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Review article

Protocols for sperm cryopreservation in the domestic cat: A review

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ABSTRACT

The main objectives of sperm cryopreservation in domestic cats are to preserve these gametes for future use, especially in valuable domestic cat breeds and to use knowledge-gained for developing sperm preservation techniques in wild felids that are threatened with extinction. To achieve acceptable quality of post-thaw sperm and results after insemination, sperm samples must be properly handled, cryopreserved and thawed by using appropriate protocols. In this paper, cryopreservation protocols of domestic cat sperm that have been reported previously are described. The subtopics include sources of sperm, freezing extenders, methods of sperm dilution, freezing storage vessels, methods of sperm cryopreservation, thawing temperature, and thawing extenders. In addition, comparisons of sperm quality results for different treatments within the same studies and between different studies are also presented.

1. Introduction

Among 37 feline species, the domestic cat (*Felis catus*) is the only species that is not threatened by extinction (Baillie et al., 2004). It seems suitable, therefore, to use the domestic cat to study different aspects of other felids including sperm cryopreservation. The development of valid protocols for sperm cryopreservation in domestic cats is not only useful for application of cryopreservation techniques to wild felids but also for use in breeding of domestic cats (Rijsselaere and Van Soom, 2010).

The objective of this paper is to compile protocols for cryopreservation of domestic cat sperm which have been reported from 1978 to 2016. The subtopics sources of sperm, freezing extenders, methods of sperm dilution, freezing storage vessels, methods of sperm cryopreservation, thawing temperature, and thawing extenders are covered.

2. Sources of sperm

To obtain male gametes in the domestic cat, various sperm collection techniques have been used such as the artificial vagina (AV), electroejaculation (EE), collection from the epididymides or the testicles, and a newly developed technique: urethral catheterization after pharmacologically induced sedation (Platz and Seager, 1978; Lengwinat and Blottner, 1994; Spindler and Wildt, 1999; Zambelli et al., 2008; Chatdarong et al., 2016). Among these methods, collection of sperm using an AV, along with manual stimulation (Tsutsui et al., 2000; Thiangtum et al., 2009; Lambo et al., 2012) and EE (Spindler and Wildt, 1999; Baran et al., 2004; Tebet et al., 2006; Chatdarong et al., 2010a) is most commonly used in the cat and results in ejaculates with acceptable quality. The AV technique is, however, suitable for some tomcats that have been trained to mount a “teaser” queen (Sojka, 1986). The EE technique is considered more suitable for sperm collection in this species even though this technique requires an approval of Animal Ethics Committees and is not approved for use in all countries (Zambelli et al., 2008). Using EE, the cats have to be generally anesthetized and thereafter ejaculate by electrical current stimulation delivered through a probe inserted into the rectum (Rijsselaere and Van Soom, 2010). The

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Table 1
Characteristics (mean ± SEM) of fresh domestic cat semen obtained by electroejaculation (EE; $n = 6$) and by artificial vagina (AV; $n = 6$) (Platz et al., 1978).

Technique	Semen volume (μL)	Total number of sperm/ejaculate (x10^6)	Motility (%)
EE	223.6 ± 49.8	29.7 ± 9.0	70.4 ± 2.6
AV	33.8 ± 4.8	60.7 ± 12.9	82.5 ± 2.7
Significance	$P < 0.01$	$P < 0.05$	$P < 0.01$

volume and alkalinity of semen obtained when using this method is greater compared with semen collected with use of an AV, presumably as a result of increased accessory sex gland secretions in response to electrical stimulation (Platz et al., 1978; Dooley and Pineda, 1986). Moreover, Platz et al. (1978) found that total number and motility of sperm were greater ($P < 0.05$) for samples collected with use of an AV than those collected with use of EE (Table 1). In addition to use of an AV and EE, collections of cat sperm from the cauda epididymides and the ductus deferens ("epididymal sperm") have also been reported. The collection methods of epididymal sperm include flushing by use of a 29-gauge needle (Bogliolo et al., 2001; Siemieniuch and Dubiel, 2007), compressing by using anatomic forceps (Luvoni et al., 2002; Martins et al., 2009), cutting/mincing/slicing by a scalpel blade or scissors (Kashiwazaki et al., 2005; Thuwanut and Chatdarong, 2009; Vizuete et al., 2014), and puncturing with a 30-gauge needle (Stachecki et al., 1994). Comparing these methods, retrieval of epididymal sperm by cutting/mincing/slicing of the epididymides into small pieces is most frequently performed although sperm samples obtained are inevitably contaminated with erythrocytes, leukocytes and other cells (Chatdarong et al., 2010b) which are detrimental to survival and fertilization capacity of cryopreserved sperm (Rijsselaere et al., 2004; Verberckmoes et al., 2004). Selection and purification of epididymal sperm immediately after collection either by swim-up or centrifugation through silica colloids, and thereafter resuspension of the pellet with sperm extender should, therefore, improve post-thaw sperm quality (Chatdarong et al., 2010b). Regardless of the method, collection of cat sperm from the epididymides is quite easy to perform and is frequently conducted after castration for the purpose of research and sometimes undertaken in animals that have recently died for the purpose of conserving genetic materials. Recovery and cryopreservation of epididymal sperm, nevertheless, require specific media and equipment which are not always available. It is, therefore, desirable to keep the testes attached to the epididymides or maintain the excised epididymides at controlled temperatures until recovery and preservation of epididymal sperm. The maximum time suitable for storage of the whole tissues prior to sperm recovery without adversely affecting the frozen-thawed sperm characteristics was rather short. In case of keeping the epididymides at 4–5 °C, the maximum time varied from less than 12 (Hay and Goodrowe, 1993) to 24 (Ganan et al., 2009; Toyonaga et al., 2010, 2011b; Table 2) h. At room temperature (20 °C), the maximum storage time of epididymides was only 12 h (Toyonaga et al., 2011a). The motility, viability and normal morphology of epididymal sperm decreased ($P < 0.05$) in the 24-h compared with those in the 0-, 6- and 12-h storage groups when both pre-freeze and post-thaw sperm samples were assessed (Toyonaga et al., 2011a). Chatdarong et al. (2009) reported, however, that sperm

Table 2
Quality after thawing of cat sperm from the cauda epididymides stored at low temperatures for different time periods.

Sperm characteristics (%)		Storage temperature (°C)	Storage time (h)					References
			0	12 (overnight)	24	48	96	
Motility	Pre-freeze	5	76	72				Hay and Goodrowe (1993)
	Post-thaw		52[a]	34[b]				(Data are mean)
Motility	Pre-freeze	4	80.0 ± 4.6[a]		66.0 ± 8.4[a,b]	50.0 ± 12.7[b]		Toyonaga et al. (2010)
	Post-thaw		38.0 ± 2.2[a]		36.0 ± 5.7[a]	14.0 ± 5.1[b]		(Data are mean ± SEM)
Viability	Pre-freeze		86.8 ± 3.1		78.5 ± 7.1	66.0 ± 11.9		
	Post-thaw		54.8 ± 5.3[a]		44.3 ± 3.8[a,b]	29.3 ± 5.0[b]		
Abnormal morphology	Pre-freeze		3.7 ± 2.1		7.6 ± 1.6	11.1 ± 7.5		
	Post-thaw		7.1 ± 1.6[a]		16.2 ± 2.6[a,b]	39.0 ± 8.5[b]		
Motility	Post-thaw	4	38.6 ± 6.9		31.4 ± 16.8			Toyonaga et al. (2011b)
Viability	Post-thaw		59.2 ± 11.3		50.3 ± 22.7			(Data are mean ± SD)
Abnormal morphology	Post-thaw		8.6 ± 3.0		15.4 ± 10.0			
Pregnancy rate*			28.6		28.6			
Motility	Pre-freeze	5	70.0 ± 6.5				64.6 ± 4.0	Chatdarong et al. (2009)
	Post-thaw		28.1 ± 4.6				31.9 ± 5.8	(Data are mean ± SEM)
Viability	Pre-freeze		70.2 ± 1.9				72.0 ± 1.8	
	Post-thaw		43.1 ± 3.2				49.3 ± 4.1	
Acrosome integrity	Pre-freeze		43.0 ± 4.3				42.8 ± 5.9	
	Post-thaw		18.7 ± 1.8				16.0 ± 3.3	

Different superscripts (a,b) indicate differences among storage times ($P < 0.05$).

* Pregnancy rates (%) determined with a real-time, B-mode ultrasonography at 15 days following unilateral intrauterine artificial insemination (UIUAI) of frozen-thawed epididymal sperm.