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Enhancing Fertility and Genetic Progress in Poultry through Artificial Insemination: Current Trends and Innovations

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ABSTRACT

Artificial insemination (AI) has transformed poultry reproduction by enabling genetic improvement, enhancing productivity, and addressing challenges such as disease transmission and mating inefficiencies. This method involves manually depositing semen into the female reproductive tract, maximizing the utilization of genetically superior males. AI is particularly valuable in overcoming size disparities in broilers, optimizing mating in specialized breeds, and preserving endangered avian species. Semen is collected using techniques like abdominal massage, gloved-hand methods, and electroejaculation, with abdominal massage being the most widely used for its simplicity and non-invasive nature. Collected semen is evaluated for parameters such as volume, sperm concentration, motility, viability, and genetic integrity. Nutritional interventions and optimal management practices enhance semen quality, while extenders like BPSE improve viability for up to 24 hours at 5°C. Cryopreservation protocols, including the Pellet and Straw methods, enable long-term genetic preservation at -196°C, while tailored thawing protocols restore sperm functionality for effective use in AI. Timely insemination, typically after oviposition, minimizes oviduct obstructions and increases fertility rates, with doses of 100–500 million sperm cells session^{-1} ensuring high success rates. Technological advancements, including cryopreservation, genomic selection, and automation, have further improved AI's efficacy. Despite challenges such as storage-induced sperm damage and ethical concerns, AI remains indispensable for sustainable poultry reproduction, significantly contributing to genetic diversity, enhanced productivity, and global food security through the integration of advanced technologies and sustainable practices.

KEYWORDS: Artificial insemination, acrosome integrity, CASA, cryopreservation, sperm motility

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1. INTRODUCTION

Artificial insemination (AI) has revolutionized poultry reproduction by enhancing genetic improvement, productivity, and disease control. This technology involves the manual deposition of semen into the female reproductive tract, ensuring efficient utilization of genetically superior males to propagate desirable traits (Getachew et al., 2016; Hafez and Hafez, 2013). AI addresses challenges such as physical incompatibilities, random genetic selection inefficiencies, and disease transmission risks, making it indispensable for specialized breeds. The process begins with semen collection, commonly achieved through non-invasive methods like abdominal massage, which minimizes stress and ensures the collection of high-quality ejaculates (Bezerra et al., 2024). Following collection, semen is evaluated for volume, sperm concentration, motility, and viability-parameters critical to fertility (Rajput et al., 2024). Factors such as breed, age, nutrition, and environmental conditions significantly influence semen quality. For instance, chickens typically produce 0.2–0.5 ml of semen ejaculation^{-1}, with sperm concentrations ranging from 3 to 8 billion milliliter^{-1} (Mkpughe and Bratte, 2015). Nutritional interventions, including diets enriched with vitamin E, selenium, and omega-3 fatty acids, have been shown to enhance sperm motility and viability (Salas et al., 2019). Optimal housing and management practices are equally important, as stress, temperature fluctuations, and inadequate conditions adversely impact semen quality (Singh et al., 2023). To preserve semen viability, extenders such as Beltsville Poultry Semen Extender (BPSE) and Lake's Extender are widely used, allowing sperm to remain viable for up to 24 hours at 5°C (Sun et al., 2022). Timing insemination after oviposition, typically in the afternoon when hens have completed egg-laying, minimizes obstructions in the oviduct and improves fertilization rates (Getachew et al., 2016). AI facilitates selective breeding, enabling controlled mating to propagate traits such as enhanced egg production, disease resistance, and feed efficiency. Commercial layers developed through AI can produce over 300 eggs annually, significantly boosting productivity (Oliveira et al., 2022). AI also supports conservation efforts by allowing cryopreservation of semen from endangered avian species, preserving genetic diversity for future use (Bolton et al., 2022). Additionally, AI overcomes reproductive challenges in broilers, where size disparities between males and females hinder natural mating (Decuypere et al., 2010). Technological advancements have further enhanced AI's efficacy, particularly through innovations in cryopreservation, genomic selection, and automation. Cryopreservation techniques using advanced cryoprotectants and freezing protocols have improved the survival rates of sperm after thawing, ensuring the preservation of valuable genetics (Mohammad et al., 2021). Genomic selection and molecular markers have accelerated genetic improvement by allowing precise identification of superior traits (Sharma et al., 2024). Automation in semen collection and deposition has streamlined large-scale operations, reducing labor demands and improving consistency (Quelhas et al., 2023). Advances in semen extenders and storage techniques have optimized sperm viability during transportation, further enhancing AI's practical applications (Waberski et al., 2019). Despite its numerous advantages, challenges persist in maintaining semen quality during extended storage, optimizing fertilization rates, and addressing genetic bottlenecks in commercial lines. Ethical concerns and animal welfare issues related to intensive breeding practices highlight the need for more sustainable AI methods (Farstad, 2018). Integrating AI with emerging technologies like CRISPR gene editing, advanced phenotyping, and big data analytics offers promising opportunities for precise genetic improvements, enhanced biosecurity, and sustainable production systems (Olaniyan et al., 2024). These innovations aim to overcome current limitations while ensuring ethical and efficient poultry production. AI remains a cornerstone of modern poultry reproduction, contributing significantly to improving productivity, preserving genetic diversity, and addressing global food security challenges. By continuing to innovate and implement sustainable practices, AI will play a crucial role in shaping the future of poultry reproduction and genetics.

2. ANATOMY OF MALE POULTRY

The male reproductive system in poultry is specialized to produce, store, and deliver spermatozoa for fertilization. It comprises paired testes, epididymides, vas deferens, and a rudimentary phallus (Froman et al., 2000). Testes Located near the kidneys along the dorsal body wall, the bean-shaped testes are responsible for sperm and hormone production. Their size fluctuates with sexual activity, enlarging during the breeding season. Notably, the left testis is often larger than the right testis (Lake, 1957). Epididymis Adjacent to each testis, the epididymis is a tubular structure where spermatozoa mature before being transported. Vas Deferens These highly coiled ducts connect the epididymides to the cloaca, facilitating the passage of mature sperm. They also serve as storage sites for spermatozoa until ejaculation (Bakst, 1986). Phallus Unlike mammals, most male birds lack a well-developed copulatory organ. In species like chickens, the phallus is rudimentary, and fertilization occurs through cloacal contact during mating (El et al., 2021).

3. COLLECTION METHODS

3.1. Abdominal massage method

This non-invasive technique, introduced by Burrows and Quinn in 1937, is the most commonly used method for

Table 1: The reported sperm concentration and volume of semen ejaculate

Breed	Range of semen volume (Ml)	Range of sperm concentration ($\times 10^9$ ml^{-1})	References
Thai native chickens	0.22–0.35	3.13–3.29	(Mussa et al., 2023)
Local breeder cocks	0.36–0.50	2.5–3.0	(Bah et al., 2001)
Broiler breeder cockerels	0.30–0.40	2.0–2.5	(Abioja et al., 2022)
Light chicken breeds	0.05–0.5	Data not specified	(Benoff et al., 1981)

semen collection in poultry. It involves restraining the male bird and gently stroking the back from behind the wings toward the tail with firm, rapid strokes. This stimulation leads to the erection of the phallus, allowing semen to be collected from the external papilla of the vas deferens (Getachew, 2016, Lake, 1957).

3.2. Electroejaculation

While more commonly used in larger mammals, electroejaculation has been adapted for use in avian species, particularly in research settings. This method involves inserting a probe into the cloaca to deliver electrical stimuli, inducing ejaculation. Due to the potential for stress and injury, its use in poultry is limited and typically reserved for cases where the abdominal massage method is ineffective (Frediani et al., 2019).

3.3. Gloved-hand technique

Predominantly used in species like turkeys, this method requires the handler to manually stimulate the male's cloacal region using a gloved hand to induce ejaculation. While effective, it demands significant skill and experience to ensure the safety and comfort of the bird (Getachew et al., 2016).

3.4. Two-man method

In this technique, one person holds the bird while another strokes the back and collects semen into a funnel. This method requires coordination between two individuals and is used in certain settings (Shanmugam and Mahapatra, 2021).

Table 2: Advantage and limitations of different methods of semen collection

Methods/ Techniques	Advantage	Limitations	References
Abdominal massage method	-Simple and non-invasive. - No need for special equipment. - Suitable for most poultry species.	- Requires trained personnel. - Can be stressful for the bird if not done properly. - Not effective in certain species (e.g., turkeys).	(Girndt et al., 2017 and Kanatiyanont et al., 2012)
Electro ejaculation	-Suitable for males with handling difficulties or low libido. - Non-invasive; avoids physical handling stress. - Effective in cases where manual methods fail.	- Requires specialized equipment and expertise. - May cause stress or discomfort to the bird due to electrical stimulation. - Risk of injury or reduced semen quality due to excessive stimulation.	(Frediani et al., 2019)
Gloved-hand technique	-Simple, quick, and cost-effective. - Can be performed frequently without specialized tools. - Provides good semen quality and quantity.	- Requires proper training to avoid injury and stress to the bird. - Suitable mainly for cooperative birds, limiting use in aggressive or untrained males. - Requires practice to master the appropriate pressure and handling technique.	(Gee et al., 2004)
Two-man method	- Useful for uncooperative or larger poultry species. - Allows better control over handling and restraint. - Suitable for birds with aggressive tendencies.	- Requires two trained handlers, increasing labor requirements. - Can lead to stress in birds if not handled carefully, affecting semen quality. - Risk of injury due to improper restraint during collection.	(Burrows and Quinn, 1937)

4. POULTRY SEMEN EVALUATION

4.1. Semen volume and concentration

Semen volume and sperm concentration are fundamental metrics. Volume varies by species and breed, with roosters typically producing between 0.2 to 1.0 ml ejaculate^{-1}. Sperm concentration can reach up to 5 billion sperm ml^{-1}. These parameters are assessed using graduated collection tubes and spectrophotometric methods, respectively (Rajput et al., 2024).

4.2. Sperm motility

Sperm motility is a critical indicator of fertilization potential. Traditional assessments involve subjective microscopic evaluation, while advanced methods utilize computer-assisted sperm analysis (CASA) systems for objective measurements. CASA provides detailed data on motility patterns, enhancing the accuracy of fertility predictions.

4.3. Sperm viability and morphology

Viability assessments determine the proportion of live spermatozoa, commonly using staining techniques such as eosin-nigrosin. Morphological evaluations identify structural abnormalities that may impair function. These analyses are vital for understanding semen quality and its impact on reproductive success (Bansal et al., 2024).

4.4. Acrosome integrity

The acrosome is essential for oocyte penetration. Assessing its integrity, particularly in fresh versus frozen-thawed semen, is crucial. Giemsa Staining has been effective in evaluating acrosome status, aiding in the assessment of semen preservation methods (Andraszek et al., 2018).

4.5. Genetic integrity

Evaluating DNA fragmentation and chromatin structure provides insights into genetic integrity. Techniques such as the sperm chromatin structure assay (SCSA) are employed, as genetic defects can lead to reduced fertility and embryonic development issues (Evenson, 2022).

4.6. Sperm-egg interaction assays

In vitro assays, like the sperm-egg interaction test using the inner perivitelline layer of chicken eggs, assess the functional capacity of sperm to bind and penetrate the oocyte. This method evaluates multiple sperm characteristics and correlates highly with fertility outcomes (Bongalhardo et al., 2024).

5. INSEMINATION PROCEDURE

Before insemination, all equipment must be properly cleaned and dried. Insemination is most effective when performed after the majority of hens have finished laying, as the presence of a hard-shelled egg in the oviduct can obstruct the process and reduce fertility. Studies suggest that inseminating chickens after 3 PM yields better results. It is also challenging to inseminate non-laying hens. Typically, insemination begins when the flock achieves 25% egg production. Hens are inseminated twice in the first week, followed by weekly intervals. Experimental data indicates fertility rates of up to 90% when hens are inseminated every 3 days with 400–500 million frozen-thawed sperm cells. For chickens, insemination doses typically range from 100–200 million sperm cells session^{-1}. Given lower sperm concentrations and reduced fertility duration, 0.05 mL of undiluted pooled semen administered every 7 days is recommended. The hen's squatting behavior signals receptivity and readiness for insemination. Fertility declines later in the season, necessitating either more frequent inseminations or higher sperm doses as hens age. The hen is held upright with the left hand supporting the legs and tail tucked back against the operator's chest. Pressure is applied to the abdomen, particularly on the left side, causing the cloaca to evert and the oviduct to protrude. A syringe or plastic straw is then inserted approximately 2.5 cm (1 inch) into the oviduct, depositing semen near the vagina-uterus junction. Releasing pressure around the vent aids in retaining sperm within the reproductive tract (Bakst et al., 2013).

6. CRYOPRESERVATION PROTOCOL

Both freezing methods, the Pellet Method and the Straw Method, offer unique advantages and challenges. The Pellet Method involves placing small droplets of semen directly onto dry ice or into the vapor of liquid nitrogen, where the semen forms pellets. This method is relatively simple and cost-effective but can pose challenges related to hygiene and potential contamination. Additionally, handling frozen semen pellets requires more precision during thawing to ensure sperm viability. In contrast, the Straw Method is more standardized and widely used. Semen is loaded into small plastic straws, which are then sealed and subjected to controlled freezing in liquid nitrogen vapor. The straws are typically suspended about 6.4 cm (2.5 inches) above the liquid nitrogen, where they remain for about 10 minutes before being plunged directly into the liquid nitrogen for storage. This method offers better hygiene and provides more consistent dosing, as straws allow for easier handling, better protection of semen, and less variability in sperm concentration. It is also easier to implement on a large scale, making it more common for industrial semen storage in poultry breeding programs (Svoradová et al., 2021).

7. STORAGE

Once frozen, semen is stored in liquid nitrogen at a temperature of -196°C. This ultra-low temperature

ensures that the sperm remains viable indefinitely, provided that storage conditions are stable. Regular monitoring of liquid nitrogen levels is critical to prevent the sperm from thawing due to a drop in liquid nitrogen volume, which would result in loss of fertility. The key to long-term preservation is maintaining consistent storage temperatures and preventing contamination. Various storage containers, such as cryovials or cryotubes, are often used to ensure semen samples are stored securely. Regularly inspecting and refilling liquid nitrogen reservoirs is essential to prevent accidents or degradation of stored semen (Donoghue et al., 2000).

8. THAWING

Thawing frozen poultry semen requires careful attention to the thawing process to restore sperm function and viability. The method of thawing depends on the freezing technique used and the cryoprotective agent (CPA) incorporated into the semen during freezing. For example, semen frozen with Dimethylacetamide (DMA) is commonly thawed by immersing the straw or pellet in a 50°C water bath for about 20 seconds. This rapid thawing helps minimize sperm damage that may occur during thawing (Bellagamba et al., 1993). Proper thawing ensures that the sperm cells are not exposed to thermal shock, which can significantly reduce motility and fertilization potential. After thawing, semen is typically evaluated for motility, viability, and concentration before being used in insemination. The post-thaw evaluation is crucial because it directly impacts the success rates of artificial insemination (Çiftci and Aygün 2018).

9. CONCLUSION

Artificial insemination has revolutionized poultry reproduction, enabling genetic improvement, disease control, and conservation. By integrating advanced techniques like cryopreservation, genomic selection, and automation, AI optimizes productivity, enhances biosecurity, and supports sustainability. Despite challenges in semen quality maintenance and ethical concerns, innovations such as CRISPR gene editing and big data analytics offer solutions. The adoption of sustainable AI practices ensures its continued role in improving fertility, preserving genetic diversity, and addressing global food security challenges in the poultry industry.

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MM and KNB wrote the manuscript DJ,MG and PK analyze the manuscript AKM and SJ arrange the manuscript as per journal guidelines.

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