POLICY FOR IMPLEMENTATION OF IVF TECHNOLOGY

1. Background:

1.1 With the rising coverage of artificial insemination (AI) and thereby increasing demand for high genetic merit bulls (HGM) for semen production, the dairy industry is craving for multiplying elite germplasm faster to produce superior progenies. The situation is challenging particularly in case of indigenous cattle, where availability of elite animals of various breeds is very sparse. Over the years organised breeding programmes have been initiated in the country in the form of progeny testing and pedigree selection. To aid these efforts, we require efficient use of different assisted reproductive technologies (ART) for faster multiplication of the elite bovine population. IVF technology is commonly used ART in developed dairy nation for production of elite bulls and multiplication of elite animals. Cloning is another ART practiced for multiplication of elite animals, the technology has limited application as its extensive use causes inbreeding and is also costly technique as compared to IVF technique.

1.2 During last two decades, in vivo embryo production is carried out using multiple ovulation and embryo transfer (MOET) process in India. However, the technology requires costly hormones for super-ovulation and requires a significant resting period before using the same animal again. During last couple of years, IVF technology has emerged as replacement to in vivo embryo production technique. It is envisaged that use of this technology to multiply the superior bovine germplasm can change the face of dairying in India.

1.3 In spite of being global leader in milk production our average productivity is only 2079 kg per animal per year. This is indicative of poor yield among indigenous milch animals in the country. In indigenous cattle (both non descript and high yielding indigenous breeds) the average productivity is 1292 kg per year; while for crossbred cattle it is 3077 kg per year. Thus there is urgent need to enhance productivity and this is only possible through extension of AI coverage using semen of high genetic merit bulls. For production of high genetic merit bulls IVF technology coupled with genomics will be crucial. Therefore, there is a urgent need to promote IVF technology in the country.

1.4 There is requirement of 8,800 HGM bulls including 3,023 bulls of indigenous breeds for enhancing AI coverage from present level of 30% to 70% of the breedable bovine females. At present only 1096 bulls of indigenous breeds are available at semen stations thus there is short fall of 1927 bulls (176%) by taking semen production as 25,000 doses per bull for indigenous breeds and taking conception rate as 3 doses per conception.

1.5 Availability of elite animals is limited in the country, as at present only 33 Gir cows are available in the country with lactation yield above 4500 kg/ lactation, 23 Sahiwal cows with lactation yield above 4500 kg, 8 Red Sindhi cows with lactation yield above 4000 kgs and 6 Rathi cows with lactation yield above 4500 kgs. These animals can only be propagated through IVF technology for production of HGM bulls

2. **Objectives:**

(i) Multiplication and propagation of elite animals in an exponential manner.

(ii) Enhanced availability of disease free high genetic merit bulls for use in artificial insemination programmes

(iii) Increased availability of elite animals of indigenous cattle and buffalo breeds.

(iii) Enhancement of milk production and productivity by attaining higher genetic rate.

3. Technology:

3.1 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. In OPU oocytes are aspirated directly from the ovaries of elite donor animals using trans-vaginal probe guided by ultrasonography (USG). In vitro embryo production (IVEP) includes in vitro maturation (IVM), in vitro fertilization (IVF) and in vitro culture (IVC). The whole procedure including OPU henceforth will be referred as IVF technology in this document. Details are given at Annexure-I.

3.2 The IVF technique is totally harmless to the donor animal and tends to be more consistent than Embryo Transfer Technology (ETT). It allows repeatable embryo production without using exogenous hormones and without altering the reproductive cycle of donor. Since the technology is more efficient than in vivo embryo production (ETT), its use in bovine has gained momentum worldwide for commercial embryo production.

3.3 The prominent benefits of this technology are:

3.3.1 It can increase the number of progenies from an elite female bovine animal during its lifetime. Normally, one can get one calf from an elite bovine female in a year. However, by applying IVF technology, one can get 80-100 calves from an elite bovine female in a year. The average calves born are 30 calves/ donor per year.

3.3.2 Genetic gain per year, apart from other factors, depends on intensity of selection. By using this technology we can produce more number of calves from selected elite animals, thereby increasing the selection intensity and speeding up the rate of genetic improvement. Several superior bulls can be used in the same cycle to produce calves from different sire dam combinations.

3.3.3 Pre-pubertal elite heifers selected through genomic selection can be used for production of calves which will reduce the generation interval and thereby increase genetic gain per year.

3.3.4 Exceptionally high producing female bovines with kink cervix and having other reproductive problems such as blocked fallopian tubes could also be used to produce embryos. Dams with non-functional udder and teats, which are normally culled, could also be used to produce embryos.

3.3.5 Use of sex sorted semen in IVF can further improve the genetic gain by producing offspring of desired sex in higher number using this technology. This technology offers the most efficient use of sexed semen and also of rare superior bull's semen because required number of spermatozoa is comparatively very less.

3.3.6 In future estimating genomic breeding value through embryo biopsy and detecting genetic diseases etc. in the embryos, even before transfer is also on the anvil.

4. Selection of Donors

4.1 All donors to be used in IVF technology should be disease free as per the protocol prescribed in MSP for semen production.

4.2 Only donors (dams/ Cows/ Heifers) among the top 20% in the country to be used in embryo production. The protocol for donor selection should be reviewed for upward revision every year by Department of Animal Husbandry and Dairying, Government of India (DAHD), so that genetic gain progress is maintained. For the current year standards and specifications of donors is enclosed in Annexure-II.

4.3 All donors (dams/cows/heifer) should genomically tested using induschip/buff or other chip developed for genomic testing of indigenous breeds. Only donors among the top 10% of the positive breeding value should be used in embryos production.

4.3 Dams below MSP are never to be used as donor in IVF embryo production. All CCBFs should also adhere to these standards and specifications.

5. Selection of semen:

Semen for IVF should be obtained from A graded semen stations and bulls available at semen station should be selected on the basis of their breeding values, dams lactation yield, sire dams lactation yield. Bulls to be used in the programme should be free from IBR and genomically tested using suitable genomic chip.

5.1 Only semen of top 1% of the bulls available in the country should be used in IVF.

6. Bull Production Programme:

6.1 Reverse sex sorted semen from top 1% of the bulls available in the country should only be used in IVF for production of male calves as prospective bulls for semen production.

6.2 All Central Cattle Breeding Farms should use only reverse sex sorted semen of top 1% bulls available in the country for production of male calves for use in semen production.

6.3 Central cattle Breeding Farms or any State cattle Breeding Farms may not produce animals for natural service.

7. Farmers Incentives

7.1 Animal available with farmer meeting aforementioned standards and specifications may be used as donor cow/buffalo:

Farmers having donors above MSP may be given incentives for providing their animals as donors. Higher quality animals should be given more incentive.

7.2 All bulls produced under this programme would come from top 20% of elite animals and 1% top bull semen would belong to finest bulls in the country and would constitute part of bull production programme. All such bulls would be purchased with a confirmed buyback programme by the LDBs/ semen stations. The farmers to whom the surrogate cow / recipient belongs should be incentivised and the cost paid to IVF service provider shall also be paid to the farmer by LDB / semen station.

7.3. All the bulls after induction will be tested using genomic chip and genomic breeding value will be calculated. If genomic breeding value is among the top 10% additional incentive will be given to the farmer and in case of top 1% even higher incentive will be given/ made available. Cost of genomic testing will be borne by the LDBs.

8 Distribution of bulls:

8.1 While distribution not more than 1 bull from a donor should be given to any semen station in order to limit inbreeding.

Annexure-I

IVF Technique

1. IVF technique is a non-invasive procedure. In this, oocytes are aspirated directly from the ovaries of elite donor animals using trans-vaginal probe guided by ultrasonography (USG). In vitro embryo production (IVEP) includes in vitro maturation (IVM), in vitro fertilization (IVF) and in vitro culture (IVC).

2. Collected oocytes are evaluated under the stereo zoom microscope and good quality oocytes are selected, matured and fertilized using fresh/ frozen thawed semen in petri dishes in the controlled environment inside special incubator. Embryos are then grown by culturing in a special medium up to the blastocyst stage and embryos so produced are either transferred as fresh or cryopreserved for use in future.

3. Basic principles involved in the techniques:

3.1 Ovum Pick-Up (OPU):

After evolving for many years since development of ultrasound guided collection of oocytes from live cattle, the technique is now standardized. The basic technique is to aspirate oocytes by manoeuvring ovaries per rectum with the help of trans-vaginal ultrasound probe and needle. This technique has become widely known as ovum pick-up (OPU) or transvaginal oocyte aspiration.

3.2 In vitro maturation (IVM):

Aspiration of an immature oocyte from follicle and culturing it in a conducive environment results in maturation of oocyte. The basic purpose of In vitro maturation is to mimic the physiological changes with the addition of reproductive hormones growth hormones or epidermal growth factors and few other components. Generally oocytes aspirated from small to medium sized follicles mature after 20-24 hr.

3.3 In vitro Fertilization (IVF):

In order to fertilize the matured oocytes, they are incubated in special type of incubator with spermatozoa for around 18 hr. Spermatozoa are needed to undergo capacitation in vitro before they will be able to fertilize. The major challenge for those performing IVF is to mimic these conditions by providing spermatozoa with an environment that supports capacitation including the media and temperature of incubation.

3.4 In vitro Culture (IVC):

Generally, 6 days of culture after completion of IVF (7 days from the day of IVF) is required to attain blastocyst stage from fertilized oocyte. The basic purpose is to provide suitable culture condition for passing through different stages of embryos development. The requirement can be fulfilled through using a single media or a sequential media (media changes according to the stage of embryos development).

Sequential systems aim to mimic the physiological changes that in vivo zygotes encounter as they move down the oviducts and enter into the uterus during the first 6 or 7 days of development.

Annexure-II

Minimum Standard and specifications for Donors

Sr. No.	Breed	1st lactation yield	Best lactation yield	Breeding value, if available	Gnomically estimated breeding value, (mandatory)
	Major Breeds				
1.	Gir	3000	4000	+Ve	+Ve
2.	Sahiwal	3000	4000	+Ve	+Ve
3.	Red Sindhi	2500	3500	+Ve	+Ve
4.	Kankrej	2500	3500	+Ve	+Ve
5.	Tharparkar	2500	3000	+Ve	+Ve
6.	Hariana	2500	3000	+Ve	+Ve
7.	Rathi	3000	3500	+Ve	+Ve
8.	Murrah	3500	4000	+Ve	+Ve
9.	Nili Ravi	3500	4000	+Ve	+Ve
10.	Mehsana	3000	3500	+Ve	+Ve
11.	HF Pure	10000	11000	+Ve	+Ve
12	Jersey	7000	8000	+Ve	+Ve
13	HF cross	6000	7000	+Ve	+Ve
14	Jersey Cross	5000	6000	+Ve	+Ve
	Minor Breeds				
15.	Ongole	1500	2000	+Ve	+Ve
16	Deoni	1500	2000	+Ve	+Ve
17	Sunandini	4000	5000	+Ve	+Ve
18	Jaffrabadi	3500	4500	+Ve	+Ve
19	Surti	2000	2500	+Ve	+Ve
20	Banni	3000	3500	+Ve	+Ve
21	Bhadawari	2000	2500	+Ve	+Ve
22	Pandharpuri	2000	2500	+Ve	+Ve