Motility, Viability and Fertilizing Ability of Avian Sperm Stored Under in Vitro Conditions

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ABSTRACT
In physiological condition, avian sperm can be stored within the sperm storage tubules of female reproductive tract and may able to fertilize eggs up to 15 weeks. The long-term viability and fertilizing ability of sperm is reduced when avian sperm are stored in vitro conditions. The motility, viability and fertilizing ability of avian sperm depends on in vitro storage conditions. Many factors can affect in vitro sperm motility, viability and fertilizing ability such as storage temperature, pH of extenders, osmolarity, sperm dilution rate, and seminal plasma. Researchers are trying to extend longevity of avian sperm during in vitro condition by applying the knowledge of in vivo sperm storage mechanism(s) and sperm biology. This paper reviews the sperm motility, viability and fertilizing ability of main poultry species stored in vitro conditions. This study will help to understand a scenario of in vitro avian sperm motility, viability and their fertilizing ability.

Keywords
avian sperm, fertilizing ability, in vitro storage, motility, viability

1. Introduction
In physiological condition, the capacity of sperm to store in the female reproductive tract is relatively commonplace in reptiles, fishes, birds and amphibians (Holt, 2011). In mammals, some species of bat can also store sperm in the female reproductive tract for several months (Holt and Lloyd, 2010). Most mammalian females possesses a specialized reproductive structure for sperm storage in vivo condition (Suarez, 2010). Depending on the species, the capacity of sperm storage period and fertilizing ability varies from several hours to years. In avian species, ejaculated sperm can be stored in the lumen of sperm storage tubules (SSTs) and may retaining fertilizing ability up to 15 weeks at body temperature 41 °C (Sasanami et al., 2013). The process how sperm remains in the female reproductive tract for longer period of time still not fully understood. We have limited information regarding long-term sperm storage regulatory mechanism in vivo condition. The researchers are eagerly trying to discover the mechanisms underlying long-term sperm storage in vivo condition. Research previously conducted by Matsuzaki et al. (2015) found that sperm remains in the lumen of SSTs in quiescent state of motility at low pH (<6.0) under hypoxic condition. This unique feature may prolong sperm storage in the SSTs and enables female poultry to produce a series of fertile eggs following a single copulation event or artificial insemination (AI).

Except cryopreservation, the biotechnologists have not yet been able to develop any artificial method of achieving the goal of long-term sperm viability in vitro condition. In contrast to domestic livestock species, cryopreservation is not a reliable source for storing avian germplasm due to low fertilizing ability of frozen/thawed sperm (Long, 2006; Long et al., 2014). Unlike mammals, the avian sperm is probably not a reliable source due to differences in sperm shape, membrane fluidity and high amount of polyunsaturated fatty acids present in avian
sperm (Çiftci and Aygün, 2018). Due to cryopreservation, the plasmalemma of avian sperm damages and spermatozoa becomes abnormal (Bakst and Sexton, 1979). Cryopreservation damages avian sperm, decreases sperm survivability and morphological integrity which is a major limitation of avian sperm storage (Blesbois et al., 2005). As avian sperm can be stored in the reproductive tract for a considerable period of time, this fact has been a source of interest and researchers are attempting to improve in vitro sperm survivability by applying the knowledge of in vivo sperm survival mechanisms.

The goal of modern poultry industry is to provide high quality meat and eggs. Nowadays, artificial insemination (AI) is being used widely in the breeding farm to propagate next generations. AI is often limited due to poor survival of cryopreserved avian sperm (Ushiyama et al., 2016). In this review, motility, viability and their fertilizing ability of sperm stored in vitro liquid conditions are reviewed with the knowledge of in vivo sperm survival mechanism(s). Moreover, other factors that may affect in vitro sperm motility, viability and fertilizing ability are also considered in this study. Probably, no study has been conducted earlier which considered most of these factors related with sperm motility, viability as well as fertilizing ability in vitro conditions. This study will help to understand an overall scenario of in vitro avian sperm motility, viability and their fertilizing ability.

2. Motility, viability, and fertilizing ability of avian sperm in different physiochemical conditions

2.1 Poultry semen extenders

Poultry semen extender is liquid in nature that protect the sperm and extends their longevity in vitro with a view to maintaining sperm fertilizing ability. The ingredients and composition of extenders varies from one extender to another. There are some well-known extenders that are using widely in poultry industry. Among the commercial extenders, Beltsville poultry semen extender, EK extender and Lake extenders are widely used in poultry industry (Sexton, 1977; Sexton and Fewlass, 1978; Sexton and Giesen, 1982; Giesen and Sexton, 1983a; Giesen and Sexton, 1983b; Sexton and Giesen, 1983; Morrell et al., 2005; Siudzińska and Łukaszewicz, 2008). Sperm viability and fertilizing ability varies from one extender to another extender. The breeders compared different poultry semen extenders with different poultry species to find out the suitable poultry semen extender (Sexton, 1988; Iaffaldano et al., 2016; Rakha et al., 2016). Still now, there is no reliable extender that can fully protect the sperm in vitro condition, as sperm quality deteriorate on storage time. For achievement of better fertility, two-stages extender also developed in chicken. The first stage reduces sperm metabolism and motility during storage time and second stage for achieving motility prior to insemination (Ameha et al., 2007). To maximize the keeping quality of sperm, the researchers are trying to develop most appropriate semen extender by applying the knowledges of in vivo sperm storage mechanism(s) and sperm physiology.

2.2 Dilution of sperm

The ejaculate volume and sperm concentration varies among avian species (Garcia-Herreros, 2016). Generally, avian sperm are highly concentrated, low in volume and containing 6 (roosters) to 12 (toms) billion sperm/ml (Donoghue and Wishart, 2000). During in vitro storage condition, the percentage of viable sperm is lower in undiluted semen as compare to semen diluted with extender (Dumpala et al., 2006). So, the undiluted sperm needs to be diluted for in vitro storage or AI purpose. The sperm dilution rate, keeping quality and fertilizing ability varies among avian species. In broiler breeder, dilution of semen prior to storage is important for maintaining sperm quality index and semen diluted at 8 to10-folds maintained sperm fertilizing ability (Parker and McDaniel, 2004). It is also important to be noted that sperm fertilizing ability declines at higher dilution rate and,
especially, fertility is drastically reduced at dilution rate of 1:10 or 1:20 (Wilcox, 1958). The guineafowl which is
known for lower fertility rate as compare to other avian species, they can also maintain sperm survivability and
fertilizing ability at low dilution rate (1:2) (Hudson et al., 2016). Probably, most detrimental effect of sperm
dilution is known as dilution effect. It is possible that extensive dilution may destabilize sperm membrane which is
detrimental for sperm motility and viability (Maxwell and Johnson, 1999). For achieving the maximum fertility in
chicken, dilution effects was adjusted with sperm number (Sexton, 1981). So, the adjustment of sperm dilution is
important in avian species for achieving maximum viability and fertilizing ability \textit{in vitro} condition.

2.3 Effect of temperature

In avian species, sperm production, transportation and maintenance in the male reproductive tract occurs at
body temperature 41°C (Beaupré et al., 1997). During mating, the sperm enters into SSTs of female reproductive
tract, and the sperm can maintains its fertilizing ability at body temperature 41°C (Sasanami et al., 2013). The
sperm of male reproductive tract (testis, epididymis and ductus deferens) are almost immotile at 40 °C as undiluted
sperm, but restart motility when reduces the temperature to 30 °C or diluted with diluents (Ashizawa and Sano,
1990). Sperm motility, viability and fertilizing ability of different poultry species varies with different storage
temperature. Sperm motility, viability and fertilizing ability pattern in different poultry species are also somehow
different. Effect of temperature on motility, viability and fertilizing ability of avian sperm have been shown in
Table 1. Briefly, \textit{in vitro} avian sperm motility, viability and fertilizing ability is related with sperm storage
temperature. Although sperm survives at body temperature 41 °C in both male and female reproductive tract, but
this ability is lost within a few hours when sperm are incubated at 41 °C \textit{in vitro} condition. Generally, sperm
motility is low at both high and low temperature. The sperm motility is high at temperature between 20-37 °C. In
case of sperm viability study, high percentage of viable sperm usually found at low temperature (approximately
4 °C). It is possible that metabolic activity of spermatozoa decreases at low temperature and this condition might
be associated with long-term sperm viability \textit{in vitro} condition. Sperm storage at 5 °C for 18 h provides similar
fertility like fresh semen in turkey and decreased fertility at 15 °C, 25 °C and 35 °C (Giesen and Sexton 1983a).

2.4 pH of extender

Intracellular pH regulation is important for functioning of sperm (Nishigaki et al., 2014). In avian species, the
optimum semen pH usually ranges between 7.0 and 7.4 (Siudzińska and Łukaszewicz, 2008; Ondho, 2014;
Getachew, 2016). The pH in the lumen of Japanese quail SSTs is < 6.0, where sperm remains in a quiescence state
of motility (Matsuzaki et al., 2015). During \textit{in vitro} sperm storage condition, sperm motility and velocity
decreases at lower pH and increases the sperm motility at higher pH in avian species. The effect of pH on motility,
viability and fertilizing ability of avian sperm have been shown in Table 2. Motility, viability and fertilizing ability
of avian sperm is affected by pH of extender. pH of semen is likely to be alkaline in state and the sperm transferred
into SSTs where acidic state prevails. Sperm activity increases in alkaline pH, whereas activity decreases in acidic
pH. At low pH, sperm flagellar quiescence happens with low sperm motility, and this condition might be
associated with long-term sperm viability. Thus, a decrease in pH could play an important role in the process of
long-term sperm viability \textit{in vitro} condition.
2.5 Effect of osmolarity

Osmotic condition is associated with the intactness of sperm plasma membrane. In avian species, sperm viability was studied with wide range of osmolarity at different storage conditions. It reveals that very high and low osmolarity compare to isotonic conditions hampers sperm survivability. In chicken and turkey, sperm viability adversely affected when osmolarity was ≥ 800 mOsm/kg. Even after short storage period of 10 min, sperm survivability was significantly low in chicken and turkey at 50 mOsm/kg and ≥ 800 mOsm/kg (Blanco et al., 2000). In hypo-osmotic condition, sperm below 200 and 140 mOsm/kg adversely affected the fertility of chicken and turkey spermatozoa, respectively (Bakst, 1980). Turkey sperm osmotolerance was slightly improved by reducing the incubation temperature from 21 °C to 4 °C (Blanco et al., 2008). In emu species, spermatozoa can tolerate to osmolarities as high as ≈ 1400 mOsm/kg but lost the motility score after > 700 mOsm/kg (Sood et al., 2011). Fertility and hatchability of chicken sperm stored under hypertonic (460 mOsm/kg) or isotonic (340 mOsm/kg) condition did not differed after 24 h of sperm storage (Van Wambeke, 1977). The osmolarity of avian seminal

Table 1: Effect of temperature on motility, viability and fertilizing ability of avian sperm

| Species       | Temperature dependent sperm functions                                                                 | References                        |
|---------------|--------------------------------------------------------------------------------------------------------|-----------------------------------|
| Chicken       | sperm motility high at 20-37 °C                                                                          | Ashizawa and Nishiyama, 1978      |
|               | sperm motility almost inhibited at 5 °C and 41°C                                                         |                                   |
| Chicken       | high percentage of death sperm and low quality of sperm index at 41 °C as compare to 4 °C and 21 °C      | Dumpala et al., 2006              |
| Chicken       | sperm motility and fertility high at 5 °C and low at 41 °C                                               | Clarke et al., 1982               |
| Chicken       | sperm viability similar at 5 °C and 15 °C after 24 h storage                                              | Sexton, 1978                      |
| Chicken       | low fertility at 25 °C as compare to 10 °C                                                               | Wilcox, 1960                      |
| Chicken, Duck | complete inhibition of sperm motility at 40 °C compare to 30 °C                                         | Wishart and Wilson, 1999          |
| Turkey        | sperm motility decreases at 40 °C compare to 30 °C                                                        | Wishart and Wilson, 1999          |
| Turkey        | Sperm quality high at 15 °C as compare to 10 °C                                                           | Carter et al., 1957               |
| Turkey        | sperm motility and fertility low at 41 °C                                                                | Clarke et al., 1982               |
|               | sperm motility high at 5 °C but fertility high at 25 °C                                                    |                                   |
| Japanese quail| sperm motility almost similar at 30 °C and 40 °C                                                          | Wishart and Wilson, 1999          |
| Ostrich       | sperm motility increases at 40 °C compare to 20 °C                                                         | Bonato et al., 2012               |

Table 2: Effect of pH on motility, viability and fertilizing ability of avian sperm

| pH       | Species          | Conditions applied | Roles                                                                 | References                  |
|----------|------------------|--------------------|----------------------------------------------------------------------|-----------------------------|
| 5.8, 6.8, 7.1 and 7.4 | Chicken | sperm storage at 5 °C for 24 h | most satisfactory fertility was found at pH 6.8 or 7.1 | Lake and Ravie, 1976        |
| 6.0, 7.0 and 8.0 | Chicken | sperm storage at 7-9 °C for 48 h | motility highly preserved at pH 6.0 | Bogdonoff and Shaffiner, 1954 |
| 5.0, 6.0, 7.0, 8.0 and 9.0 | Chicken, Turkey and Quail | sperm storage at 30°C for 3 h | sperm were immotile up to pH 6 and motility increases with pH | Holm and Wishart, 1998 |
| 5.5, 6.0 and 6.5 | Turkey | sperm storage at 15 °C for 6 h | motility and fertilizing capacity decreases at pH 5.5 compare to pH 6.0 and 6.5 | Sexton and Giesen, 1983 |
| 6.0, 7.0, 8.0 and 9.0 | Ostrich | sperm storage at 20 °C and 40 °C for 10 min | sperm viability decreases with the increase of pH | Bonato et al., 2012 |
plasma is like to be isotonic (330.4±50.4) (Siudzińska and Łukaszewicz, 2008; Dietrich et al., 2010). Conclusively, avian sperm may survive in a range of osmolarity between 250 and 450 mOsm/kg.

3. Addition of additives to sperm storage conditions

3.1 Role of seminal plasma

Seminal plasma (SP) is a complex fluid (Poiani, 2006), varies among species and plays an essential role for sperm functioning in male and female reproductive tract (Juyena and Stelletta, 2012). Most components of SP are secreted from primary sex glands (seminal vesicle, prostate gland and bulbourethral gland) in mammals (Mann and Lutwak-Mann, 1951; Maňásková et al., 2002; Duncan and Thompson, 2007) and insects (Happ, 1984; Gillott, 1996). Unlike mammals and insects, avian species lack the prostate gland, seminal vesicle and bulbourethral gland. Some avian species produces lymph-like fluid and/or foam at the time of ejaculation (Fujihara, 1992). Male quail produces large quantities of cloacal foam at the time of ejaculation (Seiwert and Adkins-Regan, 1998). Thus, in avian species, the fluid adds with the sperm mainly comes from the male reproductive tract and cloacal region. As an avian species, the sperm production, processing and transportation in male Japanese quail have been shown in Fig. 1.

The roles of SP in in vitro sperm survivability and fertility are well studied in mammalian species (Thaler, 1989; Killian et al., 1993; Manjunath and Thérien, 2002; Moura et al., 2006; Manjunath et al., 2007; Koppers et al., 2011; Juyena and Stelletta, 2012; Crawford et al., 2015; Viana et al., 2018) and insects (Bertram et al., 1996; Lung et al., 2001; Bloch Qazi and Wolfner, 2003; Holman, 2009). The studies related to in vitro sperm survivability and fertilizing ability in avian species are limited and are shown in Table 3. Probably, the first proteomic analysis of SP was investigated in rooster semen (Marzoni et al., 2013). The role of SP in avian sperm viability and fertilizing ability study seems to be contradictory. Blesbois and de Reviers, (1992) reported that fowl SP contains the fraction of higher molecular weight (>50 kDa) favours sperm fertilizing ability whereas fractions of lower molecular weight (<1 kDa) are toxic to sperm in vitro condition. In turkey, whole SP reduces fertility (Douard et al., 2005), whereas dialyzed SP at 12-14 kDa is beneficial for in vitro sperm viability (Iaffaldano and Meluzzi, 2003). Sexton, (1977) reported that removal of seminal plasma by centrifugation had no significant effect on fertilizing capacity of chicken sperm at 5 °C for 24 h. It is possible that SP contains several components and all components are not beneficial for sperm viability and fertilizing ability. Proteomic analysis of SP and the application of SP components in sperm viability test may give clearer scenario about the role of SP in avian species.
Figure 1: Sperm production and transportation process in male Japanese quail. The figure is prepared from the findings of Clulow and Jones (1982). Sperm produces in testis, matures in epididymis and briefly store in ductus deferens. Sperm production, maturation and transportation is relatively quicker in Japanese quail. During ejaculation, cloacal foam is added with the semen. Testicular and epididymal sperm show low motility compare to sperm of ductus deferens and ejaculates.

| Type of SP | Species | Conditions applied | Roles | References |
|------------|---------|--------------------|-------|------------|
| Fraction of SP (M_r <1000) | Chicken | washed spermatozoa at 41 °C for 15 min | stimulate sperm motility and oxygen consumption | Ashizawa and Okauchi, 1984 |
| Fraction of SP (M_r <<1 kDa and >50 kDa) | Chicken | sperm stored at 4 °C for 24 h | <1 kDa decreases fertility, >50 kDa increases fertility | Blesbois and de Reviers, 1992 |
| SP + PBS (1:2) | Chicken | sperm stored at 0 °C for 24 h | inhibit endogenous lipid peroxidation in sperm and improve fertility | Fujihara and Koga, 1984 |
| SP albumin (1 or 4 mg/ml) | Chicken | sperm stored at 4 °C for 24 h | sperm mobility stimulating factor | Blesbois and Caffin, 1992 |
| Whole SP | Turkey | sperm stored at 4 °C for 24 h | reduces fertility | Douard et al., 2005 |
| SP dialyzed at 12-14 kDa | Turkey | sperm stored at 5 °C for 24-48 h | improve sperm viability, membrane integrity and sperm motility | Iaffaldano and Meluzzi, 2003 |

3.2 Role of antioxidants

Naturally, avian semen contain antioxidants and enzymatic defenses that prolongs the sperm longevity in vivo and in vitro condition (Bréque et al., 2003; Partyka et al., 2012). The amount of antioxidants and antioxidant enzyme present in avian seminal plasma and sperm have been shown in Table 4. Avian sperm also containing beta-defensins,
an antimicrobial peptide which is probably associated with the protection of sperm from microbial infection in the male and even in female reproductive tract (Shimizu et al., 2008; Das et al., 2011). However, avian sperm contains high amount of polyunsaturated fatty acids which is susceptible to reactive oxygen species and promotes lipid peroxidation (Khan, 2011). During in vitro storage condition, total lipid contents of spermatozoa decrease significantly at 2-5 °C after 48 h in chicken. It is possible to occur lysis of lipid and peroxidation; and can modify the structure of spermatozoa (Blesbois et al., 1999). In turkey, phospholipid content of spermatozoa decreases after 48 h sperm storage at 4 °C (Douard et al., 2000). To overcome the problem of lipid peroxidation and achieving better fertility, experts tried to add different antioxidants as feed additives (Rengaraj and Hong, 2015; Tufarelli and Laudadio, 2016; Surai et al., 2019). Here, effect of different antioxidants on in vitro sperm storage are discussed only. Poultry breeders are using different kinds of antioxidants in vitro sperm storage condition to improve the sperm quality. The effect of antioxidants on motility, viability and fertilizing ability of avian sperm have been shown in Table 5. It can be said that antioxidant improves motility, viability and fertilizing ability of avian sperm in a dose dependent manner.

### 3.3 Effect of serum

Serum, especially bovine serum albumin (BSA) has been used widely in mammalian sperm viability study in vitro condition. BSA plays important role in sperm capacitation (Xia and Ren, 2009) and acrosome reaction (Hossain et al., 2007) in mammals. BSA improves motility and viability of frozen-thawed ram spermatozoa (Uysal and Bucak, 2007), extend sperm longevity and maintains sperm plasma membrane integrity (Tvrdal et al., 2010; Sarıözkan et al., 2013) and can be substituted for egg-yolk in ram semen diluents (Matsuoka et al., 2006). The studies related to effect of serum in sperm viability and fertilizing ability is limited in avian species. In turkey, addition of BSA with diluents significantly improve the sperm motility and velocity at 7 °C after 24 h of sperm storage (Bakst and Cecil, 1992). BSA increases the viability of rooster sperm in vitro condition (Kim et al., 2017).

| Parameters | Chicken | Duck | Turkey | Goose | Guinea fowl | Ostrich |
|------------|---------|------|--------|-------|-------------|---------|
| Total antioxidant activity in seminal plasma (µM Trolox/ml) | 0.62 ± 0.02a | 2.10 ± 0.14a | 13.15 ± 1.11a | 2.13 ± 0.11a | 0.97 ± 0.04a | 0.8 ± 0.3b |
| Superoxide dismutase activity in seminal plasma (U/ml) | 46.20 ± 2.44a | 32.27 ± 3.11a | 77.71 ± 0.07a | 42.05 ± 3.06a | 65.98 ± 1.54a | - |
| Superoxide dismutase activity in sperm (U/10⁹ sperm) | 22.04a | 59.91a | 16.97a | 77.74a | 21.91a | - |
| Glutathione peroxidase activity in spermatozoa (U/10⁹ sperm) | 44.46a | 72.57a | 31.75a | 178.68a | 11.83a | - |
| Glutathione peroxidase activity in seminal plasma (mU/ml) | 12.7 ± 3.0c | - | - | 28.7 ± 7.8c | - | - |
| Vitamin E in seminal plasma (ng/ml) | 163.7 ± 6.5d | 32.4 ± 2.6d | - | - | - | - |
| Vitamin E in sperm (ng/10⁹ sperm) | 182.48 ± 8.5d | 48.1 ± 2.4d | - | - | - | - |

Source of information: a; Surai et al., 1998, b; Ciereszko et al., 2010, c; Partyka et al., 2012 and d; Surai et al., 2000
3.4 Effect of cloacal gland secretions

Cloacal foam is secreted from the cloacal gland of adult male Japanese quail and introduced into the female reproductive tract during natural mating. It was reported that removal of cloacal gland secretions from the male before mating reduced the fertility, but the supplementation of cloacal gland secretion rescued the subfertility (Sasanami et al., 2015). In vitro sperm motility study was conducted with the cloacal gland secretion and found that 5% cloacal gland secretion enhanced quail sperm motility for short time of 2-3 h at room temperature (Biswas et al., 2010). Interestingly, the sperm motility was inhibited at high concentration (25%) of cloacal gland secretions (Biswas et al., 2010). It is reported that cloacal gland secretion promotes sperm motility and acts as a medium to facilitate sperm transport in the oviduct (Singh et al., 2011; Cheng et al., 1989).

3.5 Effect of utero-vaginal junction extract

Avian sperm can be stored in the specialized structure of SSTs, which is situated inside the utero-vaginal junction (UVJ) of oviduct (Bakst, 2011) and may protect themselves from adverse conditions in SSTs (Das et al., 2006). It is known that sperm remains in the SSTs as a quiescence state in motility (Matsuzaki et al., 2015). The molecular mechanism behind the long-term sperm storage in the SSTs and retention of fertilizing ability after a single insemination still mystery. It is believed that SSTs contain some molecules and they can have some mechanism(s) that facilitate sperm storage and fertilizing ability in vivo condition. In this regard, in vitro sperm motility and viability was conducted with the fluid collected from reproductive tract. Fluid from ovarian pocket of hen stimulate sperm motility at body temperature and the fluid may facilitate the fertilization process (Ashizawa and Wishart, 1992). Albumin and transferrin were isolated from UVJ of quail and their effects on in vitro sperm survivability was significantly high when compared with control (Matsuzaki et al., 2019).

4. Conclusions

Conclusively, it can be said that sperm motility, viability and fertilizing ability in avian species depends on some physiochemical properties. Generally, combination of low pH and low temperature may reduce motility and metabolic activity of sperm. The addition of additives may improve the sperm quality in a dose dependent manner. Moreover, further studies are needed to find out the mechanism(s) behind sperm storage in vivo condition. Application of research findings into in vitro storage conditions may enrich sperm motility, viability and fertilizing ability in avian species.
REFERENCES

Ameha N, Moudgal RP and Asmare A (2007) Development of two stages cock’s semen extender for room temperature storage at laboratory. J. Poult. Sci., 44: 78–84.

Ashizawa K and Nishiyama H (1978) Effects of temperature on the vigour of motility, oxygen consumption and duration of motility of fowl spermatozoa under aerobic conditions. J. Poult. Sci., 15: 264–266.

Ashizawa K and Okauchi K (1984) Stimulation of sperm motility and oxygen consumption of fowl spermatozoa by a low molecular weight fraction of seminal plasma. J. Reprod. Fertil., 71: 593–598.

Ashizawa K and Sano R (1990) Effects of temperature on the immobilization and the initiation of motility of spermatozoa in the male reproductive tract of the domestic fowl, Gallus domesticus. Comp. Biochem. Physiol., 96(2): 297–301.

Ashizawa K and Wishart GJ (1992) Factors from fluid of the ovarian pocket that stimulate sperm motility in domestic hens. J. Reprod. Fertil., 95(3): 855–860.

Asmarawati W and Yuwanta T (2010) The effect of adding vitamin C and E in native chicken semen extender stored at temperature 4 °C on semen quality and egg fertility. The 5th International Seminar on Tropical Animal Production. 308–313.

Bakst MR (2011) Physiology and endocrinology symposium: Role of the oviduct in maintaining sustained fertility in hens. J. Anim. Sci., 89(5): 1323–1329.

Bakst MR (1980) Fertilizing capacity and morphology of fowl and turkey spermatozoa in hypotonic extender. J. Reprod. Fertil., 60(1): 121–127.

Bakst MR and Cecil HC (1992) Effect of bovine serum albumin on motility and fecundity of turkey spermatozoa before and after storage. J. Reprod. Fertil., 94: 287–293.

Bakst MR and Sexton TJ (1979) Fertilizing capacity and ultrastructure of fowl and turkey spermatozoa before and after freezing. J. Reprod. Fertil., 55(1): 1–7.

Beaupré CE, Tressler CJ, Beaupré SJ, Morgan JLM, Bottje WG and Kirby JD (1997) Determination of testis temperature rhythms and effects of constant light on testicular function in the domestic fowl (Gallus domesticus). Biol. Reprod., 56: 1570–1575.

Bertram MJ, Neubaum DM and Wolfner MF (1996) Localization of the Drosophila male accessory gland protein Acp36DE in the mated female suggests a role in sperm storage. Insect Biochem. Mol. Biol., 26: 971–980.

Biswas A, Ranganathasamy R and Mohan J (2010) The effect of different foam concentrations on sperm motility in the Japanese quail. Vet. Med. Int., Article ID 564921.

Blanco JM, Gee G, Wildt DE and Donoghue AM (2000) Species variation in osmotic, cryoprotectant, and cooling rate tolerance in Poultry, Eagle, and Peregrine Falcon spermatozoa. Biol. Reprod., 63: 1164–1171.

Blanco JM, Long JA, Gee G, Donoghue AM and Wildt DE (2008) Osmotic tolerance of avian spermatozoa: Influence of time, temperature, cryoprotectant and membrane ion pump function on sperm viability. Cryobiology, 56: 8–14.

Blesbois E and Caftin JP (1992) ‘Serum like’ albumin of fowl seminal plasma and effects of albumin on fowl spermatozoa stored at 4 degrees C. Br. Poult. Sci., 33: 663–670.

Blesbois E and de Reviers M (1992) Effect of different fractions of seminal plasma on the fertilizing ability of fowl spermatozoa stored in vitro. J. Reprod. Fertil., 95: 263–8.

Blesbois E, Grasseau I and Blum JC (1993) Effects of vitamin E on fowl semen storage at 4 degrees C. Theriogenology., 39(3): 771–779.

Blesbois E, Grasseau I and Hermier D (1999) Changes in lipid content of fowl spermatozoa after liquid storage at 2 to 5 degrees C. Theriogenology., 52(2): 325–34.

Blesbois E, Grasseau I and Seguinurin F (2005) Membrane fluidity and the ability of domestic bird spermatozoa to survive cryopreservation. Reproduction., 129(3): 371–378.

Bloch Qazi MC and Wolfner MF (2003) An early role for the Drosophila melanogaster male seminal protein Acp36DE in female sperm storage. J. Exp. Biol., 206: 3521–3528.

Bogdonoff PD and Shaffner CS (1954) The Effect of pH on in vitro survival, metabolic activity, and fertilizing capacity of chicken semen. Poult. Sci., 33: 665–669.

Bonato M, Cornwallis CK, Malecki IA, Rybnik-Trzaskowska PK and Cloete SWP (2012) The effect of temperature and pH on the motility and viability of ostrich sperm. Anim. Reprod. Sci., 133: 123–128.

Brequé C, Surai P and Brillard JP (2003) Roles of antioxidants on prolonged storage of avian spermatozoa in vivo and in vitro. Mol. Reprod. Dev., 66(3): 314–323.
Carter RD, McCartney MG, Chamberlin VD and Wyne JW (1957) The effect of storage time and temperature on fertilizing capacity of turkey semen. Poult. Sci., 36: 618–621.
Cheng K, Hickman A and Nichols C (1989) Role of the proctodeal gland foam of male Japanese quail in natural copulations. Auk Ornithol. Adv., 106: 279–285.
Ciereszko A, Rybnik PK, Horbanczuk JO, Dietrich GJ, Deas A, Slowinska M, Liszewska, E and Malecki IA (2010) Biochemical characterization and sperm motility parameters of ostrich (Struthio camelus) semen. Anim. Reprod. Sci., 122: 222–228.
Çifçi HB and Aygün A (2018) Poultry semen cryopreservation technologies. Worlds. Poult. Sci. J., 74: 699–709.
Clarke RN, Sexton TJ and Ottinger MA (1982) Effects of holding temperature and storage time on respiratory rate, motility, and fertility of chicken and turkey semen. Poult. Sci., 61(9): 1912–1917.
Clulow J and Jones RC (1982) Production, transport, maturation, storage and survival of spermatozoa in the male Japanese quail, Coturnix coturnix. J. Reprod. Fertil., 64: 259–266.
Crawford G, Ray A, Gudi A, Shah A andHomburg R (2015) The role of seminal plasma for improved outcomes during in vitro fertilization treatment: review of the literature and meta-analysis. Hum. Reprod. Update., 21(2): 275–284.
Das SC, Isobe N, Nishibori M and Yoshimura Y (2006) Expression of transforming growth factor-β isoforms and their receptors in utero-vaginal junction of hen oviduct in presence or absence of resident sperm with reference to sperm storage. Reproduction., 132(5): 781–790.
Das SC, Isobe N and Yoshimura Y (2011) Expression of toll-like receptors and avian β-defensins and their changes in response to bacterial components in chicken sperm. Poult. Sci., 90(2): 417–425.
Dietrich GJ, Deas A, Slowinska M, Liszewska E and Malecki IA (2010) Biochemical characterization and sperm motility parameters of ostrich (Struthio camelus) semen. Anim. Reprod. Sci., 122(3-4): 222–228.
Donoghue AM and Donoghue DJ (1997) Effects of water- and lipid-soluble antioxidants on turkey sperm viability, membrane integrity, and motility during liquid storage. Poult. Sci., 76: 1440–1445.
Donoghue AM and Wishart GJ (2000) Storage of poultry semen. Anim. Reprod. Sci., 62(1-3): 213–232.
Douro D, Hermier D and Blesbois E (2000) Changes in turkey semen lipids during liquid in vitro storage. Biol. Reprod., 63: 1450–1456.
Douro V, Hermier D, Labbe C, Magistrini M and Blesbois E (2005) Role of seminal plasma in damage to turkey spermatozoa during in vitro storage. Theriogenology, 63: 126–137.
Dumpala PR, Parker HM and McDaniel CD (2006) The effect of semen storage temperature and diluent type on the sperm quality index of broiler breeder semen. Int. J. Poult. Sci., 5: 838–845.
Duncan MW and Thompson HS (2007) Proteomics of semen and its constituents. Proteomics Clin. Appl., 1(8): 861–875.
Fujihara N (1992) Accessory reproductive fluids and organs in male domestic birds. Poult. Sci., 71: 385–390.
Fujihara N and Koga O (1984) Prevention of the production of lipid peroxide in rooster spermatozoa. Anim. Reprod. Sci., 7: 385–390.
García-Herreros M (2016) Sperm subpopulations in avian species: A comparative study between the rooster (Gallus domesticus) and Guinea fowl (Numida meleagris). Asian J. Androl. 18(6): 889–894.
Getachew T (2016) A review article of artificial insemination in poultry. Worlds Vet. J., 6(1): 25–33.
Giesen AF and Sexton TJ (1983a) Beltsville poultry semen extender. 9. Effect of storage temperature on turkey semen held eighteen hours. Poult. Sci., 62(7): 1305–1311.
Giesen AF and Sexton TJ (1983b) Beltsville poultry semen extender. 7. Comparison of commercial diluents for holding turkey semen six hours at 15 C. Poult. Sci., 62(2): 379–381.
Gillott C (1996) Male insect accessory glands: Functions and control of secretory activity. Invertebr. Reprod. Dev., 30: 199–205.
Parker HM and McDaniel CD (2004) The optimum semen dilution for the sperm quality index that is most predictive of broiler breeder fertility. Int. J. Poult. Sci., 3: 588–592.
Happ GM (1984) Structure and development of male accessory glands in insects. In: King R.C., Akai H. (eds) Insect Ultrastructure. Springer, Boston, MA, pp 365–396.
Holm L and Wishart GJ (1998) The effect of pH on the motility of spermatozoa from chicken, turkey and quail. Anim. Reprod. Sci., 54: 45–54.
Holman L (2009) Drosophila melanogaster seminal fluid can protect the sperm of other males. Funct. Ecol., 23: 180–186.
Holt WV (2011) Mechanisms of sperm storage in the female reproductive tract: An interspecies comparison. Reprod. Domest. Anim., 46: 68–74.

Holt WV and Lloyd RE (2010) Sperm storage in the vertebrate female reproductive tract: How does it work so well? Theriogenology, 73: 713–722.

Hossain MS, Hyeong LJ, Miah AG and Tsujii H (2007) Effect of fatty acids bound to bovine serum albumin-V on acrosome reaction and utilization of glucose in boar spermatozoa. Reprod. Med. Biol., 6(2): 109–115.

Hudson GH, Omprakash AV and Premavalli K (2016) Effect of semen diluents, dilution rates and storage periods on live and abnormal spermatozoa of pearl guinea fowls. Asian J. Anim. Vet. Adv., 11(7): 411–416.

Iaffaldano N and Meluzzi A (2003) Effect of dialysis on quality characteristics of turkey semen during liquid storage. Theriogenology, 60: 421–427.

Iaffaldano N, Rosato MP, Manchisi A, Centoducati G and Meluzzi A (2016) Comparison of different extenders on the quality characteristics of turkey semen during storage. Ital. J. Anim. Sci., 4(Suppl.2): 513–515.

Juyena NS and Stelletta C (2012) Seminal plasma: An essential attribute to spermatozoa. J. Androl., 33(4): 536–551.

Khan RU (2011) Antioxidants and poultry semen quality. Worlds. Poult. Sci. J., 67: 297–308.

Killian GJ, Chapman DA and Rogowski LA (1993) Fertility-associated proteins in Holstein bull seminal plasma. Biol. Reprod., 49: 1202–1207.

Kim SW, Kim MS, Yu Y, Kim Chan-Ian Jeon IS and Kim Chongdae (2017) The Effects of supplementation of BSA or fatty acid free BAS on the motility of fresh or cryopreserved rooster spermatozoa. Korean J. Poult. Sci., 44: 59–65.

Koppers AJ, Reddy T and O'Bryan MK (2011) The role of cysteine-rich secretory proteins in male fertility. Asian J. Androl., 13: 111–117.

Lake PE and Ravie O (1976) Effect on fertility of storing fowl semen for 24 h at 5 degrees C in fluids of different pH. J. Reprod. Fertil., 57(1): 149–155.

Long JA (2006) Avian semen cryopreservation: What are the biological challenges? Poult. Sci., 85: 232–236.

Long JA and Kramer M (2003) Effect of vitamin E on lipid peroxidation and fertility after artificial insemination with liquid-stored turkey semen. Poult. Sci., 82: 1802–1807.

Long JA, Purdy PH, Zuidberg K, Hiemstra SJ, Velleman SG and Woelders H (2014) Cryopreservation of turkey semen: Effect of breeding line and freezing method on post-thaw sperm quality, fertilization, and hatching. Cryobiology., 68: 371–378.

Lung O, Kuo L and Wolfner MF (2001) Drosophila males transfer antibacterial proteins from their accessory gland and ejaculatory duct to their mates. J. Insect Physiol., 47: 617–622.

Maňásková P, Ryšlavá H, Tichá M and Jonáková V (2002) Characterization of proteins from boar prostate. Am. J. Reprod. Immunol., 48(4): 283–290.

Mangiagalli MG, Marelli SP and Cavalchini LG (2007) Effect of lycopene on fowl sperm characteristics during in vitro storage. Arch. fur Geflugeldk., 71: 25–29.

Manjunath P, Bergeron A and Fan J (2007) Seminal plasma proteins: functions and interaction with protective agents during semen preservation. Soc. Reprod. Fertil. Suppl., 65: 217–228.

Manjunath P and Thérien I (2002) Role of seminal plasma phospholipid-binding proteins in sperm membrane lipid modification that occurs during capacitation. J. Reprod. Immunol., 53: 109–119.

Mann T and Lutwak-Mann C (1951) Secretory function of male accessory organs of reproduction in mammals. Physiol. Rev., 31(1): 27–55.

Marzoni M, Castillo A, Sagona S, Citti L, Rocchiicchioni S, Romboli I and Felicioli A (2013) A proteomic approach to identify seminal plasma proteins in roosters (Gallus gallus domesticus). Anim. Reprod. Sci., 140: 216–223.

Matsuoka T, Imai H, Kohno H and Fukui Y (2006) Effects of bovine serum albumin and trehalose in semen diluents for improvement of frozen-thawed ram spermatozoa. J. Reprod. Dev., 52(5): 675–683.

Matsuzaki M, Mizushima S, Dohra H and Sasanami T (2019) Expression of transferrin and albumin in the sperm-storage tubules of Japanese quail and their possible involvement in long-term sperm storage. J. Poult. Sci., ID: 0190049.

Matsuzaki M, Mizushima S, Hiyama G, Hirohashi N, Shiiba K, Inaba K, Suzuki T, Dohra H, Ohnishi T, Sato Y, Kohsaka T, Ichikawa Y, Atsumi Y, Yoshimura T and Sasanami T (2015) Lactic acid is a sperm motility inactivation factor in the sperm storage tubules. Sci. Rep. 5: 17643.

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Maxwell WMC and Johnson LA (1999) Physiology of spermatozoa at high dilution rates: The influence of seminal plasma. Theriogenology., 52(8): 1353–1362.
Morrell JM, Persson B, Tjellström H, Laeskker A, Nilsson H, Danilova M and Holmes PV (2005) Effect of semen extender and density gradient centrifugation on the motility and fertility of turkey spermatozoa. Reprod. Domest. Anim., 40(6): 522–525.
Moura AA, Chapman DA, Koc H and Killian GJ (2006) Proteins of the cauda epididymal fluid associated with fertility of mature dairy bulls. J. Androl., 27: 534–541.
Nishigaki T, José O, González-Cota AL, Romero F, Treviño CL and Darszon A (2014) Intracellular pH in sperm physiology. Biochem. Biophys. Res. Commun., 450(3): 1149–1158.
Ondho YS (2014) Comparative studies of semen quality on different breed of chicken in poultry breeding center Temanggung-central Java. Int. Ref. J. Eng. Sci., 3: 94–103.
Parker HM and McDaniel CD (2003) Semen dilution prior to analysis influences the ability of the sperm quality analyzer to predict fertility whether inseminating with a constant number of sperm or a constant volume of semen. Poult. Sci., 82(11): 1808–1815.
Partyka A, Łukaszewicz E and Nizański W (2012) Lipid peroxidation and antioxidant enzymes activity in avian semen. Anim. Reprod. Sci., 134(3-4): 184–190.
Poiani A (2006) Complexity of seminal fluid: A review. Behav. Ecol. Sociobiol., 60: 289–310.
Rakha BA, Ansari MS, Hussain I, Anwar M, Akhter S. and Blesbois E (2016) Comparison of extenders for liquid storage of Indian Red Jungle Fowl (Gallus gallus murghi) spermatozoa. Avian Biol. Res., 9(3): 207–212.
Rengaraj D and Hong YH (2015) Effects of dietary vitamin E on fertility functions in poultry species. Int. J. Mol. Sci., 16(5): 9910–9921.
Sarriózkan S, Türk G, Cantürk F, Yay A and Akay A (2013) The effect of bovine serum albumin and fetal calf serum on sperm quality, DNA fragmentation and lipid peroxidation of the liquid stored rabbit semen. Cryobiology., 67: 1–6.
Sasanami T, Izumi S, Sakurai N, Hira TA, Mizushima S, MatsuMaki M, Hiyama G, Yoshimura T, Ukena K and Tsutsui K (2015) A unique mechanism of successful fertilization in a domestic bird. Sci. Rep., 5: 7700.
Sasanami T, MatsuMaki M, Mizushima S and Hiyama G (2013) Sperm storage in the female reproductive tract in birds. J. Reprod. Dev., 59: 334–338.
Seiwert CM and Adkins-Regan E (1998) The foam production system of the male Japanese quail: characterization of structure and function. Brain. Behav. Evol., 52: 61–80.
Sexton TJ (1977) A new poultry semen extender. 1. Effects of extension on the fertility of chicken semen. Poult. Sci., 56(5): 1443–1446.
Sexton TJ (1978) A New poultry semen extender 3. Effect of storage conditions on the fertilizing capacity of chicken semen stored at 5 °C. Poult. Sci., 57(1): 285–289.
Sexton TJ (1981) Sperm number required for maximum fertility of chicken semen processed for freezing. Reprod. Nutr. Dev., 21(6B): 1043–1048.
Sexton TJ (1988) Comparison of commercial diluents for holding turkey semen 24 hours at 5 C. Poult. Sci., 67(1): 131–134.
Sexton TJ and Fewlass TA (1978) A new poultry semen extender 2. Effect of the diluent components on the fertilizing capacity of chicken semen stored at 5 degrees C. Poult. Sci., 57(1): 277–284.
Sexton TJ and Giesen AF (1982) Beltsville poultry semen extender: 6. Holding turkey semen for six hours at 15 C. Poult. Sci., 61: 1202–1208.
Sexton TJ and Giesen AF (1983) Beltsville poultry semen extender. 8. Factors affecting turkey semen held six hours at 15 C. Poult. Sci., 62: 1063–1068.
Shimizu M, Watanabe Y, Irobe N and Yoshimura Y (2008) Expression of avian β-defensin 3, an antimicrobial peptide, by sperm in the male reproductive organs and oviduct in chickens: An immunohistochemical study. Poult. Sci., 87(12): 2653–2659.
Singh RP, Sastry K, Singh KB, Mohan J and Moudgal RP (2011) Cloacal gland foam enhances motility and disaggregation of spermatozoa in Japanese quail (Coturnix japonica). Theriogenology., 75(3): 563–569.
Siudzińska A and Łukaszewicz E (2008) Effect of semen extenders and storage time on sperm morphology of four chicken breeds. J. Appl. Poult. Res., 17: 101–118.
Sood S, Malecki IA, Tawang A and Martin GB (2011) Response of spermatozoa from the emu (Dromaius novaehollandiae) to rapid cooling, hyperosmotic conditions and dimethylacetamide (DMA). Anim. Reprod. Sci., 129: 89–95.
Suarez SS (2010) How do sperm get to the egg? Bioengineering expertise needed! Exp. Mech., 50: 1267–1274.
Ciereszko A, Rybnik PK, Horbanczuk JO, Dietrich GJ, Deas A, Slowinska M, Liszewska, E and Malecki IA (2010) Biochemical characterization and sperm motility parameters of ostrich (Struthio camelus) semen. Anim. Reprod. Sci., 122:222–228.

Surai PF, Blesbois E, Grasseau I, Chalah T, Brillard JP, Wishart GJ, Cerolini S and Sparks NHC (1998) Fatty acid composition, glutathione peroxidase and superoxide dismutase activity and total antioxidant activity of avian semen. Comp. Biochem. Physiol. - B Biochem. Mol. Biol. 120: 527–533.

Surai PF, Brillard JP, Speake BK, Blesbois E, Seigneurin F and Sparks NHC (2000) Phospholipid fatty acid composition, vitamin E content and susceptibility to lipid peroxidation of duck spermatozoa. Theriogenology., 53(5): 1025–1039.

Surai PF, Kochish II, Romanov MN and Griffin DK (2019) Nutritional modulation of the antioxidant capacities in poultry: the case of vitamin E. Poult. Sci., 98(9): 4030–4041.

Tabatabaei S, Batavani R, Ayen E, Sciences F and Agriculture R (2011) Effects of vitamin E addition to chicken semen on sperm quality during in vitro storage of semen. Vet. Res. Forum, 2: 103–111.

Thaler CJ (1989) Immunological role for seminal plasma in insemination and pregnancy. Am. J. Reprod. Immunol., 21: 147–150.

Tufarelli V and Laudadio V (2016) Antioxidant activity of vitamin E and its role in avian reproduction. J. Exp. Biol. Agric. Sci., 4(3S): 266–272.

Tvrdù E, Knazicka Z, Massanyi P, Stawarz R, Formicki G and Lukac N (2010) Bovine serum albumin as a potential protein supplement for in vitro cultivation of spermatozoa. Mendelnet, 2010: 964–969.

Ushiyama A, Ishikawa N, Tajima A and Asano A (2016) Comparison of membrane characteristics between freshly ejaculated and cryopreserved sperm in the chicken. J. Poult. Sci., 53: 305–312.

Uysal O and Bucak MN (2007) Effects of oxidized glutathione, bovine serum albumin, cysteine and lycopene on the quality of frozen-thawed ram semen. Acta Vet. Bmo., 76: 383–390.

Van Wambeke F (1977) The effect of tonicity of storage media for fowl semen on the occurrence of neck-bending spermatozoa, fertility and hatchability. Br. Poult. Sci., 18(2): 163–168.

Viana AGA, Martins AMA, Pontes AH, Fontes W, Castro MS, Ricart CAO, Sousa MV, Kaya A, Topper E, Memili E and Moura AA (2018) Proteomic landscape of seminal plasma associated with dairy bull fertility. Sci. Rep., 8: 1–13.

Wilcox FH (1960) Effect on fertility of temperature, handling methods, Luke’s solution and the addition of egg white, egg yolk, and sugars to the diluted used in storing chicken semen. Poult. Sci., 39: 459–467.

Wilcox FH (1958) The effect of dilution and concentration of chicken semen on fertility. Poult. Sci., 37: 1357–1362.

Wishart GJ and Wilson YI (1999) Temperature-dependent inhibition of motility in spermatozoa from different avian species. Anim. Reprod. Sci., 57: 229–235.

Xia J and Ren D (2009) The BSA-induced Ca2+ influx during sperm capacitation is CATSPER channel-dependent. Reprod. Biol. Endocrinol., 7: 119.