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Livestock Improvement through Artificial Insemination

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Preface

Fueled by technological change and supported by infrastructure and institutions, India's livestock sector has emerged as an engine of agricultural growth over the past few decades leading to improvement in nutritional security and reduction in rural poverty. From the data available, the country's milk production increased by over 1100 % as against an increase of 24% in the population of cattle and 153% in the population of buffaloes between the period from 1951 to 2019. One of the most important reproductive technologies in the dairy industry is artificial insemination (AI) that not only increases the use of genetically superior sires to improve performance of the herd but also reduces the incidence of sexually transmitted diseases. However, the potential of artificial insemination has not been fully exploited in India. The overall AI coverage in bovines *(cattle and buffaloes)* is only 30%, with a conception rate of 35%. It is, therefore imperative to critically examine the reasons for the limited outreach of AI in terms of its coverage and conception rate.

Keeping in view the national importance of the issue, the National Academy of Agricultural Sciences organized a Brain Storming Session on *Livestock Improvement through Artificial Insemination* on December 06, 2019. It was attended by a galaxy of eminent scholars and a set of policy measures and recommendations emerged from their thoughtful presentations and discussion. This policy paper is the outcome of the deliberation in this Brain Storming Session. I am optimistic that this policy paper would signal a paradigm shift in livestock genetic improvement through artificial insemination. I gratefully acknowledge the contribution of Prof A.K. Srivastava, Convener and Dr A. Kumaresan, Co-convener of the Session, distinguished participants and reviewers. I am also thankful to Dr Kusumakar Sharma and Dr P.S. Birthal for their editorial support

(Trilochan Mohapatra)
President, NAAS

Livestock Improvement through Artificial Insemination

1. INTRODUCTION

India is endowed with huge livestock population reared under diverse production systems and agroclimatic conditions. The country shares 15, 58, 18 and 7% of world's cattle, buffalo, goat and sheep population, respectively. Increasing pressure for ensuring food and nutritional security to expanding human population on one hand, and shrinking resources (land, water and other inputs) on the other hand, has made it obligatory to obtain more production per unit of land or animal. Traditionally, livestock production in India has been in the hands of smallholders, keeping a small number of different animal species to sustain their livelihoods and to manage their day to day consumption needs. The animals also provide an insurance during economic crisis. However, now the scenario is changing; maintaining low-producing animals in resource constrained situations is no longer economically viable. As such the animal production system is changing towards semi-intensive to intensive mode of production, wherein these get linked to the input supply system as well as marketing channels. Thus, such a transformation in livestock production system has already started and is expected to continue in the years to come. This shift will require superior germ plasm, feed and fodder, and good health care. Since the availability of superior male germplasm, one of the most vital inputs, is limited in the country, the viable option is to use them as optimally as possible so that their superior quality is replicated in large number of females in a shorter period. In order to achieve this objective, the reproduction scientists including the biotechnologists will have to play the major role. Recent developments in reproductive bio-techniques have revolutionized and opened up new avenues for manipulating the reproductive process in livestock for improving their reproductive efficiency.

The reproductive biotechnology that has played an unequivocal and very important role in genetic improvement and production enhancement is Artificial Insemination (AI) with cryopreserved semen. The impact of this technology on economic developments of developed nations is very much evident. However, the potential of this technology has not been fully harnessed in several countries, including India. It is an established fact that the technology of *artificial insemination using cryo-preserved semen* can address major problems being faced by farmers in *managing a bull* for breeding of their animals. In developed countries, AI is the most common method of intensive breeding in dairy cattle *(approximately 80% in Europe and North America),* pigs *(< 90% in Europe and North America)* and turkeys (~ 100%; Thibier and Wagner, 2002). In India, AI is mostly restricted to cattle and buffaloes. Despite having one of the largest networks for livestock breeding, the overall AI coverage in cattle and buffaloes in India is only 29.7 % with an overall meagre conception rate of 35%. The reasons behind the failure to exploit full potential of AI are many. Difficulties in timely delivery of AI, absence of mechanisms to ensure use of semen from certified semen stations, non-adherence to state breeding policy, absence of a mandatory system of animal identification and data retrieval, and poor control over AI technicians are few limitations that need immediate redressal.

With this backdrop, National Academy of Agricultural Sciences (NAAS) organized a brainstorming session on *Livestock Improvement through Artificial Insemination* on December 6, 2019 to evolve the suitable national strategies and policies for effective application of AI technology to harness its full potential for genetic improvement of Indian livestock.

2. AI: A MAJOR CONTRIBUTOR TO PRODUCTIVITY IMPROVEMENT

In the artificial insemination technology, semen is collected from the male, processed and evaluated, stored at ultra-low temperature and artificially introduced into the female reproductive tract at proper time for conception. The primary objectives of AI in farm animals are accelerated genetic improvement and increase in the productivity. This is accomplished with the use of semen from highly superior males with proven genetic merits for insemination of several thousands of females. The adoption and preference for AI by the stakeholders is higher as compared to other reproductive techniques, because AI offers the scope for genetic improvement and productivity, allows maintenance of a close herd, prevents sexually transmitted diseases, avoids maintenance of a bull for service and offers scope for organized breeding management and record keeping.

With the developments in artificial insemination, the Government of India started recognizing cross breeding of indigenous/non-descript cattle with European breeds as a possible option for improving milk production. Cross breeding of non-descript Indian cattle at field level started in 1964 with the launch of the *Intensive Cattle Development Projects (ICDP)* and it became the policy of the government by 1969 for increasing the milk production (Singh, 2016). As a result of this, India occupies the first position in world in milk production. Visibly, AI has proven to be very effective for improving the genetic potential of animals for higher production and continues to be the backbone of all breeding programmes in India. Using this technology several cross-breeds/strains like Karan Swiss, Karan Fries, Frieswal, Sunandini, Phule-Triveni and Vrindavani cattle with high milk production potential have been developed. These and other crossbreds and upgraded cattle and buffaloes have propelled growth in Indian dairy industry. Further, there is a direct positive correlation between milk production and AI observed in the dairy sector of the country (Fig. 1)

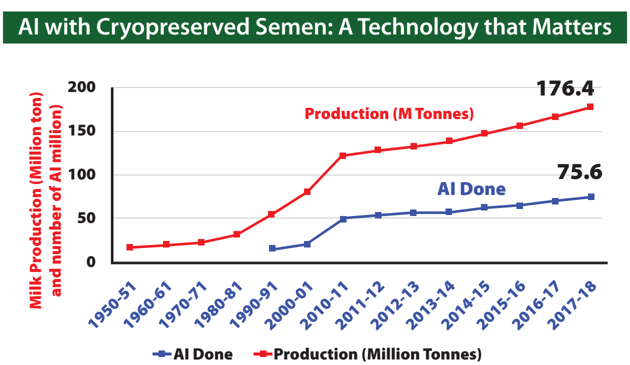

Fig. 1: Positive co-relation between milk production (million tonnes) and number of AI (million)

During the last few years, the milk production in the country has been growing at the rate of more than 6.5% AGR against 1% AGR during 1950-70 and at around 4% AGR during 1970-90. As given in Fig.2, the cattle and buffalo population was 155.3 and 43.4 million in 1951, respectively, while the total milk production was only 17 million tonnes. In 2019, the total cattle and buffalo population increased to 192.5 and 109.9 million, respectively, and the total milk

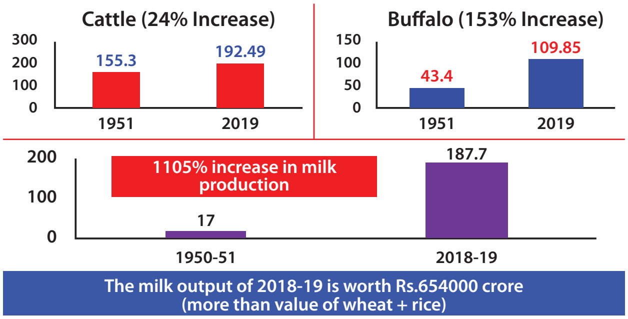

Fig. 2: AI technology led milk production in India

production reached a record level of 187.7 million tonnes (DAHDF Annual Report 2018-19). While there was 23.9 and 153.2% increase in cattle and buffalo population, respectively between 1951 and 2019, the milk production increased by 1105%. These data clearly indicate that the increased population of dairy animals is not the only reason for the phenomenal increase in milk production. Analysis of milk production grounded on *animal population-based increase and animal productivity-based increase* indicate that a significant portion of enhancement in milk production was contributed by the productivity-based increase. The cow milk yield was 423.53 kg/year in 1961, which increased continuously to reach 1191.54 kg/year in 2011. Similarly, the average milk yield of buffaloes also showed a significant increase, from 889.59 kg/year in 1961 to 1700.78 kg/year in 2011. The individual animal milk productivity in goats increased over the period to reach 150.16 kg/year in 2011 from 100 kg/year in 1961 (Srivastava *et al.,* 2013 and 2015). In 2017-18, milk productivity in cross bred cattle, indigenous cattle and buffalo further increased to 7.71, 2.93 and 5.47 kg/day as compared to their corresponding values of 4.19, 1.29 and 3.37 kg/day, respectively in the year 1990-91 (Basic Animal Husbandry and Fisheries Statistics 2019).

3. DEVELOPMENTS IN AI TECHNOLOGY AS A TOOL FOR BREEDING

AI technology was born from the research performed before the World War II, and the progress and outcome of this technology have been spectacular in several countries. Although the history of AI traces back to 17th century, its commercial use started only during 1930s. First cooperative artificial breeding association was established in Denmark in 1936 (Foote, 2002; Ombelet and Robays, 2015). In USA, AI was not much practiced until 1942 but after 1945 this technology was adopted there. In India, AI was done by National Dairy Research Institute, Bangalore, *then Imperial Institute of Animal Husbandry and Dairying,* for the first time in August 1939, when Dr Sampath Kumaran inseminated a good number of Hallikar cows with Holstein Friesian semen and obtained pregnancies at Palace Dairy Farm, Mysore. In 1942, a pilot project was taken up by Indian Veterinary Research Institute, Izatnagar under the guidance of Dr P Bhattacharya to study the feasibility of implementation of AI for breeding of cattle and buffalo, which was later expanded to four more centers, viz., Calcutta, Bombay, Patna and Bengaluru. The first buffalo calf through AI was born in 1943 at the Allahabad Agricultural Institute. Although AI was first performed in India in 1939, it was intensified only in the third phase of *Operation Flood* in 1985. Under Phase III of Operation Flood (1985-1996), veterinary first-aid health care, feed and artificial insemination services were extended to milk cooperative members. The bedrock of Operation Flood has been the village milk producers' cooperatives, which procure milk and provide inputs and services to members. In the services provided, the unique service to cows was the AI programme with exotic semen. As a strategy to increase milk yield, cross breeding with exotic cows like Jersey and Holstein Friesian was aggressively promoted. It is very important to note that the success in enhancement of buffalo milk yield and production is also of the same order as in the case of cow, although in buffaloes there is no AI with exotic germplasm (Singh, 2016).

In developed countries, AI is the most common method of breeding of domestic livestock. AI is also increasing in horses, goats and sheep, and has been reported in other domestic species such as pigs and dogs. It has also been used occasionally in *conservation breeding* of rare or endangered species of animals, e.g., primates, elephants and wild felids (Morrel, 2011). The protocol for cryopreservation of bull semen is well established and thus, frozen semen is used for AI in cattle throughout the world. However, few countries like New Zealand use fresh semen doses within 24 h of semen collection. In India, cryopreserved semen is used for artificial breeding of both cattle and buffalo. Globally, majority of research on buffalo semen cryopreservation is contributed by Indian scientists. AI in sheep and goats is traditionally performed with fresh or cooled spermatozoa, with acceptable fertility results. In India, use of frozen semen in artificial breeding of sheep and goats is very limited owing to the difficulties in cryopreservation of semen in these species. Even AI with fresh or cooled semen is also limited because of the anatomical barrier in reproductive tract of these species, *i.e.,* the cervix is tightly folded, making the insertion of the insemination catheter difficult. In pigs, use of frozen semen is limited because AI with cryopreserved boar semen results in lower farrowing rates and litter sizes than with cooled stored semen. First successful attempt for cryopreservation of boar semen and AI of pigs with cryopreserved semen for breed improvement resulted in birth of 9 normal piglets/litter at ICAR Research Complex for NEH Region, Barapani, Meghalaya in 2009 (Kumaresan *et al.,* 2009). In case of equines, initially, fresh semen was used for AI shortly after its collection, but nowadays the use of cooled semen has largely replaced the use of fresh semen. In general, the use of cryopreserved semen for AI in horses is in nascent stage owing to lack of established standard methods for cooling and freezing of stallion semen, lack of information on minimum sperm concentration required in one insemination dose and paucity of data on quality evaluation of frozen/thawed spermatozoa (Morrell, 2011).

4. CURRENT SCENARIO OF FROZEN SEMEN PRODUCTION AND AI

In India, the use of frozen semen for AI in cattle and buffaloes for genetic improvement has taken good pace from 1985 onwards. During the period, several frozen semen production stations were developed and the AI network was broadly expanded. India has one of the largest networks for livestock breeding (Table 1) with 12099 veterinary hospitals/polyclinics, 25263 veterinary dispensaries, 27628 veterinary aid centers, and 99239 AI centers (Basic Animal Husbandry & Fisheries Statistics, 2018).

Table 1. Artificial Insemination Network in India (2018)

Institutions	Numbers
State Livestock Development Board	28
Veterinary hospitals/polyclinics	12099
Veterinary dispensaries	25263
Veterinary aid centres	27628
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	56
Frozen semen banks	226
Liquid nitrogen plants	92
AI centers*	99239

**Each AI centre caters 681 inseminations/year (~1.86/day)*

Generally, breeding bulls are selected based on pedigree selection, progeny testing and sometimes through Embryo Transfer Technology. Production of quality frozen semen doses could be achieved by using high genetic merit bulls that are free from diseases and also by adhering to the standards and complying with the bio-security measures. In this direction, the Ministry of Fisheries, Animal Husbandry and Dairying (FAHD), Government of India has brought out a clear-cut Minimum Standard Protocol (MSP) for frozen semen production after consultation with experts from NDDB, ICAR Institutes and Veterinary Universities, which delineates each and every step to ensure quality semen production (NDDB Annual Report 2018-19). Currently, the breeding bulls are being produced based on the MSP and Standard Operating Procedures laid down by FAHD for progeny testing (PT) through Government approved PT programmes in the absence of genomic selection. If such males are not available and if there are no PT programmes for certain breeds, the procurement of bulls is based on the dam's standard lactation yield. Breed-wise dam's lactation requirements are also indicated in the MSP. Once the males are earmarked based on this criteria, they are subjected to Breeding Soundness Evaluation (BSE), comprising of physical examination, disease screening, karyotyping and testing for genetically transmitted diseases and semen evaluation. The bull is inducted into semen station for semen collection and cryopreservation in case the animal qualifies all these criteria. Under National Dairy Plan – I, strengthening of semen stations across the country resulted in large infrastructure for genetic evaluation and production of bulls with high genetic merit. This is reflected in the number of breeding bulls maintained in the semen station. The number of breeding bulls in semen stations were 2780 during 2010-11, which increased to 4338 during 2017-18. Correspondingly, the frozen semen production also increased from 33.79 million doses in 2003-04 to 115.9 million in 2016-17 by 53 semen stations in the country. The breakup in composition of bulls included 1173 exotic breed *(mostly Holstein Friesian and Jersey*), 988 crossbreds *(mostly crosses of Holstein Friesian and Jersey)*, 594 indigenous and 1403 buffalo bulls.

The contribution of exotic, crossbred, indigenous and buffalo bulls to total production of frozen semen doses during 2016-17 were 33.3, 24.8, 13.3 and 28.6%, respectively. During the last decade, the annual growth rate in frozen semen doses' production has been more than 10%. During 2000-01, the total number of AI performed in India was 19.77 million, which increased to 75.6 million during 2017-18 (Fig. 3).

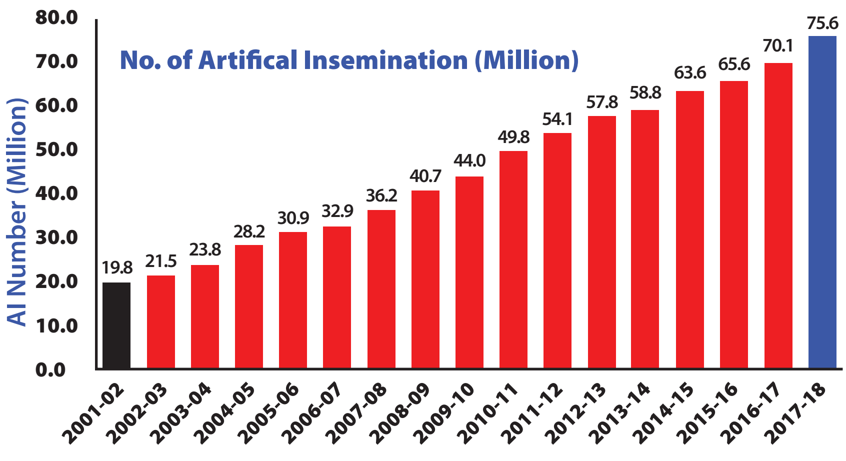

Fig. 3: Current status of AI in India

In 2017-18, there were 130.5 million breedable cattle and buffaloes in the country. Against this backdrop, the total AI done were 75.6 million with an overall AI coverage and conception rate of 29.7 and 35%, respectively. The data indicate that despite several positive developments, the coverage of AI is still far less than the desired. While the national average of AI coverage of breedable bovine population is around 30%, there are several states with less than 10% AI coverage (Table 2). Artificial breeding of goat, sheep, horse, pig etc. is very limited, mostly restricted to research farms or few commercial farms and being done in the field very nominally.

Table 2: State wise AI coverage (%) in cattle and buffaloes, 2017-18.

AI Coverage	States
Less than 10%	Arunachal Pradesh (0.97), Assam (3.88), Manipur (6.04), Meghalaya (3.26), Sikkim (9.89)
>10 but<20	Chhattisgarh (10.71), Bihar (16.1), Jharkhand (16.54), Madya Pradesh (12.76), Mizoram (17.78), Nagaland (18.1), Rajasthan (17.27)
>20 but <30	Maharashtra (27.38), Odisha (20.05), Telangana (24.5), Tripura (23.24), Uttar Pradesh (27.44), Uttarakhand (24.19)
>30 but <40	Andra Pradesh (37.59), Himachal Pradesh (37.94), Jammu & Kashmir (30.28), West Bengal (30.80)
>40 but <50	Goa (42.59), Gujarat (41.80)
>50 but <60	Haryana (55.80), Karnataka (53.94), Punjab (59.09)
>75	Tamil Nadu (75.42), Kerala (100)

5. MAJOR CONSTRAINS AND ISSUES

While most of the gains of using AI technology have occurred in the developed countries, there are considerable opportunities to increase the use efficiency of AI in developing countries including India. Considering the average achievements of artificial insemination in India, as compared to the gains derived in many developed countries, now a time has come to examine the issues hindering its largescale adoption for getting the desired genetic improvement in the livestock population. After a careful assessment, following issues emerged as the important ones to be addressed to realize the most gains out of the AI technology.

- ***Low coverage of AI:*** At present, animal husbandry departments are the primary providers of AI services (45.7% *of the total*) followed by Dairy Cooperatives (24.3%) and other agencies (29.9%). Therefore, the present AI delivery system needs to be strengthened and modernized to ensure a targeted coverage of 50% breedable females in the coming years. The major hindering factor is distance of AI services to farmers. The distance to veterinary institutions providing the service either delays or discourages farmers to access quality AI services. The cooperatives are better equipped to reach farmers in time at their door-steps, provide services more efficiently and receive required feedback effectively. The services of other agencies are largely dependent on the funds received from the state governments and other sponsoring agencies. The major issues related to current AI delivery system include difficulties in timely delivery of AI, lack of mechanisms to ensure use of semen from certified semen stations, non-adherence to state breeding policy, absence of a mandatory system of animal identification and data retrieval, and poor control over AI technicians (Gupta *et al.,* 2017). While attempting to answer the query of expanding the AI coverage to 100 percent breedable population, there is an imminent need to examine the economic feasibility and necessity to broaden the AI delivery system to remote areas of the country to cover very low or poor producing nondescript animals. Further, the biggest question before the country is to evolve an effective strategy to reduce the population of low and non-producing animals. Avoiding breeding such animals could be a viable option. Against this view, another option could be to intensify the breeding programme and bring the faster genetic improvement in these animals. However, it is pertinent to mention here that it took almost 3-4 decades to cover only 30% breedable bovine population under AI. Therefore, the possibilities of undertaking such an exercise may need further debate in the face of shrinking resources.

- ***Poor conception rates with AI:*** In India, the fertility rate from AI is comparatively lower than in other major milk producing countries, where the fertility rates through AI range from 60-72% with an average non-return rate of 60 days (Vishwanath, 2003). Against this, the average conception rate in India still hovers around 35%. Poor conception rate may be one of the major reasons for poor adoption of AI by farmers. Studies have shown that the acceptance level of AI is lower in areas where the farmers complain about poor conception rate through AI relative to natural insemination. Further, detection of estrus (heat) in animals is another challenge to farmers. Each missed estrus in a dairy cow producing an average of 10 liters of milk a day, leads to a direct loss of more than Rs. 8400/- to the farmer in 21 days, before the animal comes in next heat. Each day of an extended calving interval results in huge economic loss (Rs 281 and Rs. 368 in Zebu and crossbred cattle, respectively per day) to the farmers because of not getting milk from these animals but paying the maintenance costs of non-pregnant cow (Abdulla *et al.,* 2017). Inseminating the animals without proper heat confirmation is a major factor for reduced conception rate under field conditions. It has been reported that the percentage of cattle and buffaloes wrongly detected as in oestrus by visual observations were 11.05 and 20.75 per cent, respectively (Kumaresan et al, 2001). About 19% of the inseminations were performed when the plasma level of progesterone was high and the cows were pregnant. Such an insemination of pregnant cows led to an estimated 17% induced embryonic death and/or abortion (Sturman *et al.,* 2000). The present system of AI delivery, cannot ensure the service at right time due to (i) stationery nature of most AI centers with fixed working hours, (ii) lack of effective communication between farmers and AI service providers, (iii) limited number of AI service providers and (iv) poor training of inseminators.

- ***Limited availability of bulls with high genetic merit and high-fertility:*** In order to realize the full potential of AI for livestock improvement, it is essential that the semen used for artificial breeding should be from bulls of high genetic merit. The number of breeding bulls required is 8847 as against the present number of 4158 to meet out the current requirement (2020-21) of frozen semen doses for AI (DAHDF Annual Report 2018-19). Another problem lies in the selection of bulls for replacement, as the tool(s) for selection of males at young age with good fertility are yet to be evolved. The semen production ability of a bull is identified after rearing the male calf for 2-2.5 years and subjecting it to semen evaluation. At this point the bulls with inferior semen quality are discarded. Further to know the fertility status of the bull, one has to wait for years together after the production and supply of sufficient doses of frozen semen for field fertility assessment. Based on the conception rate observed after insemination of a large number of females, a bull is categorized as *above-average*, average or below-average. Keeping a bull for such a long duration in breeding programme and then identifying it as *below-average* leads to huge costs to the semen stations as well as to farmers. During such evaluation trials, semen from infertile/sub-fertile bulls often delays the conception and consequently cows remain open for several days leading to loss of milk production and productive life. The cost of raising a bull from birth to 18 months was calculated to be approximately US$1188 and the total annual expenses during breeding period were between US$1820 and US$2110 (Valergakis *et al.,* 2007). In brief, a very effective tool(s) and mechanisms(s) for the selection of males at very early age for high genetic merit and high-fertility is needed.

- ***Frozen semen quantity and quality:*** The National Action Plan aims to increase the milk production to 300 million tonnes by 2023-24. To achieve this target, the requirement of the total number of bovines and frozen semen doses will be very high. Accordingly, both the bull and the semen dose production facility in the country will have to be scaled up to almost double the existing numbers. Although male and female both contribute to the end result of AI, the role of males is far greater because semen from a single male is used to breed several thousands of females. Thus, ensuring the post-thaw quality of semen assumes immense significance in improving the reproductive capabilities of animals. Although there have been several developments in the process of semen analysis, the developments in cryopreservation, extenders and the method of insemination, have not been translated into reality. Poor understanding of the semen biology is one of the limiting factors for successful cryopreservation of spermatozoa. Even today, traditional method of semen analysis is being followed that gives only an idea about few pre-requisite characteristics of spermatozoa to fertilize an oocyte. These tests do not indicate about other important sperm functional requirements. The sperm motility, viability, acrosomal and membrane integrity are mostly assessed to certify the suitability of frozen semen; however the results of such tests do not always correlate with field outcomes (Rodriguez Martinez, 2013). Further, to maintain the frozen semen quality, adequate quantity of liquid nitrogen at required interval is essential, which is also a major problem at gross root level.

- ***Limited or non-availability of frozen semen for non-bovine species:*** Frozen semen of non-bovine animals *(sheep, goats, pigs, mares etc.)* is literally not available at large scale, expect in some pockets wherein few institutes produce cryopreserved semen doses and in very small numbers. Although in India, AI in sheep was introduced in late 1940's and early 1950's, AI in small ruminants is yet to be undertaken at commercial scale. In small ruminants, either fresh or fresh + diluted + chilled or frozen semen can be used for AI. However, the AI technique must be selected and standardized on the basis of the type of semen planned to ensure its successful use. At present technological backstopping and policy are not available for use of AI in other species including small ruminants and pigs. Few countries even adopt laparoscopic insemination for sheep with good results. Difficulties in semen cryopreservation, estrus detection and insemination procedure are few constraints in the adoption of the AI technology in small ruminants.

- ***Use of Sexed semen for AI:*** Recently, the demand and desire for use of sexed semen in dairy cattle is increasing. Several Indian states have already started using the sexed semen for AI in dairy cattle. A single dose of sexed semen straw costs Rs. 1500-2000, however, states are making it available at a subsidized rate. Until recently, the sexed semen doses were imported from other countries, but now under Rashtrya Gokul Mission, sexed semen production facilities are being developed at 10 A-grade bovine semen stations. It is targeted to produce 30 lakhs Sexed/Sex Sorted Semen doses annually from these semen stations. The sexed Semen Production has already started at 4 centers i.e. ULDBs Rishikesh, BAIF Pune, ABS Chitale and Mehsana Semen Station. Here, it is pertinent to note that the current average conception rate in dairy cattle when inseminated with 20 million spermatozoa is around 35% in India, which may further go down when 2 million spermatozoa in one sexed semen dose will be used for insemination. So far a total of 9133 AIs has been done in five districts with an overall conception rate of 24.9% under Pilot Project on AI with Sex Sorted Semen. Thus, there is an urgent need to standardize the dose and the site of reproductive tract for insemination with sex sorted spermatozoa in indigenous and crossbred animals. Further, it is also essential to develop the expertise and train the inseminators with *low-dose insemination procedures* to improve the conception rate with sexed semen. Once sexed semen is available, majority of stakeholders may like to use it for producing females that may again add to decreased availability of quality males. Hence, a stringent policy needs to be framed for using sexed semen, otherwise uncontrolled use of sex sorted semen might skew the sex ratio towards one sex and lead to unforeseen problems in Indian dairy sector.

6. RECOMMENDATIONS

6.1 Policy

In the given situation, genetic improvement of livestock through artificial insemination could be achieved by (i) enhancing the coverage of AI in the bovine, (ii) use of quality assured semen from bulls of high genetic merit and fertility, (iii) improving the conception rates with AI by proper detection of heat, (iv) by expanding AI services to entire breedable bovine population and non-bovine species and (v) selecting the bulls of indigenous bovine breed for AI, strictly as per breeding policy of Govt. of India and respective State Govt. The key recommendations in this direction include:

Implementation of Bovine Breeding Act: Any efficient AI delivery system should ensure that their AI technicians are using the semen doses as per the breeding policies of the respective states. To improve the productivity of bovines, regulating all the breeding activities such as use of breeding bulls for production of semen, and processing, storage, sale and distribution of frozen semen, and providing artificial insemination as well as natural breeding must continue concomitantly. Several animals belonging to well-defined breeds in their native tract also get inseminated, often inadvertently, with exotic semen causing considerable loss of indigenous germplasm. Further, the issues like limiting the blood levels of exotic inheritance and adherence of breeding policy of the states need legal provisioning for proper implementation. In this direction, the Bovine Breeding Act needs to be implemented in all the states. This will enforce the strict implementation of breeding policies, breed conservation, selection of quality semen, and also farmers' right to choose the best germplasm. Currently, the farmer's right in selecting semen from preferred bulls for breeding is limited. Very few platforms provide basic information on pedigree details of bulls being used for breeding by dairy farmers. Further, the information on comparative performances of bulls of semen stations in terms of conception rate is also limited in Sire Directory. Therefore, it is essential to legally bind the semen distributing agencies to provide such data to farmers while following the bull rotation schedules in the area.

Regulation of AI delivery agencies: In recent years, the accuracy and ease of obtaining breeding information from AI have been compromised by the overlapping distribution of frozen semen from several agencies, coupled with indiscriminate insemination. The presence of multiple AI delivery systems with no or different semen distribution plans in a given area, poses both a threat as well as an opportunity. The threat is in terms of difficulties in maintaining proper breeding records as each operator has different recording systems. The opportunity is in terms of healthy competition among various AI service providers to extend better services to farmers. As such, a policy measure for strict regulation of the AI delivery systems needs to be evolved. Further, the *next generation AI delivery system* may be developed by designing a farmer friendly Mobile App including location tracker for animal as well as AI technician. This will help farmer to invite a nearby AI technician, who has high scores based on the beneficiary's ratings and avail the AI service for his/her animal and can also pay online. The AI recording system for every state and integration of all data at national level by using *Cow-side AI delivery System*, with Smartphone/TAB based applications could also be a viable option. Further, incentivising AI workers based on calf-born rate, which is currently being practiced by few states, can be extended to all other states.

Mechanism for animal identification, data retrieval and analysis: The current AI delivery system does not necessarily mandate individual animal identification and tracking either for breeding purpose or for epidemiological surveillance, except in some pockets. Although Government of India is insisting to have a uniform unique identification system for all the bovines and has provided funds for this purpose, the pace of its adoption by states is rather very slow. There is also resistance from farmers for tagging their animals under unique identification system. The adoption has been reported to be good in the areas where progeny testing and pedigree selection projects are being implemented under NDP-I and the Information Network for Animal Productivity & Health (INAPH) developed by NDDB is being used. The AI needs to be brought under such animal identification and data retrieval system for tracking of the animal and quality of semen for managing successful breed improvement programmes in the country. Moreover, implementation of such technology aided tracking system would also help in addressing the emerging diseases and zoonosis.

Licensing of AI technicians: The AI delivery system in India needs a major shift in its approach. In India, a major proportion of AI is being carried out by AI technicians who are not well trained. As the skill of inseminator is very important to achieve high conception rates, increasing the number of skilled AI technicians is the need of the hour. A mechanism should be in place for rigorous training of AI technicians. The AI workers should be trained under a nationally *(approved by DAH&DF/VCI)* recognized training centre. Further, all the AI workers need to be registered with a specific registration numbers at state and/or at national level, and a separate registry should be maintained. These AI workers should be called as *Registered AI Workers* with issuance of a *License ID Card* with QR code for each individual. Only the registered AI workers should be allowed to undertake AI and also to enter all the data on-spot by using their QR code. These data may finally be linked to all India database, where the breed and location specific AI delivery system can be monitored and analysed. In addition, this would ensure the identification of genuine AI workers by the farmers and also facilitate the government agencies to monitor and assess the performance of AI workers. The next generation AI delivery systems should ensure timely services at farmers' doorsteps, maximise the efficiency *(penetration in remote places, conception rate, data retrieval, etc.),* comply with the breeding policies and goals of respective states, and be cost effective. Moreover, in line with government policies, it should also have only online or e-payment options to ensure transparency in charging (Gupta *et al.,* 2017). Presently, the policies and protocols related to AI under field conditions are completely ignored and sometimes decided by inseminators only. As such, for enforcing the breeding policies, the licensing system of AI technicians is expected to help implementation of the SOPs.

Continuing education programme for AI technicians: In India, most animals, that are brought for insemination, are inseminated by the inseminators based on farmers' observation on visual signs of heat. However, in Israel, inseminators are extensively trained to detect the cows in estrus. These inseminators reject about 16% of the cows submitted for re-insemination with an accuracy of 95% of rejection because the cows were pregnant (Sturman *et al.,* 2000). In this line, continued education programme for AI technicians appears to be of great significance in achieving the optimum conception rates and getting dependable field data for accurate breeding value estimation and selection of future bulls. Further, an online feedback system from farmers regarding adherence to Standard Operating Procedures by technicians while performing AI will not only help in assessment of their efficiency but also in identification of desired area of training for skill improvement. It will also help the farmers to get educated on right SOPs for AI so that they may monitor the procedure during the next AI. The online breeding advice platforms may be conceived for both AI technicians as well as farmers. Use of estrus detection aids, like Crystoscope, may be promoted under field conditions for estrus confirmation.

Timely treatment of infertile/sub-fertile animals and repeat breeders: Although the number of field veterinarians is significantly less, the availability of para veterinary staff is by and large satisfactory in some states (Rao *et al.,* 2015). The posts of veterinary officers are lying vacant in most districts, resulting in limited scope for validation of the factors for failure of conception in animals. Qualified veterinarians are not available in required numbers to diagnose and treat the repeat breeders in time, which is the main deterrent to successful penetration of AI in rural areas. Employing mobile veterinarians *(as in Gujarat)* and odd-hour veterinary service *(as in Kerala)* can be replicated in other states. Further, implementing state wide estrous synchronization and AI could help in reducing the problems of conception failure and repeat breeding. In the recent past, several protocols have been developed and/or modified to allow timely inseminations so as to circumvent the practical difficulties associated with estrus detection.

Use of semen from bulls of high genetic merit: The genomic selection is not practiced in India at present. However, a few genomic selected exotic bulls have recently been imported and their semen is in use for AI. It is imperative to study the performance and potential of these genomic selected exotic bulls under Indian field conditions. A robust AI networking and feedback reporting mechanism is required to generate perfect data on performance of these bulls, in terms of fertility and daughters' milk production under diverse agroclimatic conditions. Providing semen from proven bulls with good conception rate is most important in popularizing AI and expanding AI coverage. The Government Animal Husbandry Department, Non-Governmental Organizations and Cooperatives mostly use frozen semen from A or B graded semen stations following the SOPs for AI, whereas the majority of private AI technicians neither bother about getting frozen semen doses from A and B graded semen stations nor follow the Standard Operating Procedures. The use of progeny tested semen from registered sources can assure increased milk production across the generations, in addition to the disease control. In this direction, the semen supply chain may be restricted to only from A or B graded semen stations having progeny testing and quality assurance protocols to control the quality of semen. The frozen semen straw should also be printed with QR code for easy scanning using Mobile App for recording the details of the straw and animal used for AI service. Further, the bull mother farms should be encouraged to adopt the Assisted Reproductive Technologies (ATR), especially OPU-IVF technology for faster multiplication of elite breeding bulls. Registered Breed Societies for indigenous breeds should be formed for their genetic improvement through artificial insemination and conservation. Breed specific AI delivery system need to be strictly followed to prevent unscrupulous breeding.

Technology based semen analysis to be inducted at semen stations: In developed countries, high throughput semen analysis technology like flow cytometry is being routinely used in semen analysis laboratories. With the developments in science and semen technology, it is now possible to assess every fine characteristic of spermatozoa. It is high time for India to shift from *traditional semen analysis to technology based semen quality control tests.* Recently, it has been shown that the estimation of few functional attributes of spermatozoa using fluorescent microscopy or flow-cytometry, in addition to sperm motility, could fairly predict the fertility in cattle and buffaloes (Singh, 2016). These tests can be adopted by the semen stations to ensure the fertility of frozen semen doses. In addition, the human resources available at semen station quality control section need to be trained in these upstream, sensitive and accurate tests of semen analysis.

Standard Operating Procedures for sexed semen usage: Unlike normal AI with frozen semen, the use of sexed semen for AI requires specific SOPs and skills like selection of animals with adequate body condition score for the given age, optimization of the diet, application of estrus synchronization/ Timed artificial insemination (TAI) protocol, proper handling of sexed semen straws, apt insemination *(either deep cornual or in uterine body)* etc. As such it is very essential to develop the SOPs for using sexed semen so that good conception rates could be achieved.

Expanding AI to non-bovine species: In order to increase the coverage of AI in other food producing animals, priorities should be given to expand the AI coverage to small ruminants and pigs. With the developments in technologies and establishment of several commercial units of small ruminants and pigs, now it is high time to expand the realized benefits of AI in bovines to these species. However, it requires technological backstopping and establishment of an effective AI delivery mechanism. Replicating the same model being adopted for bovine may not hold good because of the variation in semen quality, freezability and the method of insemination. It is time to start AI in sheep, goat and pigs at least with liquid semen. To facilitate the expansion of AI in these species, more AI centres need to be established alongwith the development of nucleus breeding herd for elite buck and ram production. AI program in these animals should be implemented large scale at the commercial as well as individual farms. Further, efficient protocol for semen freezing and capacity building for AI in sheep and goat needs to be developed.

6.2 Research

- ***Fertility prediction tools***: Presently, the method for testing the bull fertility is based on insemination of many fertile females with the semen of said bull. However, this method is time-consuming, expensive, and allows only a limited number of bulls to be tested at any given time. Thus, there is an urgent need to develop an alternative method to predict the bull fertility. Research should be directed to develop the tools/methods and technology for prediction of fertility of male at young age.
- ***Sperm number reduction in frozen semen doses***: Currently, 20 million spermatozoa are cryopreserved in one frozen semen dose for AI in cattle and buffalo, with the objective that at least 10 million motile spermatozoa per straw would be available after freezing and thawing. Off late, it is understood that only the number of sperms alone will not yield the desired result of fertility. Several developed countries are currently using 10-12 million sperms per frozen semen dose and are obtaining very good conception rates. In insemination with sexed semen with 2 million spermatozoa, expected to have around 1 million motile sperm after freezing and thawing, comparable good conception rates have been achieved across the globe. Research efforts are required to develop the technology and procedure to reduce the number of spermatozoa to 10-12 million in a given dose, without compromising the fertility.
- ***Estrus detection tools***: There is a huge economic loss associated with failure in proper estrus detection in breedable females. Although the visual observation for estrus signs is a gold standard for estrus detection, this method has many constraints and difficulties including lack of trained and competent manpower, decreasing duration and intensity of estrus signs, silent estrus in buffaloes and less scope for automation. Thus, a *cow-side on-spot estrus detection* method/tool assumes significance and research efforts need to be continued in this direction.
- ***Detection of sub-clinical uterine infection***: Subclinical uterine infection is one of the major causes for conception failure in cattle and buffaloes. Recent developments in OMIC(s) technology *(genomics, transcriptomics, proteomics and metabolomics)* offer scope for identification, characterization and quantification of uterine fluid molecules that are specific

to uterine infection or differentially expressed during sub-clinical uterine infection. Research efforts are required to identify such molecules so that an *on the-spot kit* can be developed.

- ***Early detection of pregnancy in animals***: Identification of pregnant animals at an early stage would help in reducing the pregnancy losses and improving the calving rates. It is established that developing concept secretes several molecules, which are very specific to pregnancy. Identification of these bio-molecules and development of an assay to detect them at an early age could be a viable option to diagnose early pregnancy.

- ***Room temperature/refrigerated long-term storage of semen***: At present, maintaining the proper liquid nitrogen chain is essential for ensuring the sperm quality and fertility of frozen semen. However, it is constrained by inadequate availability of liquid nitrogen at right time and difficult accessibility to several areas. Although the lyophilization/freeze drying of semen has been tried for long term sperm storage, the success was not in the desired range. As such research on alternate methods of sperm storage has to be taken up on priority to find out a viable solution.

- ***Alternate to antibiotics for semen preservation***: Antibiotics are currently being added in semen extenders during freezing of semen to control bacterial contamination in semen arising at the time of collection and processing. However, their efficacy against the semen microorganisms is now being questioned, as microorganisms are being detected in semen even after adding the antibiotics. A number of plant extracts having antimicrobial effects have already been identified, but their effect on spermatozoa is yet to be tested. Research in this direction is warranted.

- ***Prolonged sperm release***: AI at improper time is one of the reasons for reduced fertility. Since the frozen-thawed sperm live for a shorter time as compared to freshly ejaculated spermatozoa, the early insemination with frozen semen, in relation to ovulation, might result in loss of sperm viability and functionality leading to fertilization failure or embryonic mortality. The sperm encapsulation technique has shown the release of spermatozoa relatively for a prolonged period. Accordingly, such technologies need to be developed and used in cryopreservation procedure so that infertility due to early insemination or prolonged estrus could be addressed, especially in crossbred cattle.

- ***Alternate method for sperm sexing***: Till date, flow cytometry-based sex sorting is the only commercially viable option available for sexing of spermatozoa. Now intensive research efforts are needed to develop the alternate indigenous method(s) for differentiating the X and Y chromosomes bearing spermatozoa and also to sort at high speed along with high accuracy and fertility. In this line, the identification of sperm surface markers specific for X or Y bearing spermatozoa and using them in sorting or killing of unwanted sex bearing spermatozoa could be an option. In future, development of designer bulls to produce either X or Y spermatozoa will be possible by knocking out the undesired.

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