5% of the body weight.
- In addition to this mineral mixture at the rate of 50 g per day should be given.
Semen collection

Collection arena

- The floor of the collection yard shall be made of cement concrete up to about a depth of one foot from ground level. A mixture of sand and limestone shall be used to fill up above this level and pressed firmly to have proper footing for the bulls.

- Alternately good quality rubber mat with interlocking shall be put in concrete groove for adequate cushioning effect. Over rubber mat coir mat can be used.

- After collection of semen the area shall be thoroughly cleaned with odorless disinfectant solution (Colloidal iodine).

- The floor of the collection yard shall not be dusty.

- There shall be sufficient plantation around collection arena which will serve as wind breakers preventing dust.
False Mounts
False Mounts
Semen Collection

- On the day of collection and previous evening the bulls shall be thoroughly washed and groomed. The prepuce and adjoining abdomen shall be cleaned with sterilized napkin, soaked in warm normal saline to remove any dust particles.

- A separate napkin shall be used for each bull.

- On the previous evening of the semen collection, the preputial cavity shall be washed with sterile normal saline, about 200 ml of saline can be pushed with the help of sterile Folley’s catheter and held by closing orifice with one hand. A mild massage from lower to upper side can be given and then the saline can be drained out. For each bull separate catheter shall be used.

- Sterilized bull apron shall be tied to the bull when he enters the semen collection arena, and then the bull shall be given two to three false mounts and restraint of about two minutes to stimulate him thoroughly for donation of good quality semen ejaculate.
Semen collection (continued)

- Preferably a veterinarian or trained livestock supervisor shall collect the semen.
- However, veterinarian shall supervise the semen collection process. The person collecting the semen should understand the psychology of bulls. He should be friendly with bulls and not beat or shout.
- The semen collector shall wash his hand before every collection with mild disinfectant and use separate napkin for drying hands or shall use the disposable hand gloves. He shall not touch the penis, touching the penis will cause shyness and bull dismounts.
- Immediately after semen collection the AV shall be kept immersed in a tub with neutral detergent lotion.
AVs for Cattle & Buffaloes
Prepared AVs with special cone
Semen collection (continued)

- Before taking the semen collection, the semen collector shall check that the semen collection tube is attached to the cone and properly protected with the warm water jacket and/or felt cap and covered by insulation bag and the open end of the AV is protected by aluminum foil.

- Preferably AV of 10 inches for bulls and 9 inches for buffalo bulls be used (Goat AV is good enough for buffalo bulls).

- As soon as the semen is collected the collection tube shall be labeled and handed over in the laboratory for further processing.
Ascertaining correct AV temp
Semen being collected from a bull
• The cone of the AV shall be of “Neoprene” rubber, which will facilitate for fast draining of semen directly in the collection tube without any loss in the folds etc which normally happens in latex cone.

• Use of lubricant shall be avoided as far as possible. If required only K.Y. Jelly shall be used with separate lubricating glass rod for each AV. For each ejaculate separate AV shall be used. Even when the bull introduces penis without ejaculation the AV shall be discarded.

• The semen collector shall use the protective clothing while collecting the semen. He shall use barn coat (preferably of mild green or gray color, bright colors are not liked by the bulls), Gum boots and disposable hand gloves. The bull handlers also shall have the uniform of similar color.

• Two ejaculates with the interval of half an hour to one hour shall be taken.
Water jacket covering Collection tube
Hands cleaned in detergent soln between collection
Used AVs are soaked in detergent solution.
Semen processing

• **Laboratory**

• The semen evaluation, glycerolization, printing, filling and sealing shall be carried out in one room fully air conditioned and maintaining 20 to 22°C.

• In adjoining room also maintained at 20 to 22°C racking of the filled and sealed straws and equilibration and freezing shall be carried out. The filled and sealed straws shall be transferred through a pass box provided for this purpose.

• The building material used for laboratory construction shall give no cracks and crevices. The walls of semen processing laboratory shall be fixed with glazed tiles to avoid dusting from the walls. The floor shall be made of epoxy or vinyl to avoid retention of infection and ease in wet mopping and thorough cleaning.
Shoes removed before entering Lab
Apron rack & Lab shoes
Indoor quality of air is a complex multifaceted issue.

Contaminants come with dust air and visitors as well as originate inside and threaten the quality of environment.

Acceptable indoor quality can be achieved by following the fundamental principles:

a) Contaminant source control

b) Proper ventilation

C) Humidity management

Adequate filtration
Before starting of lab work

• The empty laboratory should have:
  • a) less than 35 colony forming units (CFU) of bacteria/m$^3$ of air
  • b) Less than 1 CFU of pathogenic bacteria in 30 m$^3$
  • c) During working less than 180 CFU/m$^3$ of air ultra clean laminar flow in the lab
  • d) Less than 20 CFU/m$^3$ in the periphery of the lab and less than 10 CFU/m$^3$ at the center
Air Lock Function

• Provides barrier against loss of pressurization and against entry/exit of contaminated air in/out of the isolation room when the door to the airlock is opened

• Provides a controlled environment in which protective garments can be donned without contamination before entry into the processing laboratory room

• Provides a controlled environment in which equipment and supplies can be transferred from isolation room without contaminating the surrounding areas
Control of Humidity

- In presence of low humidity microorganisms ride on dust particles. Low humidity is reported to be suitable for Klebsiella pneumoniae activity.
- High humidity enhances the danger of growth of pseudomonas aeruginosa.
- Relative humidity should be between 40 to 65 percent.
- The air change rate of 10 air changes per hour reduces the level of any contamination present in the air by about 99 percent. 15 to 20 air changes per hour should be sufficient for comfort, to ensure pressurization in the room and to maintain considerable control of airborne microorganisms.
Air Curtain
The laboratory

• The laboratory shall not have any sink or drain and shall not store any extraneous material adding dust to the lab. atmosphere.

• In fact the laboratory tables shall not have any drawers for storage of material and the edges and corners of the tables should be rounded off.
Semen processing (continued)

- The semen processing laboratory shall be provided with air lock or anti room so that there shall not be direct entry of the persons. The persons working in the laboratory shall wear aprons, laboratory foot wares, caps and masks before entering the laboratory and will keep wearing till they are in the laboratory. While going out they will remove the laboratory wares and deposit in the laboratory.

- The aprons, caps and masks shall be washed in the premises in the washing machine and will not be sent for washing outside.

- The laboratory windows shall be made of single glass panel and covered with blue film to avoid radiation effect. The doors and windows will be in aluminum frames and glass. Visitors shall not have entry in the laboratory and can view from glass windows if so desired.
Semen Processing (continued)

• The semen extender shall be prepared in laminar flow station with the help of standard chemicals. The chemicals used for preparation of extender shall be either G.R. or A.R. quality, coming from reputed firms.

• The eggs used for the preparation of egg yolk shall be from known poultry farm and shall be fresh.

• Only fresh semen extender shall be used; the pH of the leftover extender will change on storage and the extender then may not remain suitable for extending semen.

• The combination of antibiotics in the extender should be such that can control contaminants, like mycoplasma and other ubiquitous organisms. This can be effectively done by a combination of Tylosin, Lincospectin and Gentamycin, alternatively crystalline penicillin, Streptomycin can be used.
Preparation of Dilutors
Preparation of Dilutor
Ph of Dilutor is checked
Waterbath & Photometer
20 micron semen taken in 0.5 ml dilutor
Semen being diluted in laboratory
Neat semen being evaluated in laboratory
Semen processing (continued)

• The laboratory shall be fumigated twice a week. On Wednesday the laboratory shall be fumigated with 5% formaldehyde and on Saturday with 12% formaldehyde for half an hour to two hours.

• Parts of equipment handled during processing shall be cleaned with 70% alcohol before starting the work. The laboratory shall be wet mopped twice a day before starting and after finishing the work with disinfectant having glutaryldehyde like Lysol.

• The Laminar flow stations shall be cleaned with 70% alcohol and shall be tested for DOP for every six months and should have annual maintenance contract.

• The cold handling cabinets shall be also cleaned with 70% alcohol and shall be under annual maintenance contract, they should maintain 4 to 5°C temperature for equilibration.
Diluted semen being packed in straws using automatic filling sealing machine
Straws being printed through automatic jet printer
Packed straws being kept in cold handling cabinet for equilibration
Straws being frozen through computerised bio-freezer
Semen Freezing Room
Semen processing (continued)

• The semen shall be packaged in mini French straws (0.25ml) and shall have proper identification. The prints shall have Name of the organization, Bull name/number with breed /species and batch number (day of freezing and year).

• For different breeds and species different color codes can be used as specified in MSP.

• Each semen straw shall have 20 million sperm.

• No semen sample showing less than 70% initial motility and less than 500 millions spermatozoa per ml shall be processed.
Frozen straws being segregated for storing
Frozen semen straws in a container
Equipment

• All major equipment should be under annual maintenance contract.
• The washing and sterilization of glass ware and rubber ware should be carried out in separate rooms.
• There shall be a separate room for AV preparation and washing and separate room for glass ware washing and sterilization.
• Distillation of water for preparation of diluent and milli-Q equipment shall be in separate room.
• There shall be separate arrangement for quality control of the frozen semen, away from the semen processing and freezing complex.
Quality control

- After freezing the straws shall be stored in separate container and post thaw motility of the semen shall be examined after 24 hours of storage under liquid nitrogen.

- No semen sample showing less than 50% post thaw forward motility shall be preserved. In order to avoid the bias the post thaw motility shall be seen by the quality control officer and not the person who processes the semen.

- Each batch of frozen semen shall be tested randomly for bacterial load by standard plate count. As per OIE standard, 5000 colony forming units (CFU) are permissible per ml. All bulls shall be covered in a quarter.
Quarantine of semen
Other quality control tests to be carried out are:

- Percent intact acrosome in frozen thawed spermatozoa
- Hypo osmotic swelling test for frozen thawed spermatozoa.
- Incubation test for frozen thawed spermatozoa

All these tests shall be done covering all the bulls in a quarter

- Validation of photometer once in six months
- Young bulls before they are included in regular collection schedule should be tested for morphological abnormalities of spermatozoa, live/dead count, acrosome integrity and should be tested frozen for studying the freezability at least for six consecutive ejaculates
Semen Dispatch
Frozen semen doses being dispatched
Information System

• The semen stations shall install suitable software so that online data can be fed. It is easy to generate reports on bull’s performance month wise and year wise for doses stored, doses discarded, percent discard etc.

• Information on sales of frozen semen bull and breed wise/recovery state wise and/or institute/party wise wise etc

• Fertility information bull wise and region or state wise etc

• Information on quality tests, bacterial load of frozen semen etc
Information system
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Bull attendants—one person per 7 to 8 bulls
The man power should be dedicated and committed to the work.

The officer in charge should be at least post graduate in animal reproduction and specialized in semenology.

The veterinary officers should be graduate in veterinary science and should have training in semenology (Frozen semen technology) at reputed institution.

The lab technician should be bachelor in science or microbiology.

Lab attendants should be high school and should have aptitude for working in laboratory.
Consumables and equipment purchase

- The Critical equipment required for laboratory should be purchased from reputed companies dealing in the equipment and should not be purchased by tenders.

The essential equipment are:

- Phase contrast microscope with biotherm & CCTV
- Slide warmer
- IMV Photometer
- Water bath
- Laminar airflow unit
- pH meter
- Auto filling and sealing machine
- Electronic weighing machine
- Triple glass water distillation plant
- Incubator
- Cold handling cabinets
- Millipore water purifying equipment
- AV sterilizers
- Magnetic stirrer
- Freezing racks and distribution ramp

Straw printing machine / Inkjet printer
Biological freezer
Hot air oven
Autoclave
Bulk frozen semen storage containers
Frozen semen storage containers 10 to 12000 straws capacity.
Liquid nitrogen storage containers
Mini French straws
PC, printer and UPS
Fumigator
Refrigerators
Artificial Vaginas for bulls and buffalo bulls
Air conditioners
Long steel forceps, tweezers, test tube stands, Scissors etc.
- Miscellaneous equipment
AV liners
AV cones
Hemocytometer,
Trolleys
Auto pipette stands
AV stand,
Pump for filling air in AV
Liquid Nitrogen Storage tank
Thank you