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A Review Article of Artificial Insemination in Poultry

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ABSTRACT

The objective of this review is to discuss the history, importance of Artificial Insemination (AI), semen collection and deposition techniques, physiology of cockerel reproduction, characteristics and chemical components of chicken semen, semen storage, and evaluation of semen, semen extenders and behavior of sperm in the oviduct. The first AI in poultry was reported in 1936. All of the avian male reproductive system is inside the bird unlike the males of mammalian species. Avian semen contains energy source to help the viability of semen for AI. For the purpose of in vitro storage semen extenders with appropriate osmolarity and source of energy can be used. Abdominal massage method is the most common technique to collect semen. The technique involves restraining the male and gently stroking the back of the bird from behind the wings towards the tail with firm rapid strokes and gentle squeeze to extract the semen. There are two methods of semen deposition in poultry. These methods are the Intra-peritoneal insemination and vaginal insemination. Vaginal insemination is the commonly used method. It involves everting (turning inside out) the cloaca to expose the reproductive tract of the female.

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INTRODUCTION

Assisted Reproduction Technologies (ART's), such as Artificial Insemination (AI) contribute to increase poultry production, as it allows a wider use of genetically superior cockerels with a high productive performance. The development of AI technique has allowed the rapid dissemination of genetic material from a small number of superior sires to a large number of females (Vishwanath and Shannon, 1997). The impacts of AI on genetic improvement and control of venereal diseases has been the greatest (Foote, 2002). Poultry producers over the years have used genetic selection and improved nutritional management practices as a result there has been a steady and rapid increase of the growth rate in poultry production which in turn has certain detrimental effects on reproduction (Bramwell, 2002).

Due to the sharp increase in chicken meat consumption it has also become important to increase the production of layers to meet the demand. Assisted reproduction technologies (ART's), such as AI contribute to increase poultry production, as it allows a wider use of genetically superior cockerels with a high productive performance (Benoff et al., 1981). On the other hand, ART's have the potential benefit of allowing the preservation of semen collected from these cockerels for future use and for export if necessary.

Artificial insemination has been considered as a valuable technique in the poultry industry (Benoffet al., 1981). One of the advantages of this technology over natural mating is the efficient use of males. This in turn, decreases the cost of AI directly by reducing the number of cocks needed (Benoffet al., 1981). When fertility in the broiler breeds continues to decline due to the fact that males are selected for growth coupled with compatibility problems between large and smaller breeds, AI may become effective in broiler breeder management and in solving compatibility problems (Reddy, 1995). In addition to its breeding significance, AI is important in controlling venereal diseases. Birds present special challenges in disease control (Blanco and Hofle, 2004).

Artificial insemination in poultry

AI in poultry was first successful in 1899 when Ivanov produced fertile chicken eggs using semen recovered from the duct us deferens after killing a cock (Lunak, 2010). The most widely used technique of intravaginal insemination was first reported by Quinn and Burrows in 1936. The avian male reproductive system is all inside the bird unlike the males of mammalian species which have their reproductive systems outside of the body cavity (Brooks, 1990). This is one of the really remarkable things about birds where the sperm remain viable at body temperature. Mammalian sperm does not remain viable at body temperature which is the reason the male reproductive organs are found on the outside of the body (Brooks, 1990).

According to Aisha and Zain (2010) AI in poultry is the process by which semen from male bird is collected and then introduced to females for the purpose of fertilizing eggs. The main objectives of AI in poultry are to place the

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required dose of semen into the oviduct of the female so that it is deposited near the sperm storage glands and to carry out the AI process with due regard to the best health and welfare of the breeder females thereby, achieving the highest fertility levels possible. During insemination, the volume of semen required is generally less than 0.1 ml, within a minimum of 100 to 200×10^6 viable sperm per insemination within the hen's vagina (Gordon, 2005).

Biologically**,** after deposition of semen in the oviduct the semen will enter the sperm storage gland, situated at the junction of the vagina and the shell gland and from here the spermatozoa will make their way up the oviduct to a second storage site situated at the junction of the magnum and infundibulum (Aisha and Zain, 2010). The passage of an ovum into the infundibulum stimulates spermatozoa activity and fertilization of the ovum by one sperm takes place (Aisha and Zain, 2010).

Controlling diseases

Birds present special challenges in disease control (Blanco and Hofle, 2004). The semen diluents may be another common source of contamination, especially for E. coli and pseudomonas (Van Eck and Goren, 1980). These agents can trigger significant sperm mortality in raw or diluted ejaculate and when used for AI may cause both systemic disease and infertility (Van Eck and Goren, 1980). This problem is commonly addressed by adding antibiotic to diluents (i.e., penicillin, Gentamicin and streptomycin) although these drugs may adversely impact on sperm viability (Donoghue et al., 2004).

Collectively, results to date strongly emphasize the need to minimize ejaculate contamination by focusing on sanitary semen collection and processing (including using appropriate and prudent doses of broad spectrum antibiotic and antifungal) as well as protecting birds from pathogens. This includes maintaining thorough pathogen monitoring protocols and even strict isolation/ quarantine practices for breeder populations (Turin et al., 1999). Once infectious situations arise, rapid mitigation is mandatory, although it is challenging to alleviate certain viruses (i.e., West Nile virus) from infected birds (Turin et al., 1999).

Poultry semen collection techniques

In 1937 Burrows and Quinn described a non-invasive method, the abdominal massage method for collection of semen from roosters. The technique involves restraining the male and gently stroking the back of the bird from behind the wings towards the tail with firm rapid strokes. The male responds with tumescence erection of the phallus, at which time the handler gently squeezes the cloaca extracting semen through the external papillae of the duct us deferens (vas deferens) collecting the semen into a container.

As stated by Burrows and Quinn (1937), the techniques of AI actually begin prior to the procedure. It includes housing the male poultry away from the hens maximizes the amount of available semen. Because the bird's phallus is located in the same duct as his anus, removing food 12 hours prior to collection will help prevent fecal contamination of the semen. Roosters and tom turkeys need to be routinely primed for semen collection for several days prior to the actual AI procedure to guarantee that each bird is fertile with a microscopic examination of the sperm. According to Burrows and Quinn (1937), as with semen collection of other farm animals, one must stimulate the bird's sexual organ to extend outside of his body. One person can handle this procedure with small birds such as chickens or quail; it normally takes two people with a large turkey or a goose.

Physiology of cockerel reproduction

Sperm Production is initiated by adequate secretion of Gonadotropin Releasing Hormone (GnRH) from the hypothalamus, the secretion of Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH) by the anterior lobe of the pituitary and the secretion of the gonadal steroids (testosterone and estrogen). LH acts on the Leydig cells within the testes to stimulate the production of progesterone, which is converted to the male sex hormone testosterone (Senger, 2003). Testosterone within the seminiferous tubules is essential for spermatogenesis, while the Leydig cells become unresponsive sustaining high levels of LH (Senger, 2003).

The testes are surrounded by a layer of connective tissue containing the seminiferous tubules and Leydig cells. Several androgens are produced in the interstitial cells of the testes, but the major hormone in the blood, is testosterone. Testosterone is essential for the development of the secondary sex characteristics and for normal mating behavior in the males. It is also necessary for the functioning of the accessory glands, sperm production and the maintenance of the male duct system. This hormone also aids in spermatocytogenesis, the transport of sperm and deposition of sperm in the female reproductive tract (Beardonet al., 2004). As the cockerel reaches maturity, the production of testosterone is stimulated by the increasing concentration of circulating gonadotropins (Etches, 1996).

The major gonadotropins involved are FSH and LH, which are also called the Interstitial Cell Stimulating Hormone (ICSH) in males. Both of the gonadotrophic hormones are secreted by the anterior pituitary (Salisbury et al., 1978). FSH as such, acts on the germinal cells in the seminiferous tubules of the testes and supports spermatogenesis to the

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secondary spermatocytes stage. LH stimulates the Leydig cells to produce testosterone and other androgens (Hafez and Hafez, 2000).

Spermatogenesis

Spermatogenesis is the process of division and differentiation by which sperm are produced in the seminiferous tubules of the testes and consists of two phases, namely spermatocytogenesis and spermiogenesis (Gordon, 2005). According to the previous author, spermiogenesis is a metamorphic process in which no cell division is involved and a string of events result in the formation of the sperm tail. Alteration in the sperm morphology can be seen in the nuclear proteins, cellular size, cellular shape and the position of the acrosomal granules and localization of the centrioles. The number of sperm produced is dependent on the number of Sertoli cells and Leydig cells present. The Golgi apparatus is one of the cell organelles, located near the sperm nucleus and which give rise to the subcellular organelle known as the acrosome. The acrosome develops and forms a cap over the anterior portion of the nucleus and spreads until it covers two-thirds of the anterior nucleus (Senger, 2003). During the maturation phase, the spermatids are completely differentiated with the final formation of the flagella (principal and endpiece), assembly of mitochondria (midpiece), the neck piece and complete condensation and shaping of the nucleus (Beardon et al., 2004).

Factors affecting semen production

There are inherent variations in semen production between different species of poultry and between individuals within strains and breeds (Lake, 1983). Other than in the mammals, cockerel sperm is generally immotile before ejaculation (Hafez and Hafez, 2000). According to Anderson (2001) there are many factors that may influence the production of semen and a thorough knowledge of the physiology of cockerel reproduction is essential to enable an understanding of male fertility. There are also many external and internal factors that may affect the male and may influence the production of semen. The reproductive functions in the male are endocrine controlled by the pituitary, testes and to a certain extent external factors.

The certain external factors affecting reproductive efficiency in the cockerel can be grouped into two categories firstly, the direct influence of the diet, management, and the normal physiological processes that regulate the activities of spermatogenesis and secondly, factor that influence the degree to which the male will respond to the massage technique during semen collection (Maule, 1962).

Characteristics and chemical components of chicken semen

In the male, semen is composed of sperm and seminal plasma secreted by the epididymis and vas deferens. The sperm are produced in the testes, and in the case of the avian species the seminal fluid is also produced in the testes. All these secretions in the testes are controlled by the endocrine hormones carried to them in the blood stream. The pituitary FSH and LH regulates the testes, which in turn produce testosterone, which controls the testicular development and secretions (Hafez, 1974).

Table1. Characteristics and the mean chemical components of semen in cockerel

Characteristics and Components	Cockerel
Ejaculate volume (ml)	0.2-0.5
Sperm concentration ($\times 10^6$/ml)	3000-7000
Sperm/ejaculate ($\times 10^9$)	0.06-3.5
Motile sperm (%)	60-80
Morphologically normal sperm (%)	85-90
Protein (g/100 ml)	2.8
pH	7.2-7.6
Fructose (mg/100ml)	4
Sorbitol (mg/100ml)	0-10
Inositol (mg/100ml)	16-20
Glycerylphosphoryl choline (GPC) (mg/100ml)	0-40
Ergothioneine (mg/100ml)	0-2
Sodium (mg/100ml)	352
Potassium (mg/100ml)	61
Calcium (mg/100ml)	10
Magnesium (mg/100ml)	14
Chloride (mg/100ml)	147

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Gross evaluation of semen

Determination of the viability of spermatozoa after semen storage is important for several reasons. First, it provides an estimate of semen quality. If inseminated with poor quality semen, it reduces fertility, increases embryo mortality and forces the hen to rely on spermatozoa from previous inseminations (Thurston, 1995). More traditional semen evaluation procedures include determination of semen volume, color, concentration, motility, viability and morphology of spermatozoa. Many of these assessments correlate with the fertilizing capacity of spermatozoa when fresh semen is evaluated (Wishart, 1995). Histological and fluorescent stains have been used to determine live/dead sperm ratios and metabolic activity (Lake and Stewart, 1978a; Lake and Stewart 1978b and Chaudhuri et al., 1988).

Semen color

The color of semen is generally an indicator of the density of the ejaculate. The semen of the domestic fowl varies from a dense opaque suspension to a watery fluid secreted by various reproductive glands. It ranges from a relative high sperm density or degrees of clear to milky white, with declining sperm numbers (Peters et al., 2008). The color of semen may depend on the species of bird used, but generally semen should be creamy which indicates a high sperm concentration (Cole and Cupps, 1977). Color could also serves as an indicator of contamination by e.g. feces or urine and thus become brown or green in color (Lake, 1983). Sometimes flakes of blood may be present, which may be a result of excessive force during the collection process or injury. Semen samples that are contaminated by feces do not have to be discarded, but diluted with antibiotics e.g. penicillin and dihydrostreptomycin or neomycin to reduce the loss of sperm. This however is not recommended. Antibiotics can also increase fertility when used as a diluent in semen (Bearden et al., 2004).

Ejaculate volume

The cockerel produces between 0.1 ml and 1.5 ml per ejaculation, with 0.6 ml being the average ejaculate volume recorded (Cole and Cupps, 1977). Different cockerels of the same species often produce different volumes of semen at different times (Anderson, 2001).

The average volume ejaculated using the abdominal massage technique is approximately 0.25ml (Gordon, 2005). Bah et al. (2001) found the mean semen volume to be 0.28 ± 0.14ml. However, the recorded semen volume was found to range between 0.37 ± 0.02 and 0.73 ± 0.01 ml (Peters et al., 2008). It is important to realize that semen volume and sperm concentration (volume multiplied by the concentration) will determine the total number of sperm collected per ejaculation. This could facilitate the determination of the number of insemination doses that can be prepared (Senger, 2003).

Semen pH

The semen pH varies slightly between different breeds and bird species. The optimum semen pH ranges between 7.0 and 7.4. Sperm motility is generally high between a pH of 7.0 and 7.4 (slightly alkaline) and also increases the fertilizing ability, compared to a pH of 6.4 (acidic), which is not suitable for semen preservation, as it may cause damage to the plasma membrane of the sperm cell (Latif et al., 2005). Contrary, Donoghue and Wishart (2000) reported several trials that indicate that chicken sperm can tolerate a pH range of 6.0 to 8.0. Peters et al.(2008) also found the semen pH of the cockerel to be slightly alkaline, with a mean of 7.01 ± 0.01, while Bah et al.(2001) recorded a semen pH ranging between 7.54 ± 0.04 to 7.80 ± 0.03.

This variation in semen pH may be due to many factors. The pH, especially that of ejaculated semen is dependent on several secretions involved. Poor quality semen generally contains large amounts of fluid from the accessory glands, which increases the semen pH (Salisbury et al., 1978). The pH of semen is likely to decrease as the time between collection and measurement increases, and the semen collection tubes are narrow in shape causing sperm to break down fructose in the semen to lactic acid under anaerobic conditions. Semen samples that contain many dead sperm may evolve to ammonia, which will also increase the pH (Salisbury et al., 1978).

Microscopic evaluation of cockerel semen

Motility

Sperm motility assessment is indicative of the viability of sperm and the quality of the semen sample. Evaluation of sperm motility is conducted with fresh and extended semen, and generally analyzed under the light microscope (10× magnifications) (Hafez and Hafez, 2000). Evaluation of raw semen gives the performance of the sperm in its own accessory gland fluid, which is often hindered when higher sperm concentrations make it difficult to distinguish individual sperm motility patterns (Table 2). Hence an aliquot of semen is usually extended prior to evaluation (Hafez and Hafez, 2000).

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Table 2. Motility patterns of sperm from sub fertile or infertile cockerels

Pattern of sperm motility	Sperm tail	Sperm head	Sperm movements and progression
Vibratory circular	Slow or rapid quivering from side to side, vibrations of various types and frequency bent in curved shape, immotile	Immotile or vibrating in one place	Motility without progression, perpendicular, obliqueor horizontal clockwise or counterclockwise motion
Darting	Vibration with high velocity	Irregular, propelling, no rotation	Minimal and erratic, wandering path
Rotating	Undulations of small amplitude pass down tail	Whole sperm rotates around its axis, periodic flashing effect	Rapid forward progress in a straight line
Asymmetric head and /or flagella	Amplitude of tail wave is asymmetric at both sides	Irregular, propelling, usually no rotation	Circular orbits if rotational motile is absent

Sperm morphology

Normally the sperm cell consists of a head, midpiece and tail portion. The head contains the nucleus, containing the genetic material, which is the sire's genetic contribution to the offspring (Tuncer et al., 2006). The post-nuclear cap which covers the posterior part of the nucleus and acrosome which covers the anterior part of the nucleus both protect the nucleus. If the acrosome is malformed or damaged the sperm cell will not be able to fertilize the ova by penetrating the zona pellucida. Acrosome, sperm head, middle piece and tail deformations in fresh white leghorn cockerel ejaculates have been recorded to be 0.62 ± 0.04%, 1.34 ± 0.05%, 2.47 ± 0.05% and 2.89 ± 0.08% respectively (Tuncer et al., 2006). While Tuncer et al., (2008) also recorded values of acrosome, sperm head, middle piece and tail deformations 0.39 ± 0.03%, 1.06 ± 0.03%, 2.32 ± 0.05% and 2.53 ± 0.04% respectively in cockerels.

Blesbois (2007) described an eosin-nigrosin stain technique to assess the morphology of cockerel semen. Semen was mixed with 1.6% eosin and 6% nigrosin, diluted in 20μm Beltsville Poultry Semen Extender (BPSE), diluted semen in 2ml stain solution and incubated for 2 minutes before being spread on a microscope slide. The stain is dried and observed under a light microscope (1000× magnification). Sperm morphology can serve as an indicator of semen quality and shortcomings in the male. The success of this evaluation technique depends on how the stain was prepared and used, while other more advanced laboratories use a computer analysis system for sperm evaluation. Eosin-nigrosin is a die commonly used in laboratories to determine abnormalities and smears are made, immersed in oil and observed under the light microscope. Viable, non-viable, properly formed, live, and damaged sperm can be determined using this evaluation (Lukaszewicz et al., 2008).

The sperm morphology of poultry semen differs from that of mammals. However a difference also exists between domestic birds, even though the shape and size of the sperm cell are similar. In poultry the sperm cell is surrounded by the cytoplasmic membrane and the acrosome has an inner spine surrounded by a conical shaped cap. The head of the sperm contains the nuclear material of the gamete, while the midpiece consists of the cylindrical centrioles surrounded by a sheath of mitochondria (Hafez, 1974). The midpiece of cockerel sperm is considerably longer, compared to other species, approximately one quarter longer and this property makes poultry sperm to have more midpiece bending than other species. According to Alkanet al., (2001) the in vitro assessment morphological sperm defects of cockerel semen include; neck bending (mid piece bending), mid piece damage, acrosome damage (bending, swelling, knotting or rounding), total head swelling and tail defects.

Semen concentration

Gordon (2005) stated semen collected from domestic cockerel contains an average sperm concentration of 5000 $\times 10^6$ sperm/ml. On the other hand the report of Hafez and Hafez (2000) stated that semen collected from domestic cockerel contains an average sperm concentration of 3000-7000 $\times 10^6$ sperm/ml.

Factors affecting semen quality (motility, morphology and viability)

The assessment of semen quality characteristics of poultry birds gives an excellent indicator of their reproductive potential and has been reported to be a major determinant of fertility and subsequent hatchability of eggs (Peters et al., 2004). According to Hafez (1978), the differences in volumes and sperm concentration of the domestic fowl semen depends largely on the relative contribution of the various reproductive glands, the number of spermatozoa that could be obtained from a breed/strain and the extent to which the genetic potentials can be exploited.

Breed and seasonal differences in semen production of cocks was reported by Saeed and Al-Soudi (1975) while Egbunike and Oluyemi (1979) showed that breed and time of semen collection affects cock semen. Omeje and Marine (1990) observed that significant genotype differences affected body size and semen characteristics of cocks, except the pH value. In addition, age by genotype interaction effect was important only for semen volume.

Only morphologically normal spermatozoa are capable of ascending through the vagina of the hen to the region where the sperm storage tubules are located (Bakst et al., 1994). Sperm motility is a primary determinant of fertility in domestic fowl (Donoghue et al., 1998). Sperm mobility is a function of the product of motile concentration and the

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proportion of motile sperm with a straight line velocity >30 m/s (Froman et al., 2003). The hormone level in seminal plasma is a direct reflection of male testicular endocrine activity. The relationships between semen quality and concentration of testosterone in avian seminal plasma have been discussed (Zeman et al., 1986). High quality semen determined by the condition of normal spermatogenesis. Estrogen, in addition to testosterone plays a role in the development and function of the testis and male reproductive tract (Rivas et al., 2002 and Akingbemi, 2005).

Semen extenders

The use of AI in poultry can be enhanced with the improvement of diluents and method of storing semen (Mian et al., 1990). The advantage of semen dilution includes the maximum use of good quality semen in short supply; reduction in the ratio of males to female and valuable sires with low semen quantity can be used for many females. On the contrary, it is difficult to handle the desired very small volume of undiluted semen and expel it from a tube because of its viscous nature. However, diluents make it possible thereby enabling the spread of semen over many more hens (Mian et al., 1990).

Diluents are buffered salt solutions used to extend semen, they maintain the viability of spermatozoa in vitro, and maximize the number of hens that can be inseminated. Semen diluents are based on the biochemical composition of chicken and turkey semen (Lake, 1995). Addition of various components to semen maintains motility, fertilizing capacity and preserve sperm membrane integrity (Riha et al., 2006 and Sarlos et al., 2002). Glutamic acid, the most prominent anionic constituent of avian seminal plasma, became a standard component of diluents (Lake and Mc Indoe, 1959). Egg yolk is generally accepted to be an effective agent in semen extenders for protection of spermatozoa against cold shock and the lipid phase transition effect (Aboagla and Terada, 2004). However, the use of chilled stored semen diluted in egg yolk based semen extenders is limited by its relatively short time fertilization capacity and individual differences in egg yolk due to different period of egg storage (Aurichet al., 1997).

Both hypertonic and hypotonic extenders reduce the metabolic activity of the sperm, and could disrupt the cell membrane integrity that leads to the clumping of the sperm (Latif et al., 2005). Glutamic acid is the most prominent anionic constituent in avian seminal plasma, and is a standard component of all semen diluents. Basic characteristics common to all diluents include the maintenance of pH, osmolarity and the provider of energy for the sperm. The motility and metabolic rate of sperm can thus be altered by decreasing the diluent below pH 6.0. So for example a low pH reduces the sperm motility and a high pH increases the metabolic rate in vitro (Donoghue and Wishart, 2000). Modified Ringer's solution with the following composition of sodium chloride (68g), potassium chloride (17.33g), calcium chloride (6.42g), magnesium sulphate (2.50g), sodium bicarbonate (24.50g) and distilled water, can be used to dilute poultry semen (Martin, 2004).

According to Jones and Mann (1973) and Jones et al. (1979) spermatozoa is extremely sensitive to oxidative damage. Lipid peroxidation plays a leading role in aging of spermatozoa, shortening its life spanin vitro and affecting the preservation of semen for AI. The process of peroxidation induces structural alterations; particularly in the acrosomal region of the sperm cell, a fast and irreversible loss of motility, a deep change in metabolism and a high rate of release of intracellular components. Lipid peroxidation has been defined as an important aspect of oxidative stress in mammalian spermatozoa for many years (Jones et al., 1979).

Semen osmotic pressure in poultry

Latif et al., (2005) concluded that an increase in the osmotic pressure can be ascribed to the contamination of broiler semen with urine and bacteria, which in turn results in the clumping of sperm. A 375mOsm/kg osmotic pressure is optimum for the short term storage of semen. However the recommended osmolarity of the Blom stain technique is lower and was quantified as 220 mOsmol/kg dissolved in diluents, with composition similar to that of seminal plasma. However these hypo osmotic conditions resulted in the swelling of the sperm head (Lukaszewicz et al., 2008). The semen diluents must be isotonic, as the osmotic pressure created by the solution may be detrimental to the sperm cell (Senger, 2003).

Short term poultry semen preservation

Semen diluents are currently being used for both short and long term storage of domestic fowl semen. These extenders are being commercialized to improve the general reproductive effectiveness of the cockerels and lower the cost of AI. The development of semen diluents initially began with the use of NaCl (saline) solutions. Now complex diluents containing different osmotic regulators, energy sources and buffers are being used (Bootwalla and Miles, 1992). The most common practice for short term fowl semen storage (hours to days at a temperature of -4°C) requires the suspension of sperm in a suitable extender to maintain the sperm viability, in vitro. Assessment of diluted and undiluted stored cockerel semen revealed that the application of extenders is essential to sustain sperm quality (Bootwalla and Miles, 1992).

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It was established that diluted fowl semen could be stored for up to 24 hours, without impairing the viability and fertilizing capacity of the sperm (Siudzinska and Lukaszewicz, 2008). Several other factors play a role in sustaining the quality of semen during storage over time e.g. the diluents used in semen extension and storage conditions e.g. time, aeration and storage temperatures. It is known that sperm motility and the fertilizing capacity of undiluted raw fowl semen stored in vitro usually decreases within 1h after collection (Dumpala et al., 2006). Therefore, to store cockerel semen, the type of diluents and storage temperature is very crucial.

Generally an extender will facilitate semen handling procedures, particularly during collection and evaluation, by maintaining the sperm viability, but preventing their activation. For semen maintained at 41°C and diluted BPSE or Minimum Essential Medium (MEM) there were quadratic and linear increases in the percentage dead sperm over time, while a drastic linear increase existed for undiluted semen. There was thus a linear decrease in Sperm Quality Index (SQI) for undiluted and semen diluted in MEM over time (4°C). However, for semen diluted with only BPSE, there was a linear increase in SQI (Dumpala et al., 2006). Extenders serve to also protect the sperm cells from chemical and physical changes and contamination in their environment and provide more favorable conditions for fertilization (Chulhong and Chapman, 2005).

Poultry AI technique

Generally there are two methods of semen deposition in poultry. These methods are the intra peritoneal insemination and vaginal insemination. The most reliable and successful routine for insemination of poultry, is by depositing semen directly in the mid vaginal area (Cole and Cupps, 1977).

Intra peritoneal insemination

This technique of AI is not reliable and has been used periodically for many years. In this technique a sharp needle is punched through the abdominal wall and the cannula inserted to deposit semen in the region of the ovary (Cole and Cupps, 1977).

Vaginal insemination

This is the most commonly used AI procedure and two personnel are required for this operation. The technique was developed in the 1930s and involves applying pressure to the hen's abdomen and everting (Turn inside out) the vaginal orifice through the cloaca (Quinn and Burrows, 1936; Cole and Cupps, 1977). This procedure is also referred to as cracking, venting or everting the hen. Semen is deposited 2–4 cm into the vaginal orifice concurrently with the release of pressure on the hen's abdomen. Insemination is accomplished with sterile straws, syringes or plastic tubes. In large scale commercial operations, automated semen dispensers using individual straws loaded with a set AI dose are commonly used.

Because poultry semen loses viability within 1 hour, hen insemination should begin immediately after collection (Aisha and Zain, 2010). You begin by holding the hen upside down against your body in the same way you held the rooster or tom. Exerting firm pressure on the left side of the vent causes the cloaca (the urogenital opening in birds) to evert, you need to use your thumb and forefinger to expose the oviduct (vagina). According to the same author (2011), the oviduct is the opening on the left side of the cloaca next to the anus. You can insert the insemination tube as far as possible up the oviduct, then squeeze out the semen and release the pressure on the cloaca at the same time. Relaxing the cloaca draws the semen further into the hen's body. Chicken inseminations can be completed within two consecutive days and then once a week after that. Because most hens will carry an egg in their oviducts in the morning, thus obstructing the route of the sperm to the ovary, insemination should occur in the afternoons after laying (Aisha and Zain, 2010).

Behavior of sperm in the oviduct of the hen

Froman and Feltmann (2005) reported that the hen's Sperm Storage Tube (SST) is located between the vagina and shell gland of the oviduct. Previously sperm residing in the SST were considered to be immotile; however it is likely that storage depends on moving against a generated by the SST epithelial cells. Cockerel sperm are motile at a body temperature of 41°C for an interval of days to weeks following ejaculation. How the sperm enter, survive, and exit these SST however is not known. Movement of sperm to the uterovaginal region is fast, however only viable sperm enter the SST. Current evidence suggests that the release of stored sperm is episodic, although it was first thought to be associated with oviposition.

Movement of sperm through the oviduct is achieved by smooth muscle contractions and/or ciliary activity and accumulates in the mucosal folds and short tubular glands at the lower end of the infundibulum (Hafez and Hafez, 2000). According to Hafez and Hafez (2000) the sperm in mammals spend a relatively short time in the female tract, while in chickens and the turkey sperm can spend a much longer period of time in the oviduct before fertilizing the egg yolk cell, up to 32 days in the chicken and 70 days in the turkey. Tabatabaei et al. (2009) stated that although the process of

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prolonged sperm storage is not known, it is thought to include a reversible suppression of respiration and motility of the sperm, as well as stabilization of the plasma membrane and maintenance of the acrosome.

According to Mauldin (2000), sperm are released from the SST to fertilize the sequentially ovulated ova at regular intervals. After release the sperm are taken to the ovum by contraction of the hen's oviduct, and sperm motility is no longer critical. Within 5 to 10 minutes after ovulation, sperm has already moved to the genital disc on the surface of the ovum. The sperm that make contact with the perivitelline layer of the ovum undergo an acrosome reaction and, presumably by the action of the trypsin-like enzyme acrosin, hydrolyze the perivitelline layer. Theoretically only one sperm fertilizes the ovum, but polyspermy has been observed in the hen ovum with many holes hydrolyzed in the perivitelline (Hafez and Hafez, 2000).

Competing interests

The authors have no competing interests to declare.

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