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Review Article

Artificial Insemination and Spermatologic Parameters in Poultry: A Review

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ABSTRACT

Artificial insemination is defined as the process of collecting semen from a male animal and artificially inseminating a female under suitable conditions. Achieving high success in artificial insemination requires a solid understanding of the physiology of both the male and female reproductive systems, comprehension of reproductive endocrinology, and accurate evaluation of sperm parameters. Although artificial insemination is less comm on in poultry than in mammals, it remains an important biotechnological tool in breeding programs and genetic resource preservation through semen storage. Additionally, artificial insemination allows the widespread use of a limited number of superior genetically valuable animals. With the continuous advancement of reproductive biotechnology and technologies, their application in poultry should be expanded and more research conducted. This review provides general information on artificial insemination and spermatological parameters in poultry species.

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Kanatlı Hayvanlarda Suni Tohumlama ve Spermatolojik Parametreler: Derleme

ÖZET

Suni tohumlama uygulaması kısaca erkek hayvandan elde edilen spermanın uygun koşullar altında yapay yolla dişi hayvana nakledilmesi olarak tanımlanmaktadır. Suni tohumlama uygulamasında yüksek başarı sağlanması için erkek üreme sistemi ve dişi üreme sistemi fizyolojisine hâkim olunmalı, üreme endokrinolojisi kavranmalı, kullanılan spermaya ait spermatolojik parametre muayeneleri doğru yapılmalıdır. Kanatlı hayvanlarda suni tohumlama uygulamaları memeli hayvanlar kadar yaygın olmasa da özellikle ıslah çalışmalarında ve sperma saklama yöntemleri ile genetik kaynakların korunması kapsamında önemli bir biyoteknolojik uygulama olarak karşımıza çıkmaktadır. Ayrıca az sayıda üstün genetik yapıya sahip hayvandan yararlanarak daha geniş alanlara ulaşım imkânı sunmaktadır. Sürekli olarak gelişen ve büyüyen üreme biyoteknolojisi ve teknolojileri kanatlı hayvanlar içinde uygulanmalı ve daha çok çalışmalar yapılmalıdır. Bu derleme makalesinde kanatlı hayvanlarda suni tohumlama ve spermatolojik parametreler ile ilgili genel bilgilere yer verilmiştir.

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INTRODUCTION

To ensure the continuity of life, all living organisms must perform reproductive and proliferative functions. The organs involved in carrying out these functions are referred to as reproductive organs. The system composed of organs responsible for reproduction and those secreting hormones actively involved in the reproductive process is known as the reproductive system. The reproductive systems of male and female animals differ according to their respective functions. Reproductive organs also differ between males and females in terms of shape, location, and structure (Kandil, 2016).

Compared to mammals, the male reproductive system in poultry has a simpler structure. It consists of a pair of testes, small epididymides, long-coiled ductus deferens, erectile organs, ejaculatory structures, vascular structures, and the phallus (Akbalık et al., 2016). In roosters, puberty starts at 20 weeks. GnRH from the hypothalamus stimulates the pituitary to release FSH and LH. FSH promotes development of seminiferous tubules for spermatogenesis, while LH stimulates Leydig cells to produce testosterone. Testosterone levels rise and stabilize by 30-35 weeks. Testosterone then reduces FSH and LH, but their levels rise again as testosterone drops. This cycle maintains the balance of spermatogenesis and testosterone (İleri et al., 2002). The female reproductive system in poultry, similar to that in mammals, comprises ovaries and the oviduct. However, in poultry, only the left ovary develops while the right one regresses. The oviduct is composed of five distinct regions: infundibulum, magnum, isthmus, uterus, and vagina. The functional characteristics of these regions show differences when compared to those in mammals (Yılmaz, 1999). Unlike mammals, poultry does not exhibit an estrous cycle, have significantly larger oocytes, undergo physiological polyspermic fertilization, and possess notably different female reproductive anatomy. These characteristics have limited the diversity of reproductive studies in poultry. Moreover, fertilization occurs within 15 minutes in the infundibulum; sperm can remain viable in the oviduct for up to 70 days; egg-laying occurs approximately every 25 hours; and spermatozoa do not undergo capacitation—traits that distinguish them from mammals (Nizam & Selçuk, 2019).

In artificial insemination practices in poultry, semen is collected from males and evaluated for spermatological parameters. Determination of these parameters is essential due to their direct impact on fertilization rates and hatchability outcomes. Once these procedures are completed, the semen is deposited into the female reproductive tract. Artificial insemination allows for the broader utilization of males with superior genetic traits. It also makes it possible to use male animals that are incapable of natural mating. Moreover, artificial insemination plays a key role in controlling diseases transmitted through mating. Since only a limited number of male animals are required, it also reduces overall costs. Another advantage of artificial insemination is the possibility of storing semen for future use (Vishwanath & Shannon, 1997; Blanco & Hofle, 2004; Kharayat et al., 2016; Getachew, 2016; Mohan et al., 2018).

HISTORY of ARTIFICIAL INSEMINATION

The first documented efforts to develop a practical procedure for artificial insemination, including in poultry, were initiated by Ivanow in Russia in 1899 (Ivanow, 1922). That year, Ivanow successfully produced fertile chicken eggs by transferring semen obtained from the reproductive tracts of roosters post-mortem (Lunak, 2010).

The process of semen collection for artificial insemination in chickens was facilitated by devices developed by Ishikawa in 1930. Subsequently, Burrows and Quinn introduced the abdominal massage technique for roosters, which they termed "male milking" (Burrows & Quinn, 1937). This innovative method has since been widely adopted across various poultry species (Quinn & Burrows, 1936; Mohan et al., 2018).

The technique was widely adopted in countries such as Israel (Thumin, 1951), Australia (Skaller, 1951), and the United States (Moultrie, 1956) to improve broiler chicken productivity. Its large-scale use in breeding farms was facilitated by the simplicity of semen collection and the close housing of hens, which allowed for efficient use of fresh semen. Since the 1960s, artificial insemination has become essential in turkey reproduction and remains a key practice in commercial poultry production (Mohan et al., 2018).

ARTIFICIAL INSEMINATION in POULTRY

Artificial insemination in poultry involves several key stages: the collection of semen from the male, the evaluation of semen quality in terms of motility, viability, and concentration, and the subsequent deposition of semen into the female reproductive tract. Both roosters and hens typically reach sexual maturity at approximately 18 weeks of age. Semen collected from a single rooster is sufficient to inseminate around 20 hens. Due to the high sensitivity of poultry semen to procedures such as cryopreservation, artificial insemination is generally conducted using fresh semen (Kharayat et al., 2016).

Artificial insemination and related reproductive technologies have enabled the widespread use of genetically superior and highly fertile roosters. Advances in artificial insemination techniques have facilitated the transmission of desirable genetic traits from a limited number of elite males to a broad population of females (Vishwanath & Shannon, 1997). Additionally, roosters with superior genetics that are unable to mate naturally can still be effectively utilized. From an economic perspective, artificial insemination helps reduce production costs by minimizing the number of roosters required (Mohan et al., 2018). For instance, in response to increasing global demand for poultry meat, the strategic distribution of semen from high-yield roosters can significantly enhance production capacity. Another key advantage is the potential to preserve semen for future use (Getachew, 2016). In advanced poultry production, artificial insemination has become increasingly widespread due to its contribution to high reproductive efficiency, genetic improvement, and reduced management costs (Sun et al., 2019).

Moreover, artificial insemination contributes significantly to disease prevention by helping control the spread of infectious and venereal diseases, which are often difficult to manage under natural mating conditions (Blanco & Hofle, 2004). Consequently, artificial insemination is regarded as the foremost biotechnological approach employed in poultry production to enhance both productivity and genetic quality (Mohan et al., 2018).

Table 1. *The key advantages of artificial insemination in poultry for enhancing reproductive efficiency and its role in genetic management (Kharayat et al., 2016)*

Advantage	Description
Increased Mating Efficiency	While a rooster can naturally mate with only 6 to 10 hens, this number can be quadrupled through artificial insemination, significantly improving reproductive efficiency.
Utilization of Aged Superior Males	Semen from older males with superior genetics and performance can still be used via artificial insemination, even across generations. In contrast, natural mating limits the economic reproductive lifespan of males.
Use of Injured Males	Artificial insemination allows the continued use of genetically valuable males that are unable to mate naturally due to physical injury.
Elimination of Mating Preferences	Artificial insemination eliminates selective mating behavior, thereby avoiding fertility issues that may arise from such preferences among hens and roosters.
Facilitation of Hybridization	In natural mating, roosters involved in crossbreeding programs may be rejected by hens of different breeds or colors. Artificial insemination enables successful reproduction in such hybridization efforts by overcoming behavioral barriers.

Semen Collection

Semen collection is a critical step for the success of artificial insemination, requiring consistent retrieval of clean semen in sufficient volumes. In the earliest attempts, natural mating between the rooster and hen was allowed, after which the hen was sacrificed, and semen was surgically extracted from the oviduct. Due to the invasive and harsh nature of this method, Burrows and Quinn (1937) developed the abdominal massage technique. This technique has since become widely used for semen collection in chickens, turkeys, and pigeons (Klimowicz et al., 2005).

During the collection process, the bird is positioned either on the collector's knee or on a table, held securely by the legs, and gently pulled downward from the chest. The legs are restrained either by an assistant or with

the aid of leg clamps. One hand is placed firmly on the abdominal area near the cloaca, while the other hand performs a massage from the back toward the base of the tail feathers. Depending on individual variation among males, the phallus typically enlarges after 3 to 6 massage movements, at which point the collector must be prepared. The cloaca is then gently compressed between the thumb and forefinger from top and bottom, and the semen is collected directly from the phallus into a sterile tube. If a second collection is required, the process can be repeated. This method allows semen to be collected 4 to 6 times per week (Kharayat et al., 2016).

Figure 1. *a. Ensuring proper environmental conditions and correct positioning to optimize the collection of rooster semen, b. Application of massage techniques for effective semen ejaculation in roosters, c. Collection of rooster semen into sterile containers ensuring contamination prevention.*

Semen collection may begin at 16 weeks of age in roosters and at 28 weeks in turkeys. However, optimal semen yield is usually attained at 26–28 weeks in roosters and 32–36 weeks in turkeys. Since poultry generally exhibit mating behavior in the afternoons, semen collection is recommended during this period to obtain higher volumes. While sperm fertilization capacity remains relatively stable in roosters up to three years of age, semen volume tends to decline with age. To obtain high-quality samples, male birds should be acclimated to the collection procedure in advance. As the phallus is located in the cloacal region near the anus, there is an increased risk of fecal contamination. To reduce this risk and ensure semen cleanliness, feed should be withheld for at least 12 hours prior to collection (Mohan et al., 2018).

Semen Dilution

Semen diluents are nutrient media specifically formulated to support the viability of spermatozoa, protect them from environmental conditions, preserve their fertilizing capacity, prevent potential damage to the female reproductive tract, and increase the overall semen volume (Pabuccuoğlu, 2013). The preservation of semen for artificial insemination is made possible through the use of appropriate diluents and storage techniques. By diluting high-quality semen, limited ejaculate volumes can be extended, allowing a greater number of females to be inseminated using fewer male birds. Furthermore, the naturally viscous consistency of undiluted semen can hinder handling and transfer, making dilution a practical necessity (Getachew, 2016).

Semen diluents are buffered salt solutions designed to enhance semen volume and maintain sperm viability by providing a physiologically suitable environment. The composition of diluents used for avian semen is ustomized to the biochemical characteristics specific to poultry. These formulations aim to replicate the osmotic pressure and pH of seminal plasma. Initial diluents were based on simple NaCl solutions, which were later enhanced through the inclusion of osmotic regulators, energy substrates, and buffering agents (Mohan et al., 2018). Commonly used diluents contain components such as sodium glutamate, glucose, fructose, and specialized buffers that stabilize pH near 7.0 and maintain osmolarity around 400 milliosmoles. For storage exceeding 4–6 hours, the addition of glutamate is especially critical (Kharayat et al., 2016).

While semen cryopreservation protocols differ among species, the core principles remain largely the same. Commonly used additives include egg yolk, glycerol, and milk. Carbohydrates like glucose and fructose provide essential energy, whereas buffering agents such as citrate, Tris, phosphate, and citric acid help maintain pH and osmotic balance. To reduce the risk of microbial contamination, additives of sterile origin are preferred.

Glycerol-based cryoprotectants continue to be widely used in modern cryopreservation techniques. (Coşkun & Karaca, 2016).

The dilution ratio and the type of diluent significantly influence factors such as sperm motility, ionic balance, gas exchange, and ATP consumption. A moderate dilution ratio—typically two- to threefold—is considered optimal for the in vitro storage of chicken semen. Dilution ratios exceeding fivefold generally lead to decreased sperm motility and reduced shelf life. Conversely, under low-temperature conditions, a dilution ratio of 1:1 or lower may negatively affect sperm viability. Due to the naturally high concentration of avian semen, low-dilution preparations can bring normal and morphologically damaged spermatozoa into close proximity, potentially resulting in harmful interactions that compromise the integrity of healthy cells (Mohan et al., 2018).

ARTIFICIAL INSEMINATION TECHNIQUE in POULTRY

Generally, two distinct artificial insemination techniques are employed in poultry: intraperitoneal insemination and vaginal insemination. Of these, the most widely used and successful method for fertilization in poultry involves the deposition of semen into the mid-vaginal region (Getachew, 2016).

Intraperitoneal Insemination

Although this technique is generally regarded as unreliable, it involves the insertion of a cannula through an incision in the abdominal wall to deposit semen near the ovary (Getachew, 2016). This method has notable drawbacks, including its adverse effects on egg production and the time-intensive nature of the procedure (Brown et al., 1963). As a result, it is not routinely employed in modern poultry production.

Vaginal Insemination

Developed in the 1930s, the technique is currently the most widely used method of artificial insemination in poultry and requires two individuals for implementation. This procedure involves applying pressure to the hen's abdominal area, causing the vaginal opening to evert outward through the cloacal tract. The hen is held in an inverted position, and firm pressure is applied to the left side of the abdomen, which helps straighten the cloaca. Considering that sperm in poultry loses its viability within approximately one hour, insemination should be performed immediately after semen collection. Insemination is performed using sterile pipettes, syringes, or plastic tubes. In large-scale commercial operations, automated semen dispensers are commonly employed. The insemination tube should be inserted as deeply as possible into the oviduct. After semen is deposited, the pressure on the cloaca is released to allow the semen to move inward within the reproductive tract. Artificial insemination should initially be performed in hens on two consecutive days, followed by once-a-week applications thereafter. It is recommended to schedule insemination in the afternoon, when the oviduct is empty, to maximize success (Bakst & Dymond, 2013; Getachew, 2016; Bekele et al., 2023).

Figure 2. *Application of vaginal artificial insemination in laying hens to enhance fertilization success.*

SHORT-TERM STORAGE of SEMEN

Storing semen at low temperatures, such as 5°C, effectively slows metabolic activity in spermatozoa, thereby extending their lifespan and preserving their functional and structural integrity. While spermatozoa lose their fertilizing ability within a few hours at room temperature, when diluted and stored at 5°C, they can maintain their fertilizing capacity for up to 72 hours (Pabuccuoğlu, 2013).

Under appropriate short-term storage conditions, diluted semen can remain viable for several hours to a few days. For this purpose, storage is typically performed at 4–5°C (Kulaksız, 2016). It is crucial to dilute sperm using appropriate extenders to maintain fertility. Studies have shown that diluted rooster semen retains viability for a longer period compared to undiluted semen (Getachew, 2016). Brillard (2009) recommended storing semen at temperatures ranging from 4°C to 10°C for extended preservation, while temperatures below 0°C should be avoided.

Donoghue and Wishart (2000) also reported that, to preserve the fertilizing potential of semen, storage should be maintained within the temperature range of 2–8°C. Similarly, in a study on turkeys, Slanina et al. (2015) demonstrated that turkey semen could be effectively stored within the range of 4–8°C. Blank et al. (2021) examined the effects of various storage temperatures (5, 25, and 37°C) and durations (0, 2, 4, 8, and 24 hours) on rooster spermatozoa. The results indicated that the most significant damage occurred at 37°C after 24 hours, whereas no substantial changes were observed at 5°C over a 24-hour period. Furthermore, the study suggested that spermatozoa could be preserved at 25°C for up to 4 hours.

LONG-TERM STORAGE of SEMEN (CRYOPRESERVATION)

Cryopreservation refers to the process of storing living cells or tissues by cooling them to subzero temperatures, thereby halting their biological activity and preserving them for future use (Tunalı, 2014). The primary objective of the freezing process is to store living cells or tissues for extended periods with minimal damage during exposure to low temperatures.

In long-term storage, spermatozoa are frozen at extremely low temperatures, typically around -196°C, in specialized extenders containing cryoprotectants (Pabuccuoğlu, 2013). Cryoprotectants are used to minimize damage to spermatozoa during freezing. These agents provide protection against cold shock injury, intracellular ice crystal formation, recrystallization during thawing, and subsequent membrane destabilization (Çoşkun & Karaca, 2016). The most commonly used cryoprotectants in the freezing of semen, embryos, oocytes, and somatic cells include glycerol, DMSO (dimethyl sulfoxide), ethylene glycol, and propylene glycol (Pabuccuoğlu, 2013). In relation to this topic, Stanishevskaya et al. (2021) reported that the addition of trehalose at different concentrations (4.8 mM and 9.5 mM) to the Leningrad Cryoprotective Medium reduced the detrimental effects of cryopreservation on rooster spermatozoa and increased the fertilization rate compared to the control group. In a parallel study, Mehdipour et al. (2021) investigated the effects of varying concentrations of Type III antifreeze protein (AFP3) in Lake extender on post-thaw semen quality and fertility parameters in breeder rooster sperm. The authors reported that AFP3 had beneficial effects and identified the optimal dose range to be between 0.1 and 1 µg/mL. Furthermore, Elomda et al. (2024) examined the impact of different cryoprotectants—dimethylacetamide (DMA), dimethyl sulfoxide (DMSO), and ethylene glycol (EG)—on rooster spermatozoa during the freeze-thaw process. Their findings indicated that cryopreservation adversely affected sperm motility, quality, antioxidant biomarkers, and fertility. However, among the cryoprotectants tested, DMA was found to be more suitable in terms of preserving sperm quality and fertility. In another study, Nizam et al. (2025) thawed frozen rooster semen either in a water bath at 37 °C for 30 seconds or using a device that provided dry thawing. They reported that dry thawing, compared to the water bath method, improved sperm viability and motility while reducing the proportion of abnormal sperm and DNA damage.

In poultry, variations in semen quality contribute to different sensitivities during the freezing process. Consequently, cryopreservation protocols may vary significantly (Kulaksız, 2016). Morphological differences between avian and mammalian spermatozoa are a major factor contributing to the relatively low post-thaw viability of poultry semen. For example, the cylindrical shape of the sperm head, the long and narrow tail, and the limited cytoplasmic content in avian spermatozoa restrict motility and increase sensitivity to freezing (Akçay et al., 2007).

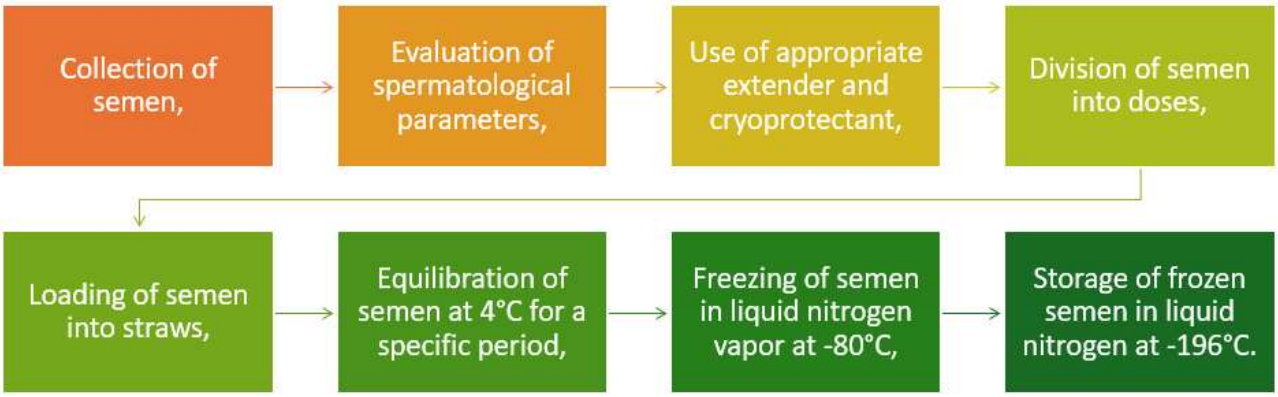

Figure 3. *Primary steps in protocols for long-term semen preservation techniques.*

SPERMATOLOGICAL PARAMETERS

Determining sperm quality in poultry is an essential aspect, as it directly impacts egg fertilization rates and hatchability. Evaluating sperm quality is essential not only for maintaining males with high-quality sperm in the flock but also for determining sperm concentration and optimizing artificial insemination doses (Mohan et al., 2018). In areas where artificial insemination is routinely practiced, the most employed analyses for semen evaluation include volume, concentration, motility, and viability (Silyukova et al., 2022).

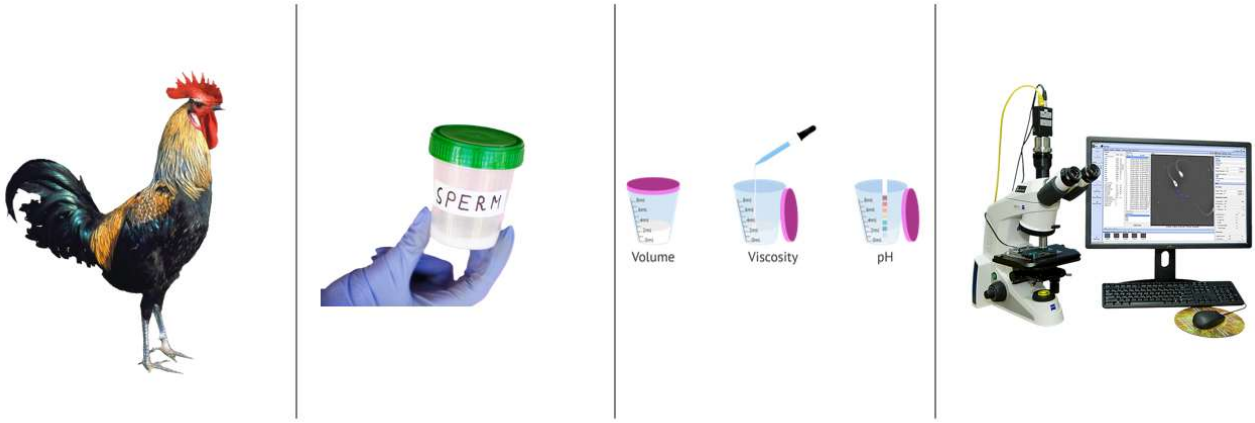

Figure 4. *Steps of some analyzes used to determine semen quality in roosters.*

Semen Color

Semen color generally indicates the concentration of the ejaculate and can vary between poultry species. A creamy color typically indicates a high semen concentration. The color can also indicate contamination within the semen. For example, contamination with feces or urine can cause the semen to appear brown or green (Getachew, 2016). High-quality rooster semen typically appears pinkish-white and has a thick consistency (Keskin et al., 1995).

Semen pH

Rooster and turkey semen can tolerate pH values between 6.0 and 8.0. Low pH reduces oxygen uptake, lactic acid production, motility, and metabolic rate of spermatozoa, while high pH increases the in vitro metabolic rate (Uysal et al., 2011). Studies measuring pH in roosters have shown varying results: Keskin et al. (1995) reported an average pH value of 7.4±0.1, Keskin et al. (1997) found an average pH of 7.5±0.1, Bah et al. (2001) recorded pH values ranging from 7.54±0.04 to 7.80±0.03, Peters et al. (2008) found an average pH of 7.01±0.1, and Uysal et al. (2011) reported an average pH of 6.97±0.3.

Table 2. *Reported semen pH values in roosters.*

Species	Value	Reference
Rooster	7.4 ± 0.1	Keskin et al., 1995
Rooster	7.5 ± 0.1	Keskin et al., 1997
Rooster	7.54 ± 0.04 – 7.80 ± 0.03	Bah et al., 2001
Rooster	7.01 ± 0.1	Peters et al., 2008
Rooster	6.97 ± 0.3	Uysal et al., 2011

Ejaculate Volume

The volume of semen is a critical parameter for determining spermatological characteristics, particularly in the context of artificial insemination procedures (Şeber, 2002). Roosters typically produce between 0.1 and 1.5 ml of ejaculate per collection, with an average of 0.6 ml. Even the same rooster may produce varying volumes of semen at different times (Getachew, 2016). Studies on ejaculate volume have yielded varying results: Keskin et al. (1995) reported an average ejaculate volume of 0.6±0.1 ml in roosters, Keskin et al. (1997) reported 0.3±0.0 ml for five Ross breed roosters, Akçay et al. (2007) reported an average of 0.28±2.8 ml in turkeys, Uysal et al. (2011) reported an average of 0.8±0.2 ml for seven different purebred roosters, and İnanç et al. (2017) reported ejaculate volumes of 0.39±0.22 ml, 0.52±0.35 ml, 0.52±0.16 ml, and 0.51±0.23 ml for four different groups. Sun et al. (2019) reported a value of 0.486 ± 0.046 (n=15) ml in high-motility sperm samples and 0.293 ± 0.046 (n=15) ml in low-motility samples. Boz et al. (2021) found the volume of geese semen to be between 0.18 and 0.24. Prabakar et al. (2022) performed monthly measurements over a 12-month period and reported that the ejaculate volume ranged from 0.14 to 0.26 ± 0.01 ml. Di Iorio et al. (2024) investigated ejaculate volume in 145 roosters from 13 breeds, reporting values ranging from 0.03 ± 0.1 to 0.61 ± 0.1 mL, with a mean of 0.18 ± 0.1 mL.

Table 3. *Reported ejaculate volume (ml) in roosters and turkeys.*

Species	Value (ml)	Reference
Rooster	0.6 ± 0.1	Keskin et al., 1995
Rooster	0.3 ± 0.0	Keskin et al., 1997
Turkey	0.28 ± 2.8	Akçay et al., 2007
Rooster	0.8 ± 0.2	Uysal et al., 2011
Turkey	0,22 ± 0,01	Kuzlu, 2015
Rooster	0.39 ± 0.22 – 0.52 ± 0.35	İnanç et al., 2017
Rooster	0.486 – 0.293 ± 0.046	Sun et al., 2019
Geese	0.18-0.24 ± 0,004	Boz et al., 2021
Turkey	0.14-0.26 ± 0.01	Prabakar et al., 2022
Rooster	0.18 ± 0.1	Di Iorio et al., 2024

Sperm Motility

Sperm motility serves as an indicator of sperm viability and semen quality. Sperm motility evaluation is typically carried out using fresh or diluted semen under a light microscope with 100x magnification. As motility analysis is more challenging with undiluted sperm, sperm are typically diluted with appropriate diluents prior to analysis (Hafez & Hafez, 2000). In other words, sperm motility refers to the ratio of sperm moving in the same direction and with strength compared to those that are immobile or exhibit erratic movements. This parameter reflects the fertilizing capacity of sperm (Şeber, 2002). Several studies on sperm motility have yielded the following results: Keskin et al. (1995) reported an average sperm motility of 65.0±2.9%, Keskin et al. (1997) reported sperm motility ranging from 10-40% in 26-week-old roosters, which later increased to 95%, with an average of 72.1%, Tuncer et al. (2006) found the average sperm motility for Denizli roosters (a Turkish native breed) to be 72.33±0.80%, Tuncer et al. (2008) found the average sperm motility for Gerze roosters (another Turkish native breed) to be 74.28±0.73%, Uysal et al. (2011) reported an average sperm motility of 86.2±9.3%, and Kuzlu (2015) found an average sperm motility of 77.0±9.56% in turkeys. Sun et al. (2019) reported a value of 67.84 ± 2.83 (n=15) in high-motility sperm samples and 38.85 ± 2.83 (n=15) in low-motility samples. Boz et al. (2021) performed a study on geese and analyzed various

semen characteristics. They reported that sperm motility rates ranged between 46.77 and 50.13%. Rafalska et al. (2025) reported sperm motility rates of 68.43 ± 3.58% in white turkeys and 57.43 ± 3.91% in yellow turkeys.

Table 4. *Reported sperm motility rates (%) in roosters and turkeys.*

Species	Value (%)	Reference
Rooster	65.0 ± 2.9	Keskin et al., 1995
Rooster	72.1	Keskin et al., 1997
Rooster	72.33 ± 0.80	Tuncer et al., 2006
Rooster	74.28 ± 0.73	Tuncer et al., 2008
Rooster	86.2 ± 9.3	Uysal et al., 2011
Turkey	77.0 ± 9.56	Kuzlu, 2015
Rooster	67.84-38.85 ± 2.83	Sun et al., 2019
Geese	46.77- 50.13	Boz et al., 2021
Turkey (white)	68.43 ± 3.58	Rafalska et al., 2025
Turkey (yellow)	57.43 ± 3.91	Rafalska et al., 2025

Sperm Concentration

Rooster sperm concentration is generally measured using a hemocytometer. Several studies on sperm concentration have reported varying results: Keskin et al. (1997) found an average sperm concentration of 3.0x10⁹/ml, Akçay et al. (2007) reported an average sperm concentration of 5.2±0.55x10⁹/ml in turkeys, Uysal et al. (2011) found an average sperm concentration of 3.2±1.0x10⁹/ml, and Kuzlu (2015) found an average sperm concentration of 3.5±1.3x10⁹ sperm/ml in turkeys. Boz et al. (2021) found sperm concentration to range between 220.47 and 370.19 ± 5.9 × 10^6 mL^{-1} in their study on geese. Prabakar et al. (2022) reported sperm concentration in turkeys ranging from 4.70 ± 0.33 to 7.05 ± 0.16 over a 12-month period. Di Iorio et al. (2024) investigated sperm concentrationin 145 roosters from 13 breeds, reporting values ranging from 0.42 ± 0.08 to 5.37 ± 0.08 mL, with a mean of 2.71 ± 0.08 mL. Rafalska et al. (2025) reported sperm concentration of 3.51 ± 0.12 in white turkeys and 2.18 ± 0.13 in yellow turkeys.

Table 5. *Reported sperm concentration (×10⁹/ml) in roosters and turkeys.*

Species	Value (10^9/ml)	Reference
Rooster	3.0	Keskin et al., 1997
Turkey	5.2 ± 0.55	Akçay et al., 2007
Rooster	3.2 ± 1.0	Uysal et al., 2011
Turkey	3.5 ± 1.3	Kuzlu, 2015
Geese (nx10^6 mL^{-1})	220.47-370.19 ± 5.9	Boz et al., 2021
Turkey	4.70 ± 0.33-7.05 ± 0.16	Prabakar et al., 2022
Rooster	2.71 ± 0.08	Di Iorio et al., 2024
Turkey (white)	3.51 ± 0.12	Rafalska et al., 2025
Turkey (yellow)	2.18 ± 0.13	Rafalska et al., 2025

Abnormal Spermatozoa Ratio

The determination of the abnormal spermatozoa ratio is a crucial criterion in assessing sperm quality. Normally, a certain proportion of abnormal spermatozoa is present in fresh semen, but this ratio can increase due to individual factors or during the freezing process. Abnormal spermatozoa lack fertilizing ability and, therefore, together with motility, have a direct effect on fertility (Keskin et al., 1997). Several studies have reported the following abnormal sperm percentages: Keskin et al. (1997) found an average abnormal sperm ratio of 4.8% in 47 ejaculates, Tuncer et al. (2006) reported an average abnormal sperm ratio of 7.33±0.18%, Tuncer et al. (2008) found an average abnormal sperm ratio of 6.32±0.10%, and Uysal et al. (2011) reported an average abnormal sperm ratio of 3.9±3.2%. Sun et al. (2019) reported a value of 10.68 ± 0.90 (n=15) in high-motility sperm samples and 11.19 ± 0.90 (n=15) in low-motility samples. Prabakar et al. (2022) reported that the abnormal spermatozoa ratio in turkeys ranged from 5.61 ± 0.38 to 9.00 ± 0.46 over a 12-month period.

Table 6. *Reported abnormal spermatozoa rates (%) in roosters.*

Species	Value (%)	Reference
Rooster	4.8	Keskin et al., 1997
Rooster	7.33 ± 0.18	Tuncer et al., 2006
Rooster	6.32 ± 0.10	Tuncer et al., 2008
Rooster	3.9 ± 3.2	Uysal et al., 2011
Rooster	10.68-11.19 ± 0.90	Sun et al., 2019
Turkey	5.61 ± 0.38-9.00 ± 0.46	Prabakar et al., 2022

Dead-to-living Ratio of Spermatozoa

The dead-to-living sperm ratio is an important parameter in determining the fertility potential of semen (Şeber, 2002). Studies on the dead-to-living sperm ratio have reported the following results: Tuncer et al. (2006) reported a dead-to-living sperm ratio of 21.65±0.81%, Tuncer et al. (2008) found an average dead-to-living sperm ratio of 19.71±0.73%, and in a different study, İnanç et al. (2017) determined the dead-to-living sperm ratio using an eosin-nigrosin staining method. After preparing smears on heated slides and applying the dye mixture, dead spermatozoa were identified by the purple coloration, while live spermatozoa were recognized by the absence of dye. They reported dead sperm ratios of 9.50±8.73%, 7.98±3.58%, 9.31±8.51%, and 9.31±6.86% across four groups. Prabakar et al. (2022) reported that the dead-to-living sperm ratio in turkeys ranged from 6.59 ± 0.46 to 10.17 ± 0.57 over a 12-month period.

Table 7. *Reported dead-to-living spermatozoa ratios (%) in roosters.*

Species	Value (%)	Reference
Rooster	21.65 ± 0.81	Tuncer et al., 2006
Rooster	19.71 ± 0.73	Tuncer et al., 2008
Rooster	9.50 ± 8.73 – 9.31 ± 6.86	İnanç et al., 2017
Turkey	6.59 ± 0.46-10.17 ± 0.57	Prabakar et al., 2022

CONCLUSION

Artificial insemination is an assisted reproductive technology that has been used in farm animals for many years, favored due to its high repeatability, allowing for the widespread use of males with desired genetic traits across large areas. The artificial insemination studies, which began in 1899 by Russian researcher Ivanov in various farm animals, continue to develop rapidly today. Over the years, numerous studies have been conducted, and numerous new techniques and methods have been developed.

In poultry, artificial insemination is mostly used in breeding programs. Although the procedure is easy to apply in commercial poultry operations, its use has not become widespread due to the labor required. However, it is commonly used in turkey farms due to the difficulties associated with natural mating. The primary reasons for preferring artificial insemination in breeding programs are its ability to allow for the proper recording of parent lines, to enable the insemination of approximately four times more animals compared to natural mating, and to ensure that semen of appropriate quality is used through sperm examination and analysis. Artificial insemination also facilitates the long-term preservation of genetic material, ensuring that the superior genetic traits of both male and female animals are passed on. Finally, it guarantees the pairing of genetically superior individuals, ensuring the continuity of breeding without the issues that may arise from natural mating.

Considering the potential benefits of artificial insemination, which remains limited in use among poultry species, it is clear that much more research is required in this field. To ensure that artificial insemination becomes more effective and widely adopted in poultry production, it is necessary to develop and standardize application protocols specific to different species and breeds. Important parameters such as insemination frequency, timing, semen volume, dilution rate, and the composition of diluents should be optimized and clearly defined within these protocols. Moreover, further research is essential for improving long-term semen preservation and the success of insemination using frozen-thawed semen. This includes studies on extenders, cryoprotectants, thawing temperatures and durations, and advanced freezing technologies. Greater attention

should also be given to biotechnological approaches, particularly the use of fertility-related molecular markers in the selection of genetically superior males, as well as the discovery of new markers. Since insemination must be performed as quickly as possible after semen collection, the development of automated systems is crucial to improve both efficiency and reduce labor requirements. These studies will significantly contribute to the advancement of artificial insemination in poultry, supporting genetic improvement efforts and promoting high-quality and sustainable animal production.

ETHICAL APPROVAL

The study, entitled " Artificial Insemination and Spermatologic Parameters in Poultry: A Review", was conducted in accordance with the relevant scientific, ethical and citation rules. No falsification was made of the collected data, and this study has not been sent to any other academic media for evaluation. As it does not require ethics committee approval, it can be considered to be in accordance with the relevant ethical standards.

CONFLICT OF INTEREST

The authors declare no conflict of interest in this study.

AUTHOR CONTRIBUTION

All authors contributed equally.

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