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Egg-cellent Breeding: The Science of Poultry AI

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Abstract: -

Artificial insemination (AI) is an important assisted reproductive technology that has revolutionized modern poultry breeding by improving reproductive efficiency and accelerating genetic improvement. It enables the efficient use of superior males, reduces the number of breeding cockerels, overcomes mating difficulties in heavy breeds, and minimizes disease transmission. This article provides an overview of the reproductive physiology of male birds, semen collection and evaluation, semen processing and storage, artificial insemination techniques, and sperm storage in the hen's oviduct. It also discusses the major advantages and limitations of AI, highlighting its significance in enhancing productivity and promoting sustainable poultry production.

Keywords: Poultry; Semen; Artificial Insemination; Reproductive Technology etc.

Introduction:

Poultry farming is one of the fastest growing livestock sectors worldwide, providing affordable, high-quality animal protein in the form of eggs and meat while contributing significantly to food security and rural livelihoods. Continuous genetic selection has greatly improved growth rate and egg production in modern poultry. However, these improvements have also led to reproductive challenges, particularly in heavy broiler breeders where natural mating efficiency declines because of reduced mating activity,

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body size differences, and advancing age of breeding males. Artificial Insemination (AI), an important Assisted Reproductive Technology (ART), has emerged as an effective solution to overcome these limitations. AI involves collecting semen from a male bird and depositing it directly into the reproductive tract of a female using a syringe or insemination tube, eliminating the need for natural mating. This technique enables the use of genetically superior males for breeding a larger number of females, thereby accelerating genetic improvement, improving fertility, reducing the number of breeding males required, lowering production costs, and minimizing the spread of venereal diseases. The concept of AI in poultry dates back to 1899 when Ivanov successfully produced fertile chicken eggs using semen collected from a rooster. The technique became practical after Burrows and Quinn developed the abdominal massage method for semen collection in the 1930s, a simple and non-invasive procedure that remains widely used today. Since then, AI has become an indispensable breeding tool, especially in commercial turkey and broiler breeder operations, while also facilitating semen preservation, transport, and conservation of valuable poultry genetic resources.

Reproductive Physiology of Male Bird and Semen Production

Unlike mammals, the testes of male birds are located deep within the abdominal cavity, close to the kidneys,illustrated in (figure.1) and function efficiently at the normal body temperature of about 41°C. As cockerels approach sexual maturity (around 18 weeks of age), the testes enlarge rapidly, reaching approximately 15–20 g in weight. Although semen production may begin as early as 12 weeks depending on breed, body weight, and lighting programme, optimum semen quality and fertility are generally achieved only after sexual maturity. Sperm production (spermatogenesis) is regulated by the hypothalamic–pituitary–gonadal axis. The hypothalamus secretes Gonadotropin-Releasing Hormone (GnRH), which stimulates the anterior pituitary to release Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH). FSH promotes spermatogenesis within the seminiferous tubules, while LH stimulates the Leydig cells to produce testosterone, the principal male sex hormone. Testosterone is essential for sperm production, development of secondary sexual characteristics, mating behaviour, and maintenance of the male reproductive tract (Senger, 2003).The testes contain numerous seminiferous tubules, where sperm cells are produced, and interstitial (Leydig) cells, which synthesize testosterone. In birds, both spermatozoa and seminal plasma are produced

within the testes. On average, approximately 100 million sperm are produced daily per gram of testicular tissue, irrespective of mating or semen collection frequency. Spermatogenesis occurs in two successive phases: spermatocytogenesis, during which germ cells undergo repeated cell division, and spermiogenesis, in which immature spermatids are transformed into mature spermatozoa. During spermiogenesis, the acrosome develops from the Golgi apparatus, the nucleus condenses, the mitochondria arrange themselves in the midpiece, and the tail (flagellum) is formed, producing a fully functional sperm capable of fertilization (Gordon, 2005).Several factors influence semen production and reproductive efficiency in male birds, including genetics, age, nutrition, health status, management practices, season, and lighting programme. In addition, the temperament and responsiveness of individual males to the abdominal massage technique can significantly influence the efficiency of semen collection.

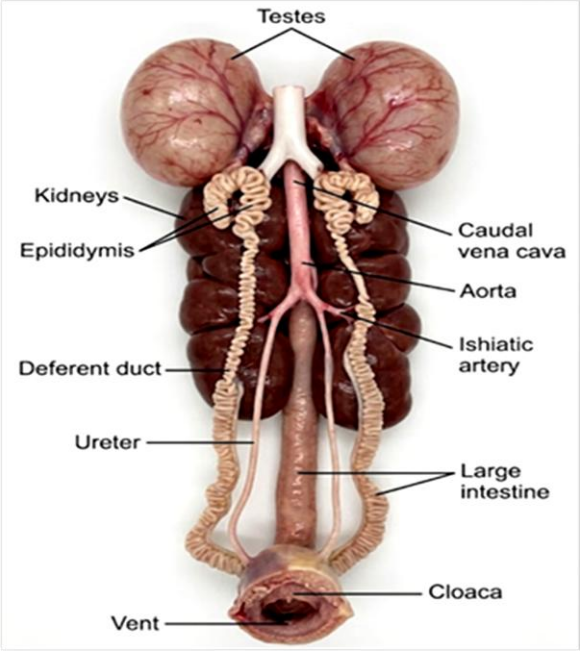

Figure.1: Comprehensive Reproductive & Urinary system of male avian

Semen Collection from Male Birds

Successful artificial insemination depends on obtaining a **clean, uncontaminated, and adequate quantity of high-quality semen**. Earlier, semen was recovered from the female reproductive tract after natural mating, but this cumbersome method was replaced by the **abdominal massage technique** developed by **Burrows and Quinn (1937)**. This simple, non-invasive method is now widely used for semen collection in chickens, turkeys, and pigeons.In this technique, a healthy, sexually mature, and well-trained male bird is gently restrained while the abdomen and back are massaged from the wings towards the tail to stimulate ejaculation. As the phallus protrudes, gentle pressure is applied around the cloaca, and the semen is collected directly into a clean, sterile tube, avoiding contamination with faeces or urine.For best results, males should be trained for semen collection several days before the start of an AI programme. Feed is usually withdrawn for about **12 hours** before collection to reduce faecal contamination. In chickens, semen can be collected **three times**

a week without affecting fertility. Semen collection is often more productive in the afternoon, coinciding with the birds' natural mating behaviour.Semen yield varies among species and breeds. Chickens produce approximately **0.05–0.90 ml** of semen per collection, while turkeys produce **0.08–0.33 ml**. Although chickens generally produce a greater semen volume, turkey semen has a higher sperm concentration.

Semen Quality Evaluation

The success of artificial insemination largely depends on the quality of semen used. Evaluating semen helps identify healthy breeding males, estimate the number of insemination doses that can be prepared, and predict fertility. Semen evaluation includes **gross (macroscopic)** and **microscopic** examination.

1. Colour

Fresh, good-quality rooster semen is **creamy to pearly white**, indicating a high sperm concentration. Watery or transparent semen usually suggests a low sperm count, while **yellow, green, or brown** colour indicates contamination with urine or faeces. Blood-stained semen may result from injury during collection (Cole and Cupps, 1977).

2. Volume

Semen volume is measured immediately after collection. In chickens, the ejaculate generally ranges from **0.05 to 0.9 ml**, although breed, age, and collection frequency may influence the volume. Semen volume, together with sperm concentration, determines the number of insemination doses that can be prepared (Hafez and Hafez, 2000).

3. pH

Normal poultry semen is **slightly alkaline**, with a pH of **7.0–7.4**. This range provides optimum conditions for sperm survival and motility. Delayed evaluation may reduce the pH due to lactic acid production, adversely affecting sperm quality (Latif *et al*., 2005).

4. Sperm Concentration

Sperm concentration indicates the number of sperm cells present per millilitre of semen and is used to calculate the correct dilution rate for AI. Good-quality rooster semen contains approximately **3–7 billion (3,000–7,000 million) sperm/ml**. It can be determined using a haemocytometer, spermatocrit, or photometer (Hafez and Hafez, 2000).

5. Sperm Motility

Motility refers to the percentage of sperm moving actively in a forward direction and is considered the **most important indicator of fertility**. Semen with **60–80% progressively motile sperm** is generally regarded as suitable for artificial insemination. Motility is usually assessed under a light microscope (Donoghue *et al*., 1998).

6. Sperm Viability

Viability indicates the proportion of **live spermatozoa** in a semen sample. It is commonly evaluated using the **eosin–nigrosin staining technique**, where live sperm remain unstained, whereas dead sperm take up the eosin stain and appear pink. High sperm viability is essential for maintaining fertility during semen storage.

7. Sperm Morphology

Morphology refers to the **shape and structural integrity** of spermatozoa. Normal avian sperm possess a slender head, elongated midpiece, and long tail. Sperm with bent heads, damaged midpieces, coiled tails, or defective acrosomes have reduced fertilizing ability. Ideally, **85–90% of sperm should be morphologically normal** (Peters *et al*., 2008).

Processing of poultry semen

Semen Dilution

Fresh poultry semen rapidly loses its motility and fertilizing ability after collection. Therefore, it is diluted immediately using specially prepared **semen extenders (diluents)**, which increase the volume of semen, provide an optimum environment for sperm survival, and ensure uniform distribution of spermatozoa in each insemination dose. Semen dilution also allows a single ejaculate from a superior male to be used for inseminating a larger number of females, thereby improving the efficiency and reducing the cost of artificial insemination.

Initially, simple normal saline was used as a diluent, but modern poultry semen extenders are more complex and contain buffers, osmotic regulators, energy substrates, and protective agents that help maintain sperm viability. Commonly used extenders include **Lake's Diluent, Modified Ringer's Solution, Beltsville Poultry Semen Extender (BPSE), EK Extender, Tselutin Extender**, and the recently developed **CARI Poultry Semen Diluent**. Most extenders contain sodium glutamate, glucose, fructose, and specialized buffers to maintain a pH of **7.0–7.4** and an osmolarity of about **400 mOsm**. Sodium glutamate, a major constituent of avian seminal plasma, serves as an important energy source and improves sperm survival during storage. In chickens, a dilution ratio of **1:2 to 1:3** is generally considered optimum for maintaining semen quality and fertility.

Semen Storage

Undiluted rooster semen begins to lose its motility and fertilizing capacity within **one hour** of collection. However, when diluted with a suitable extender, semen can be stored under refrigeration (**4–10°C**) for up to **24 hours** without significant loss of viability or fertility. Temperatures close to **0°C** should be avoided because poultry sperm are highly

susceptible to cold shock, which damages the sperm membrane and reduces fertility. During storage, extenders not only provide nutrients and maintain pH but also protect sperm from physical, chemical, and oxidative damage.

Cryopreservation

For long-term preservation, poultry semen can be cryopreserved in liquid nitrogen. Cryopreservation is particularly useful for conserving elite breeding lines and valuable poultry germplasm. However, compared with mammals, poultry sperm are highly sensitive to freezing and thawing. Structural damage to the plasma membrane, acrosome, mitochondria, and tail during the freeze–thaw process often results in reduced motility and fertility. Cryoprotectants such as **glycerol** are added at low concentrations to minimize freezing injury, although excessive glycerol may adversely affect fertility in hens. Continuous improvements in semen extenders, antioxidants, and freezing techniques are helping to enhance the storage life and fertilizing ability of poultry semen.

Artificial Insemination in Female Birds

Artificial insemination in poultry involves depositing semen into the female reproductive tract without natural mating. Two methods of semen deposition have been described: **intraperitoneal insemination** and **vaginal insemination**. Among these, vaginal insemination is the most practical and widely adopted technique because of its simplicity, safety, and high fertility (Cole and Cupps, 1977).

Intraperitoneal Insemination

Intraperitoneal insemination is an older and less commonly used technique in which semen is deposited into the abdominal cavity near the ovary using a fine needle and cannula. Because of its invasive nature, lower reliability, and risk of injury, this method has largely been replaced by vaginal insemination in commercial poultry production (Cole and Cupps, 1977).

Vaginal Insemination

Vaginal insemination is the standard method used in chickens and turkeys. The procedure requires two trained persons. One person gently restrains the hen and applies pressure to the left side of the abdomen near the vent, causing the vaginal opening to evert through the cloaca as shown in Figure.2. This process is commonly referred to as **venting, cracking**, or **everting** the hen (Quinn and Burrows, 1937).

Figure.2: A.I. in Hen

The second person immediately deposits the semen 2–4 cm into the vaginal opening using a sterile straw, syringe, or plastic insemination tube. Once the semen is deposited, the abdominal pressure is released, allowing the cloaca to return to its normal position and helping draw the semen further into the reproductive tract. In large commercial hatcheries, automated semen dispensers are often used for rapid and uniform insemination.

Timing and Frequency of Insemination

Since poultry semen rapidly loses its fertilizing ability after collection, insemination should be carried out as soon as possible. The ideal time for insemination is between 2:00 and 4:00 p.m**.**, after most hens have laid their daily egg. Morning insemination is generally avoided because the presence of an egg in the oviduct may interfere with sperm transport .In chickens, hens are usually inseminated on two consecutive days at the beginning of the breeding programme and subsequently once every seven days to maintain fertility. Turkeys are generally inseminated every 10–14 days. The recommended insemination dose is approximately 0.05 ml of undiluted pooled semen in chickens and 0.025 ml in turkeys. Under proper management, fertile eggs can be obtained from the second day after insemination and fertility may persist for two weeks or more.

Sperm Storage and Behaviour in the Hen's Oviduct

One of the most remarkable features of avian reproduction is the ability of the hen to store viable sperm for an extended period after insemination. Following vaginal insemination, millions of sperm move rapidly through the oviduct, but less than 1% enter the specialized Sperm Storage Tubules (SSTs) located at the uterovaginal junction between the vagina and shell gland. Only healthy and viable sperm are able to enter these storage tubules (Froman and Feltmann, 2005).Within the SSTs, sperm remain alive for several days to weeks**,** allowing a single insemination to fertilize multiple eggs. Under normal conditions, sperm may survive for up to 32 days in chickens and 70 days in turkeys, a unique feature that reduces the need for frequent insemination (Hafez and Hafez, 2000). The exact mechanism of prolonged sperm survival is not fully understood, but it is believed to involve temporary suppression of sperm motility and metabolism, stabilization of the plasma membrane, and maintenance of acrosomal integrity (Tabatabaei *et al*., 2009).Stored sperm are released gradually from the SSTs and transported by muscular contractions and ciliary movements of the oviduct towards the infundibulum, where fertilization occurs. Some sperm may also be temporarily stored in

sperm nests located near the junction of the magnum and infundibulum. Following ovulation, the released sperm rapidly reach the ovum, undergo the acrosome reaction, and penetrate the perivitelline layer to achieve fertilization. Although only one sperm ultimately fertilizes the ovum, multiple sperm may penetrate the outer egg coverings, a phenomenon known as physiological polyspermy, which is a normal feature of avian fertilization (Hafez and Hafez, 2000).

Artificial insemination has become an indispensable reproductive tool in modern poultry production. It improves breeding efficiency, facilitates rapid genetic improvement, and overcomes many of the limitations associated with natural mating, particularly in heavy broiler and turkey breeds. However, its successful implementation requires proper management, skilled personnel, and suitable infrastructure.

Advantages

- *Efficient use of superior males*: A single high-quality cockerel can inseminate many more hens than under natural mating, reducing the number of breeding males required and lowering production costs.
- *Rapid genetic improvement:* AI enables widespread use of genetically superior sires, accelerating selective breeding and improving flock performance.
- *Useful for heavy breeds*: It overcomes mating difficulties in large broiler breeders and turkeys, where excessive body weight often reduces natural mating efficiency.
- *Facilitates crossbreeding:* Controlled mating allows planned crossbreeding programmes and the production of desirable hybrid strains.
- *Improved fertility:* Accurate semen deposition reduces the effects of preferential mating and helps achieve more consistent fertility and hatchability.
- *Reduced disease transmission:* Since direct mating is avoided, the risk of spreading certain reproductive and venereal diseases is minimized.
- *Semen preservation and transport:* Diluted or cryopreserved semen can be stored, transported, and exchanged between farms, reducing the need to move live breeding birds.
- *Conservation of valuable germplasm:* AI supports the preservation of elite breeding lines and indigenous poultry genetic resources.
- *Reduced stress and injury*: It minimizes injuries to hens that may occur during natural mating, particularly in heavy breeds.

Limitations

- *Requires skilled personnel:* Successful semen collection, evaluation, and insemination demand trained and experienced technicians.
- *Labour-intensive:* Large-scale AI programmes require considerable labour and careful coordination.
- *Limited semen storage:* Poultry semen has a short storage life, and cryopreservation still results in lower fertility compared with fresh semen.
- *Higher initial investment:* Equipment, semen extenders, storage facilities, and training increase the initial cost of adopting AI.
- *Strict hygiene requirements:* Poor handling or contamination during semen collection and insemination can reduce fertility and increase the risk of infection.
- *Not always economical for small flocks:* In small backyard poultry systems, the cost and labour involved may outweigh the benefits.

Conclusion

Artificial insemination (AI) has become an indispensable reproductive technology in modern poultry production, offering an efficient alternative to natural mating. It enables the widespread use of genetically superior males, improves fertility, reduces the number of breeding cockerels required, minimizes disease transmission, and overcomes mating difficulties in heavy breeds such as broilers and turkeys. The success of AI depends on proper semen collection, quality evaluation, dilution, storage, and timely insemination, together with the remarkable ability of hens to store viable sperm for extended periods.In India, where poultry farming is a rapidly growing source of livelihood for millions of farmers, AI offers significant economic and genetic advantages by improving breeding efficiency while reducing production costs. At the same time, successful implementation requires trained personnel, hygienic handling, and careful attention to bird welfare during semen collection and insemination.Although further research is needed to improve semen extenders, long-term storage, cryopreservation techniques, and the conservation of valuable indigenous and endangered avian germplasm, artificial insemination remains a powerful tool for enhancing productivity, genetic improvement, and sustainable poultry production. With continued technological advances and responsible management, AI is expected to play an increasingly important role in meeting the growing global demand for high-quality poultry meat and eggs.

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