Minimum Standards

For

PRODUCTION AND TRANSFER OF BOVINE EMBRYOS

2025

DEPARTMENT OF ANIMAL HUSBANDRY AND DAIRYING
MINISTRY OF FISHERIES, ANIMAL HUSBANDRY AND DAIRYING
GOVERNMENT OF INDIA
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MINIMUM STANDARDS FOR PRODUCTION AND TRANSFER OF BOVINE EMBRYOS

Introduction

With the rising coverage of artificial insemination (AI) and thereby increasing demand for high genetic merit Donors/ Recipients (HGM) for semen production, the dairy industry is craving for multiplying elite germplasm faster to produce superior progenies. The situation is further demanding in case of indigenous cattle, where availability of elite animals of various breeds is very sparse. Under National Dairy Plan I, large scale field based genetic improvement programmes were implemented to identify and multiply elite bovine population. Considering the importance of such programme for genetic improvement of dairy animals, Government of India (GoI) continued implementation of the same programmes under Rashtriya Gokul Mission (RGM). To aid these efforts, there is requirement of efficient use of different assisted reproductive technologies (ART) for faster multiplication of the elite bovine population. Embryo production, both *in vivo* and *in vitro*, is an ART deployed to produce more number of offspring from a female animal during its lifetime. The non-elite inferior animals are used as recipient (surrogate) for the embryos to produce better progenies.

There is strict requirement of maintaining quality standards of the embryos produced by either of the methods to provide quality service to the farmers. The present minimum standard document is intended to put forth guiding norms for production, grading, packing and identification norms for production and transfer of embryos produced through both *in vivo* and *in vitro* methods.

1. Standards for Genetic Merit of Donors and Sires

1.1 Donors:

Donors should meet the breed characteristics and production standards laid down in the present document. Following criteria should be maintained for selection of donors:

a. Only donors (Dams/ Cows/ Heifers) meeting the pedigree standards prescribed in the Minimum Standards Protocol for Semen Production issued by Department of Animal Husbandry and Dairying, Government of India (DAHD), GoI are to be to be used in embryo production. Current standards of donors is mentioned in Table 1. The same would stand revised up on revision of Minimum Standards Protocol for Semen Production.
b. The milk production record of the donors should be as per the milk recording system

approved for Progeny Testing/Pedigree Selection programmes under RGM project and registered in NDLM.

c. Heifers based on positive Genomic Estimated Breeding Value (GEBV) for milk production for the breeds wherein GEBV estimation is feasible can be used as donors.
d. Heifers produced from imported embryos can be used as donors.
e. For selection of donors whose record is not available in NDLM, endorsement of donor by a committee, constituted by DAHD, would be mandatory.
f. Donors from farmers can also be used if meeting above criteria.
g. For using imported embryos, the standards for import of germplasm as prescribed in the "Guidelines for export/ import of bovine germplasm" issued and amended from time-to-time by DAHD, Ministry of Fisheries, Animal Husbandry and Dairying, GOI shall be applicable.

1.2 Sires:

a. Semen should be obtained from A graded Embryo Production Facility and Donors/ Recipients available at Embryo Production Facility should be selected on the basis of their breeding values, dam's lactation yield, sire dam's lactation yield.
b. Donors/ Recipients should be of high genetic merit meeting the pedigree standards prescribed in the Minimum Standards Protocol for Semen Production issued by Department of Animal Husbandry and Dairying, Government of India (DAHD), GoI. Current standards of sire's dam's lactation yield is mentioned in Table 1. The same would stand revised up on revision of Minimum Standards Protocol for Semen Production.
c. For imported Donors/ Recipients or semen the standards for import of germplasm as prescribed in the "Guidelines for export/ import of bovine germplasm" issued and amended from time-to-time by DAHD, Ministry of Fisheries, Animal Husbandry and Dairying, GOI shall be applicable.

Table: 1 Standards for Dam's lactation

Breed	Dam's Lactation yield (kg)		
	First	Best	Fat %
Holstein Friesian	7000	10000	3.5
Jersey	5000	6000	5.0
Sahiwal	3000	3500	4.0

Red Sindhi	3000	3500	4.5
Gir	3000	3500	4.5
Kankrej	2500	3000	4.5
Tharparkar	2500	3500	4.0
Hariana	2000	2500	4.0
Rathi	2500	3000	4.0
Ongole	1100	1600	4.0
Deoni	2000	2500	4.0
Khillar	380	500	4.0
Dangi	400	530	4.0
Amritmahal	400	500	4.0
HF Cross- F2	5000	6000	4.0
Jersey Cross- F2	3500	4500	4.5
Sunandini	2500	3000	3.5
Frieswal	4000	4500	3.5
Murrah	3500	4000	7.0
Mehsana	2400	3000	7.0
Nili Ravi	3000	3500	7.0
Jaffrabadi	3000	3500	8.0
Surti	1600	2000	7.0
Banni	3000	3500	7.0
Bhadawari	1300	1600	8.0
Pandharpuri	1300	1600	7.0

The standard for Dam's lactation yield for F1 cross Donors/ Recipients will be the same as that of respective indigenous Donors/ Recipients dam *i.e.* Gir, Sahiwal, Kankrej, Red Sindhi, etc.

For Breeds not mentioned in above table, concerned state government may notify the min. Dam's lactation details and Breed code.

For imported Donors/ Recipients, semen and embryos, the standards for import of germplasm as prescribed in the "Guidelines for export/ import of bovine germplasm" issued and amended from time-to-time by DAHD, Ministry of Fisheries, Animal Husbandry and Dairying, GOI shall be applicable.

2. Biosecurity of donors and recipients

2.1 Quarantine of donors and recipients

a. A minimum quarantine period of 60 days is compulsory before bringing new donors/ recipients into a facility. Preferably the donors/ recipients procured from same source should be housed together in smaller groups. Necessary infrastructure should be created in quarantine station for the same to avoid any physical contact between animals of different groups. Only after favourable results from the health control point, the donors/ recipients shall be admitted to the facility. Relevant definitions are given in Annexure 1.

b. In the quarantine station, new animals shall be housed for a minimum of 60 days in a place which is effectively separated and away from (preferably at a distance of 5 km) the main facility where donors and recipients are housed. Manpower deployed and all equipment used in handling, feeding, watering and cleaning the new donors/ recipients shall not be shared with the resident herd(s).

c. Each new animal in quarantine station will be tested against major contagious diseases before its entry to resident herd namely Tuberculosis (TB), Johne's disease (JD), Brucellosis, Campylobacteriosis, Trichomoniasis, Infectious Bovine Rhinotracheitis and Bovine Viral Diarrhoea. All tests shall be done by an accredited agency or disease diagnostic laboratory as indicated in Annexure 2.

d. During the quarantine period, the donors/ recipients 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 in the area.

e. Once the quarantine period is over, all donors/ recipients shall be introduced to main facility.

*The procedure and duration for quarantine in different situations is given in Annexure 3A, 3B and 3C.

2.2 Testing of donors/ recipients

The testing protocols/ procedures for donors/ recipients against TB, JD, Brucellosis, Campylobacteriosis, Trichomoniasis, Infectious Bovine Rhinotracheitis and Bovine Viral Diarrhoea are given in Annexure 4 to 10. The donors/ recipients should be free from above mentioned diseases. Though JD is not a sexually transmitted disease but being a chronic, infectious and incurable disease, it has been included and the donors/

recipients found positive for JD need to be removed. The donors/ recipients in the quarantine station and the main herd should go through periodical testing and vaccinations as per the schedule listed.

2.3 Vaccination Schedule

The donors/ recipients 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 in the area.

Exotic and crossbred donors/ recipients shall be vaccinated against Theileriosis once in every three years.

The facility shall arrange for carrying out ring vaccinations of all cloven footed animals including swine against FMD within a radius of 10 km around the Embryo Production Facility. Vaccinations against HS and BQ shall be carried out in the areas having incidence of these diseases.

2.4 Culling of Donors/ Recipients due to Specific Diseases

The Embryo Production Facility must remove donors and recipients (within 48 hours) which are positive for Brucellosis, TB, JD, IBR and persistently infected BVD.

Guidelines issued by the Department of Animal Husbandry and Dairying (DAHD), Ministry of Fisheries, Animal Husbandry and Dairying for progressive IBR/ BVD control and as amended from time-to-time shall be followed in letter and spirit by all embryo production facilities.

3. Identification and Grading of embryos

Identification, grading and certification are critical components in production, transfer and freezing of the bovine embryos, particularly in commercial breeding companies. A high level of trust is endowed on the embryologist and it is believed that the embryologist would work in the most professional and ethical manner. The present chapter is based on the Manual of International Embryo Technology Society (IETS), 5th Edition. For details please refer Volume 1, Chapter 9 on “Certification and identification of embryos” in the Manual of International Embryo Technology Society (IETS), 5th Edition.

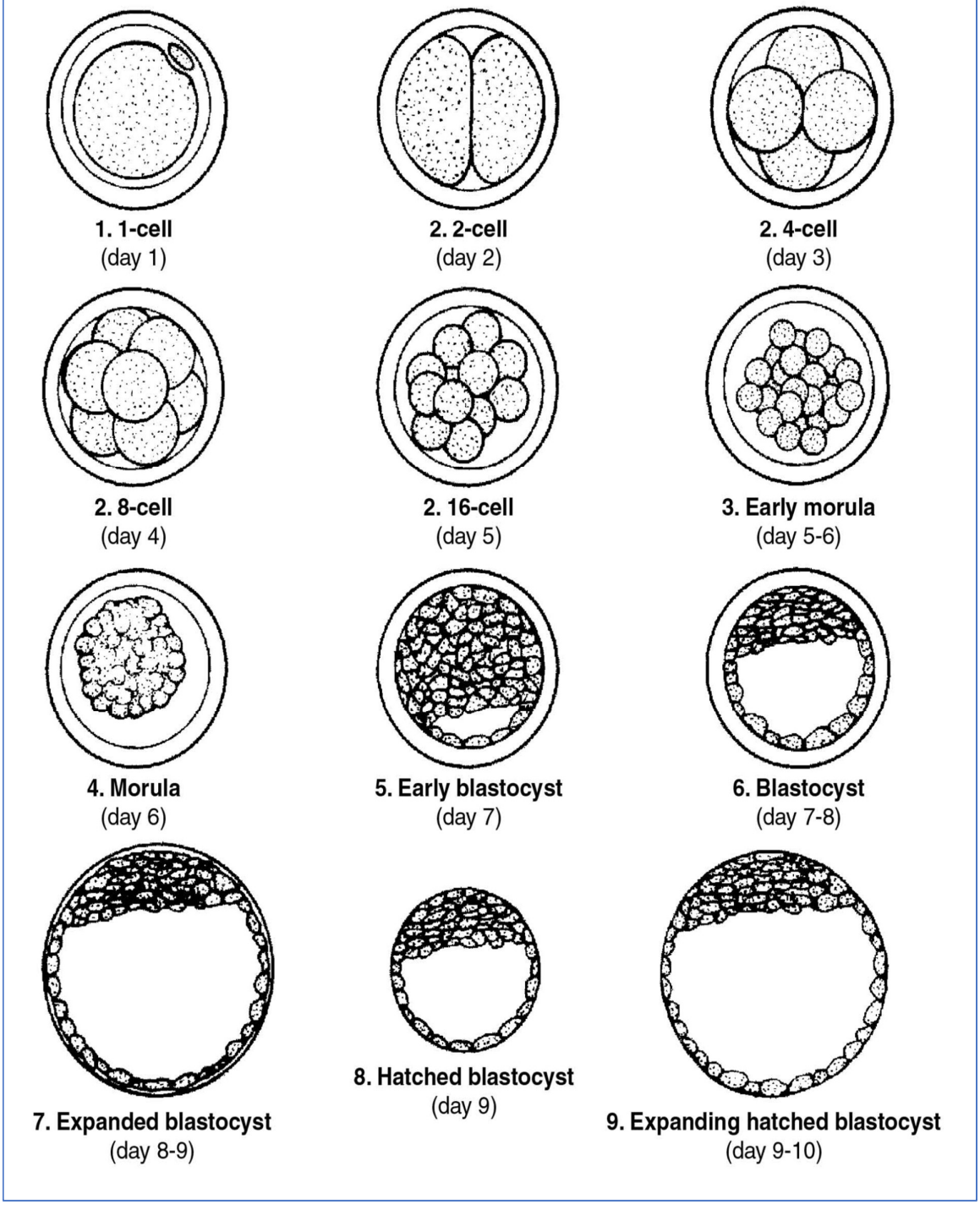
Following issues are associated with proper identification and grading of the embryos:

1. Improper identification may lead to parentage error and breach of international rules for movement of embryos in terms of health certificate.
2. Improper grading may lead to lower pregnancy rate, in case a lower grade embryo is graded as higher.
3. Improper grading of embryo may lead to freezing of unsuitable embryos and discard of suitable one.

The International Embryo Technology Society (IETS) developed a system to standardize record keeping aimed at assuring accuracy and confidence that embryos are precisely identified. The IETS Board of Governors approved the basic system in January 1986, and has approved all subsequent additions and modifications. The committee that developed the recording system is known as the IETS Subcommittee for Forms and Certification and functions under the parent committee of the IETS Health and Safety Advisory Committee (HASAC). Subcommittee members are from academia, regulatory agencies, recording agencies, and veterinary practice (Manual of the IETS, 5th Edition).

Codes for different stage of development (Manual of the IETS, 5th edition):

Stage of development	Code
Unfertilized embryo or 1–cell embryo *(day 1)*	1
Embryos with 2 to 16 cells *(days 2 to 5)*	2
Early morula *(day 5–6)*	3
Morula *(day 6)*	4
Early Blastocyst *(day 7)*	5
Blastocyst *(day 7–8)*	6
Expanded Blastocyst *(day 8–9)*	7
Hatched Blastocyst *(day 9)*	8
Expanding hatched blastocyst *(day 9–10)*	9


1. 1-cell
(day 1)
2. 2-cell
(day 2)
2. 4-cell
(day 3)
2. 8-cell
(day 4)
2. 16-cell
(day 5)
3. Early morula
(day 5-6)
4. Morula
(day 6)
5. Early blastocyst
(day 7)
6. Blastocyst
(day 7-8)
7. Expanded blastocyst
(day 8-9)
8. Hatched blastocyst
(day 9)
9. Expanding hatched blastocyst
(day 9-10)

Grading of embryos

Standardized grading of the quality of the embryo has been prescribed by IETS depending upon the cell mass, developmental stage compared to age of the embryo, percentage of cell extrusion from the embryo and overall appearance of the embryo. Though the evaluation is subjective and vary between embryologists but if the IETS guidelines are followed, there should not be very high variation between grading. The code for embryo quality is also numerical and is based on morphological integrity of embryos. The evaluation of in vitro produced (IVP) embryos generally follows that of in vivo-derived embryos, as described above. However, despite continuing efforts to improve culture conditions, some differences remain. First, differences in stage of development must be recognized. Day 0 with in vivo-derived embryos is considered to be the onset of estrus in the donor, whereas Day 0 for IVP embryos is the time of fertilization (approximately 24 hr later). Therefore, a Day 7 IVP embryo should be close to the same stage of development as a Day 8 in vivo derived embryo.

Characteristics of different grade of embryos (as per manual of the IETS, 5th edition)

Grade of embryos	Characteristics
Code 1: Excellent or Good	1. Symmetrical and spherical embryo mass with individual blastomeres (cells) that are uniform in size, colour, and density. 2. This embryo is consistent with its expected stage of development. Irregularities should be relatively minor, and at least 85% of the cellular material should be an intact, viable embryonic mass. 3. This judgment should be based on the percentage of embryonic cells represented by the extruded material in the perivitelline space. 4. The zona pellucida should be smooth and have no concave or flat surfaces that might cause the embryo to adhere to a petri dish or a straw.
Code 2: Fair	1. Moderate irregularities in overall shape of the embryonic mass or in size, colour, and density of individual cells.

	2. At least 50% of the cellular material should be an intact, viable embryonic mass
Code 3: Poor	1. Major irregularities in shape of the embryonic mass or in size, colour, and density of individual cells. 2. At least 25% of the cellular material should be an intact, viable embryonic mass.
Code 4: Dead or degenerating	1. Degenerating embryos, oocytes, or 1–cell embryos 2. Nonviable

Critical consideration for grading

1. The grading is a subjective evaluation and should be learnt initially under qualified technician.
2. It should be taken in consideration that there will be difference in the system used for grading.
3. One single photo of embryo is sometime deceptive in grading and it should always be thought of a three dimensional structure.
4. Grading should be done quickly to avoid exposing the embryos outside for long before transferring or freezing.

Identification of embryo straw, cane and goblets

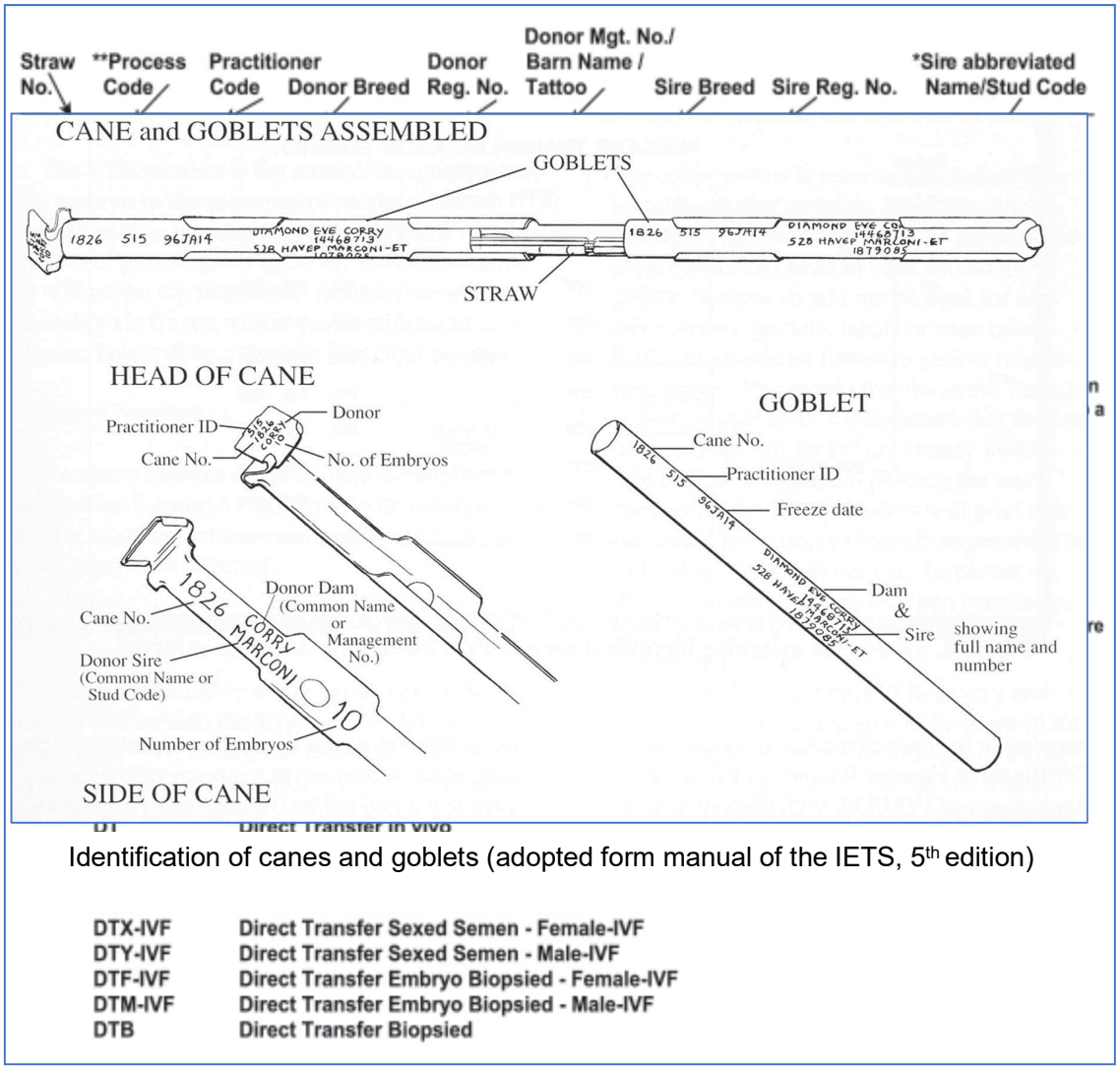
Identification of embryos needed to be performed with high precision and care to avoid any confusion about the identity of the embryo. IETS has certain recommendation for labelling the straw, goblet and cane for storing the embryos. Briefly the methods are presented below and for details please refer chapter 9 of the manual of IETS, 5th Edition.

The information may be printed by hand or by machine on the straw containing the embryo, on the extension of a straw or plug, or on a gummed label wrapped around a straw and inserted within a 0.5 ml extension of the straw containing the embryo. It is recommended that 2 lines be used for labelling straws. If a third line must be used, it is important that the order of items on the label remains the same.

Embryos frozen for direct transfer should be packed in **yellow colour straws**. Further, the extension of the straw or plug carrying the label or identification of embryos and goblets and tops of canes should be yellow. However, it is acceptable to use clear straws provided the extension of the straw or plug carrying the label or identification of embryos and goblets and tops of canes are yellow. The letters “DT” should follow the straw number on the label.

Vendors will print this on yellow translucent straws if requested. The individual straw number may be placed on the second line of the label when necessary, but it should not be separate from the “DT” that follows it.

The **colour light blue** is reserved exclusively for vitrified embryos. Therefore, vitrified embryos should be in light blue straws and the extension of the straw or plug carrying the label or identification of embryos and goblets and tops of canes should be light blue. Similar to DT embryos, it is acceptable to use clear straws with similar conditions.



Identification of canes and goblets (adopted form manual of the IETS, 5th edition)

DTX-IVF	Direct Transfer Sexed Semen - Female-IVF
DTY-IVF	Direct Transfer Sexed Semen - Male-IVF
DTF-IVF	Direct Transfer Embryo Biopsied - Female-IVF
DTM-IVF	Direct Transfer Embryo Biopsied - Male-IVF
DTB	Direct Transfer Biopsied

Embryo identification on straw (adopted form manual of the IETS, 5th edition)Different stages of development of embryos and their numeric code (adopted form manual of the IETS, 5th edition)

Different coding system for identification of embryos (as per manual of the IETS, 5th Edition):

Stage of development

No.	Stage
1 –	Unfertilized
2 –	2–12 cells
3 –	Early Morula
4 –	Morula
5 –	Early Blastocyst
6 –	Blastocyst
7 –	Expanded Blastocyst
8 –	Hatched Blastocyst
9 –	Expanded Hatched Blastocyst

Quality of embryos

Code	Grade
1 –	Excellent or good
2 –	Fair
3 –	Poor
4 –	Dead or Degenerating

Manipulation of embryos

Code	Manipulation
N –	Not manipulated
D –	Divided
F –	Female by biopsy
M –	Male by biopsy
U –	Sex undetermined by biopsy

Codes for month

Code	Month
JA –	January
FE –	February
MR –	March
AP –	April
MY –	May
JN –	June
JY –	July
AU –	August
SE –	September
OC –	October
NO –	November
DE –	December

Sexed Semen

X –	Female
Y –	Male

4. Information System

The facility shall use Bharat Pashudhan to record data pertaining to various activities. Maintaining following record are must (few indicative formats are placed as Annexure 12:

a. Date of OPU/ flushing
b. Donor and sire details
c. Embryo production details
d. Embryo Transfer details
e. Embryo freezing/ vitrification details
f. Details of sale of embryos

5. Minimum manpower requirement for embryo production and transfer

Then facility should have following manpower in place:

	Number of embryos produced and transferred			
	<1000	**1000-3000**	**3000-5000**	**5000-10000**
Minimum number of manpower	In charge: 1 Veterinarians: 2 Embryologists: 1 Lab attendant: 1 Farm supervisor: 1 Farm worker: 1 in every 10 animals	In charge: 1 Veterinarians: 4 Embryologists: 2 Lab attendant: 1 Farm supervisor: 1 Farm worker: 1 in every 10 animals	In charge: 1 Veterinarians: 5 Embryologists: 3 Lab attendant: 2 Farm supervisor: 1 Farm worker: 1 in every 10 animals	In charge: 1 Veterinarians: 5 Embryologists: 4 Lab attendant: 2 Farm supervisor: 2 Farm worker: 1 in every 10 animals

In case of field embryo transfer, the numbers would vary depending on the area covered and animal density.

The manpower structure suggested above is meant only for embryo production and transfer. For other activities, manpower may be positioned as per the need. After recruitment, all new persons shall be trained at any of the recognized institutes. Once trained, they shall continue to work in the Embryo Production Facility at least for five years. As embryo production and transfer activity is a highly professional/ technical work, job rotation of personnel could be detrimental. If it is inevitable, a proper replacement should be identified at least six months in advance and shall be trained. Technical exposure of the personnel working in the embryo production and transfer must be arranged compulsorily once in two to three years at reputed institutions. It is recommended that In-charge should have at least 05 years of experience in bovine embryo production or transfer.

Definitions for use in the Health Protocol

Donor	Animal used for embryo production
Recipients	Animal used for embryo transfer
Known health status	Animals originating from an embryo production facility that is strictly complying with the guidelines mentioned in the MSP.
MSP diseases	MSP diseases are the set of diseases – the causative organism of which should not be present in the donors and recipients (when animals are housed in embryo production facility). These diseases include IBR, Bovine Brucellosis, Tuberculosis (TB), Paratuberculosis (JD), Bovine Genital Campylobacteriosis, Trichomoniasis and Foot and Mouth Disease (FMD).
Quarantine station	A farm where donors and recipients are isolated and examined to assess the health status before shifting to the embryo production facility. A series of clinical and laboratory examinations, vaccinations and medications etc. are undertaken during quarantine.
Embryo production facility	A farm along with embryo production laboratory where donors/ recipients are housed for embryo production and transfer. A series of clinical and laboratory examinations, vaccinations and medications etc. are undertaken during the stay of donors/ recipients in the Embryo production facility to maintain their health status.
Unknown health status	Animals originating from village or farm where all the animals of the farm or the village have not been tested against the MSP diseases

Details of the tests to be conducted			
Disease	**Test**	**Sample**	**Tested by officers of**
Brucellosis	ELISA	Serum	CDDL/RDDL/ NDDB/ State Veterinary Universities
TB*	DTH- Tuberculin PPD	Intra-dermal on the Donors/ Recipients	Embryo Production Facility/ CDDL/RDDL/ NDDB/ State Veterinary Universities
JD*	DTH- Johnin PPD	Intra-dermal on the Donors/ Recipients	Embryo Production Facility/ CDDL/RDDL/ NDDB / State Veterinary Universities
Trichomoniasis	Agent identification	Vaginal washings	CDDL/RDDL/ NDDB/ State Veterinary Universities
Bovine Genital Campylobacteriosis	Agent identification	Vaginal washings	CDDL/RDDL/ NDDB/ State Veterinary Universities
FMD	ELISA	Serum	PD-FMD, Mukteshwar and its laboratories/ NDDB /State Veterinary Universities
IBR	ELISA	Serum for ELISA (9 months age)	CDDL/RDDL/ NDDB/ State Veterinary Universities
BVD	ELISA 2 times at 30 days interval (RT-PCR up to 6 months age)	Serum	CDDL/RDDL/ NDDB/ State Veterinary Universities

* TB and JD testing at Quarantine Station shall be performed by the officers of the Embryo Production Facility. However, the testing at the Embryo Production Facility shall be done by the Officers of the CDDL/RDDL/NDDB/NABL accredited State Veterinary Universities and approved by CMU.

Quarantine Guidelines

A. Quarantine of adult Donors/ Recipients of unknown health status		
Quarantine period	Minimum 60 days or long enough to allow at least two tests for MSP diseases to be performed during quarantine with a minimum interval of 30 days between the two tests. In case of TB and JD the interval between the two tests should not be less than 42 days.	
Shifting of Donors/ Recipients from the quarantine	Within 30 days from the date when the last test was performed and all Donors/ Recipients were found negative.	
Action on finding a positive result	Brucellosis, TB, JD, Bovine Genital Campylobacteriosis, Trichomoniasis, IBR, BVD (PI)	Cull / remove the positive Donors/ Recipients and put all the remaining Donors/ Recipients under extended quarantine.
Extended quarantine	For a period of minimum 60 days or long enough to allow atleast two tests for the diseases mentioned above to be performed, from the day last positive Donors/ Recipients was culled/ removed. Perform one test within the last 30 days of the extended quarantine.	
Action on finding a positive during extended quarantine	During Quarantine, if the Donors/ Recipients are housed and managed a. Individually - Remove only the positive Donors/ Recipients. b. In groups (not more than 5 animals in each group) – Remove all Donors/ Recipients in the group in which positive was detected. c. Free and not in groups- Remove all the Donors/ Recipients.	
B. Quarantine of adult Donors/ Recipients of known health status		
Quarantine period	Minimum 30 days or long enough to allow at least one test for all MSP diseases	
Shifting of Donors/ Recipients from the quarantine	Within 30 days of the last negative test	
Action on finding a positive result	Same as in Annex- 3A	
Extended quarantine	For a period of minimum 30 days from the day last positive Donors/ Recipients was culled/ removed. Perform one test within the last 30 days of the extended quarantine.	
Action on finding a positive during extended quarantine	Same as in Annex- 3A	

C. Quarantine of adult Donors/ Recipients to be shifted between the farms managed by the same administration	
Quarantine period	Minimum 30 days or sufficient to allow at least one test for MSP diseases
Shifting of Donors/ Recipients from the quarantine	Within 30 days of the last negative test
Action on finding a positive result	Same as in Annexure 3A
Extended quarantine	For a period of 30 days from the day last positive Donors/ Recipients was culled/ removed. Perform one test within the last 30 days of the extended quarantine.
Action on finding a positive during extended quarantine	Same as in Annexure 3A

<table>
<tr><td colspan="3">D. Quarantine of calves above 2 months of age</td></tr>
<tr><td>Quarantine period</td><td colspan="2">Minimum 60 days or sufficient to allow at least two tests for each of the MSP diseases to be performed with a minimum interval of 30 days between the tests. In case of TB and JD the interval between the two tests should not be less than 42 days.</td></tr>
<tr><td>Shifting of calves from quarantine</td><td colspan="2">Within 30 days of negative results.</td></tr>
<tr><td rowspan="3">Action taken
on finding
positive calf</td><td>TB, JD</td><td>Remove the positive calf and put all the remaining calves under extended quarantine.</td></tr>
<tr><td>Bovine Genital Campylobacteriosis and Trichomoniasis</td><td>Tests conducted only on calves older than 6 months. Remove the positive calf and put all the remaining calves under extended quarantine.</td></tr>
<tr><td>Brucellosis,
IBR, BVD
(PI)</td><td>Remove the positive calf irrespective of age and put all the remaining calves under extended quarantine.
OR
For Brucellosis and IBR: If the positive calf is less than 9 months old, isolate the calf till it is 9 month old and retest. Calf positive at retesting should be removed.</td></tr>
<tr><td>Extended quarantine</td><td colspan="2">For a period of minimum 60 days from the day last positive calf was removed. Perform one test within the last 30 days of the extended quarantine.</td></tr>
<tr><td>Action on finding a positive during extended quarantine</td><td colspan="2">Same as in Annexure 3A</td></tr>
</table>

Disease testing and management of Bovine Tuberculosis in Embryo Production Facility

Name of test	Delayed Hypersensitivity – Single Intra Dermal (SID) Test
Reagent used	Bovine tuberculin PPD
Manufacturer	IVRI, Izatnagar
Testing done	On site, where animals are housed
Result criteria	Positive: Increase in skin thickness of 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. 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 4mm, absence of above clinical signs, 72 hours post-inoculation. Donors/ Recipients with inconclusive result should be immediately isolated. Only if the animal is negative during the testing in isolation, it should be brought back to the Embryo Production Facility.
Eligible animals	Animals above 2 months of age
Action to be taken on Positive animal	Immediate isolation and removal from herd (within 2 days)
Positive herd testing	Testing not before 42 days after culling of last positive animal.
Negative herd testing	Six monthly (± 1 week) testing after last whole herd negative testing.
TB free herd	Herd found negative on two consecutive tuberculin tests carried out at an interval of 6 months, the first being performed 6 months after the culling of last affected animal. If frequency of testing is more than two in a year, the testing should establish that all animals in the herd have been negative for the last 6 months beginning from 6 months after culling the last affected animal.

Disease testing and management of Para tuberculosis (JD) in Embryo Production Facility

Name of test	Delayed Hypersensitivity – Single Intra Dermal (SID) Test
Reagent used	Johnin PPD
Manufacturer	IVRI, Izatnagar
Testing done	On site, where animals are housed
Result criteria	Positive: Increase in skin thickness of 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. 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 4mm, absence of above clinical signs, 72 hours post-inoculation. Donors/ Recipients with inconclusive result should be immediately isolated. Only if the animal is negative during the testing in isolation, it should be brought back to the Embryo Production Facility.
Eligible animals	Animals above 2 months of age
Action to be taken on Positive animal	Immediate isolation and removal from herd (within 2 days)
Positive herd testing	Testing not before 42 days after culling of last positive animal.
Negative herd testing	Six monthly (± 1 week) testing after last whole herd negative testing.
JD negative herd	Herd found negative on two consecutive Johnin tests carried out at an interval of 6 months, the first being performed 6 months after culling of the last affected animal. If frequency of testing is more than 2 in a year, the testing should establish that all animals in the herd have been negative for the last 6 months beginning from 6 months after culling the last affected animal.

Disease testing and management of Bovine Brucellosis in Embryo Production Facility

Name of test	Enzyme Linked Immunosorbent Assay (ELISA)
Sample required	Serum
Eligible animals	All animals. However, animals up to 9 months of age may have maternal antibodies.
Action to be taken on the positive animal	Immediate isolation and removal from herd after castration (within 2 days)
Positive herd testing	Testing 30 to 60 days after culling of last positive animal.
Negative herd testing	Six monthly (± 1 week) testing after last whole herd negative testing.
Brucellosis free herd	Herd found negative on two consecutive annual tests. If the frequency of testing is more than one in a year, the testing should demonstrate that the herd has been negative for the last one year

Disease testing and management of Bovine Genital Campylobacteriosis (BGC) in Embryo Production Facility

Name of test	Agent –Identification
Sample required	Vaginal washings
Eligible animals	Donors/ Recipients
Positive animal	Refer Annexure 3A

Only to be tested in quarantine and refer Annexure 3A

Disease testing and management of Bovine Trichomonosis in Embryo Production Facility

Name of test	Agent –Identification
Sample required	Vaginal washings
Eligible animals	Donors/ Recipients
Positive animal	Refer Annexure 3A

Only to be tested in quarantine and refer Annexure 3A

Testing and management of Infectious Bovine Rhinotracheitis (IBR) at Embryo Production Facility

Name of the test	Enzyme Linked Immuno absorbent Assay (ELISA)
Sample (s) required	Serum for ELISA
Induction of new animals into herd/Embryo Production Facility	Only negative animals will be inducted. All the animals to be inducted irrespective of their age should be put on hold and inducted only if found test negative after the age of 9 months.
Sero positive Donors/ Recipients at IBR positive Embryo Production Facility	Action in order of priority:- (i) Immediately cull sero-positive animals (ii) Test all the animals at three months interval.
Action to be taken on Donors/ Recipients at the IBR free Embryo Production Facility**	(i) All positive Donors/ Recipients culled immediately. (ii) Retest remaining Donors/ Recipients at 30 - 60 days after culling last positive animals. Repeat (i) & (ii) until the remaining herd is tested negative. Thereafter test at 6 monthly interval. (iii) The negative herd should be tested at 6 monthly interval.

**Please refer to the Guidelines for progressive IBR/BVD control issued by DAHD, Govt. of India and as amended from time-to-time; If the donors/ recipients are vaccinated against IBR, DIVA ELISA to be used for testing the animals.

Testing and management of Bovine Viral Diarrhoea (BVD) at Embryo Production Facility

Name of the test	Enzyme Linked Immuno absorbent Assay (ELISA) for detection of antigen (Ag-ELISA)/real time PCR (rt PCR)
Sample	Serum
Induction of new animals into herd/Embryo Production Facility	Test the animal for Persistent Infection (PI) by testing two times at an interval of at least 30 days by Ag- ELISA. Test by rt-PCR instead of Ag-ELISA for animals up to 6 months of age. If the animal is positive on both the tests, the animal is considered positive for PI. Only PI negative animals shall be inducted.
Action to be taken for PI positive animals	Immediately isolate and cull

Only to be tested in quarantine and refer Annexure 3A
Please refer to the Guidelines for progressive IBR/BVD control issued by DAHD, Govt. of India and as amended from time-to-time.

Management of Foot & Mouth Disease (FMD) in Embryo Production Facility

FMD outbreak in Embryo Production Facility	
Immediate action to be taken	Immediate disinfection of premises and fomites. Destruction of contaminated feed & fodder by burning.
Action to be taken on FMD infected animal	a. Isolate the affected Donors/ Recipients immediately b. Affected Donors/ Recipients is treated and rested for 90 days after recovery from clinical symptoms.
Animals in the farm not affected by FMD	No embryo production and transfer from healthy Donors/ Recipients during the outbreak and no embryo production and transfer up to one month after the last case has recovered.
FMD outbreak in areas surrounding the SS	
Ring vaccination	Arrange immediate ring vaccination within a radius of 10 Km around the focus of infection starting from the perimeter towards the focus.
Disinfection	Disinfection of the roadsides adjacent to the farm on a daily basis.
Movement of fodder	Stop all fodder movement through areas of infection.
Animal movement	Stop animal movement of Embryo Production Facility through areas of infection.

OPU Routine

Date of OPU:

Donor Id: Breed: Donor Type: Cow/ Heifer

Date of last Heat of Donor:

Date of last OPU of Donor:

Stimulation: Yes/ No

Stimulation Hormone: _____ Dose and Schedule _____

Coasting Period (Hrs.):

Start time of OPU:
End time of OPU:
Anesthesia used (in ml): Status of Anesthesia: Satisfactory/Unsatisfactory Remarks (if any):
Temperament of donor: Calm/ Restless/_____
Status of Internal Genitalia, excluding Ovaries:
Right Ovary (size and other observation): CL: Yes/ No Type of CL:
Left Ovary (size and other observation): CL: Yes/ No Type of CL:
Pump pressure (mmHg):
OPU Media used (Make, Batch and Quantity used):
No. of follicles aspirated: Right Ovary: _____ Left Ovary: _____ Total: _____ Oocyte Recovered: _____ Zona Recovered: _____ Total: _____ Recovery (%):_____
Oocyte put to IVM: Yes/ No
OPU Done by:

IVEP Routine

Donor Id:	**Breed:**	**Date:**

- ***In Vitro* Maturation** **IVM Media Batch:**

Volume of media used (µl): Incubator slot number:

No. of oocytes for IVM (drop wise): Plate Drop I _____ II _____ III _____IV _____ Total ____

Plate Drop I _____ II _____ III _____IV _____ Total ____

Plate Drop I _____ II _____ III _____IV _____ Total ____

Start IVM (date & time): End IVM (date & time):

Duration:

Temperature of Incubator: °C/ °C CO_2 level: %/ %

Responsible: Signature:

- ***In Vitro* Fertilization** **IVF Media Batch:**

Semen Details (Breed/Bull Id/Batch):

Sexed/Conventional semen: SS / CS Incubator slot number:

Sperm PTM: Percoll volume: upper layer _____ lower layer _____

Centrifugation speed for 1st wash (rpm/rcf): Time:

Centrifugation speed for 2nd wash (rpm/rcf): Time:

Sperm Motility post 2nd sperm wash:

Sperm concentration in the IVF drop (sperms/ml):

Volume of media used: _____ µl media, _____ µl with oocyte, _____ µl sperm suspension

No. of oocytes in IVF (drop wise): Plate Drop _____ Plate Drop _____ Plate Drop ___

Start IVF (date & time): End IVF (date & time):

Duration of IVF:

Temperature of Incubator: °C/ °C CO_2 level: %/ %

Responsible: Signature:

- ***In Vitro* Culture IVC Media Batch:**

Wash Media Batch:

No. of presumptive zygotes in IVC (drop wise): Plate Drop _____ Plate Drop _____ Plate Drop _____ Incubator slot number:

Start IVC (date & time):

Volume of media used (µl):

Temperature of Incubator: °C/ °C CO_2 level: %/ % O_2 level: %/ %

Cleavage rate (No. & %) Blastocyst rate (No. & %):

Responsible: Signature:

Development and Cleavage Evaluation (72 h post IVF):

Drop No.	**Oocytes Kept**	**2-3 cell embryos**	**4-8 cell embryos**	**≥ 8-cell embryos**	**Unfertilized (UFO)**

Embryo Development:

Stage/Grade	**Day 6**					**Day 7**					**Day 8**					**Day 9**				
	I	II	III	IV	**To**	I	II	III	IV	**To**	I	II	III	IV	**To**	I	II	III	IV	**To**
Mo																				
EBI																				
BI																				
ExBI																				
HBI																				
Total																				
T___F____V____D___						T___F____V____D__ _					T___F____V____D__ _					T___F____V____D___				

To – Total, T – Transfer, F – Frozen, V – Vitrified, D – Discarded

Embryo Transfer & Calving

Place: **Date & time of transfer:**

Recipient details:

Recipient id:			
Breed:			
Lactation number:			
Attempt No.			
Date and Time of Estrus:			
Type of estrus: Natural/ Synchronized			
Day of Cycle:			
PG/ CIDR etc. details (if synchronized)			
Date of administration:			
Trade name:		Batch No.:	
Dose:			
CL side:	(with cavity/ without cavity)	CL grade:	Recipient Grade:

Embryo details:

Donor id:		Donor Breed:	
Sire id:		Sire breed:	
Embryo No:	Stage:	Grade:	Age in Days:
Fresh/ Frozen		Conventional/ Sexed semen	

Transfer details:

Transfer site:
Transfer Grade:
Transfer done by:
Remarks (if any):

Pregnancy Diagnosis (PD) details:

Date of return in heat (if repeated):
PD outcome (post 45 days):
PD outcome (post 60 days):
Early embryonic death details/ abortions (if happened):
Remarks (if any):

Calving details:

Date & Time:	Type of calving: Normal/ Required some assistance/ Dystocia	
Calf born: Live or still (dead)	Presentation: Anterior/ Posterior	
Sex: Male/ Female	Calf Tag id:	Birth Weight:

Remarks (if any):

Embryo Freezing

Date & time of freezing:

Embryo details:

Donor id	Donor Breed	Sire id	Sire breed	Embryo No	Stage	Grade	Age in Days

Freezing details:

Freezing media details (Name, batch no., expiry): Freezing machine used: Protocol used: Embryo loading temperature: °C Time difference between loading and seeding: Seeding temperature: °C Post seeding hold _____ minutes Cooling rate post seeding: Plunging in LN_2 done at temperature: °C Time taken for completion of freezing: minutes Person Responsible: Remarks (if any)

Storage details:

Embryo No.	Container id	Canister id	Cane id	Used or Supplied on	Used at or Supplied to

Performed by:

Calving Report

Place:

Date of Calving:

Time of Calving:

Recipient details:

Recipient id:
Breed:
Lactation number:

Embryo details:

Donor id:	Donor Breed:		
Sire id:	Sire breed:		
Embryo No:	Stage:	Grade:	Age in Days:
Fresh/ Frozen		Conventional/ Sexed semen	

Calving details:

Type of calving:
Calf born: Live or still (dead)
Sex: Male/ Female
Calf Tag id:
Birth Weight:
Remarks (if any):