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OVERVIEW OF TURKEY SEMEN STORAGE: FOCUS ON CRYOPRESERVATION – A REVIEW

Nicolaia Iaffaldano[1♦], Michele Di Iorio[1], Silvia Cerolini[2], Angelo Manchisi[1]

[1]Department of Agricultural, Environmental and Food Sciences, University of Molise, via De Sanctis snc, 86100 Campobasso, Italy
[2]Department of Health, Animal Production and Food Safety, University of Milan, via Trentacoste 2, 20134 Milan, Italy
*Corresponding author: nicolaia@unimol.it

Abstract

This review updates the current state of technologies available for turkey semen storage (hypothermic-liquid storage and cryopreservation), with special attention paid to cryopreservation. Liquid semen can be stored for up to 24 or 48 h at temperatures around 5°C, while cryopreservation allows long-term storage at –196°C. The possibility of using frozen turkey semen for artificial insemination (AI) would have practical benefits for turkey production. Reported fertility rates in response to AI using frozen/thawed semen range from 15.8 to 84.3%. Unsatisfactory fertility may be attributed to an inability of turkey spermatozoa to successfully survive the freezing/thawing process, and this, along with the high variability observed, makes this technique unacceptable for commercial breeding programs. There is therefore a need to standardize the whole freezing and thawing process to improve the post-thaw quality of turkey semen and minimize variability in results. Finding an efficient freezing protocol for turkey semen will allow for the creation of a sperm cryobank, improving current prospects for the commercial use of frozen turkey semen and also for the long-term conservation of the genetic diversity of this bird.

Key words: turkey semen, cryopreservation, semen storage, semen quality, fertility

The cryopreservation and storage of germplasm has long been valued for the indefinite preservation of genetic material, especially in cases of high-risk populations. An immediate need for this practice was identified for research using unique poultry lines (Long and Kulkarni, 2004). Today, however, semen cryopreservation seems to be the only effective method of storing reproductive cells for the *ex situ* management of genetic diversity in birds (Blesbois, 2011; Kowalczyk and Łukaszewicz, 2015). Successful semen cryopreservation has enabled the creation of semen banks for several wild and some poultry species (Saint Jalme et al., 2003; Blackburn, 2006; Woelders et al., 2006; Blesbois, 2007; Blanco et al., 2009; Kowalczyk et al., 2012).

However, research efforts have not yet served to create a turkey semen cryobank. The possibility of using turkey semen in frozen form for artificial insemination (AI), besides maintaining and ensuring the long-term conservation of this bird's genetic diversity, would have practical benefits for turkey production. Turkeys are the only commercial poultry species that depend entirely upon AI for fertile egg production. This is because the difference in size between males and females of commercial strains, resulting from genetic selection, makes it impossible for turkeys to naturally mate (Donoghue and Wishart, 2000; Iaffaldano et al., 2010). When freshly collected semen is used, AI yields exceptional fertility rates. However, this practice requires that toms and hens are kept on the same farm, meaning that a large number of birds have to be managed (Long and Bakst, 2007; Rosato et al., 2012). The turkey industry would therefore greatly benefit if semen could be stored after collection and used for subsequent AI (Rosato et al., 2012). The technologies available for semen storage are essentially: 1) hypothermic-liquid storage (refrigeration), which enables the storage of semen for up to 24 or 48 h at chilling temperatures around 5°C (Wishart, 2009); and 2) cryopreservation, whereby semen can be long-term stored at –196°C, the temperature of liquid nitrogen. In this review, we update the current state of these technologies, with special attention paid to the cryopreservation of turkey semen.

Hypothermic-liquid storage

During liquid storage at reduced temperature, sperm metabolism is not completely arrested. Using this method, domestic turkey semen cannot be stored longer than 6 h without losing its fertilizing ability, even when oxygenated and stored with appropriate diluents (Thurston, 1995). Recently, the research focus has been on defining the optimum diluent and improving storage systems addressing the composition of seminal plasma and sperm metabolic requirements or through the use of antioxidant supplements with biotechnologies designed to optimize the quality of stored turkey semen and consequent fertility (Donoghue and Wishart, 2000; Douard et al., 2000; Neuman et al., 2002; Douard et al., 2003; Iaffaldano and Meluzzi, 2003; Long and Kramer, 2003; Douard et al., 2004, 2005; Iaffaldano et al., 2005; Dimitrov et al., 2007; Iaffaldano et al., 2008; Zaniboni and Cerolini, 2009; Rosato et al., 2012). The results of these studies, although variable, have indicated the compromised survival of chilled turkey semen determining a reduction both in its quality and fertilizing ability (Table 1). The most popular extender used for stored turkey semen is BPSE (Beltsville Poultry Semen Extender). Extenders are buffers that promote the immediate survival of spermatozoa because they provide a similar osmotic pressure (330–400 mOsm) and pH (7.0–7.5) to that of seminal plasma, and are also a source of energy due to substrates such as carbohydrates (glucose or fructose) or other components such as citrate, glutamate and acetate (Iaffaldano et al., 2005). The liquid refrigeration of semen has returned better results in terms of both quality and fertilizing ability in the chicken (Sexton and Fewlass, 1978; Blesbois et al., 1999; de Figueiredo et al., 1999; Lemoine et al., 2011) and duck (Kasai et al., 2000; Penfold et al., 2001) (Table 1). Accordingly, Donoghue and Wishart (2000) reported fertility levels comparable to inseminated fresh semen for the storage at refrigeration temperatures of chicken semen for up to 24 h and of turkey semen for up to 6 h only. Moreover, tur-

key spermatozoa are active only in aerobic conditions whereas chicken spermatozoa are active also in anaerobic conditions. Turkey spermatozoa are considered efficient because of their high oxidation rate and low lactic acid accumulation in the presence of oxygen (Sexton, 1974). Further factors affecting the storability of turkey semen are age and strain, which were found to impact the quality of both fresh semen and of semen stored in liquid form (Iaffaldano et al., 2008).

Table 1. Recovered viability, motility and fertility rates recorded for chilled turkey semen (T: 4−5°C storage time: 24−72 hours) compared to rates reported for other poultry species

Species	Extenders	Storage time (h)	Viability (%)	Motility (%)	Fertility (%)	Reference
Turkey	BPSE	48	95	56	–	Douard et. al., 2000
	BPSE	48	35–80	40–75	–	Iaffaldano et al., 2005, 2008; Rosato et al., 2012
	BPSE	48	83–95	20–50	53–76 (24 h)	Douard et al., 2005
	CE*	48	83	35	–	Zaniboni et al., 2009
	BPSE	24	50	20–30	45–50	Long and Conn, 2012
	CE*	48	–	70–75	–	Słowińska et al., 2013
Chicken	BPSE	24	–	–	50–60	Sexton and Fewlass, 1978
	BPSE	48	93	53	–	Blesbois et al., 1999
	BPSE/Lake/ CE*	24	–	–	87–95	De Figueiredo et al., 1999
	BPSE	48	84	90	–	Lemoine et al., 2011
Duck	BPSE	24	–	85	52	Kasai et al., 2000
	BPSE	48 72	98 98	84 77	80 43	Penfold et al., 2001

*CE: commercial extender.

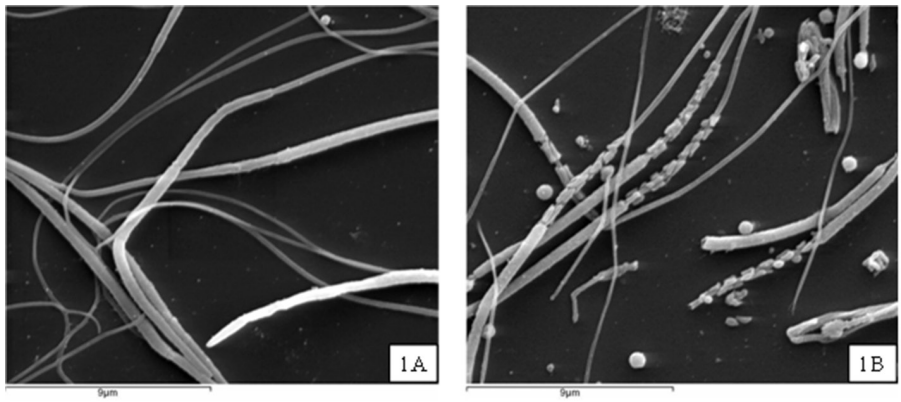
Cryopreservation

The possibility of using semen in frozen form for AI is a key factor for ensuring the long-term conservation of genetic diversity through the creation of a semen cryobank. Frozen semen also has several practical advantages for turkey production. Over 50 years ago, the discovery of glycerol's cryoprotective properties led to the development of a technology for the cryopreservation of semen in a wide range of species (Long, 2006). However, despite the good progress made in the cryopreservation of semen in cattle, this preservation method has not been as successful in avian species. The reason for this is the high cost of preparing and storing frozen ejaculates compared to the market price of day-old chicks, and also low semen quality and consequently the fertility levels achievable with frozen/thawed spermatozoa (Blesbois, 2007; Iaffaldano et al., 2011). The poor fertilization rates obtained for avian as opposed to mammalian species are attributable to the unique morphological characteristics of avian spermatozoa, such as their filiform shape, long tail and condensed nucleus, which makes them more susceptible to freezing damage (Donoghue and Wishart, 2000; Long, 2006).

In addition, membrane damage induced by cryopreservation results in impaired sperm transport and survival in the female reproductive tract with the consequent decreased duration of fertility that has been correlated with the number of spermatozoa in sperm storage tubules at the utero-vaginal junction (Pierson et al., 1988; Tajima, 2013).

Despite this, a variety of semen cryopreservation protocols involving different cryoprotective agents (CPAs), packaging methods, and freezing and thawing rates, have been developed, firstly in the chicken and then in other domesticated birds, such as turkey, duck and goose (see reviews Lake, 1986; Bellagamba et al., 1993; Hammerstedt, 1995; Surai and Wishart, 1996; Donoghue and Wishart, 2000; Blesbois, 2007, 2011).

Semen cryopreservation involves several steps, each one affecting sperm structure and function (Garner et al., 1999; Bailey et al., 2003): extension, cooling, CPA addition, freezing, and thawing (Bailey et al., 2003). Deleterious effects are the result of osmotic stress, and temperature changes produced during cooling, freezing and rewarming, ice crystal formation being one of the main biophysical mechanisms of sperm death (Swain and Smith, 2010) (Figure 1). A principal challenge for the survival of cells during cryopreservation, is the lethality of the intermediate temperature zone (–15 to –60°C), which is crossed twice during the cryogenic cycle, as cells are cooled and rewarmed (Gao and Critser, 2000; Blanco et al., 2011). Thus, the cryopreservation process causes numerous negative effects including damage to cell membranes (plasma and mitochondrial) and, in some cases, to the nucleus with devastating consequences for sperm survival (Blesbois, 2007). Following cryopreservation, metabolic damage may affect levels of adenosine triphosphate (ATP), which is essential for sperm motility (Long, 2006).



Bars represent 9 µm. (1A) Fresh semen: spermatozoa show intact membranes. (1B) Cryopreserved semen: spermatozoa show damaged membranes.
Scanning electron micrographs from the Department of Agricultural, Environmental and Food Sciences, University of Molise.

Figure 1. Pictures of fresh and frozen turkey semen, obtained by SEM (scanning electron microscopy)

Despite their similar morphology, the cryosurvival of sperm cells also varies among different avian species and has been correlated with the freezing procedure (i.e. speed of freezing/warming, sample volume, CPA, and CPA concentration) (Massip et al., 2004).

The decline in spermatozoa quality following semen storage is accompanied by changes in the proacrosin/acrosin system, which is involved in the acrosome reaction of spermatozoa and in phospholipids (Douard et al., 2000, 2004; Kotłowska et al., 2007; Słowińska et al., 2012, 2013). Thus, disruption of the proacrosin/acrosin system in turkey spermatozoa has been linked to a decline in semen quality during storage (Kotłowska et al., 2007; Słowińska et al., 2012). In particular, the phospholipid profile and contents of turkey spermatozoa are severely affected by *in vitro* storage, and changes in phospholipids are paralleled by the decrease in semen quality. This effect could be the consequence of endogenous metabolism of membrane phospholipid fatty acids inducing membrane destabilization. However, we cannot rule out a combination of many complex factors, including phospholipid lysis, endogenous metabolism, and lipid peroxidation (Douard et al., 2000, 2004; Kotłowska et al., 2007).

There is currently scarce information in the literature on turkey semen cryopreservation, since the freezing and thawing procedures developed for chickens or other birds are inefficient for turkey spermatozoa. Research efforts have focused on developing freezing protocols for the improved cryopreservation of turkey semen reducing the cell damage caused by freezing and thawing. Studies to date have examined the effectiveness of several cryoprotectants (glycerol, dimethylsulfoxide (DMSO), ethylene glycol, dimethylacetamide (DMA)), rapid or slow freezing-thawing procedures and the use of pellets or straws for packaging. The choice of CPA and its concentration certainly seems among the most important factors for an effective turkey semen freezing protocol. Permeable CPAs penetrate the sperm cell, increase membrane fluidity and partially dehydrate the cell, lowering the freezing point and thus reducing the formation of intracellular ice crystals, which is to be avoided because it causes physical and chemical stress. The permeable CPAs mainly used in freezing protocols for turkey semen are: glycerol, DMSO and DMA at different concentrations (Table 2). DMA and DMSO have been used as alternatives to glycerol since its discovered contraceptive effect. Glycerol has to be removed before AI which is an important drawback (Hammerstedt and Graham, 1992). Early studies examined the use of DMSO concentrations of 4% (Bakst and Sexton, 1979; Sexton, 1981). However, Iaffaldano et al. (2016) reported that 10% DMSO was better than 4%. These authors also showed (Iaffaldano et al., 2011) that a DMA concentration of 8% worked better than one of 6% when cells were frozen at high cooling rates, for example when directly plunging semen droplets into liquid nitrogen. Blanco et al. (2011) reported that 10% and 18% DMA provided more cryoprotection for turkey sperm when cryovials were used as the packing system. In this regard the effectiveness of sperm cryopreservation may also depend on the interaction between the cryoprotectant type used and the semen freezing and packaging method employed, that is the use of pellets or straws (Tselutin et al., 1999; Abouelezz et al., 2015). In addition, permeable CPAs themselves could paradoxically have a toxic effect on sperm, caus-

ing membrane destabilization and protein and enzyme denaturation; this toxicity is directly related to the CPA concentration used and the time of cell exposure (Swain and Smith, 2010).

Table 2. Freezing methods and devices used for the cryopreservation of turkey semen

CPA	Freezing methods	Packaging system	Reference
DMA (4–26%)	– rapid – slow (programmable freezer and liquid nitrogen vapor)	– pellets (50–100 µL) – straws (0.25–0.5 mL) – cryovials (100–500 µL)	Tselutin et al., 1995; Blanco et al., 2000, 2011, 2012; Labbé et al., 2003; Blesbois et al., 2005; Iaffaldano et al., 2009, 2011, in press; Long et al., 2014
DMSO (4–10%)	– slow (programmable freezer and liquid nitrogen vapor)	– straws 0.25 mL – cryovials	Bakst and Sexton, 1979; Sexton, 1981; Iaffaldano et al., 2016
Glycerol (9–11%)	– slow (programmable freezer and liquid nitrogen vapor)	– straws 0.25 mL	Pandian et al., 2011; Long et al., 2014

The addition of non-permeable CPAs to the freezing medium therefore serves to offset the cryodamage caused by permeating CPAs. At similar concentrations, these substances are less toxic than permeable CPAs and have multiple protective roles such as inhibiting ice crystal growth and helping the sperm to stabilize internal solute concentrations under osmotic stress. This reduces the amount of penetrating CPAs needed (Swain and Smith, 2010). Non-penetrating cryoprotectants are generally large molecules such as polymers, sugars, proteins or amino acids (Blanco et al., 2011; Rosato and Iaffaldano, 2013; Iaffaldano et al., 2014).

Blanco et al. (2011) tested trehalose and/or sucrose as non permeable CPAs in combination with DMA and reported the improved post-thawing motility of turkey semen compared with the use of DMA alone as CPA, which was dependent upon DMA concentration.

With regard to freezing rates, two main sperm cryopreservation techniques have been tested: slow freezing (conventional freezing) and ultra-rapid freezing (Table 2). The liquid nitrogen vapors used in conventional freezing produce a step-wise decrease in temperature, whereas ultra-rapid freezing rapidly solidifies the semen sample avoiding ice crystal formation.

As packaging systems pellets, cryovials or straws have been used (Table 2) (Sexton, 1981; Zavos and Graham, 1983; Lake, 1986; Tselutin et al., 1995; Blesbois et al., 2005; Blanco et al., 2011, 2012; Iaffaldano et al., 2011; Long et al., 2014). The semen thawing procedure (temperature and time) is also crucial for the post-freezing quality of semen. During slow thawing (low temperature, long time), the small ice crystals formed during freezing start to melt, turning into large crystals (recrystallization) that are harmful to the spermatozoa (Watson, 1995). During fast thawing (high temperature, short time) the time for recrystallization to occur is limited and this increases the survivability of spermatozoa. Using the pellet procedure, Iaffaldano et al. (2011) observed that thawing at a temperature of 75°C for 10 sec was better than 60°C for 12 sec, while when straws were used, thawing conditions of 50°C/10 sec were more efficient than 4°C/5 min (Iaffaldano et al., 2016).

Semen cryopreservation by the pellet procedure

Among the freezing systems assessed for the cryopreservation of avian spermatozoa, the pellet procedure is cheap, easily adaptable to field conditions, takes only a few seconds for cooling and warming, the cryoprotectant does not have to be removed and very fast freezing rates are achieved compared to the glycerol/slow-freeze method (Tselutin et al., 1999). The pellet procedure consists of the use of DMA and rapid cooling through direct plunging of semen droplets into liquid nitrogen to form frozen pellets. The method was developed by Tselutin et al. (1995) and subsequently adopted by others (Surai and Wishart, 1996; Blesbois et al., 2005; Iaffaldano et al., 2009). Recently, by testing different critical step combinations, we identified the best pellet procedure as: Tselutin extender, dilution rate 1:4, semen cooling 60 min; DMA 8%; equilibration time 5 min, drop volume 80 μL and thawing at 75°C for 10–12 sec (Iaffaldano et al., 2011).

This best pellet cryopreservation protocol returned recovery rates of 60% for mobility, 40% for viability (evaluated using the LIVE/DEAD Sperm Viability Kit, by fluorescence probes SYBR-14 and PI) and 42% for osmotic tolerance (using hypoosmotic swelling test). In *in vitro* studies, similar (Blesbois et al., 2005; Lemoine et al., 2011) or lower (Słowińska et al., 2012) sperm quality were observed after pellet cryopreservation following different turkey semen processing conditions. *In vivo*, Tselutin et al. (1995) obtained encouraging results using turkey semen cryopreserved by the pellet method. Rates of fertile eggs ranged from 71 to 84.3% but, unfortunately, the semen processing conditions and *in vitro* sperm quality were not specified. In later studies, however, lower fertility rates of 35–38% were observed for the pellet procedure (Labbé, 2003). The good fertility results obtained by Tselutin et al. (1995) were also confirmed in chicken and ducks (Tselutin et al., 1995). It is normally recognized that turkey sperm is much more sensitive to cryodamage than chicken sperm, and that the female reproductive tract is more stringently selective of turkey spermatozoa (Blesbois, 2007; Blanco et al., 2000; Whishart, 2009). In the review by Blesbois (2007), success rates for freezing/thawing of chicken and gander semen were higher compared to the semen of most other domestic birds including the turkey. In a prior work we observed the similar susceptibility to cryopreservation of chicken and turkey semen. Recovery rates of viable sperm were 39% and 41% respectively, while pheasant semen returned a recovery rate of viable sperm of only 20% (Cerolini et al., 2009).

Fast cooling by directly plunging of cryovials into liquid nitrogen has been found to be more detrimental for turkey sperm viability than slow cooling, whereas the reverse is true for chicken semen (Blanco et al., 2000).

Variation in the cryotolerance of male gametes among different bird species is thought to be a consequence of the bird's lipid profile, including cell membrane lipids such as cholesterol and phospholipids, whose ratio determines membrane fluidity (Blesbois et al., 2005; Blanco et al., 2000; 2008); any biochemical changes occurring during cryostorage (Long, 2006); and osmotic tolerance, which is low for turkey spermatozoa (Blanco et al., 2008). Thus, there is strong evidence to suggest that the success of a semen freezing procedure in one bird species may not translate to its success in another species (reviewed in Blesbois and Brillard, 2007; Blesbois, 2011, 2012; Tajima, 2013).

Table 3. Effectiveness of the cryopreservation of turkey semen compared to the semen of other poultry species

Species	Packaging system	CPA concentration	Viability (%)	Motility (%)	Reference
Turkey	pellet	DMA 6%	38	54	Lemoine et al., 2011
			–	24	Słowińska et al., 2012
		DMA 8%	39	–	Blesbois et al., 2005
			41	59	Cerolini et al., 2009
		DMA 6–8%	30–40	55–60	Iaffaldano et al., 2009, 2011
	straws	DMSO 10%	53	48	Iaffaldano et al., 2016
		Glycerol 11%	72–84	14.8–27.5	Long et al., 2014
		DMA 6%	50.5–61.2	12.4–19.5	
	Eppendorf	DMA 18%	35–39	40	Blanco et al., 2011, 2012
Chicken	pellet	DMA 6%	33–42.5	33–50	Tselutin et al., 1999; Blesbois et al., 2005; Cerolini et al., 2009; Mocé et al., 2010; Gliozzi et al., 2011; Zaniboni et al., 2014; Kowalczyk and Łukaszewicz, 2015
		DMA 3%	23.6	24.7	Abouelezz et al., 2015
		DMA 6%	18	19.8	
		Glycerol 11%	18.6	24	
	straws	Glycerol 11%	52	45	Seigneurin and Blesbois, 1995
		Glycerol 11%	79	–	Tselutin et al., 1999
		DMA 6%	74	–	
		Glycerol 11%	54	–	Chalah et al., 1999
		DMA 6%	47	–	
		Glycerol 11%	–	50	Mocé et al., 2010
		Glycerol 11%	73	–	Peláez et al., 2011
		DMSO 11%	28	–	
		DMA 6%	60.7	46.3	Kowalczyk and Łukaszewicz, 2015
		DMA 6%	21.1	21.3	Abouelezz et al., 2015
		Glycerol 8%	37	39	
		Glycerol 11%	47.6	43.4	
Duck	Eppendorf	DMSO 10%	–	73	Han et al., 2005
		Glycerol 8%	–	68	
		DMA 10%	–	61	
		DMF 8%	–	58	
Guinea fowl	pellet	DMA 6%	31.4	–	Váradi et al., 2013
	cryovials	EG 10%	41.1	–	Váradi et al., 2013
		DMF 6%	27.1	–	
	straws	DMF 6%	19	–	Blesbois et al., 2005
Pheasant	pellet	DMA 6%	20	17	Cerolini et al., 2009

Semen cryopreservation using the straw as packaging system

Although it was initially considered (Blesbois and Grasseau, 2002; Labbé et al., 2003) that straws were less efficient than pellets to restore the fertility of frozen turkey or chicken semen (Table 4), straws as a packaging system have benefits such as sperm traceability, and the safe transport of semen for breeding or storage in gene

banks. Straws have been widely used to freeze chicken semen using different CPAs such as glycerol, DMA or DMSO (Sexton, 1981; Williamson et al., 1981; Seigneurin and Blesbois 1995; Tselutin et al., 1999; Purdy et al., 2009; Mocé et al., 2010; Peláez et al., 2011; Santiago-Moreno et al., 2011). In a recent study in turkey, we obtained a semen quality recovery rate of about 50% using straws and 10% DMSO (Iaffaldano et al., 2016) (Table 3). Lower semen quality values were reported by Blanco (2011; 2012) using Eppendorf tubes as the packaging system (Table 3). In an *in vivo* study, Labbé et al. (2003) recorded lower fertility rates using the straw (15–20%) compared to the pellet procedure (Table 4). Long et al. (2014) reported similar fertility results ranging from 15.8 to 25% depending on the turkey line considered although these fertility results were achieved with just a single insemination of 240×10^6 sperm per hen. Higher fertility has been observed for the chicken (Seigneurin and Blesbois, 1995; Tselutin et al., 1999; Chalah et al., 1999), duck (Han et al., 2005) and guinea fowl (Seigneurin et al., 2013; Váradi et al., 2013). Post-thaw quality and fertility in the chicken tends to be lower using DMSO or DMA compared with the glycerol method (Tselutin et al., 1999; Santiago-Moreno et al., 2011; Peláez et al., 2011). It is not necessary to remove these cryoprotective agents prior to artificial insemination (Tajima et al., 1990; Tajima, 2013). Glycerol concentrations before insemination, on the other hand, should be below 0.163 M to avoid its contraceptive effect as reviewed in Tajima (2013).

Table 4. Fertility rates obtained in different avian species using frozen semen

Species	Packaging system	CPA concentration	Fertility (%)	Reference
Turkey	pellet	DMA 4%	71–84.3	Tselutin et al., 1995
		DMA 8%	35–38	Labbé et at., 2003
	straws	DMA 8%	15–20	Labbé et al., 2003
		DMA 6%	15.8–25	Long et al., 2014
Chicken	pellet	DMA 6%	93–94.4	Tselutin et al., 1995
		DMA 6%	84.7–92.7	Tselutin et al., 1999
		DMA 3%	25	Abouelezz et al., 2015
		DMA 6%	12.8	
		Glycerol 11%	4.2	
	straws	Glycerol 11%	88	Seigneurin and Blesbois, 1995
		DMA 6%	26.7	Tselutin et al., 1999
		Glycerol 11%	63.9	
		Glycerol 11%	76	Chalah et al., 1999
		DMA 6%	88	
		DMF 6.5%	79	
		DMA 6%	84.4	Kowalczyk and Łukaszewicz, 2015
		DMA 6%	10.8	Abouelezz et al., 2015
		Glycerol 8%	28.8	
		Glycerol 11%	2.1	
Duck	flasks	DMA 5%	75.1–83.6	Tselutin et al., 1995
	Eppendorf	DMSO 10%	40	Han et al., 2005
Guinea fowl	pellet	DMA 6%	63.6	Váradi et al., 2013
	cryovials	EG 10%	29.1	Váradi et al., 2013
	straws	DMF 6%	70.7	Seigneurin et al., 2013

Conclusions

In conclusion, the data reviewed indicate that the commercial use of stored turkey semen, particularly in frozen form, is still not satisfactory because of the inability of turkey spermatozoa to successfully survive freezing/thawing. According to the literature, variability in the biological material used and the multiplicity of preservation procedures has meant that it has not been possible to reproduce either the quality or fertilizing capacity of stored semen. Susceptibility to semen cryopreservation varies among poultry species (Blesbois, 2007, 2011; Iaffaldano et al., 2011), within species (Siudzińska and Łukaszewicz, 2008) and/or genetic lines (Long et al., 2010, 2014), and within breeds. Though surprising, occasionally higher fertility rates obtained for frozen-thawed turkey semen may be attributed to a higher dose of spermatozoa and a greater frequency of AI (Blesbois et al., 2008). Thus, there is a clear need to standardize the whole freezing and thawing process to minimize variability in results. In addition, we need to identify the key factors in turkey semen processing that will significantly affect the success of cryopreservation. Finding an efficient freezing protocol for turkey semen and determining the appropriate inseminating dose and frequency will allow for the introduction of a sperm cryobank and improve current prospects for the commercial use of frozen turkey semen.

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