Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2017; SP1: 307-313

Gaurang kumar Patel
Sardarkrushinagar Dantiwada Agricultural University, SK Nagar, Gujarat, India

Nilufar Haque
Sardarkrushinagar Dantiwada Agricultural University, SK Nagar, Gujarat, India

Mahesh Madhavatar
Sardarkrushinagar Dantiwada Agricultural University, SK Nagar, Gujarat, India

Ashvin kumar Chaudhari
Sardarkrushinagar Dantiwada Agricultural University, SK Nagar, Gujarat, India

Dhaval kumar Patel
Sardarkrushinagar Dantiwada Agricultural University, SK Nagar, Gujarat, India

Nikita Bhalakiya
Sardarkrushinagar Dantiwada Agricultural University, SK Nagar, Gujarat, India

Natvarbhai Jamnesha
Sardarkrushinagar Dantiwada Agricultural University, SKNagar, Gujarat, India

Pankaj Patel
Sardarkrushinagar Dantiwada Agricultural University, SK Nagar, Gujarat, India

Rajesh Kumar
C. V. Sc. & A.H, NDUAT, Faizabad, UP, India

Correspondence
Nilufar Haque
Sardarkrushinagar Dantiwada Agricultural University, SK Nagar, Gujarat, India

Artificial insemination: A tool to improve livestock productivity

Gaurang kumar Patel, Nilufar Haque, Mahesh Madhavatar, Ashvin kumar Chaudhari, Dhaval kumar Patel, Nikita Bhalakiya, Natvarbhai Jamnesha, Pankaj Patel and Rajesh Kumar

Abstract
Artificial insemination (AI) is the manual placement of semen in the reproductive tract of the female by a method other than natural mating which is one of a group of technologies commonly known as "assisted reproduction technologies" (ART), whereby offspring are generated by facilitating the meeting of gametes (spermatozoa and oocytes). AI is by far the most common method of breeding of intensively kept dairy cattle. In developed countries, advances in artificial insemination have already had a major impact on livestock improvement programmes. AI speeds up genetic progress, reduces the risk of disease transmission and expands the number of animals that can be bred from a superior parent. The acceptance of AI technology worldwide provided the impetus for developing other technologies, such as cryopreservation and sexing of sperm, estrous cycle regulation, and embryo harvesting, freezing, culture and transfer, and cloning.

Keywords: Artificial insemination, Livestock, Assisted reproduction technologies, Genetic progress.

Introduction
Artificial insemination is a technique in which sperm are collected from the male, processed, stored and artificially introduced into the female reproductive tract at proper time for purpose of conception. AI is most common method of breeding intensively kept domestic livestock, such as dairy cattle, pigs and turkeys. AI is increasing in horses, beef cattle and sheep, and has been reported in other domestic species such as dogs, goats, deer and buffalo. It has also been used occasionally in conservation breeding of rare or endangered species, for example, primates, elephants and wild felids. Artificial Insemination as a means of livestock improvement are now accepted and utilized worldwide. Hafez (1980) pointed out that artificial insemination is the most important single technique ever devised for the genetic improvement of animals. This is possible because, a few highly selected males produce enough spermatozoa to inseminate thousands of females per year, whereas only relatively few progeny per selected female can be produced per year even by embryo transfer. The increased use of outstanding proven sires to enhance production potentials, control genital diseases transmitted through natural service which aid in animal improvement results from the expanding use of Artificial Insemination.

History
Lazzaro Spallanzani (1780) reported first successful use of AI. He started experiment with dog. When one bitch manifested the signs of heat, he used semen at body temperature to inseminate the bitch. Sixty-two days later she gave birth to three pups. He is also called "Father of modern artificial insemination". In 1922, E.I. Ivanoff, a leading Russian investigator and a pioneer in artificial insemination, was the first man to undertake successfully the AI of cattle and sheep. Ivanoff worked with stud farms. He obtained successful results with 10 cows. Danish veterinarians (1937) developed the first rectovaginal/cervical fixation method of artificial insemination. In India, first time, AI was done by Sampat Kumaran (1939) at 'Palace Dairy Farm Mysore". He inseminated large number of Halliker cows with semen of Holstein Friesian and got 33 cows pregnant. Philips and Lardy (1940) developed egg yolk phosphate diluter for preserving fertility and motility of refrigerated bull spermatozoa. Salisbury *et al.* (1941) developed egg yolk citrate diluter. The first buffalo calf through AI (1943) was born at the Allahabad Agricultural Institute. Polge, Smith and Parkes (1949) discovered cryoprotective effect of glycerol in frozen semen technology. Cassou (1964) improved the straws by reducing their size and named it as medium

French straws. The size of the straw was 135 mm long and 2.8 mm diameter with 0.5 ml semen capacity. Cassou (1968) further reduced the size of the straws to the diameter of 2 mm with a capacity of 0.25 ml and named it as mini French straws. These are few important milestones in the history of artificial insemination.

The success of AI technology lies on the efficiency of semen collection and preservation. Hence, before describing the process of artificial insemination, process of semen collection, evaluation, storage etc is described stepwise here.

Method of semen collection

Sr. No.	Species	Methods commonly used for semen collection
1	Bull	artificial-vagina, electroejaculation, massage, directly from vagina
2	Ram and buck	directly from vagina, artificial vagina, electroejaculation
3	Boar	using glove hand and artificial vagina
4	Stallion	using artificial vagina
5	Dog	using digital manipulation and artificial vagina method

2. Evaluation of semen

1. **Macroscopic evaluation**
2. **Microscopic evaluation**

2.1 Macroscopic evaluation

The semen should be transferred to a water bath maintained at 35±1 °C. Visual evaluation for volume, colour, consistency/density, odour and observation for presence of foreign material (blood, pus cells, dung, hair, etc.) shall be made and recorded. If dung or hair is found in the semen, filtration with special semen filter is done.

2.2 Microscopic examination

Microscopic evaluation is done using a simple or phase contrast microscope for mass activity (wave motion) and individual motility. Determination of concentration is done with a hemocytometer or a calibrated photometer. At this point if required smears can be made for morphological studies and live/dead count. Nigrosin-eosin stain is recommended. Buffered nigrosin-eosin solution is mixed with a drop of semen and smeared on a glass slide for morphological examination. It should be dried and examined under oil immersion. Automated computerised machines for recording motility and concentration and calculating the required extensions are now frequently used in AI centres that can afford them.

Low magnification for initial assessment of semen quality

The following are guides to the values of semen characteristics in the bull that indicate good reproductive function:

- Motility (moving actively forward): > 60%,
- Concentration: > 500 million /ml,
- Live sperm: > 70%,
- Abnormal sperms: < 20% (range for bulls with good fertility is 8–12%),
- Proximal droplets: < 4 %;
- Distal droplets: < 4%,
- Tailless: < 15%;
- Singly bent tails: < 8%;
- Double bent tails: < 4%;
- Coiled tails: < 3%,
- Cells other than spermatozoa: none, or very few leucocytes or epithelial cells.

Various biochemical tests used for asses the sperm fertility

1. Methylene blue reduction test
2. Cold shock resistance test
3. Zone free hamster egg penetration test
4. Sperm mucus penetration test
5. Hypo-osmotic swelling test
6. Computer assisted semen evaluation
7. Fructolysis index
8. Oxygen utilization test
9. Millovanov's resistance test
10. Hyaluronidase contents

3. Dilution of semen

The first major improvement in the AI procedure initiated in the United States was the development of a yolk-phosphate semen extender (Phillips and Lardy, 1940) [1]. Salisbury *et al*. (1941) [2] improved the media by buffering the egg yolk with sodium citrate. Extender preserves fertilizing capacity of the spermatozoa for long period and increases the volume and thereby services to large number of females. Dilutor provide viability and fertility of spermatozoa for prolong period. Ideal semen dilutor should be isotonic with the seminal plasma, have a pH of 6.6 to 6.8 with high buffering capacity contain lipoproteins and lecithin, minerals in adequate quality and substances used for aerobic as well as anaerobic metabolism by the spermatozoa and glycerol. It should contain fructose to supply energy to sperm and antibiotic prevent microbial growth.

Dilution of raw semen

Diluted semen in water bath for controlled Cooling

The qualities of a good semen extender outlined by Samad (1985)[3]

1.	Osmotic pressure and electrolyte balance	Equipment of seminal plasma(285 millimoles)
2.	Energy source	Glucose,Fructose and Lactose in appreciable quantities.
3	Buffering capacity	Should maintain pH of diluted semen.
4.	Protection against cold shock	Lecitten,Lipoprotein as casine in milk and egg yolk Protect against cold shock
5.	Cryoprotectant	Glycerol
6.	Antibiotics	To get bacteria free semen,use penicillin and streptomycin

Extenders differ in composition depending on the species, use, temperature at which the diluted semen is to be stored; and the duration of storage desired. All extenders are based on a particular buffer, which has provided the best results for a given species (Hopkins & Evans, 1989) [4]. Buffers play an important role in modulating changes in the pH of extenders due to the products of metabolism in stored sperm. Carbohydrates (sugars) are necessary to provide the energy required for sperm glycolysis, egg-yolk based fractions provide phospholipids necessary to promote membrane stabilization at lower temperature and limit premature acrosomal membrane activation. The commonest extender used for frozen semen is skimmed milk or homogenized milk to which 10% glycerol is added. Tris citric acid fructose-glycerol yolk extender with 5% glycerol has been used with success (Merck, 1983) [5].

4. Packing

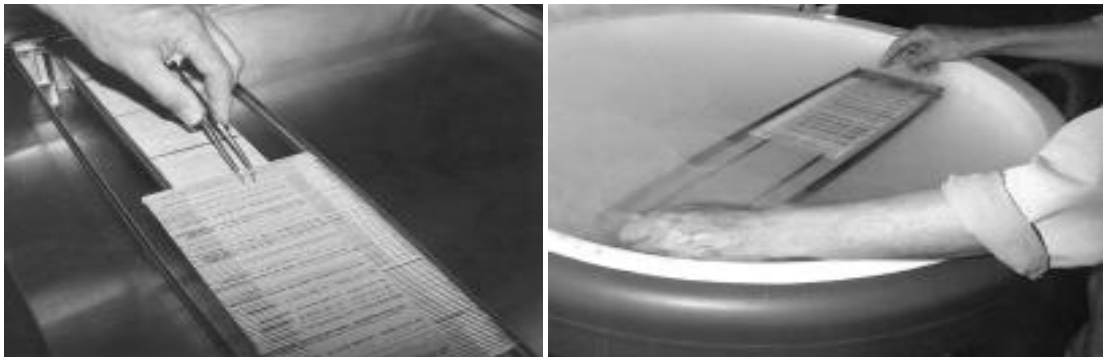
Packing of semen is done in medium straw (0.5 ml volume) and mini straw (0.25 ml volume). Medium straw have a 3 mm diameter and mini straw have a 2 mm. They are pluged at one end (double plug end) with a sealing powder which is retained between two cotton plug. By applying vacuum to this end of the straw, semen can be drawn up the tube and into contact with the sealing powder. As soon as powder becomes wet it turns into a gel to provide a very effective seal. Automatic straw filling machine use an ultrasonic pulse to seal the other end.

5. Semen preservation

Semen is used either immediately after collection ("fresh") for example turkeys, human beings; after storage at a reduced temperature ("stored") for example horses, pigs, dogs; or after freezing and thawing ("cryopreservation") for example, bulls.

Cryopreservation

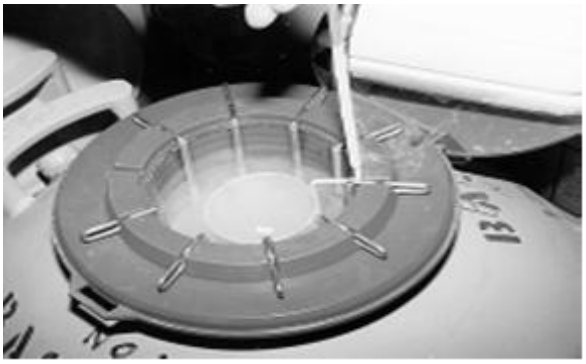
Semen is most useful for AI if it can be cryopreserved, since this method of preservation ideally enables the semen to be stored for an unlimited period without loss of quality until needed for AI.



Loading of semen straws on rack

The spermatozoa are mixed with a protective solution containing lipoproteins, sugars and a cryoprotectant such as glycerol. These constituents help to preserve membrane integrity during the processes of cooling and re-warming. However, sperm motility must also be maintained, so that the thawed spermatozoa can reach the oocytes after insemination and fertilize them. In most species, the seminal plasma is removed by centrifugation before mixing with the cryoextender, for example, stallion, boar, goat and human semen.

Straws are placed in vapour for freezing



Freezing is completed in liquid nitrogen

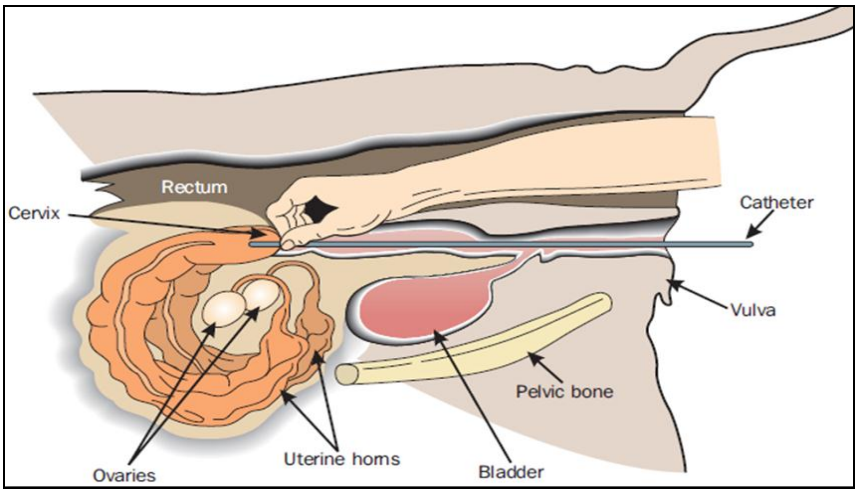
The extended semen is packed in straws and frozen in liquid nitrogen vapour before plunging into liquid nitrogen for long-term storage.

6. Thawing of semen

Thawing semen in warm-water (35-38 °C) for 40 seconds is the most commonly used thawing procedure reported by inseminators (80%). A significant increase in the conception rate (27%) was reported when thawing occurred in warm-water (33-35°C) as opposed to air (Dejarnette and Marshall, 2005)[6]. Kaproth *et al*. (2005) [7] also reported a significant increase (62.4%) in the fertility rate when thawing is done in warm-water 35 °C for 30 seconds compared to pocket-thaw.

Procedure for insemination

Firstly, it has to be ensured that the cow to be bred is truly in heat. The cow should be restrained at first and then semen should be thawed. The restraint area should be familiar to the cow and free of stressful conditions. The tail is moved and the cow is cleaned to remove any excess manure and debris from the vulva. The gun is unwrapped and then inserted at a 30°-40° angle into the cow's vulva. The left hand is inserted into the rectum to check for the location of the end of the AI gun. The cervix is grasped with the hand in the rectum of the cow and is held steadily while the AI gun is thread into the cervix of the cow. When the AI gun is all the way through the cervix, the location is checked with index finger. The AI gun should be only ½ to ¼ of an inch into the uterus. Slowly the plunger is depressed at the end where the right hand is so that ½ of the straw's contents is deposited. Recheck and deposit the remaining ½.



Cows are artificially inseminated by palpating the animal through the rectum and passing the insemination tool through the cervix.

Timing of insemination

Correct timing of insemination is as important as correct placement of semen. Field experience has shown that the best results are obtained when the insemination is performed at or near the end of oestrus. The beginning and end of oestrus are very difficult to determine. The simplest practical method of timing inseminations is to use the a.m.- p.m. rule.

Cows first seen on heat Insemination time

Morning (a.m.) Same afternoon (p.m.)
Afternoon (p.m.) Next morning (a.m.)

Two-thirds of cows commence oestrus at night and so will first be seen on heat in the morning. With heifers and some Bos indicus breeds, many authorities do not recommend the a.m.– p.m. rule. They recommend that these animals should be inseminated soon after the first observed oestrus.

Dual placement of semen

The reason that placement of semen both in the uterus and the cervix is recommended is that it helps to overcome the unpredictability of ovulation times. In cattle, ovulation can occur from 2 to 26 hours after the end of heat. Semen deposited in the body of the uterus reaches the fertilization site quickly to fertilize an ovum that is released earlier than normal. Semen deposited in the cervix survives longer. Due to its slow release from the cervix it is more likely to fertilize an ovum released later than normal.

The use of AI in different species

Despite the fact that the basic principles of AI are the same in all species, there is wide variation in the uptake of this biotechnology in different species.

AI in cattle

In cattle, frozen semen doses are used most widely in Europe and North America, since there are well-established protocols for cryopreserving bull semen. Semen doses typically contain approximately 15 million motile spermatozoa. In New Zealand, however, fresh semen doses are used instead, with AI occurring within 24h of semen collection.

AI in pigs

The porcine AI industry uses liquid semen that has been stored for one to several days at 16-18°C. In contrast, AI with cryopreserved boar spermatozoa results in lower farrowing rates and litter sizes than with cooled, stored spermatozoa, making the use of frozen-thawed sperm doses unattractive for commercial pig breeders.

AI in horses

AI has increased in horses in the last 25 years. Initially, fresh semen was used for AI shortly after semen collection, but nowadays the use of cooled semen has largely replaced fresh semen. The extended semen is cooled to approximately 5 °c, and transported in insulated containers, together with a cold pack. The fertility of the cooled semen is maintained for approximately 24h. Frozen semen doses are used infrequently, although this trend may change with the development of better freezing protocols.

AI in sheep and goat

AI in sheep and goats is traditionally performed with fresh or cooled spermatozoa, with acceptable fertility results. However, use of foreign breeds, genetic improvement and the use of "safe" semen from other countries requires the use of frozen semen, to enable analyses for contaminants or diseases in the "donor" male to be completed before the semen doses are used for AI. Although the post-thaw motility of frozen semen from goats and sheep is usually considered acceptable, low fertility has been associated with its use in AI, mainly owing to a shortened lifespan of the spermatozoa.

Advantage

1. ***Increased efficiency:*** During natural breeding, a male will deposit much more semen than is theoretically needed to produce a pregnancy. In addition, natural breeding is physically stressful. Both of these factors limit the number of natural matings a male can make. However, collected semen can be diluted and extended to create hundreds of doses from a single ejaculate. Also, semen can be easily transported, allowing multiple females in different geographical locations to be inseminated simultaneously, and semen can be stored for long periods of time, meaning that males can produce offspring long after their natural reproductive live end.

2. ***Increased potential for genetic selection:*** Because artificial insemination allows males to produce more offspring, fewer males are needed. Therefore, one can choose only the few best males for use as parents, increasing the selection intensity. Furthermore, because males can have more offspring, their offspring can be used in a progeny test program to more accurately evaluate the genetic value of the male. Finally, individual farmers can use artificial insemination to increase the genetic pool with which his or her animals can be mated, potentially decreasing effects of inbreeding.

3. ***Increased safety for animals and farmers*:** As mentioned, male animals can become large and aggressive. These factors mean that maintaining a bull on a farm may be dangerous. Also, because of the relatively larger size of adult males than females, natural mating is more likely to result accidents and injury to either the cow or the bull than is artificial insemination.

4. ***Reduced disease transmission*:** Exposure of sires to infectious genital diseases is prevented by use of AI which reduces the danger of spreading such diseases (Webb, 1992) [8]. In other way, if only males known to be free from disease are selected for semen collection, artificial insemination can play an important part in controlling diseases spread through sexual contact. Among the diseases in this group are granular vaginitis, trichomoniasis, navel ill, dourine, brucellosis and coital exanthema.

5. **Improving animals' productivity:** AI plays an important role in enhancing animal productivity, especially milk yields, in developing countries that have a well-defined breeding strategy and a sound technical base to absorb and adapt the technology to meet their needs (BBC, 2015) [9]. Daughters of AI sires produce significantly more milk than those of herd bulls sires and the income from this extra milk may cover the extra costs resulting from extended calving intervals because of low heat detection.
6. Breeding can occur in the event of physical, physiological or behavioural abnormalities;
7. AI is a powerful tool when linked to other reproductive biotechnologies such as sperm cryopreservation, sperm sexing.
8. AI can be used in conservation of rare breeds or endangered species.
9. The use of semen extenders containing antibiotics also helped to prevent the transmission of bacterial diseases.

Disadvantage

1. **Cost of AI compared to natural service:** Despite the well-known advantages of artificial insemination, a large number of dairy farmers all over the world still use natural service (NS) bulls to breed their cows. The main arguments allegedly justifying their choice are higher AI costs compared to those of keeping herd bulls and additional costs resulting from extended calving intervals because of low heat detection rates when AI is used.

2. **Impact of AI in genetic diversity:** Even though AI is highly effective in improving animals' productivity, there is also a concern that its inappropriate or unplanned usecan lead to increased rates of genetic erosion and breed extinction (Pilling *et al*., 2007) [10]. The heavy use of the best males results in a strong increase in inbreeding and a lose of genetic diversity.

3. **Difficulty of heat detection:** Among different factors that can affect conception rate per AI service, accuracy of heat (estrus) detection is the major one that determines AI program since ova remains viable for only about 12-18 hours after ovulation (Bekana, 1991) [11]. The failure to detect heat is the most common and costly problem of AI programs and the major limiting factor of reproductive performance on many dairies (Nebel and Jobst, 1998,

Dalton, 2004) [12, 13].

4. Some males shed virus in semen without clinical signs of disease ("shedders").
5. Some bacterial pathogens are resistant to the antibiotics in semen extenders or can avoid their effects by forming bio-films.
6. There has been a decline in fertility in dairy cattle and horses associated with an increase in AI.
7. The focus on certain individuals may result in loss of genetic variation.

Costing an AI program

The cost of landing an AI calf on the ground will vary tremendously. Before a program is attempted it is advisable that a budget is prepared. It is possible, but not common, for a well conducted AI program to produce calves at a cost comparable with those produced by natural service. The cost of AI calves will differ depending on the price of the semen used, the type of program to be used, (depending on whether synchronisation drugs are used) and on the results obtained from the program. Realistically all costs should be included and should at a minimum take account of the following:

1. **Labour**: Labour must be costed at a realistic level, even for self-administered programs. It takes highly skilled labour to successfully conduct an AI program.

2. **Equipment & infrastructure**: Provision and maintenance of suitable facilities for restraint and cattle handling procedures should be maintained. The yard structure should be designed to handle and hold the required number of animals and minimise the stress on both the animal and operators during the detection and drafting processes.

3. **Animal husbandry procedures**: Labour and other costs are incurred during the selection of suitable cows for inclusion in the program before it even starts. These costs need to be recognised. Costs are involved with the pregnancy testing and also with the methods used for monitoring the success after the completion of the program.

4. **Welfare and nutrition**: The provision of suitable feed and water, over and above the normal nutritional requirements for the cattle, need to be considered as part of the costs is associated with the AI program.

5. **Semen costs:** The cost of straws will range considerably. From the low cost use of an owner own bull through to the purchase of expensive imported semen.

6. **AI equipment:** The cost of purchasing, maintaining and depreciation of AI equipment (such as liquid nitrogen tanks and kit boxes), must be considered. Similarly the cost of consumables such as heat detection devices, disinfectants and paper towel must also be included.

7. **Synchronizing drugs**: The cost of drugs for the synchronization of the cycles of the cows and the administration of these drugs is considerable. The cost of the drugs will vary greatly according to the type of program attempted and programs using a number of drugs.

Faults in AI technique

1. **Overexposure of semen:** Frozen semen has a critical temperature of minus 70 °C. If the temperature of the dose is raised above this level and re-frozen, sperm will be damaged.

2. **Incorrect site of semen deposition:** Inability to pass an insemination gun through the cervix, or difficulty in doing so, is primarily due to inexperience. A greater and far more common mistake is to pass the gun too far into the uterus. This fault is prevalent, irrespective of experience, and is caused solely by inattention to detail.

3. **Poor seal between straw and sheath:** If the gun is loaded incorrectly some semen will escape into the sheath and perhaps into the barrel. This reduces the number of sperm available for fertilization.

4. **Neglecting to open the vulva:** Opening the vulva before the introduction of the gun is a hygiene precaution of major importance. Irrespective of the cleaning precautions practiced, there will be heavy bacterial contamination of the vulva. Transfer of bacteria directly into the uterus is therefore inevitable unless the vulva is opened adequately.

Conclusions

Artificial insemination is receiving renewed attention in dairy cows gradually. AI technology not only maximizes animals' productivity and harvests individual sires with traits of superior quality, but also reduces the risks of spreading sexually transmitted diseases. In-spite of the efforts made to introduce large-scale AI breeding services, growth in its use has generally not been very strong and conception rate is very low. Therefore, the desired effect in terms of animal improvement has not been achieved. Hence, governments have to design and implement clear policies for AI through alleviating the most important causes of failure. Economic incentives should be provided to farmers to breed improved animals for the successful introduction or extension of AI in developing countries. Furthermore, sound long-term breeding strategies that would improve the farmers' profits without destroying the indigenous genetic resources should be supplemented. However, further researches to create awareness for the society have to be conducted and forwarded.

References

1. Phillips PH, Lardy HA. A yolk-buffer pabulum for the preservation of bull semen. J Dairy Sci. 1940; 23:399-404.
2. Salisbury GW, Fuller HK, Willett EL. Preservation of bovine spermatozoa in yolk-citrate diluent and field results from its use. J. Dairy Sci. 1941; 24:905-910.
3. Samad SA. Diluters of the Buffalo Semen. Processing and use of Buffalo semen. *Ani. Sci. Inst. NARC*, Islamabad, 1985, 34-46.
4. Hopkins SS, Evans LE. Artificial Insemination. In: Veterinary Endocrinology and Reproduction. 4th Ed. MacDonald, L.E. and Pineda, M.H., (eds). Philadelphia, Lea and Feibeger, 1989, 354-88,
5. Merck. The Merck Veterinary Manual, 5th Ed, 1983.
6. Dejarnette JM, Marshall CE. Straw-thawing method interacts with sire and extender to influence sperm motility and conception rates of dairy cows. J. Dairy Sci.

2005; 88(11):3868-3875.
7. Kaproth MT, Rycroft HE, Gilbert GR, Abdel-Azim G, Putnam BF, Schnell SA. Theriogenology. 2005; 63:2535-49.
8. Webb DW. Artificial Insemination in Dairy Cattle, a document,DS58, one of a series of the Animal Science Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida, 1992.
9. BBC History. 'Robert Bakewell (1725-1795)' BBC Historic 2015, Figures: http://www.bbc.co.uk/history/historic_figures/bakewell_robert.shtml
10. Pilling D, Cardellino R, Zjalic M, Rischkowsky B, Tempelman KA, Hoffmann I. The use of reproductive and molecular biotechnology in Animal Genetic Resources management: a global overview Animal Production and Health Division, FAO, V. ledelle Terme di Caracalla 1, 00100 Rome, Italy. 2007; 40:1-13.
11. Bekana M. Farm animal obstetrics, Monograph, Faculty of veterinary medicine, Addis Ababa University. 1991, 1-12.
12. Nebel RL, Jobst SM. Evaluation of systematic breeding programs for lactating dairy cows: A review. J. Dairy Sci. 1998; 81:1169-1174.
13. Dalton JC, Ahmadzadeh A, Shafii B, Price WJ, Dejarnette JM. Effect of simultaneous thawing of multiple 0.5 Ml straws of semen and sequence of insemination on conception rate in dairy cattle. J Dairy Sci. 2004; 87(4):972-975.