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Poultry semen cryopreservation technologies

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Several techniques have been developed for the preservation and improvement of genetic resources to maintain genetic diversity. Among those techniques, semen cryopreservation is thought to be the best and successfully applied by dairy and beef industries, but so far, it has not been established in the poultry industry. This is because poultry sperm cells have a unique shape and membrane fluidity, differing from those of mammalian sperm. Also, poultry sperm membranes contain higher quantities of polyunsaturated fatty acids than mammalian sperm, and hence may require more antioxidant protection. Due to the peculiarity of poultry sperm cells, commonly used cryoprotectants for cryopreservation have a contraceptive or toxic effect. This renders the fertility of frozen poultry sperm to become highly variable and not reliable enough for use in commercial production or preservation of genetic resources. The average fertility of frozen/thawed poultry sperm ranges between 2-80%. Therefore, this paper reviews the possible reasons for the lower success of poultry sperm cryopreservation.

Keywords: cryopreservation; fertility; morphology; poultry; sperm

Introduction

The development of practical procedures for artificial insemination (AI) in Russia lead the establishment of dairy AI cooperatives in Europe and the United States (US) between 1933-1938 (Ombelet and Van Robays, 2015). After 1953, the dairy industry needed longer-term bull sperm storage, and this resulted in research into sperm cryopreservation (Walters *et al.*, 2009). The first important factor in sperm freezing was the discovery of glycerol by Polge *et al.* (1949) cited in Pegg (2002) and Walters *et al.* (2009). This was supported by the introduction of a simple method of preserving human sperm by using glycerol, reported by Dr Jerome K Sherman in 1955 (Ombelet and Van Robays, 2015). Sherman showed, for the first time, that frozen sperm were able to fertilise an egg after thawing, resulting in normal development. Over time, cryopreservation became an important technique for maintaining genetic diversity and the preservation of genetic resources. Several techniques have been developed to prevent the loss of genetic

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resources. Among these techniques, poultry semen cryopreservation is considered to be a challenging method to preserve reproductive cells (Blesbois, 2011; Kowalczyk and Łukaszewicz, 2015).

In most mammalian species, reasonable fertility rates have been obtained after the insemination of thawed sperm cells. In poultry, however, semen cryopreservation has not been as successful (Blesbois, 2011; 2012; Blesbois and Brillard, 2007), since fertility, after the freezing/thawing process of poultry sperm, is inconsistent and significantly lower than that seen in mammalian species (Long, 2006). This makes frozen poultry semen not reliable enough to be used in artificial insemination programs. In particular, the fertility of frozen tom turkey semen is considerably lower than that of rooster semen. Consequently, the turkey industry is more dependent on insemination with fresh semen.

The aim of this paper was to review the present condition of poultry semen cryopreservation and the possible reasons for its lower fertility.

Poultry semen cryopreservation

Currently, semen cryopreservation has only been commercially applied in the cattle industry, where semen cryopreservation has been optimised, standardised and automated. However, the extent of success in sperm cryopreservation has not been achieved for other livestock mammalians, such as pigs and sheep (Holt, 2000). In poultry, semen cryopreservation has not been successful, mainly because the poor fertility rates reported with frozen/thawed poultry, whereby sperm counts are extremely low, highly variable, and not sufficiently reliable (*Table 1*) in commercial production or the preservation of genetic resources (Donoghue and Wishart, 2000; Hammerstedt and Graham, 1992; Wishart, 1985).

Table 1 Fertility of poultry semen reported after freezing in different extenders containing different cryoprotectants in different concentrations.

Breed	Extender	Cryoprotectants	Fertility (%)	References
Rooster	Modified Beltsville poultry semen extender (BPSE)	Dimethylsulfoxide (DMSO, 4%, v/v)	38.2 ± 2.5	Ansari *et al.* (2017)
Rooster	Modified extender	DMSO, (4%, v/v).	41.6 ± 0.0	Zhandi *et al.* (2017)
Rooster	Lake and Ravie medium	Dimethylacetamide (DMA, 6%, v/v)	10.8 ± 0.0	Abouelezz *et al.* (2015)
Rooster	Lake and Ravie medium	DMA (6%, v/v)	33.6 ± 13.4	Santiago-Moreno *et al.* (2011)
Rooster	Beltsville extender	Glycerol (2% ,v/v)	29.2 ± 2.8	Shahverdi *et al.* (2015)
Rooster	Lake and Ravie medium	Glycerol (%11 ,v/v)	2.1 ± 0.0	Abouelezz *et al.* (2015)
Rooster	Lake and Ravie medium	Glycerol (8%,v/v)	31.3 ± 0.0	Abouelezz *et al.* (2017)
Gander	EK diluent	Dimethylformamide (DMF, 6% , v/v)	83.78 ± 9.5	Lukaszewicz (2002)
Turkey	Lake diluent Lake diluent	DMA (6%, v/v) Glycerol (11%,v/v)	8.6 ± 2.1 10.7 ± 2.3	Long *et al.* (2014)

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Therefore, the freezing of chicken semen is still in the experimental phase and is not yet sufficiently advanced for practical application in the poultry industry (Blesbois *et al.*, 2007; Wishart, 1985). The fertility problem with frozen semen is much more worse in turkeys (*Table 1*). Overall fertility rates of cryopreserved turkey sperm cells are considerably lower than that of frozen/thawed chicken sperm cells (Kurbatov *et al.*, 1986; Nelson *et al.*, 1980; Schramm and Hubner, 1988; Sexton, 1981; Wishart, 1989). Therefore, AI in turkey depends on the availability of fresh semen, while it is less often employed in chickens (Blanch *et al.*, 2014). Turkeys are heavy birds, and the large white strains of males can weigh over 33 kg compared to hens weighing approximately 9 kg. Therefore, natural mating results in low fertility in this strain, which is the reason for the integration of AI in commercial turkey enterprises (Donoghue and Wishart, 2000).

Why is cryopreservation of poultry semen not successful?

The fertility of frozen poultry semen is dramatically lower as compared to that of fresh semen. The reason why freezing/thawing decreases the fertility rate of poultry semen is not completely understood, but might be related to the unique morphological and physiological features of poultry sperm cells (Cecil and Bakst, 1993; Cerolini *et al.*, 1997).

DIFFERENCES BETWEEN POULTRY AND MAMMALIAN SPERM CELLS

Poultry sperm cells are morphologically different from mammalian sperm cells. The morphology of sperm cells from different poultry species (such as chickens, turkeys, drakes, and ganders) is similar, however. Therefore, the damage to their spermatozoa during freezing/thawing should be the same, although chicken and turkey sperm cells are metabolically different from each other (Lake and Wishart, 1984).

The filiform shape of the poultry sperm head is not much wider in diameter than the tail (Thurston and Hess, 1987). Therefore, sperm heads have less cytoplasmic volume, and this means they have less ability to move in the cryoprotectants (CPA). Poultry sperm have a relatively long tail (between 90-100 μm; Thurston and Hess (1987)) compared to bull spermatozoa (Salisbury and Vandemark, 1961). Such long-tailed sperm cells are easily damaged during freezing/thawing. According to previous studies, during the cryopreservation and thawing process, the ultrastructure of poultry sperm cells is damaged, especially the mitochondria, midpiece, and perforatorium (Xia *et al.*, 1988). Electron microscopy has shown that 60% of poultry sperm organelles suffered irreversible damage after cryopreservation (Bakst and Sexton, 1979).

MEMBRANE FLUIDITY

The sperm plasma membrane is composed of lipids, cholesterol and proteins. Cholesterol has major effects on sperm by regulating membrane stability, the acyl chains order of phospholipids, and the consequent regulation and control of numerous membrane properties and functions, including membrane thickness, fluidity, and permeability to water and other molecules. It influences phase transitions and maintains a favourable micro-environment for membrane proteins (Crockett, 1998; Müller *et al.*, 2008).

The resistance of sperm cells to cryopreservation has been verified by the membrane phospholipid content and fluidity (Holt, 2000). Fluidity of poultry sperm is closely related to the cholesterol/phospholipids ratio of the membrane (Holt, 2000) and is an indication of sperm freezability (Blesbois *et al.*, 2005). It has been hypothesised that a

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high cholesterol content of the sperm plasma membrane is associated with a low freezing tolerance in mammals (Müller *et al.*, 2008), while in poultry, the lower freezing tolerance of sperm cells is associated with lower concentrations of cholesterol and a lower fluidity of the plasma membrane (Blesbois *et al.*, 2005).

Membrane fluidity can be determined by the measurement of fluorescence polarisation anisotropy *via* fluorescent dye. Anisotropy values correspond to membrane rigidity and are inversely related to membrane fluidity (Blesbois *et al.*, 2005). According to previous studies, anisotropy values are highest in guinea fowl (0.205), lowest in chickens (0.155), and intermediate in turkeys (0.180). As a result, membrane fluidity is highest in chickens, intermediate in turkeys, and lowest in guinea fowl (Blesbois *et al.*, 2005).

The effect of cholesterol on membrane fluidity depends on the temperature. At temperatures above phase transition, high cholesterol in the plasma membrane decreases membrane fluidity, making sperm cells more susceptible to cold shock and reducing their ability to survive after freezing/thawing (Blesbois *et al.*, 2005; Buffone *et al.*, 2009; Darin-Bennett and White, 1977; Partyka *et al.*, 2016). At temperatures below phase transition, cholesterol increases membrane fluidity. This bimodal effect of cholesterol is more significant in membranes rich in saturated phospholipids as compared with those containing unsaturated ones (Crockett, 1998; Mocé *et al.*, 2010a). It has been reported that increasing membrane fluidity and permeability may reduce the volume of intra-cellular ice formation at temperatures above the phase transition, which can minimise freezing damage (Partyka *et al.*, 2016). In addition, the removal of cholesterol from the chicken sperm prior to freezing has resulted in the highest fluidity measured at 5°C (Partyka *et al.*, 2016).

POLYUNSATURATED FATTY ACIDS IN MEMBRANES

Cryopreservation causes a dramatic reduction in the fertility of poultry semen because of a high polyunsaturated/saturated fatty acid ratio in the plasma membrane, which makes poultry sperm relatively vulnerable to cold shock (Darin-Bennett and White, 1977; Santiago-Moreno *et al.*, 2012). Polyunsaturated fatty acids in the poultry sperm membrane can easily undergo lipid peroxidation (LPO) when reactive oxygen species (ROS) are present in the freezing medium (Cerolini *et al.*, 2006; Shahverdi *et al.*, 2015; Surai *et al.*, 1998).

Cryopreservation increases reactive oxidising species (ROS) production in the freezing media and ROS can attack the bis-allylic methylene group of plasma membrane phospholipids, leading to LPO (Agarwal *et al.*, 2014; Aitken *et al.*, 2014; Nguyen *et al.*, 2015) and subsequent sperm plasma membrane damage. Membrane damage is an important factor causing sperm death during the freezing/thawing process (Morris *et al.*, 2012). LPO causes sub-structural damage in sperm (Aitken *et al.*, 1996; Long, 2006). Increased ROS production reduces the phosphorylation of axoneme proteins, resulting in a decrease in membrane fluidity. Membrane fluidity is an indicator of sperm freezability in poultry species (Blesbois *et al.*, 2005) and is important for sperm-oocyte conjugation (Moghbeli *et al.*, 2016). Consequently, all these factors result in the immobility of sperm cells.

High amounts of ROS in the freezing media damage sperm DNA (Duru *et al.*, 2000; Fanaei *et al.*, 2011; Hu *et al.*, 2006; Khan, 2011). Experimental studies have shown that antioxidants prevent the decrease in sperm motility and DNA fragmentation (Greco *et al.*, 2005; Khan *et al.*, 2012b; Kopena *et al.*, 2011). Normally, antioxidant systems such as glutathione peroxidase, catalase, superoxide dismutase, vitamin E, and vitamin C are present in poultry semen (Khan *et al.*, 2013) to prevent the damage imposed by ROS, but the activity of such compounds is considerably decreased by cryopreservation (Partyka *et al.*, 2012).

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COMMONLY USED CPA ARE HARMFUL TO POULTRY SPERM

The first important step in successful poultry semen cryopreservation is the choice of the cryoprotectants and its use during the process. Commonly used cryoprotectans are glycerol (GLY) (Abouelezz *et al.*, 2017; Shahverdi *et al.*, 2015), dimethylacetamide (DMA) (Abouelezz *et al.*, 2015; Santiago-Moreno *et al.*, 2011), dimethylsulphoxide (DMSO) (Ansari *et al.*, 2017; Zhandi *et al.*, 2017) and dimethylformamide (DMF) (Lukaszewicz, 2002) (*Table 1*). However, these CPA are toxic (GLY, DMA and DMSO) for poultry sperm. GLY is the least toxic cryoprotectant, but it has contraceptive activity (Blesbois, 2011; Lake, 1986), although the underlying mechanism has not yet been clarified. One possible reason might be the decreased acrosome reaction of sperm. According to a previous study, a major reduction occurred in the ability of sperm cells to undergo the acrosome reaction immediately after the supplementation of CPA. The damage caused by GLY was more dramatic than that caused by DMA. The decrease in acrosome reaction was more pronounced than the decrease in motility and in the membrane integrity of sperm cells. CPA had considerably more harmful effects than cryopreservation, especially when GLY was used in combination with slow-freezing (Mocé *et al.*, 2010b). According to empirical studies, the combined introduction of sperm cells and GLY into the vagina prevents fertilisation if the final concentration of GLY is over 2% (Blesbois, 2011; Lake, 1986). Increasing the GLY concentration to 4% or greater resulted in almost no fertilized eggs (Abouelezz *et al.*, 2015) . Therefore, prior to intra-vaginal insemination, GLY has to be removed (Hammerstedt and Graham, 1992; Polge, 1951; Tajima *et al.*, 1989).

Preferred methods of GLY removal involve centrifugation through a discontinuous Accudenz column, direct centrifugation and stepwise dilution with a GLY-free medium (Purdy *et al.*, 2009). These methods are time-consuming and decrease the fertility rate of sperm cells due to the osmotic and physical damage associated with the removal of glycerol (Hammerstedt and Graham, 1992). Because of this, DMA has been proposed as an alternative cryoprotectant due to the its non-contraceptive effects (Bacon *et al.*, 1986; Blesbois *et al.*, 2007; Chalah *et al.*, 1999; Woelders *et al.*, 2006).

DMA does not need to be removed from the frozen/thawed sperm (Blesbois *et al.*, 2007; Chalah *et al.*, 1999; Woelders *et al.*, 2006), thereby avoiding sperm damage and resulting in greater fertility. However, in roosters, fertility did not differ between intra-vaginal insemination of sperm cells cryopreserved with GLY or DMA. Only intramagnal insemination of DMA-preserved sperm cells resulted in higher fertility levels than insemination of GLY-preserved sperm cells (Purdy *et al.*, 2009).

In the hen genital tract, egg yolk (EY) has a contraceptive effect (Santiago-Moreno *et al.*, 2012). Consequently, the fertility rate in chickens is reduced due to the use of EY in extenders and preservatives (Fewlass *et al.*, 1975; Harris Jr, 1968; Jasper, 1950; Wilcox, 1960). Experimental studies have shown that the dilution of rooster semen with chicken EY adequately protected rooster sperm from cold shock during cooling, freezing, and thawing, but EY-treated sperm cells could not fertilise any eggs. The biochemical mechanisms behind the contraceptive effect of EY are still not understood (Fuller, 2004; Santiago-Moreno *et al.*, 2012).

Strategies used for better fertility results

In contrast to the fertility rate of cryopreserved mammalian sperm, the fertility rate of cryopreserved poultry sperm is significantly lower (Long, 2006), constraining its commercial use. Therefore, different strategies, such as the use of different kinds of CPAs (Abouelezz *et al.*, 2015; Mocé *et al.*, 2010b), the use of different numbers of sperm

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cells (Zaniboni *et al.*, 2014), manipulation of the cryoprotectant concentration (Abouelezz *et al.*, 2015), different cooling rates (Madeddu *et al.*, 2016), packaging systems (pellets, vials, or straws) (Blesbois *et al.*, 2007; Mocé *et al.*, 2010a; Tselutin *et al.*, 1999) and the addition of antioxidants to poultry semen (Amini *et al.*, 2015a; Lotfi *et al.*, 2017; Safa *et al.*, 2016) have been used to increase the fertility rate for commercial use.

The most commonly used CPAs are GLY and the DMA (Abouelezz *et al.*, 2017). Experimental studies have shown that higher fertility rates could be obtained when the semen was mixed with DMA and frozen in a pellet form by directly plunging it into liquid nitrogen (Abouelezz *et al.*, 2017; Tselutin *et al.*, 1999). Alternatively, sperm cells could be mixed with GLY and slow-frozen in straws (Blesbois *et al.*, 2007; Long and Kulkarni, 2004; Seigneurin and Blesbois, 1995). However, the practical identification of semen samples frozen with DMA in pellets is difficult, as this method is prone to cross contamination (Wishart, 2009). Therefore, the use of straws and slow-freezing is more suitable and is required by Food and Agriculture Organization of the United Nations (FAO) guidelines (FAO, 2011).

As GLY has a contraceptive effect on avian sperm, avian semen must undergo further processing to reduce GLY before use. The mechanical damage associated with GLY removal was more harmful to fertilising capacity than the toxicity of DMA and freezing/thawing (Abouelezz *et al.*, 2017). Therefore, freezing the poultry semen both with DMA in pellets or with GLY in straw results in a small portion of thawed live sperm with a lower fertilizing ability compared with that of semen samples immediately after collection (Abouelezz *et al.*, 2017).

Poultry sperm cell membranes contain higher quantities of n-6 polyunsaturated fatty acids, including arachidonic (20:4n-6) and docosatetraenoic (22:4n-6) acids, than the membrane of mammalian sperm cells (Amini *et al.*, 2015b). This makes poultry sperm cells extremely vulnerable to LPO by ROS produced by the cellular components of semen during freezing (Nguyen *et al.*, 2015). Experimental studies have shown that LPO causes irreversible damage to DNA and decreases sperm mobility and fertility in the freezing/thawing process (Amini *et al.*, 2015a; 2015b; Safa *et al.*, 2016).

Seminal plasma from numerous animal species, including poultry, normally contains antioxidant properties (Bréque *et al.*, 2003; Cerolini *et al.*, 2006; Khan *et al.*, 2012a; Li *et al.*, 2010; Partyka *et al.*, 2012). However, antioxidant activity is dramatically decreased by cryopreservation, resulting in higher levels of LPO (Partyka *et al.*, 2012). Therefore, avian sperm cells must be supplemented with additional antioxidants in the media to overcome the effects of lipid oxidation during freezing/thawing. For this reason, numerous substances with antioxidant effects, such as vitamin C (Amini *et al.*, 2015a), vitamin E (Safa *et al.*, 2016), selenium (Safa *et al.*, 2016), catalase (CAT) (Amini *et al.*, 2015b) and hyaluronic acid (HA) (Lotfi *et al.*, 2017) have been added to semen prior to cryopreservation. Studies have shown that the addition of vitamin E, Se, CAT, and superoxide dismutase to rooster semen reduced oxidation rates and improved post-thaw sperm motility, viability, and membrane stability of the sperm cells (Amini *et al.*, 2015b; Safa *et al.*, 2016). The addition of CAT and vitamin E to rooster semen extender increased post-thaw sperm motility, activity in the mitochondria, and stability of the sperm cell membrane in contrast with the control group, but adding vitamin E and CAT to the extender had no effect on fertility rates (Moghbeli *et al.*, 2016). In another study, rooster semen supplemented with 1 and 2 mM/l carnitine reduced LPO and increased motility, membrane functionality, and viability, while lower motility and viability were observed in frozen-thawed sperm extender containing 8 mM/l carnitine (Fattah *et al.*, 2017).

HA has antioxidant effects both *in vitro* and *in vivo* (Campo *et al.*, 2004; Halicka *et al.*,

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2009). The beneficial effect of HA on sperm function depends on its concentration in the medium. The addition of 1 and 2 mM HA reduced oxidation, resulting in a higher percentage of acrosome integrity and higher fertility rates (Lotfi *et al.*, 2017).

Conclusions

Due to the lower fertility rate, cryopreserved poultry semen can currently not be used in commercial poultry production. Only fresh poultry semen is recommended in artificial insemination, especially in the turkey industry. Every poultry species has its own physiologic aspects of sperm, and significant differences exist between poultry species. However, standard cryopreservation protocols for each poultry species are not presently available. A new effective cryoprotectant for poultry semen still has to be discovered.

References

ABOUELEZZ, F., CASTAÑO, C., TOLEDANO-DÍAZ, A., ESTESO, M., LÓPEZ-SEBASTIÁN, A., CAMPO, J. and SANTIAGO-MORENO, J. (2015) Effect of the interaction between cryoprotectant concentration and cryopreservation method on frozen/thawed chicken sperm variables. *Reproduction in Domestic Animals* **50**: 135-141.

ABOUELEZZ, F., SAYED, M. and SANTIAGO-MORENO, J. (2017) Fertility disturbances of dimethylacetamide and glycerol in rooster sperm diluents: Discrimination among effects produced pre and post freezing-thawing process. *Animal Reproduction Science* **184**: 228-234.

AGARWAL, A., VIRK, G., ONG, C. and DU PLESSIS, S.S. (2014) Effect of oxidative stress on male reproduction. *The World Journal of Men's Health* **32**: 1-17.

AITKEN, R.J., BUCKINGHAM, D.W., CARRERAS, A. and IRVINE, D.S. (1996) Superoxide dismutase in human sperm suspensions: Relationship with cellular composition, oxidative stress, and sperm function. *Free Radical Biology and Medicine* **21**: 495-504.

AITKEN, R.J., SMITH, T.B., JOBLING, M.S., BAKER, M.A. and DE IULIIS, G.N. (2014) Oxidative stress and male reproductive health. *Asian Journal of Andrology* **16**: 31.

AMINI, M.R., KOHRAM, H., SHAHANEH, A.Z., ZHANDI, M., SHARIDEH, H. and NABI, M.M. (2015a) The effects of different levels of vitamin e and vitamin c in modified beltsville extender on rooster post-thawed sperm quality. *Cell and Tissue Banking* **16**: 587-592.

AMINI, M.R., KOHRAM, H., ZARE-SHAHANEH, A., ZHANDI, M., SHARIDEH, H. and NABI, M.M. (2015b) The effects of different levels of catalase and superoxide dismutase in modified beltsville extender on rooster post-thawed sperm quality. *Cryobiology* **70**: 226-232.

ANSARI, M., ZHANDI, M., KOHRAM, H., ZAGHARI, M., SADEGHI, M. and SHARAFI, M. (2017) Improvement of post-thawed sperm quality and fertility of arian rooster by oral administration of d-aspartic acid. *Theriogenology* **92**: 69-74.

BACON, L.D., SALTER, D.W., MOTTA, J.V., CRITTENDEN, L.B. and OGASAWARA, F.X. (1986) Cryopreservation of chicken semen of inbred or specialized strains. *Poultry Science* **65**: 1965-1971.

BAKST, M. and SEXTON, T. (1979) Fertilizing capacity and ultrastructure of fowl and turkey spermatozoa before and after freezing. *Journal of Reproduction and Fertility* **55**: 1-7.

BLANCH, E., TOMÁS, C., CASARES, L., GÓMEZ, E., SANSANO, S., GIMÉNEZ, I. and MOCÉ, E. (2014) Development of methods for cryopreservation of rooster sperm from the endangered breed "gallina valenciana de chulilla" using low glycerol concentrations. *Theriogenology* **81**: 1174-1180.

BLESBOIS, E. (2011) Freezing avian semen. *Avian Biology Research* **4**: 52-58.

BLESBOIS, E. (2012) Biological features of the avian male gamete and their application to biotechnology of conservation. *The Journal of Poultry Science* **49**: 141-149.

BLESBOIS, E. and BRILLARD, J. (2007) Specific features of in vivo and in vitro sperm storage in birds. *Animal* **1**: 1472-1481.

BLESBOIS, E., GRASSEAU, I. and SEIGNEURIN, F. (2005) Membrane fluidity and the ability to survive cryopreservation in domestic bird spermatozoa. *Reproduction* **129**: 371-378.

https://doi.org/10.1017/S0043933918000673 Published online by Cambridge University Press

BLESBOIS, E., SEIGNEURIN, F., GRASSEAU, I., LIMOUZIN, C., BESNARD, J., GOURICHON, D., COQUERELLE, G., RAULT, P. and TIXIER-BOICHARD, M. (2007) Semen cryopreservation for ex situ management of genetic diversity in chicken: Creation of the French avian cryobank. *Poultry Science* **86**: 555-564.

BRÉQUE, C., SURAI, P. and BRILLARD, J.P. (2003) Roles of antioxidants on prolonged storage of avian spermatozoa in vivo and in vitro. *Molecular Reproduction and Development* **66**: 314-323.

BUFFONE, M.G., VERSTRAETEN, S.V., CALAMERA, J.C. and DONCEL, G.F. (2009) High cholesterol content and decreased membrane fluidity in human spermatozoa are associated with protein tyrosine phosphorylation and functional deficiencies. *Journal of Andrology* **30**: 552-558.

CAMPO, G.M., AVENOSO, A., CAMPO, S., D'ASCOLA, A., FERLAZZO, A.M. and CALATRONI, A. (2004) The antioxidant and antifibrogenic effects of the glycosaminoglycans hyaluronic acid and chondroitin-4-sulphate in a subchronic rat model of carbon tetrachloride-induced liver fibrogenesis. *Chemico-Biological Interactions* **148**: 125-138.

CECIL, H. and BAKST, M. (1993) In vitro lipid peroxidation of turkey spermatozoa. *Poultry Science* **72**: 1370-1378.

CEROLINI, S., KELSO, K., NOBLE, R., SPEAKE, B., PIZZI, F. and CAVALCHINI, L. (1997) Relationship between spermatozoan lipid composition and fertility during aging of chickens. *Biology of Reproduction* **57**: 976-980.

CEROLINI, S., ZANIBONI, L., MALDJIAN, A. and GLIOZZI, T. (2006) Effect of docosahexaenoic acid and α-tocopherol enrichment in chicken sperm on semen quality, sperm lipid composition and susceptibility to peroxidation. *Theriogenology* **66**: 877-886.

CHALAH, T., SEIGNEURIN, F., BLESBOIS, E. and BRILLARD, J. (1999) In vitro comparison of fowl sperm viability in ejaculates frozen by three different techniques and relationship with subsequent fertility in vivo. *Cryobiology* **39**: 185-191.

CROCKETT, E.L. (1998) Cholesterol function in plasma membranes from ectotherms: Membrane-specific roles in adaptation to temperature. *American Zoologist* **38**: 291-304.

DARIN-BENNETT, A. and WHITE, I. (1977) Influence of the cholesterol content of mammalian spermatozoa on susceptibility to cold-shock. *Cryobiology* **14**: 466-470.

DONOGHUE, A. and WISHART, G. (2000) Storage of poultry semen. *Animal Reproduction Science* **62**: 213-232.

DURU, N.K., MORSHEDI, M. and OEHNINGER, S. (2000) Effects of hydrogen peroxide on DNA and plasma membrane integrity of human spermatozoa. *Fertility and Sterility* **74**: 1200-1207.

FANAEI, H., KESHTGAR, S., BAHMANPOUR, S., GHANNADI, A. and KAZERONI, M. (2011) Beneficial effects of α-tocopherol against intracellular calcium overload in human sperm. *Reproductive Sciences* **18**: 978-982.

FAO (2011) Draft guidelines for the cryoconservation of animal genetic resources, Journal, Vol. 13, pp. 1-135 (Rome, FAO).

FATTAH, A., SHARAFI, M., MASOUDI, R., SHAHVERDI, A., ESMAEILI, V. and NAJAFI, A. (2017) L-carnitine in rooster semen cryopreservation: Flow cytometric, biochemical and motion findings for frozen-thawed sperm. *Cryobiology* **74**: 148-153.

FEWLASS, T., SEXTON, T. and SHAFFNER, C. (1975) Effect of various levels of egg yolk, milk, seminal plasma or blood serum on the respiration and reproductive efficiency of chicken spermatozoa. *Poultry Science* **54**: 346-349.

FULLER, B.J. (2004) Cryoprotectants: The essential antifreezes to protect life in the frozen state. *CryoLetters* **25**: 375-388.

GRECO, E., ROMANO, S., IACOBELLI, M., FERRERO, S., BARONI, E., MINASI, M.G., UBALDI, F., RIENZI, L. and TESARIK, J. (2005) Icsi in cases of sperm DNA damage: Beneficial effect of oral antioxidant treatment. *Human Reproduction* **20**: 2590-2594.

HALICKA, H.D., MITLITSKI, V., HEETER, J., BALAZS, E.A. and DARZYNKIEWICZ, Z. (2009) Attenuation of the oxidative burst-induced DNA damage in human leukocytes by hyaluronan. *International Journal of Molecular Medicine* **23**: 695-699.

HAMMERSTEDT, R.H. and GRAHAM, J.K. (1992) Cryopreservation of poultry sperm: The enigma of glycerol. *Cryobiology* **29**: 26-38.

HARRIS, G. (Jr) (1968) Fertility of chickens inseminated intraperitoneally with semen preserved in liquid nitrogen. *Poultry Science* **47**: 384-388.

HOLT, W. (2000) Fundamental aspects of sperm cryobiology: The importance of species and individual differences. *Theriogenology* **53**: 47-58.

HU, J.-H., LI, Q.-W., LI, G., CHEN, X.-Y., HAI-YANG, H.-Y., ZHANG, S.-S. and WANG, L.-Q. (2006) The cryoprotective effect on frozen-thawed boar semen of egg yolk low density lipoproteins. *Asian-Australasian Journal of Animal Sciences* **19**: 486-494.

JASPER, A.W. (1950) Storage studies of diluted cock semen for use in artificial insemination of chickens. *Poultry Science* **29**: 812-817.

https://doi.org/10.1017/S0043933918000673 Published online by Cambridge University Press

KHAN, R. (2011) Antioxidants and poultry semen quality. *World's Poultry Science Journal* **67**: 297-308.
KHAN, R., LAUDADIO, V. and TUFARELLI, V. (2012a) Semen traits and seminal plasma biochemical parameters in white leghorn layer breeders. *Reproduction in Domestic Animals* **47**: 190-195.
KHAN, R., RAHMAN, Z., JAVED, I. and MUHAMMAD, F. (2013) Effect of vitamins, probiotics and protein level on semen traits and seminal plasma biochemical parameters of post-moult male broiler breeders. *British Poultry Science* **54**: 120-129.
KHAN, R., RAHMAN, Z., NIKOUSEFAT, Z., JAVDANI, M., LAUDADIO, V. and TUFARELLI, V. (2012b) Vitamin E: Pharmaceutical role in poultry male fecundity. *World's Poultry Science Journal* **68**: 63-70.
KOPENA, R., MARTÍN, J., LÓPEZ, P. and HERCZEG, G. (2011) Vitamin E supplementation increases the attractiveness of males' scent for female European green lizards. *PLOS ONE* **6**: e19410.
KOWALCZYK, A. and ŁUKASZEWICZ, E. (2015) Simple and effective methods of freezing capercaillie (tetrao urogallus l.) semen. *PLOS ONE* **10**: e0116797.
KURBATOV, A., NARUBINA, L., BUBLIAEVA, G., TSELUTIN, K., MAVRODINA, T., TUR, B. and KOSIANENKO, S. (1986) Cryopreservation of fowl semen. Proceedings of the 7th European Poultry Conference, M. Larbier (Ed) World's Poultry Science Assocication, Paris, France, pp. 998-1001.
LAKE, P. (1986) The history and future of the cryopreservation of avian germ plasm. *Poultry Science* **65**: 1-15.
LAKE, P. and WISHART, G. (1984) Comparative physiology of turkey and fowl semen, in: CUNNINGHAM, F., LAKE, P.Y. & HEWITT, D. (Eds) *Reproductive biology of Poultry*, pp. 151-160. British Poultry Science Ltd. (Longman Group, Harlow).
LI, Z., LIN, Q., LIU, R., XIAO, W. and LIU, W. (2010) Protective effects of ascorbate and catalase on human spermatozoa during cryopreservation. *Journal of Andrology* **31**: 437-444.
LONG, J. (2006) Avian semen cryopreservation: What are the biological challenges? *Poultry Science* **85**: 232-236.
LONG, J. and KULKARNI, G. (2004) An effective method for improving the fertility of glycerol-exposed poultry semen. *Poultry Science* **83**: 1594-1601.
LONG, J.A., PURDY, P.H., ZUIDBERG, K., HIEMSTRA, S.-J., VELLEMAN, S.G. 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.
LOTFI, S., MEHRI, M., SHARAFI, M. and MASOUDI, R. (2017) Hyaluronic acid improves frozen-thawed sperm quality and fertility potential in rooster. *Animal Reproduction Science* **184**: 204-210.
LUKASZEWICZ, E. (2002) An effective method for freezing white Italian gander semen. *Theriogenology* **58**: 19-27.
MADEDDU, M., MOSCA, F., SAYED, A.A., ZANIBONI, L., MANGIAGALLI, M., COLOMBO, E. and CEROLINI, S. (2016) Effect of cooling rate on the survival of cryopreserved rooster sperm: Comparison of different distances in the vapor above the surface of the liquid nitrogen. *Animal Reproduction Science* **171**: 58-64.
MOCÉ, E., BLANCH, E., TOMÁS, C. and GRAHAM, J. (2010a) Use of cholesterol in sperm cryopreservation: Present moment and perspectives to future. *Reproduction in Domestic Animals* **45**: 57-66.
MOCÉ, E., GRASSEAU, I. and BLESBOIS, E. (2010b) Cryoprotectant and freezing-process alter the ability of chicken sperm to acrosome react. *Animal Reproduction Science* **122**: 359-366.
MOGHBELI, M., KOHRAM, H., ZARE-SHAHANEH, A., ZHANDI, M., SHARAFI, M., NABI, M.M., ZAHEDI, V. and SHARIDEH, H. (2016) Are the optimum levels of the catalase and vitamin e in rooster semen extender after freezing-thawing influenced by sperm concentration? *Cryobiology* **72**: 264-268.
MORRIS, G.J., ACTON, E., MURRAY, B.J. and FONSECA, F. (2012) Freezing injury: The special case of the sperm cell. *Cryobiology* **64**: 71-80.
MÜLLER, K., MÜLLER, P., PINCEMY, G., KURZ, A. and LABBE, C. (2008) Characterization of sperm plasma membrane properties after cholesterol modification: Consequences for cryopreservation of rainbow trout spermatozoa. *Biology of Reproduction* **78**: 390-399.
NELSON, D., GRAHAM, E. and SCHMEHL, M. (1980) Fertility after dialyzing frozen turkey semen. Proceedings of the 9th International Congress on Animal Reproduction and Artificial Insemination, 16th-20th June 1980. III. Symposia (free communications).
NGUYEN, T.M.D., SEIGNEURIN, F., FROMENT, P., COMBARNOUS, Y. and BLESBOIS, E. (2015) The 5'-amp-activated protein kinase (ampk) is involved in the augmentation of antioxidant defenses in cryopreserved chicken sperm. *PLOS ONE* **10**: e0134420.
OMBELET, W. and VAN ROBAYS, J. (2015) Artificial insemination history: Hurdles and milestones. *Facts, Views & Vision in ObGyn* **7**: 137.
PARTYKA, A., BONARSKA-KUJAWA, D., SPORNIAK, M., STROJECKI, M. and NIŻAŃSKI, W. (2016) Modification of membrane cholesterol and its impact on frozen-thawed chicken sperm characteristics. *Zygote* **24**: 714-723.

https://doi.org/10.1017/S0043933918000673 Published online by Cambridge University Press

PARTYKA, A., ŁUKASZEWICZ, E. and NIŻAŃSKI, W. (2012) Effect of cryopreservation on sperm parameters, lipid peroxidation and antioxidant enzymes activity in fowl semen. *Theriogenology* **77**: 1497-1504.

PEGG, D. (2002) The history and principles of cryopreservation. Proceedings of the Seminars in Reproductive Medicine, pp. 005-014.

POLGE, C. (1951) Functional survival of fowl spermatozoa after freezing at− 79°C. *Nature* **167**: 949-950.

POLGE, C., SMITH, A.U. and PARKES, A.S. (1949) Revival of spermatozoa after vitrification and dehydration at low temperatures. *Nature* **164**: 666.

PURDY, P., SONG, Y., SILVERSIDES, F. and BLACKBURN, H. (2009) Evaluation of glycerol removal techniques, cryoprotectants, and insemination methods for cryopreserving rooster sperm with implications of regeneration of breed or line or both 1 2 3. *Poultry Science* **88**: 2184-2191.

SAFA, S., MOGHADDAM, G., JOZANI, R.J., KIA, H.D. and JANMOHAMMADI, H. (2016) Effect of vitamin e and selenium nanoparticles on post-thaw variables and oxidative status of rooster semen. *Animal Reproduction Science* **174**: 100-106.

SALISBURY, G.W. and VANDEMARK, N.L. (1961) Diluents and extension of semen, in: SALISBURY, G. W. & VANDEMARK, N.L. (Eds) *Physiology of reproduction and artificial insemination of cattle*, pp. 434-443 (San Francisco and London, W.H. Freeman & Company).

SANTIAGO-MORENO, J., CASTAÑO, C., TOLEDANO-DÍAZ, A., COLOMA, M., LÓPEZ-SEBASTIÁN, A., PRIETO, M. and CAMPO, J. (2011) Semen cryopreservation for the creation of a Spanish poultry breeds cryobank: Optimization of freezing rate and equilibration time. *Poultry Science* **90**: 2047-2053.

SANTIAGO-MORENO, J., CASTAÑO, C., TOLEDANO-DÍAZ, A., COLOMA, M.A., LÓPEZ-SEBASTIÁN, A., PRIETO, M.T. and CAMPO, J.L. (2012) Cryoprotective and contraceptive properties of egg yolk as an additive in rooster sperm diluents. *Cryobiology* **65**: 230-234.

SCHRAMM, G. and HUBNER, R. (1988) Effects of differentiated cryoprotectives and deep-freeze techniques on reproductive potential of turkey sperma after long-time storage. *Monatshefte für Veterinarmedizin* **43**: 426-427.

SEIGNEURIN, F. and BLESBOIS, E. (1995) Effects of the freezing rate on viability and fertility of frozen-thawed fowl spermatozoa. *Theriogenology* **43**: 1351-1358.

SEXTON, T. (1981) Development of a commercial method for freezing turkey semen. I. Effect of prefreeze techniques on the fertility of processed unfrozen and frozen-thawed semen. *Poultry Science* **60**: 1567-1573.

SHAHVERDI, A., SHARAFI, M., GOURABI, H., YEKTA, A.A., ESMAEILI, V., SHARBATOGHLI, M., JANZAMIN, E., HAJNASROLLAHI, M. and MOSTAFAYI, F. (2015) Fertility and flow cytometric evaluations of frozen-thawed rooster semen in cryopreservation medium containing low-density lipoprotein. *Theriogenology* **83**: 78-85.

SURAI, P., BLESBOIS, E., GRASSEAU, I., CHALAH, T., BRILLARD, J.-P., WISHART, G., CEROLINI, S. and SPARKS, N. (1998) Fatty acid composition, glutathione peroxidase and superoxide dismutase activity and total antioxidant activity of avian semen. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology* **120**: 527-533.

TAJIMA, A., GRAHAM, E. and HAWKINS, D. (1989) Estimation of the relative fertilizing ability of frozen chicken spermatozoa using a heterospermic competition method. *Journal of Reproduction and Fertility* **85**: 1-5.

THURSTON, R. and HESS, R. (1987) Ultrastructure of spermatozoa from domesticated birds: Comparative study of turkey, chicken and guinea fowl. *Scanning Microscopy* **1**: 1829-1838.

TSELUTIN, K., SEIGNEURIN, F. and BLESBOIS, E. (1999) Comparison of cryoprotectants and methods of cryopreservation of fowl spermatozoa. *Poultry Science* **78**: 586-590.

WALTERS, E.M., BENSON, J.D., WOODS, E.J. and CRITSER, J.K. (2009) The history of sperm cryopreservation. *Sperm banking theory and practice*, pp. 1-17 (Cambridge University Press, London).

WILCOX, F. (1960) Effect on fertility of temperature, handling methods, lake's solution and the addition of egg white, egg yolk, and sugars to the diluent used in storing chicken semen. *Poultry Science* **39**: 459-467.

WISHART, G. (1985) Quantitation of the fertilising ability of fresh compared with frozen and thawed fowl spermatozoa. *British Poultry Science* **26**: 375-380.

WISHART, G. (1989) Physiological changes in fowl and turkey spermatozoa during in vitro storage. *British Poultry Science* **30**: 443-454.

WISHART, G. (2009) Semen quality and semen storage, in: HOCKING, P. (Ed) *Biology of Breeding Poultry, Poultry Science Symposium Series*, pp. 151-178 (London, UK).

WOELDERS, H., ZUIDBERG, C. and HIEMSTRA, S. (2006) Animal genetic resources conservation in the Netherlands and Europe: Poultry perspective. *Poultry Science* **85**: 216-222.

XIA, L., LALLI, M.F., ANSAH, G.A. and BUCKLAND, R.B. (1988) Ultrastructure of fresh and frozen-thawed spermatozoa of high and low fertility lines of chickens. *Poultry Science* **67**: 819-825.

https://doi.org/10.1017/S0043933918000673 Published online by Cambridge University Press

ZANIBONI, L., CASSINELLI, C., MANGIAGALLI, M.G., GLIOZZI, T.M. and CEROLINI, S. (2014) Pellet cryopreservation for chicken semen: Effects of sperm working concentration, cryoprotectant concentration, and equilibration time during in vitro processing. *Theriogenology* **82**: 251-258.

ZHANDI, M., ANSARI, M., ROKNABADI, P., ZARE SHAHNEH, A. and SHARAFI, M. (2017) Orally administered chrysin improves post□thawed sperm quality and fertility of rooster. *Reproduction in Domestic Animals* **52**: 1004-1010.

https://doi.org/10.1017/S0043933918000673 Published online by Cambridge University Press

https://doi.org/10.1017/S0043933918000673 Published online by Cambridge University Press