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Artificial Insemination in Poultry and Possible Transmission of Infectious Pathogens: A Review

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ABSTRACT

Spallanzani's thought of Artificial Insemination (AI) has revolutionized the animal husbandry field, both in developing and developed countries, by improving the genetic potential of livestock and poultry; minimizing the managerial costs and holding the service of genetically superior males even after their death. AI in domesticated birds especially in turkey shows promising results unlike other domestic and wild animals. The advantages of AI are many which support the wide adaptation of this technique in the poultry industry to augment its growth. Making AI as an integral part of captive breeding programme for non-domesticated birds would facilitate the process of saving various endangered species of wild birds. However, there are various problems involved in case of birds which need to be addressed before implementing AI. Apart from these, AI also poses a risk of possible transmission of various infectious pathogens/diseases of poultry through semen or its contamination or during the process of insemination. Hence, careful and regular screening and monitoring of poultry will help to check the spread of such diseases. Novel methods are adopted to prevent the colonization of contaminant microbes in stored semen thereby minimizing the pathogen transfer. The recent advances in biotechnology and molecular biology need to be explored fully for early and rapid diagnosis of poultry diseases. This would help in formulating appropriate disease prevention and control strategies and thus safeguard poultry health and production. This review describes the salient facts about AI practices in poultry and possible transmission of infectious pathogens during insemination along with suitable prevention and control strategies to be adapted.

Key words: Artificial insemination, poultry, pathogens transmitted, health care, management, prevention, control

INTRODUCTION

Artificial Insemination (AI) of farm animals is very common in current animal husbandry practices and livestock and poultry breeding programmes. It is being practiced especially for breeding of dairy cattle, horses, pigs, pedigreed dogs, exotic and captive wildlife and poultry to obtain and propagate desirable characteristics/traits of one male to many progeny (Cooper, 1977; Rutz and Xavier, 1998; Foote, 2002; Gee *et al.*, 2004; Blanco *et al.*, 2009; Dhama *et al.*, 2007; Bakst and Long, 2010). It is meant to upgrade the herds or overcome breeding related problems in an economical way. Among the great biotechnological technologies artificial insemination holds upper hand for improving reproductive efficiencies of poultry and for this reason it has got an enormous impact worldwide. The impact of this technology has become the hallmark of certain other reproductive technologies like sexing of sperm; culture and transfer; as well as cloning (Donoghue and Wishart, 2000; Spasojevic, 2010).

The credit of first successful AI goes to Spallanzani and Bonnet (1784), who did it in dog which resulted to three pups 62 days later. This technique was first successfully used in birds almost a century ago when Ivanov (1907) produced fertile chicken eggs using semen recovered from the vas deferens of cock. However, phenomenal growth of AI in birds began in 1935 when researchers in USA, learned techniques to obtain semen from male fowl (Rutz and Xavier, 1998). Quinn and Burrows were the two pioneers who first reported the present day's technique of intravaginal insemination (Quinn and Burrows, 1936). Thereafter, with the support of recent advances in science and technology AI is playing a major role in up gradation of the poultry production in many countries (Surai and Wishart, 1996; Rutz and Xavier, 1998; Dhama *et al.*, 2007).

It is established now that AI in poultry expresses better fertility than natural mating and it has the ability to speed up the rate of genetic improvement by increasing selection differential, wherein one highly selected sire is mated with thousands of females (Chaudhury, 1996; Gill *et al.*, 1999; Gee *et al.*, 2004; Dhama *et al.*, 2007). This technique is practiced extensively with commercial turkeys. Continuous genetic selection for turkeys with large breast muscling has resulted in many heavy breeds which are no longer capable to properly breed naturally; because of their large size the natural mating may also result in injuries to birds, this has forced the adoption of AI in commercial turkey production. The technique of AI is also getting momentum in other poultry species. For example, as fertility in the broiler breeds continues to decline as males are selected for growth, AI may become cost effective in broiler breeder management. However, due to the presence of cloacal gland foam in quail and low semen volume in quail and guinea fowl, AI is not very ease to be performed. Similarly, AI procedure is not as simple in ducks and geese unlike chickens and turkey hens; because of the difficulties in semen collection (intromittent phallus) and oviduct eversion and therefore commercial demand for AI in these species is limited. AI technique has also been successfully adapted to wild birds such as cranes, bustards and peregrine falcon (Blanco *et al.*, 2009) and in some pet and fancy birds like pigeon and parrot (Sontakke *et al.*, 2004; Lierz *et al.*, 2013). The significance of this technique may be more important for those birds that belong to the endangered list where captive breeding is the only option to restore the population status).

With the advancement in the AI technology in poultry in near future it will be possible to increase the insemination interval to 10-14 days (instead of 7 days) with lesser concentration of sperm per insemination (Froman *et al.*, 2011). There is also greater chance of getting good quality of transgenic progeny following the insemination of sperm that carry transgenes. This review discusses about the various aspects of AI in poultry along with the microbial diseases that can interfere with this technology and pose significant health and production threats to poultry

producers. It also focuses suitable prevention and control strategies to be adopted for sexually/vertically transmitted infectious pathogens during AI which would enlighten the researchers to make a progress towards better outcomes of this technology.

ARTIFICIAL INSEMINATION (AI) IN BIRDS: FACTS AND IMPORTANCE

The poultry sector has now become a major contributor to a nation's economy as a result of the revolutionary and scientific approaches in avian health care management and efficient breeding programs. As the poultry industry is expanding, there is a true need for improving the efficiency and output of production. At this juncture, the role of artificial insemination in addition to the breeding policies need to be given due importance if we have to improve the production in heavier breeds. Poultry production is now focused towards intensive and semi-intensive production systems; so a fruitful AI program should be well supplemented with proper equipments and sufficient qualified handlers to ensure that collection of semen and insemination are done in a scientific and hygienic manner. This ensures a positive impact on hatchability of various genotypes of chickens (Paul *et al.*, 1999; Dhama *et al.*, 2007).

AI has made significant contributions to the breeding of domestic birds and other pet and captive birds. In case of chickens AI has not got wide application but in case of special breeding work it is used on routine basis (www.as.nchu.edu.tw). This technique has been successfully used in breeding programs of many rare and endangered species like perigreen falcons and others and efforts are being made to develop methods of preserving falcon semen through cryo-preservation and banking frozen semen. AI can be used to overcome behavioral problems associated with mating in such captive birds (Barna, 1995; Froman *et al.*, 2011). AI in poultry has practical impact in an economical point of view for poultry farmers/producers (Surai and Wishart, 1996).

In certain birds like the prey birds viz., turkeys and cranes; pigeons there is frequent use of collection of semen and artificial insemination subsequently. In larger psittacines in contrast there have been reports of semen collection anecdotally. Therefore, insemination has been done successfully in small psittacines. Such studies regarding AI in birds if conducted can help to protect certain endangered species of psittacine birds like large parrots (Lierz, 2008). To make it practically viable, a quiet, unhurried approach is necessary for handling of breeder birds during AI, following up of appropriate semen collection and insemination procedures, which all need to be done in a scientific manner. Prior to semen collection, the selected male birds should be examined for external parasites or infectious pathogens and treated accordingly. Clipping of feathers from around vent area will give easy access to male organ. In turkeys, the volume obtained is about 0.35-0.5 mL, with a spermatozoon concentration of 6-8 billion mL⁻¹, whereas in chickens, the total volume is 2-3 times that of turkeys, but concentration is only one-half. After collection of semen, insemination is done by simple process of transferring semen samples directly into the oviduct of the hen, manually using small diameter glass or plastic tubes (Bakst, 1992; Bakst *et al.*, 1994). Freshly collected semen samples can also be diluted with diluents to obtain desired spermatozoa concentration for multiple inseminations or for short-term and long-term storage (Birkhead *et al.*, 1994; Chaudhury, 1996).

Artificial insemination technique: Most widely practised method of AI in poultry was developed by Quinn and Burrows (1936). The technique involves combined action of applying pressure on the abdomen along with evertion of vaginal orifice. The procedure is called by different names as crackling, venting or everting the hen. With the aid of straws or syringes semen (80-100 million

spermatozoa) is deposited in the vaginal orifice (to the depth of 2-4 cm or as close as possible to the sperm storage tubules). The depth of insemination actually depends upon the species of the bird and length of vagina. Usually, AI is carried out in the late afternoon (Brillard and Bakst, 1990) because at this time the incidence of the hard-shelled eggs in the uterus of the hens is rare.

Methods of semen collection: In different birds the anatomical variation in the phallic region primarily leads to the variation in semen collection techniques. In order to have a good AI programme the first step should be to have a good quantity of semen from a good donor bird. It is necessary to develop a simple as well as effective method of collection of semen as well as insemination for making use of the potential role of birds in poultry research as well as production (Burrows and Quinn, 1937; Chelmonska *et al.*, 2008). To achieve this, semen should be collected from the donor birds in such a way that the required amount is obtained without environmental contamination. Three or four times milking of the male birds should be done in order to check quantity as well as colour of semen. Preservation of semen becomes useless in cases if there is refusal in production of semen by the male birds 10 days after handling or if there is discolouration of the semen which is normally white (Gee and Sexton, 1990; Tselutin *et al.*, 1995). Unlike domestic animals which can be trained easily to mount a dummy and ejaculate, birds are tricky especially wild birds which are usually in flight. The usual methods followed for collection of semen from birds are as follows:

- **Co-operative approach:** As the name implies this technique needs co-operation from the donor birds which can be achieved by an external stimulus which are of behavioral, for example voice, nest, food (Hamerstrom, 1970). The lead point of this technique is that there is no stress and injury free on the bird as there is no bird handling involved. The major advantage of this technique is that the quality of semen is good without contaminants like faeces and urine. However, the quantity of the semen obtained will be less which is the drawback of this technique. The co-operative approach technique showed promising results with artificial vagina in case of Muscovy ducks (Gvaryahu *et al.*, 1984), Emu (Malecki *et al.*, 1997) and with female dummy in case of Houbara bustard (Jalme *et al.*, 1994), quail (Chelmonska *et al.*, 2008) and ostrich. Uses of dummy or teaser females require a special mention in this regard (Rybnik *et al.*, 2007)
- **Electro-ejaculation:** This technique is commonly employed for ducks and geese, psittacines and pigeons. Anaesthesia is a mandatory in this technique and contamination with urine is a major drawback (Harrison and Wasmund, 1983; Betzen, 1985; Samour *et al.*, 1985)
- **Abdominal massage technique:** This is the mostly widely accepted and used technique (Burrows and Quinn, 1935) which is yet a non invasive method (Howell and Bartholomew, 1952; Birkhead *et al.*, 1995). In chickens and turkeys it involves massaging of the cloacal region in order to achieve phallic tumescence (Burrows and Quinn, 1935). Properly restrained donors are stroked gently in the back region behind the wings which stimulates most males with phallic engorgement at which state the cloaca can be squeezed to collect the semen (Cooper, 1977). There may be however damage to the phallic as well as cloacal regions due to additional cloacal strokes thereby contributing to semen contamination (Malecki *et al.*, 2008). For investigation of the physical as well as biochemical characteristics of the semen of broiler chicken attempts have been made. Abdominal massage method has been used for collection of semen of certain indigenous poultry birds like Kadaknath (Shinde *et al.*, 2012)

Semen dilution: After collection the spermatozoa start losing integrity thereby causing reduced fertility. Within 30-45 min of collection it is useful to dilute semen. There is report of excellent levels of fertility with chicken as well as turkey semen *in vitro* for 24 h or more. The volume of semen is increased by dilution thereby helping to retain integrity of cells and buffering the detrimental effects that use to arise on storage. Ideal extenders and their careful handling are required in general if semen need to be stored (Koochpar *et al.*, 2010). Semen has to get diluted with suitable diluent in order to: (i) Increase semen volume, (ii) Increase in number of birds inseminated by per unit volume of semen and (iii) Finally, it prolongs the sperm survival for both short and long term preservation of semen *in vitro*. Semen with proper diluents would be economically viable and effective for insemination in birds. Using the semen diluent, the services of a superior male can be used maximally by the AI technique. Hence, the major objective of the diluent is to improve the reproductive efficiency of cock and to reduce the cost of AI. However, variations exist between the males of different breeds/species of fowl with respect to the physico-biochemical characteristics and fertilizing ability of their spermatozoa. Hence, it is not possible to develop a master semen diluent for all the avian species. This necessitate a breed/species specific semen diluent for avian species for short term preservation (Mohan *et al.*, 2011). There are many commercially available semen diluents nowadays such as Lake's, Tyrode and BPSE etc. A simple diluent for chicken (WLH) semen for short term storage (24 h) at low temperature has also been developed (Mohan *et al.*, 2000).

Advantages of artificial insemination in birds:

- A manifold increase in mating ratio utilizing appropriate semen diluent compared to natural mating where usually one cockerel mates with six to ten hens
- Use of older males from outstanding performers for improving the genetic identity of flock can be used for several generations. Whereas, under natural mating their useful life is limited
- Valuable male birds that have been injured in the leg, unable to mate naturally, can be utilized
- Poor fertility problems caused by preferential mating (monogamous mating and color discrimination) of birds can be eliminated. Whenever fertile eggs are needed laying cages are no longer problems. AI solves the problem of using colony cages with several hens and one rooster that usually decreases the fertility rate (Surai and Wishart, 1996)
- There is reduced chance of transmission of infectious and contagious diseases, apart from those transmitted through semen, from tom (adult male of turkey) or cock to the hens as there is no physical contact (Chaudhury, 1996)
- Breeding experiments can be facilitated. Chickens, turkeys, guinea fowl, ducks, geese and quail are used in intra-species and inter-species insemination experiments. Reciprocal crossing of wild geese with domesticated geese has been performed
- Transportation of birds from one region to another is difficult but semen can be transported easily and at a large scale
- Prevention of vertically and sexually transmitted diseases can be achieved in a poultry flock if properly certified and good quality semen is used (Das *et al.*, 2004)
- AI can serve as a good means for conservation of endangered avian species (Blanco *et al.*, 2009). As of now there are approximately 1,308 species of avian which are marked either as endangered or threatened (BirdLife International, 2013)
- As far as the production of hatching eggs are concerned reduced libido in case of poultry birds is a costly problem for which AI is practiced for overcoming such problem

ARTIFICIAL INSEMINATION IN WILD BIRDS

Artificial insemination has brought a revolution in case of domestic animals and has also stepped into the field of avian husbandry but it has certain limitations in case of its implementation in wild avian species. The outmost limitations are semen collection, low volume of poor quality semen, lack of proper semen diluents and finally scanty information on reproductive physiology of wild species. Wild avian species are not usually at rest and it cross border which is the major hurdle for the implementation of AI in these species. AI is the urgent need in the present context of time in order to conserve the valuable wild avian species which are listed as endangered. AI has already been successfully implemented at a larger extent in the conservation programme of cranes, bustards and peregrine falcon (Blanco *et al.*, 2009). Research shows that by implementation of AI, fertility of eggs can be improved by additional 5-10%. Captive breeding of non-domestic birds has increased dramatically and there is every possibility that production of young birds often exceeds that of the same number of birds in their native habitat. Co-operative, massage and electroejaculation are the three methods described for semen collection. Training of birds imprinted on men forms the basis of co-operative method of AI and extensively used in some raptor programs. In order to inseminate larger number of birds the massage technique is generally used. Semen collection by electrical stimulation is however not practiced generally. In reproductively active birds, the electroejaculation is possible without prior conditioning (when properly restrained) unlike the other two methods which require behavioral co-operation by the birds. AI in wild birds achieved the highest fertility rate with inseminations of more than 10 million spermatozoa every 4-5 days. Multiple deep inseminations used to improve fertility and poor semen quality can be overcome in this manner. It is also to be kept in mind that captive breeding must be used to increase or maintain populations by releasing captive-bred individuals after AI (Gee and Temple, 1978; Seddon *et al.*, 1995; Jalme, 2002; Gee *et al.*, 2004).

POINTS FOR SUCCESSFUL AI PROGRAMME

- Semen samples should be free from transparent fluid, which is released from the lymph fold present in cloacal region. This fluid is found rich of calcium ion which causes the agglutination of the spermatozoa resulting into a rapid loss of fertilizing ability in fowl. Thus, for AI, to maintain high fertility it is advisable not to collect semen contaminated with excessive amounts of transparent fluid
- AI must be conducted with fresh semen as it has become easy to collect semen in poultry and seeing proximity of hen in large breeding farms. In certain instances cryopreservation of poultry semen is recommended but it may prove less fertile, for the purpose of this utilisation of frozen semen further experiments have been suggested (Gill *et al.*, 1999; Foote, 2002)
- Candidate birds can be fasted 5-7 h before the scheduled AI so that there will not be any faecal matter which obstructs the AI
- Handling of the birds should be done away from the nest to prevent breakage of eggs and also handling should be gentle to prevent trauma
- Conditioning of wild birds is necessary so to have a smooth AI programme because unconditioned birds may struggle which will hinder the transport of sperm to the ovum
- Immediately after AI, food item which is favourite for the bird can be provided so as to make a conditioned reflex phenomenon (Staley *et al.*, 2007)
- One/two inseminations per week prior to onset of egg laying should be carried out in chicken

PATHOGENS/DISEASES TRANSMITTED DURING ARTIFICIAL INSEMINATION IN BIRDS

Improper handling and lack of hygiene is expected to result in contamination of semen with harmful microbes (or pathogens) which could be transferred to the bird/hen and the progeny (Guy *et al.*, 1995; Lombardo, 1998; Lierz, 2008). Moreover, the unscientific management of breeder flocks kept for semen collection will result in them contracting Sexually Transmitted Diseases (STDs). For developing suitable birds for AI procedures, factors such as the health parameters of the breeder/parent stock and freedom from pathogens such as *Avian leukosis virus* (ALV), *Chicken anaemia virus* (CAV), *Mycoplasma*, *Salmonella*, *Campylobacter* and others need to be efficiently monitored (Corrier *et al.*, 1999; Buhr *et al.*, 2005; Dhama *et al.*, 2007, 2008a; Kabir, 2010).

Sexually transmitted diseases (STDs)/microbes through semen: Dual functions of excretion as well as transfer of gametes are served by the avian cloaca. During copulation between cock and hen, microbes may get directly transmitted as because intestinal microbes can get incorporated into ejaculate (Sheldon, 1993). Sexually Transmitted Diseases (STDs) may thus be important selective forces in the evolutionary process for the choice of avian mate as well as for selection of mating systems (Hamilton, 1990; Lombardo, 1998; Dhama *et al.*, 2007).

Exposure to potentially pathogenic STM from semen may influence female reproductive performance, their health status and affect survival of their offsprings. This most important factor has to be looked upon seriously during AI procedure. Chances of microbial contamination of semen due to improper and unhygienic collection practices could result in propagation of disease causing agents (viral/bacterial) to a large number of birds and in a wider way even to distant places depending upon practical application or utility of AI in poultry (Van Eck and Goren, 1980). Incidences have been reported regarding transmission of *Salmonella* (*Salmonella* Gallinarum causing Fowl Typhoid and *Salmonella* Pullorum causing Pullorum Disease) through semen; which resulted in presence of this organism in ovaries and oviducts of female chickens and these birds laid *Salmonella* contaminated eggs, leading to disease in progeny followed by mortality (Reiber *et al.*, 1995; Donoghue *et al.*, 2004; Dhama *et al.*, 2007; Kabir, 2010). These microbes present in the semen can also cause male infertility. *Mycoplasma meleagridis* in turkeys is predominantly transmitted vertically from parent stock. *M. iowae*, mostly spread in lay, occurs following unskillful artificial insemination. Exclusion of mycoplasmas from breeder flocks is the most effective way of preventing the negative economic impacts on poultry producers.

Cock semen meant for AI may contain *Campylobacter* or *Salmonella* due to contamination of the diluents (Cole *et al.*, 2004; Buhr *et al.*, 2005). This all occurs due to the unscientific collection and handling of the semen. Contaminated semen can easily transmit these bacteria during AI in turkeys as because the semen on turkey farms are pooled and subsequently are used for insemination of multiple hens. Even the tested semen extenders cannot reduce or eliminate the bacteria from semen. This gives rise to the fact that especially *Campylobacter* remains as the most predominant pathogen in turkey semen that gets transferred to hens through AI (Blaser, 1997; Byrd *et al.*, 2003; Donoghue *et al.*, 2004). *Campylobacter*-positive semen, could provide a route in addition to fecal-oral, for transmission of *Campylobacter* from rooster to the reproductive tract of hen i.e., vertical transmission between dam and offspring (Cox *et al.*, 2002; Tomar *et al.*, 2006). Diseases like avian influenza, Newcastle Disease (ND), duck plague (duck viral enteritis-caused by *duck herpesvirus 1*) and turkey rhinotracheitis (*Pneumovirus*) have also been a constant threat to

the AI program in birds; due to ease of horizontal transmission and possible semen contamination or contamination during AI procedure (Senthilkumar *et al.*, 2003; Dhama *et al.*, 2005, 2007, 2013a). Transmission of *Chicken anaemia virus* (CAV) via semen is also possible apart from avian leucosis (Hoop, 1993; Dhama *et al.*, 2007, 2008a). *Eastern equine encephalitis virus* and *Highlands J virus* have also be reported to be experimentally transmitted through semen of infected tom turkeys (Guy *et al.*, 1995). Venereal transmission of *Infectious bronchitis virus* (IBV) has also been reported recently (Gallardo *et al.*, 2011).

Semen can also be contaminated with vertically transmitted pathogens viz., *Egg drop syndrome-76 (EDS-76) virus*, *Avian encephalomyelitis* (AE) virus, Avian Reovirus (ARV), *Avian leukosis virus* (ALV) etc. (Smith and Fadly, 1994; Segura *et al.*, 1988; Senthilkumar *et al.*, 2003; Dhama *et al.*, 2007). Various other important poultry pathogens viz. *Infectious bronchitis virus* (IBV), *Newcastle disease virus* (NDV), *Avian rotavirus*, *Infectious bursal disease virus* (IBDV); *Escherichia coli*, *Pasteurella* (fowl cholera), *Chlamydia*, etc., apart from Sexually Transmitted Microbes (STMs) during route of fertility, could be transmitted as possible contaminants during AI; since cloaca in birds is the common point of secretion/excretion for digestive, urinary and reproductive system (Blanco and Hofle, 2004; Kabir, 2010; Dhama *et al.*, 2007, 2009, 2013b, c). As the semen is pooled and then used to inseminate multiple hens, contaminated semen could easily spread the pathogens throughout entire flocks via artificial insemination. Other diseases reported to be transmitted through imported poultry semen include avian Spirochetosis (*Borrelia anserina*) and Goose Parvovirus Infection. *Erysipelothrix insidiosa* is responsible for causing a disease called erysipelas primarily in turkeys and secondarily in chickens as well as ducks and geese due to application of AI techniques (www.thepoultrysite.com).

There are several hypotheses which have been proposed for explaining the phenomenon of repeated copulation of female birds with a single mate inspite of the fact that a single copulation is only required for fertilizing an entire clutch. Copulation at high frequencies along with the uses of multiple copulation partners provided, they receive a cloacal inoculation of beneficial microbes which are sexually transmitted and which can protect the birds from future encounters with pathogens (Lombardo *et al.*, 1999).

KEYS FOR A SUCCESSFUL AI PROGRAM IN POULTRY

Keeping healthy flocks is essential for a successful AI program in poultry. The male birds that are used to collect the semen should be devoid of harmful pathogens that can be transmitted through semen (STMs) or contaminate the semen as well as ruin the health status of the breeder male. For this purpose, a holistic management and disease prevention/control strategy has to be devised and followed in order to have a meaningful and fruitful result. Breeder males that are reared in a comfort zone devoid of stress and harmful pathogens are more liable to yield healthy semen (Chaudhury, 1996). To keep in check all the stressful factors and microbial pathogens as well as to improve the nutritional status of the breeder flock, basic principles regarding to the avian disease management is to be applied with top priority (Dhama *et al.*, 2003, 2007; Kataria *et al.*, 2005; Dhama and Mahendran, 2008).

Good management practices and biosecurity measures: General management strategies need to be strengthened so as to maintain stress-free and healthy flock of birds. Freedom from stress is an essential prerequisite. Pneumonia and heat stress in birds is a common problem due to lapses in proper management. For reducing the stress in birds, vitamin C and mineral

supplementation (Zinc) along with appropriate nutritional modulation is necessary. Provision of nutritionally balanced feed is inevitable for the production of a healthy semen production in breeder toms and cockerels. Lack of vitamins and minerals in diet may predispose to various deficiency diseases and result in general weakness and reduced performance in birds kept for breeding purposes (Das, 2002).

The personnel who are involved should have adequate knowledge of proper collection methods that may help in excluding microbial agents. The addition of antimicrobials at appropriate concentrations and proper storage and transportation of semen are also essential for the development of a successful AI program in poultry. Eradication of the sexually transmitted bacterial diseases of poultry depends largely on the selection of antibiotics along with improved hygiene and managemental practices. Use of various groups of antibiotics viz., tetracyclines, macrolides, quinolones and tiamulin can help to prevent the diseases caused by stringent pathogens (e.g., *Mycoplasma*) (Stipkovits and Kempf, 1996). Good managemental practices along with the use of bacterins are useful in controlling disease like Erysipelas in poultry (www.thepoultrysite.com).

Strict biosecurity principles need to be followed so as to keep the pathogens away (Dhama *et al.*, 2003; Sharma, 2010; Conan *et al.*, 2012). Biosecurity refers to the methods adopted to secure a disease free environment by preventing the exposure of the birds/flock to disease causing organisms by reducing the introduction and spread of pathogens into and between farms. It involves an understanding of the principles of epidemiology and economics and requires teamwork to maximize benefits. Awareness as well as resources and perception of higher risk as well as loss of profit form the basis of implementing biosecurity. As rearing poultry is often considered as a secondary activity, basic biosecurity implementation especially in villages is not seen as a priority. But when the question of artificial insemination comes, biosecurity measures are considered as the first priority (Conan *et al.*, 2012). Key principles of biosecurity are (1) Isolation, which is the confinement of birds within a controlled environment, (2) Traffic control, which includes both the traffic onto your farm and the traffic patterns within the farm and (3) Sanitation, which addresses the disinfection of materials, people and equipment entering the farm and the cleanliness of the farm personnel (Dhama *et al.*, 2003, 2007). A clean sanitized environment is a good insurance against disease outbreak and hence, a high standard of hygiene is required in the poultry farms. When there is threat of break down of biosecurity norms automatically the introduction of new stock of birds poses risk of great importance to the health of birds. Managing such factor is therefore a top priority in a poultry farm. At the same time, economics as well as common sense and relative risk should be considered while taking into consideration biosecurity measures for proper and successful AI program in poultry (Sharma, 2010). Effective and targeted use of biosecurity measures, high levels of management along with environmental control can reduce the infections like salmonellosis and campylobacteriosis to a greater extent (Reiber *et al.*, 1995; Cole *et al.*, 2004). There is thus need for formulating norms for the maintenance and use of quality poultry semen in AI.

Diagnosis, surveillance and monitoring of infectious pathogens: One should also be aware of the essential prerequisites for developing healthy and disease-free flocks, for which early and accurate diagnosis of pathogens/disease is very crucial. Familiarity with symptoms of the disease as well as lesions and follow up of proper diagnostic procedures helps in adopting appropriate disease prevention and control program against poultry pathogens. Epidemiologic surveillance is critical to detect new outbreaks and contain them timely before they could cause an outbreak that

affects the breeder toms, cockerels or the hens. Proper attention should be paid towards early diagnosis and prevention of diseases of poultry. The flocks should be tested regularly for salmonellosis, mycoplasmosis and campylobacteriosis, using serological tests like Rapid Plate Agglutination Test (RPAT), immunodiffusion (Agar Gel Precipitation Test, AGPT) tests and cultural procedures (Cox *et al.*, 2002; Kataria *et al.*, 2005; Tomar *et al.*, 2006; Dhama *et al.*, 2007). Surveillance and monitoring of major viral pathogens like AIV, NDV, ALV, IBDV, IBV, CAV, EDS-76 virus etc. should be followed regularly to know the disease status. The serological tests commonly employed for the detection of viral diseases of birds are haemagglutination (HA), Haemagglutination Inhibition (HI), Agar Gel Immunodiffusion (AGID), Serum Neutralization (SN), Complement Fixation Test (CFT), Indirect Fluorescent Antibody Technique (IIFT) and Enzyme Linked Immunosorbent Assay (ELISA). The conventional procedures involving virus or bacterial isolation and detection methods like cultural techniques, staining and biochemical procedures, cell cultures and various diagnostics available need to be used optimally.

The use of nucleic acid based diagnostics has increased exponentially in the recent years and has redefined the level of information available for poultry disease prevention and control programs (Kataria *et al.*, 2005; Dhama and Mahendran, 2008; Dhama *et al.*, 2012a). After the introduction of nucleic acid hybridization techniques, researchers put forward a novel technique called Polymerase Chain Reaction (PCR), which amplifies a desired part of the genome of a microbe. Various oligonucleotide primers have been designed to amplify a specific portion of the bacterial, viral or fungal pathogen, thus enabling its specific detection. Nowadays, PCR is widely employed for the detection of almost all microbial pathogens of poultry (Kataria *et al.*, 2005; Dhama and Mahendran, 2008; Ongor *et al.*, 2009; Menghistu *et al.*, 2011). A newer and more sophisticated version of PCR called real time PCR also emerged in recent times and is capable of detecting the presence of pathogen in no time. This technique is based on the principle of fluorescent resonance energy transfer and uses fluorescent probes in the process. Multiplex PCR and quantitative PCR are other useful versions for detecting, differentiating and viral load estimations. Modern tools and technologies for detection of pathogens like loop-mediated isothermal amplification of DNA (LAMP), nanobiotechnology, microarrays, biochips and biosensors also need to be applied to their full potential (Dhama and Mahendran, 2008; Dhama *et al.*, 2012a, 2014). Laboratory techniques based on nucleic acid detection methods have increased in popularity among microbiologists, over the last decade as it is very sensitive, rapid and easy to perform. The molecular detection of microbial pathogens has the advantage of rapid screening of poultry flocks for the presence of disease causing pathogen. This could also help to identify the subclinical disease status and identify the carrier birds, which need to be eliminated to prevent the spread of pathogens to healthier birds and thus keep the flock disease free. Regular sero-surveillance and monitoring for various bacterial, viral and fungal pathogens is essential in order to maintain a disease-free flock. A rapid and specific detection of microbial pathogens in the semen will help to remove the infected stocks/birds and improve the practical feasibility of AI program. Altogether, this would help preventing diseases transmitted via semen and contamination, thus checking the multiple and wide dissemination of poultry pathogens via artificial insemination (Kataria *et al.*, 2005; Dhama *et al.*, 2007).

Immunization programs: Appropriate immunoprophylaxis/vaccination schedules need to be implemented against major pathogens of poultry. Judicious vaccination is a major strategy used by poultry producers for prevention of major poultry pathogens that may affect the flocks kept for

semen collection. The breeder males should always be vaccinated against the diseases that are endemic to a particular region. Also other poultry flocks should be vaccinated appropriately using killed or live vaccines available (Kataria *et al.*, 2005; Dhama and Mahendran, 2008). If properly and timely done on a priority basis, vaccination helps to get healthy and disease free flock maintenance. With the present disease scenario in indigenous poultry flocks, the birds should be primarily protected against Marek's Disease (MD), ND, fowl pox, IB, IBD and also for preventing vertically transmitted diseases like EDS-76 and CAV (Hoop, 1993; Dhama *et al.*, 2007).

Recent advances in diagnosis, vaccine and therapeutics: Advances in developing rapid and confirmatory diagnostic tools along with strengthening of surveillance and monitoring systems for detecting poultry pathogens are to be exploited to their optimum applications (Kataria *et al.*, 2005; Dhama and Mahendran, 2008; Dhama *et al.*, 2013d). Recent developments for finding safer and effective vaccines (DNA vaccines, plant based vaccines, reverse genetics based vaccines etc.) must be explored in the right directions to prevent various poultry diseases (Dhama and Mahendran, 2008; Dhama *et al.*, 2008b; 2013e). Several novel/alternative therapeutic and immunomodulatory regimens (avian egg antibodies, phages, cytokines, herbs, panchgavya, probiotics and others) are nowadays available, which need to be implemented widely to counter poultry pathogens (Dhama *et al.*, 2011, 2012a, b, 2013f-h; Mahima *et al.*, 2012; Lateef *et al.*, 2013; Tiwari *et al.*, 2011, 2014a, b; Karthik *et al.*, 2014). Besides all these, suitable and effective disease prevention and control strategies inclusive of strict biosecurity and good management practices to lessen the burden of infectious microbes need to be applied very practically and holistically (Kataria *et al.*, 2005; Dhama *et al.*, 2007; Dhama and Mahendran, 2008). The emerging scenario of antibiotic resistance need to be kept in mind and antibiotics should be prescribed after proper antibiotic susceptibility testing (Tiwari *et al.*, 2013). The issues of emerging and re-emerging diseases, global warming and one health concept also needs due attention so as to follow appropriate and timely disease diagnosis, prevention and control strategies for safeguarding overall poultry health and productivity (Dhama *et al.*, 2013i).

Selection and breeding programs: For all agriculture based breeding companies genomic selection is becoming a new paradigm. In order to perform genetic evaluation and selection of elite poultry population genomic selection will act to reshape the whole program. Healthy male chicks of good breeds should be procured from disease free sources for the purpose of starting an AI operation. These sources should maintain strict biosecurity, good sanitation and hygiene and appropriate disease prevention/control programmes. Poultry geneticists have to include various selection procedures, in order to evolve disease resistant breeds. Research and development efforts are required to develop suitable genotypes with better immuno-competence, production potential and functional traits (Dhama and Mahendran, 2008; Dhama *et al.*, 2012b). Marker Assisted Selection (MAS) is nowadays used to develop genetically superior breeding stock (Paul *et al.*, 1999). Genetic selection has identified disease resistance loci in Major Histocompatibility Complex (MHC) gene for Marek's disease and avian leucosis. The BL-bII genotype has been found to be associated with disease resistance capabilities and may be used as marker in selection for the immunocompetence (Rutz and Xavier, 1998). The association of Class II and Class-IV sub region of MHC in contributing resistance for parasitic and viral diseases has also been reported. Moreover, the advantages of the indigenous poultry breeds especially the disease resistant abilities could also

be transferred to generations using a scientifically managed AI program. Although, there is dramatic decrease in cost of genotypes relatively they are still expensive when the value of individual bird is taken into consideration (Fulton, 2012).

CONCLUSION

A rational Artificial Insemination (AI) program with certified semen is capable of revolutionizing the poultry farming sector by targeting genetic improvement along with appropriate health strategies. Chickens, guinea fowl, turkeys, ducks, geese, quail, pet and fancy birds including pigeons are nowadays used for the purpose of AI. AI in domesticated birds can be used as a major tool for the genetic improvement of poultry as it is considered superior to natural mating in many aspects. The innovations in AI technology in poultry can be brought about by developing new species-specific semen diluents and performing extensive research on poultry sperm biology. To effectively utilize the potential of AI technology, manifold factors are to be taken into consideration. The first and the foremost factor of importance is the production of neat, healthier and pathogen free semen from the breeder flocks. For this to materialize, various strategies have to be framed in order to evolve a successful outcome. The presence of various microbes in the semen is detrimental to the whole production unit and will result in the total failure of the AI system. A rapid and specific detection of microbial pathogens in the semen will also help to remove the infected stock and improve the feasibility of AI programme, as well helping in prevention of diseases transmitted via imported/exported semen. The presence of the bacterial or viral pathogens in semen can be avoided by keeping healthy and properly monitored flocks as well as practicing strict sanitation and hygienic measures along with strict biosecurity principles in and around the rearing area. Healthy flock maintenance will largely depend on timely detection of the incidence of pathogenic microbes in the flocks by either conventional diagnostic tools, sero-monitoring or by using latest molecular biology detection tools. Immunization programs against major viral pathogens are of paramount importance while considering appropriate disease prevention and control strategy.

Apart from the detection of the presence of extraneous pathogens in semen, general management strategies are also to be devised so as to maintain stress free and healthy flock. The role of biosecurity and general management can never be undermined while intending to keep healthy flocks for any breeding program. For reducing the stress in birds, vitamin C and mineral supplementation along with appropriate nutritional modulation is necessary as it may induce a good immune status to the bird kept for the AI program. Healthy male chicks of good breeds should be procured from disease free sources for the purpose of starting an AI operation. Researchers have to include various selection procedures, in order to evolve disease resistant breeds. Apart from these, the advances in development of vaccines and therapeutics need to be utilized fully for safeguarding health and production of poultry. The development in avian genetics now has paved way for the generation of disease resistant breeds especially towards specific pathogens. Various selection procedures have also made it possible to develop birds with more functional potential. Regarding the processes involved before and after collection of the semen, the personnel who are involved should have adequate knowledge regarding proper collection methods that may help in excluding various microbial agents. The addition of antimicrobial agents at appropriate concentrations and proper storage and transportation facilities are also essential for the development of a successful AI program in poultry. There is also need for formulating norms for the maintenance and use of quality poultry semen in AI. A novel strategy, apart from the use of antimicrobial agents, has also to be developed in order to reduce the risk of colonization of pathogens in semen. Based on all these,

it can be inferred that in near future, a strategic artificial insemination programme/practice in birds will play a major role in developing and propagating economically viable poultry flocks; making poultry farming more popular and profitable and also help in the breeding programs of exotic and endangered species of birds. In conclusion, a rationale AI programme with certified semen, targeting genetic improvement along with appropriate health strategies, will help revolutionizing the poultry farming and industry.

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