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A Review on Poultry; Semen Storage and Preservation

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A REVIEW ON POULTRY; SEMEN STORAGE AND PRESERVATION

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ABSTRACT

For the improvement of genetic resources and their maintenance, new techniques of assisted reproductive technologies have been developed. Cryopreservation of semen, embryo transfer and artificial insemination in mammals are used, while in the avian technique of artificial insemination after semen cryopreservation is proved fruitful. By using this developed technique, the best fertility rate in chicken can be obtained by artificial insemination. In vitro cryopreservation can affect the motility, life of sperm, viability, fertilizing ability. From mammalian sperm cell, the avian sperm cell is different in shape, size having polyunsaturated fatty acid. The use of suitable cryo-protectants and semen extender can save the viability and morphology of sperm and can provide a better result of AI by a good fertility rate. A good fertility rate can be achieved by storing avian, turkey and chicken semen at 2-8°C, 4-8°C and 7-8°C. This paper will help to conclude the script of semen collection, cryopreservation, evaluation, diluents used to maintain sperm viability and fertility ratio. This review uniquely synthesizes recent advancements in poultry semen preservation, focusing on novel cryoprotectants, semen extenders, and optimization techniques. It integrates emerging practices and technologies to provide new insights into enhancing semen quality, fertility rates, and overall reproductive efficiency in poultry. This review uniquely synthesizes recent advancements in poultry semen preservation, focusing on novel cryoprotectants, semen extenders, and optimization techniques. It integrates emerging practices and technologies to provide new insights into enhancing semen quality, fertility rates, and overall reproductive efficiency in poultry

Keyword: Avian**,** cryopreservation; diluents, evaluation, fertility.

INTRODUCTION

The demand for poultry eggs and meat is increasing by the constant increase of the global population. Developed assisted reproductive technology (ART) of semen production, collection and preservation, and inseminate it to the female helps to increase the production efficiency at low costs (Lukaszewicz et al., 2020). Assisted reproduction technologies (ART's), such as artificial insemination (AI) have a great role in increasing poultry production, by using genetically superior quality cockerel having great productive performance (Getachew, 2016; Benoff et al., 1981). In poultry breeding farm, AI is widely used to procreate next generation for increasing meat and egg production but cryopreserved avian semen indicates less fertility rate that caused limited AI (Ushiyama et al., 2016). Semen quality and fertility rate of birds affected by age, season, nutrition, management, spermatogenesis and semen collection method (Mohan et al. 2016; 2018). In the poultry industry, rate of AI is increasing and helps to assort good quality semen. Better results of insemination are possible after the proper protocol of collection, preservation and AI technique. Further increasing use of AI emphasizes the need for the distribution of good

quality sperm. The freezing and thawing procedure also affect the quality of semen (Blesbois, 2011). Different studies occurred on cryopreservation of poultry semen to prevent damages of sperm cell by using a cryoprotective agent (CPA) (Abouelezz et al., 2017; Mosca et al., 2019).

The normal ejaculated volume of chicken semen is 0.2-0.5 ml with a high concentration (Getachew, 2016). In the laboratory by proper processing and using suitable semen extenders are helpful to increase the semen volume. Semen quality depends on semen extender, diluents, storage condition (time, aeration, temperature) (Das et al., 2015; Çiftci and Aygün, 2018).

Good fertilizing potential ability is achieved by using semen having live, motile sperm with correct morphological structure. In reptiles, fishes, birds and amphibian semen storage pouch is commonplace to store sperm in the female reproductive tract (Holt, 2011). In the avian female reproductive tract, sperm can remain viable and fertile for a longer period up to 15 weeks at bird body temperature (Sasanami et al., 2015). (Matsuzaki et al., 2015) reported that in female semen storage tubules, sperm cell remains viable and motile at low pH <6.0 and in low oxygen condition. Avian sperm is less viable as mammal's sperm due to the presence of polyunsaturated fatty acids (PUFA), sperm shape and membrane fluidity (Çiftci and Aygün, 2018). This review of the study summarizes to establish the semen storage and preservation techniques in commercial poultry to achieve higher production from superior breeds.

Physiology of Chicken male Reproductive System

Gonadotropin-Releasing Hormone (GnRH) from the hypothalamus activates the secretion of Luteinizing Hormone (LH), Follicle Stimulating Hormone (FSH) and steroids secretion of reproductive glands (estrogen and testosterone) by the adenohypophysis. The male sex hormone testosterone is produced by the action of luteinizing hormone on leydig cells by progesterone secretion (Senger, 2003; Thelie et al., 2018). Spermatogenesis occurs in seminiferous tubules with the major hormone testosterone (Senger, 2003; Thelie et al., 2019). Normal mating behaviour of male and secondary sex characters are activated by testosterone. Testosterone hormone enhances the accessory sex glands functioning and maintenance of the male reproductive duct system and sperm production. Testosterone hormone not only aids in spermatocytogenesis but also helps for the transport of sperm and deposition of sperm in the female reproductive tract (Johnson and Lee 2016; Hu and Zadworny, 2017; Scanes., 2019;). As the cockerel age increases and it reaches maturity, testosterone production is stimulated by the increasing concentration of gonadotropins hormone in blood circulation (Marques et al., 2000; Handelsman et al., 2018; Etches, 1996). Adenohypophysis of the pituitary gland secretes gonadotropin-releasing hormone having FSH and LH in males (Barrington and Ernest, 2020). In the secondary spermatocyte stage, FSH acts on the seminiferous tubules and supports spermatogenesis, while Leydig cells are activated by the LH for testosterone and others androgens production (Hafez and Hafez 2010; Oduwole et al., 2018).

Spermatogenesis

Spermatogenesis and spermatocytogenesis are the two phases for sperm production in seminiferous tubules of testes (Gordon and Taylor, 2005). Different authors reported that spermatogenesis is a metamorphic process in which different changes occur for sperm tail formation. Sertoli cells and leydig cells presence and their number depend on the numbers of sperm production (Gordon and Taylor, 2005; Teves et al., 2020). A single organelle (Golgi apparatus) found in the head of sperm near the nucleus and it helps to form a subcellular organelle for acrosomal reaction. After the acrosomal development, a cap is formed over the

nucleus and this cap spread over the nucleus until it covers two-third of an anterior nucleus (Lerer-Goldshtein et al., 2010; Teves et al., 2020; Yasuno et al., 2013; Senger, 2003). After sperm production, the maturation phase occurs that increases sperm quality and motility. In the maturation phase completely differentiation of spermatids (flagella formation in end piece, mitochondria in midpiece and shaping of nucleus and neckpiece formation) occurred (Lehti and Sironen 2017; Leao et al., 2021).

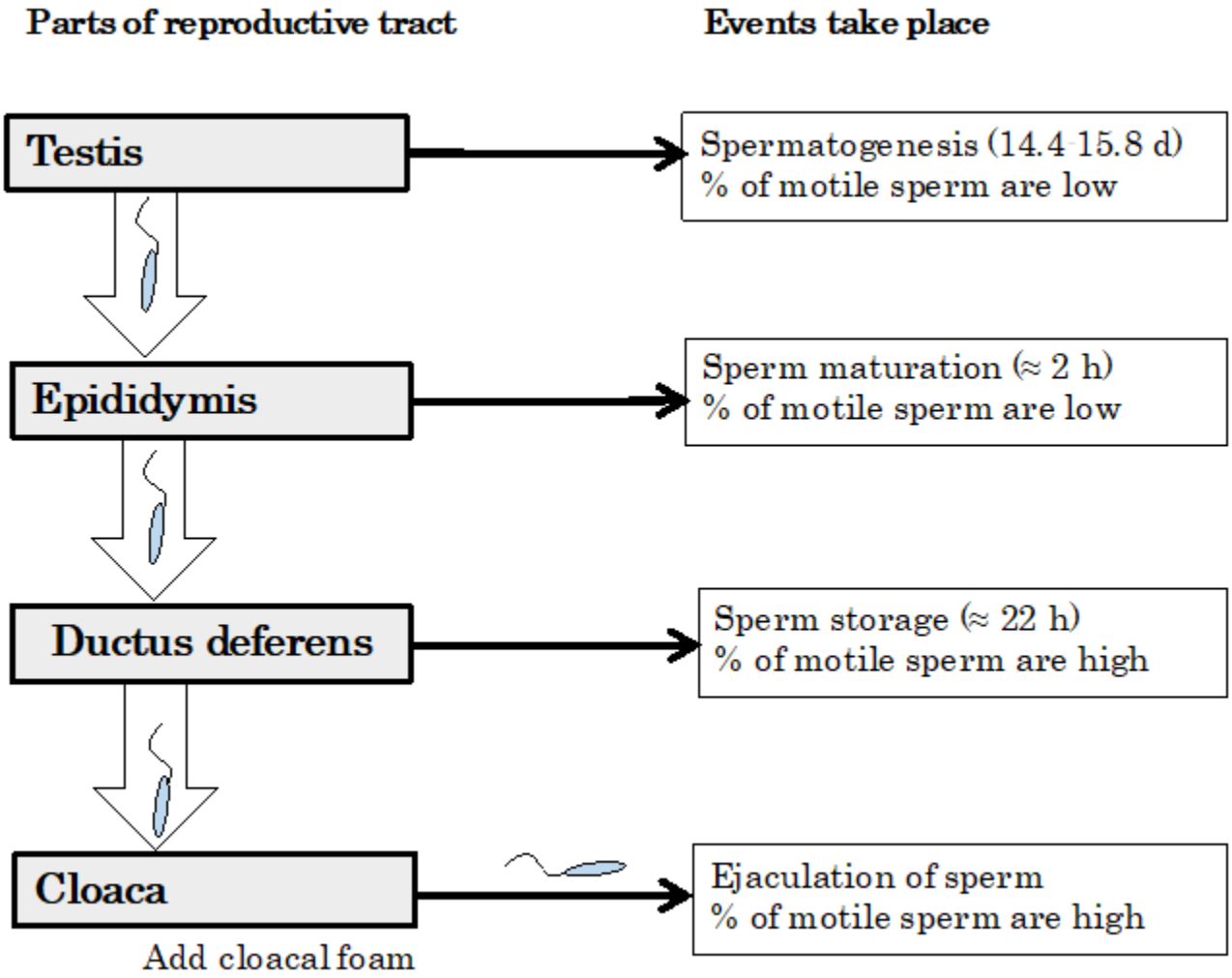

Figure 1: Sperm production and transportation process in male (Sarkar, 2020).

Characteristics and Chemical Components of Chicken Semen

In the male cockerel reproductive tract, semen containing sperm and seminal fluid is produced by epididymis, testes and vas deferens. All this occurred by the neuroendocrine reflexes. Testicular developments and secretion occur by testosterone hormone through regulation of pituitary hormones FSH and LH (Berndtson, 2014; Long, 2014). The characteristics and chemical components of semen ejaculated volume (ml) 0.2-0.5, sperm concentration 3000-7000 ($\times 10^6$/ml), motile sperm 60-8-% and 7.2-7.6 pH in cockerel (Getachew, 2016).

Gross evaluation of Semen

Semen evaluation is a technique that helps to determine the quality and quantity of semen. In this technique semen volume, colour, consistency, motility, viability and morphology of spermatozoa are determined. It helps to relate the fertilizing capacity of fresh semen with frozen semen (Vasan, 2011; Mohan et al. 2016; 2018). Semen evaluation is necessary to determine sperm quality and several sperm in a single dose (Senger, 2003). Semen evaluation has a great role to enhance the fertility rate, decrease embryo mortality (Donoghue and Wishart, 2000). Different laboratorical techniques like the fluorescent stain technique and histological stains technique are helpful to judge the metabolic activity, motility and dead ratio of sperm (Farah et al., 2013; Çiftci and Aygün, 2018). Semen volume, colour, consistency, motility and morphology are determined by traditional methods. These procedures correlate with the fertility ratio of fresh semen after ejaculation. The fertility and hatchability ratio of avian eggs depends on semen quality (SO et al., 2008). About 5 billion sperm per ml present in a single collection from domestic cockerels (Gordon and Taylor, 2005). By using a computer-assisted light microscopy technique, (Villaverde-Morcillo et al. 2015) analyzed chicken and partridge semen characters. A method by (Santiago-Moreno et al. 2012) is helpful to assessed sperm motility and concentration level. Motility of spermatozoa, non-progressive motility (active tail with no forward movement), straight line and curve line velocity of rooster semen was analyzed (Davila et al., 2015).

The milky white colour of semen shows high sperm density with low sperm numbers (Hambu et al., 2016). Different domestic bird species have variation in sperm colour due to various secretions by reproductive glands. Any contamination in sperm changes their colour like feces and urine makes semen brown and green (Johnson, 2020). Excessive force for semen collection or any injury in the reproductive tract shows blood clots or flakes in semen. Contaminated samples can be used after dilution in antibiotic like penicillin, dihydrostreptomycin that helps to reduces the loss of sperm and increases the fertility rate (SO et al., 2008).

For sperm functioning and maintenance of viability, regulation of intracellular pH is significant (Nishigaki et al., 2014). Different avian species and breeds having slight variation in semen pH. High sperm motility is seen at optimum pH 7-7.4, if pH decreases to 6.4 it becomes acidic and causes damage to the sperm cell membrane and acidic pH is not suitable for semen preservation (Latif et al., 2005; SO et al., 2008). In vivo and in vitro, naturally antioxidant have a great role to maintained or increased avian sperm life (Partyka et al., 2012). The use of antioxidant vitamin E to store chicken sperm at 4c for 24 hours enhances the sperm viability, motility and lessen the morphological defects during storage (Tabatabaei et al., 2011), while another scientist in 2010 seen better sperm motility and viability by using vitamin E for storage of sperm at 4 ^{0}C for 72 hours (Asmarawati and Yuwanta, 2010).

Greater secretion from accessory glands increases the semen pH and reduces the semen quality (Getachew, 2016). Complex secretion from accessory sex glands is seminal plasma that has an imperative role for sperm functioning in the reproductive tracts of male and female birds (Juyena and Stelletta, 2012). By collection and measurement of semen, pH can be decreased and the narrowing shape of semen collection tubes caused the breakdown of the sperm cell. Dead sperm in semen sample increases the ph due to ammonia evolution (Salisbury et al., 1978; Okoro et al., 2016).

According to studies, the eosin-nigrosin technique is helpful to determine the cockerel semen morphology. It contains 1.6% eosin and 6% nigrosin diluted with 20 µm Beltsville Poultry Semen Extender (BPSE) and containing 2 ml diluted semen solution with stain and this should be set for 2 minutes before slide preparation. Better results depend on the proper protocol used. Viable, non-viable, motile, dead and damaged sperm ratio can be easily evaluated by the eosin-ergosin technique (Lukaszewicz et al., 2008; Getachew, 2016).

The size and shape of a domestic avian sperm cell is similar, while slight variation is seen in the morphology of poultry semen according to mammals. Cytoplasmic membrane covered the sperm cell and acrosome having inner spine covered by a conical-shaped cap. Nuclear material present in a headpiece of sperm while midpiece of cockerel sperm is longer in size according to other birds sperm having centrioles embraced by the sheath of mitochondria (Alkan et al., 2002; Einarsson et al., 2009; Okoro et al., 2016). In the l ab, morphology and defects like neck bending and damage, acrosomal damage, head swelling and defects in the tail of Cockerel sperm can be assessed (Tesfay et al., 2020; Alkan et al., 2002).

Semen Collection Methods and Dilution

Two basic methods (abdominal massage method and teaser female birds) are used for semen collection in birds. In chicken and turkey, the abdominal massage method is mostly used for semen collection and some researchers used this method and elaborate it (Chelmonska et al., 2008). The abdominal massage method is a non-invasive method for semen collection from a rooster. For this, restraining and gently stroking of the back of male bird from behind the wing. After rapid stroking, the phallus (male sex organ) erect and the handler gently squeezes the cloaca and collect the sperm in a container (Farah et al., 2013; Çiftci and Aygün, 2018; Gee, 1995).

The use of different extender effects on sperm motility and viability. Different breeders use different semen extender and find out the best poultry semen extender by comparing each other in different species (Iaffaldano et al., 2016; Holt, 2011; Rakha et al., 2016; Shanmugam and Mahapatra, 2019). Avian species have variation in sperm concentration and ejaculate volume (García-Herreros, 2016). In vitro stored diluted sperm with extender have greater sperm viability than undiluted sperm. Semen dilution at 8-10 folds before storage results in the maintenance of sperm quality and fertilizing ability in broiler breeders. Dilution or extender quality and quantity effects on fertilization rate and at a high dilution rate of 1:10 and 1:20 decrease fertility (Sarkar, 2020). According to other avian species guinea fowl have a low fertility rate, and at a low dilution rate sperm viability and fertilizing ability rate of guinea fowl is kept up (Hudson et al., 2016). Beltsville poultry semen extender, Ek extender, lake extender are mostly used to extend and preserve poultry semen (Siudzińska and Łukaszewicz, 2008; Morrell et al., 2005).

Different buffer solutions are used as semen diluents for providing an optimum environment for sperm viability and increases semen volume to make more doses (Vasicek et al., 2015). According to poultry semen attributes semen diluents are used for providing optimal pH (7.0-7.4) and osmotic pressure (Getachew, 2016). According to mammals, in chickens and turkeys female reproductive tract sperm remain viable for long period (70 days in turkeys and 32 days in chicken) (Pearlin et al., 2021; Hafez and Hafez, 2010). In a female reproductive tract of chicken, sperm nest is second place for semen storage at the infundibulum and magnum junction. For fertilization, releasing of spermatozoa from sperm nest is occurred by stimulation of the ovum in the infundibulum (Slanina et al., 2014). By using 1:6 semen with diluents, 70 doses of semen can be prepared to have 38 million sperm per dose for AI. Better fertility rate produced by AI in avian species than natural mating (Mohan et al., 2016; Brillard, 2003).

Semen Preservation for Short and Long Time

It is reported that diluted fowl semen can be stored for a short period of about 24 hours without changing viability and fertility rate (Lukaszewicz et al., 2008; Siudzińska and Łukaszewicz, 2008). Semen extenders are used for short or long term semen preservation and

these extenders increase the reproductive effect of Cockerel and increase the breeding rate at a low cost by AI. Sodium chloride (saline) solution, complex diluents having osmotic regulator ability, buffer and energy source are used to preserve semen (Blesbois, 2011). After semen evaluation in a laboratory, it is reported that the use of a semen extender for semen preservation is helpful to sustain sperm quality (Ibarra et al., 2020; Benoff et al., 1981). Semen quality of stored semen depends on the quality and type of extender used, and preservation condition like time, temperature. Sperm motility and fertilizing capacity of raw fowl undiluted semen is usually decreased within an hour after collection (Dumpala et al., 2006). In rooster semen, the proteomic analysis of seminal plasma was explored first time (Marzoni et al., 2013). Great effect on quail sperm survival seen by using transferrin and albumin obtained from the uterovaginal junction of quail (Matsuzaki et al., 2015).

For broiler breeding, cryopreservation has the potential for long-term preservation of diluted semen. Glycerol and dimethylacetamide (DMA) are used for diluents for chicken semen conservation. Frozen semen with DMA pellet has a high fertility ratio according to semen stored with glycerol straw (Long et al., 2014; Rakha et al., 2016; Shanmugam and Mahapatra, 2019). The suitable temperature for long term storage of avian semen is 4-10c (Brillard, 2003). 4-8 c temperature is suitable for storage of turkey sperm (Slanina et al., 2014). A good fertility rate of chicken semen is seen after storage at 7-8c for 24 hours (Mohan et al., 2016; 2018).

Cryoprotectants

A technique to preserve sperm is cryopreservation that can alter the structure of sperm and metabolism of spermatozoa and changes seen in the motility of sperm and ATP content for survival (Long, 2006). Cryopreservation of reproductive cells of avian endangered breeds is a suitable technique for the conservation of endangered species (Rakha et al., 2016; Thelie et al., 2019). An effective and simple technique of semen cryopreservation is a fruitful way to preserve the long term genetic resource of chicken breeds (Silversides et al., 2012). Japanese and Korean breeds chicken sperms were successfully treated by cryoprotective agent N-methyl acetamide (Mosca et al., 2019; Sasaki et al., 2010; Abouelezz et al., 2015). An optimal cryoprotective agent helps to prevent sperm cell from damage during freezing (Lee et al., 2012; Miranda et al., 2018; Mphaphathi et al., 2016). In vitro preservation of germ cell is a superlative technique for the conservation of endangered breeds of avian have been developed in Europe, America and Africa (Paiva et al., 2016). In cryobanks, different semen containing sperm and seminal fluid and primordial germ cells of birds, semen of other mammalian and fishes are stored. While some cryobanks have stored some somatic cell with the hope that these cells be reprogrammed into effective germ cells (Sekita et al., 2016; Canovas et al., 2017; Mahabadi et al., 2018). For the preservation of avian genetic resources and rare breeds, semen cryopreservation is proved as a less expensive and non-invasive method (Ehling et al., 2012). Cryopreservation of primordial germ cells and goandic tissue is developed, while embryo conservation in avian is not possible. Preservation of germ cells is proved highly invasive and more economic according to semen cryopreservation. Basic key points for semen cryopreservation are external cryoprotectants (sucrose, trehalose) and internal cryoprotectants (glycerol, dimethylformamide, ethylene glycol), freezing, packing and thawing methods (Khiabani., 2017; Miranda et al., 2018; Lotfi et al., 2017).

Sperm freezing techniques were used for chicken mainly, but now it is used for turkeys, ganders, and guinea fowl (Seigneurin et al., 2013; Santiago-Moreno et al., 2017). An antioxidant like selenium, hyaluronic acid use in semen extender shows a negative effect of reactive oxygen species during cryopreservation (Lotfi et al., 2017; Ebeid, 2012; Rui et al., 2017). Motility and viability of thawed and frozen semen is increased by using metformin, an activator of 5-AMP Activated Protein Kinase (Nguyen et al., 2015). Cryopreservation

decreased sperm motility results in lessening the fertility rate and cryopreserved semen used on the chicken farm is limited (Hou et al., 2008). Semen storage at low temperature lessens the sperm cell metabolism and maintains its viability for long period (Karunakaran et al., 2007). At high temperature, approximately at 41℃ sperm become died, while it remains motile at 5, 15, and 25℃. A high egg fertility rate is seen by using fresh raw semen according to frozen semen (Blesbois et al., 2007).

CONCLUSIONS

Conclusively, it can be said that the physiochemical properties of sperm affect the viability, motility and fertilizing of sperm. Alteration in temperature and pH can alter the metabolic activity of sperm. The use of suitable diluents can enhance sperm life and quality. Improper handling, storage, and processing can reduce semen quality. Different poultry species have minor changes in sperm morphology and size, so use suitable diluents and temperature for storage. Semen evaluation methods should be strengthened for better results.

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CONFLICT OF INTEREST

All authors declare no conflict of interests.

AUTHOR CONTRIBUTIONS

All the authors are contributed to written, design, checking and approving the final version of the manuscript.

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