Chapter 3
Centrosomal Functions and Dysfunctions in Cat Spermatozoa

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Abstract The cat sperm centrosome is a paternally inherited organelle essential to successful fertilization and early embryo development. While there are structural and functional commonalities with other mammals, we have discovered original traits in the cat sperm centrosome that have offered new insights into sperm maturation as well as possible treatments for certain types of infertility. In contrast to ejaculated counterparts, cat testicular spermatozoa contain an immature centrosome preventing the formation of a large sperm aster post fertilization, which increases the incidence of early arrest in embryonic development. Emerging techniques that involve cellular desiccation or preservation in a liquid environment can adversely impact centrosomal properties of normal, mature spermatozoa. Most importantly, our investigations of the sperm centrosome in the cat model (1) re-emphasize the significance of this organelle in achieving successful reproduction and (2) are laying groundwork for overcoming centrosomal immaturity or dysfunctions using gamete micromanipulations or alternative, new in vitro sperm maturation systems.

3.1 Introduction

Our laboratory has strongly advocated for the need to expand reproductive knowledge beyond traditional animal models, especially laboratory and livestock species (Comizzoli et al. 2010; Wildt et al. 2010). Such a comparative approach is

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essential to appreciate the diversity of reproductive mechanisms and to speed the discovery of therapies that allow the more efficient treatment of infertility, including in humans. We, and others, have found a striking number of morphological and physiological phenomena in the domestic cat that are relevant to addressing fertility issues in both men and women. Among the commonalities between the cat and human are the temporal profiles of folliculogenesis and oocyte maturation (Pelican et al. 2006; Comizzoli et al. 2011) as well as the expression of teratospermia, a condition whereby >60 % of ejaculated spermatozoa are pleiomorphic (Pukazhenthi et al. 2006).

More recently, we have both characterized and examined the significance of cat sperm centrosome—the paternally inherited organelle essential to successful fertilization (via sperm aster formation) and early embryo development (Schatten and Sun 2011). The first centrosomal study in the cat occurred almost 30 years ago with transmission electron microscopy confirming the presence of a pair of sperm centrioles in the neck region with the more distal centriole clearly undergoing various degrees of degeneration compared to the intact, proximal counterpart (Sato and Oura 1984; Schmehl and Graham 1989). This ultrastructure was similar to other observations made in non-rodent mammals, including the sheep, bull, rhesus monkey, and human (for review see Manandhar et al. 2005). Interestingly, there have been few studies about centrosomal function in felids or in carnivores in general. In our recent studies (Comizzoli et al. 2006), we first confirmed that the cat sperm centrosome played a critical role in fertilization and early embryo development. Specifically, we determined that proper sperm aster formation is mainly directed by the paternal centrosome and is crucial for pronuclear migration and apposition as well as first mitotic spindle formation, as has been shown in other species (Schatten and Sun 2011). We also discovered that a larger size cat sperm aster correlates well with early embryo developmental success to the blastocyst stage (Comizzoli et al. 2006), as has been observed in bovine (Navara et al. 1996) and human (Terada et al. 2002, 2010) systems.

Besides increasing our fundamental base of knowledge, the characterizations of normal centrosomal structure and function in the cat can have applied relevance to addressing certain fertility issues. These conditions are significant in that sperm centrosomal dysfunctions have been identified to contribute to infertility in men (Van Blerkom and Davis 1995). There have been a few investigations of centrosomal functions involving heterologous human sperm injections into bovine or rabbit oocytes (Terada et al. 2002). However, the etiology of centrosomal dysfunctions remains poorly understood, and means for mitigation are still lacking (Schatten and Sun 2011; Terada et al. 2010).

The objective of this review is to highlight the value and detailed findings of sperm centrosomal studies, specifically in the domestic cat model. Such studies are providing new insights into the role of this organelle, a better understanding of its dysfunction, and potential means for overcoming such biological anomalies that may have relevance to improving human reproductive health.

3.2 Centrosomal Immaturity in Cat Testicular Spermatozoa

Spermatozoa extracted from the seminiferous tubules of testicular tissue as well as epididymal and ejaculated cat spermatozoa have a similar-shaped head region that contains highly compacted chromatin with a low incidence (<5 %) of DNA damage (Comizzoli et al. 2006). Labeling of the long midpieces with Mitotracker Green reveals that mitochondria occupy most of the area between the head and the flagellum (Fig. 3.1a). The sperm centrosome is easily detectable by the presence of centrin, a common centrosomal protein, in both centrioles located between the head and the midpiece. Centrin appears equally abundant in testicular (Fig. 3.1b) as well as in ejaculated (Fig. 3.1c) spermatozoa.

The first role of the sperm centrosome after fertilization is to organize the formation of microtubules into a sperm aster that enables both maternal and paternal pronuclei to migrate and undergo syngamy (Schatten and Sun 2011). The formation of a large sperm aster occurs about 5 h after sperm penetration into oocytes in vitro (Comizzoli et al. 2006; Jin et al. 2011; Xu et al. 2011). As previously observed in the bovine system (Navara et al. 1996), cat sperm aster morphology is highly reflective of developmental potential. Specifically, pronuclear migration is accelerated in the presence of a larger size sperm aster that, in turn, promotes the first cleavage division (no later than 26 h post-penetration) that eventually encourages embryonic development to the blastocyst stage (Table 3.1; Comizzoli et al. 2006). More specifically, we measured a 50 % increase in advanced embryo formation in the presence of a large diameter sperm aster (Comizzoli et al. 2006). The source of spermatozoa influences the capacity to develop asters of varying size. For example, a primary reason that testicular spermatozoa fail to fertilize or experience delayed first cleavage and compromised embryo development is due to inability to produce an aster or one of normal, large size (Table 3.1; Comizzoli et al. 2006). Additionally, we have routinely observed that about 25 % of all motile cat spermatozoa from ejaculates or the epididymis consistently produced small asters. Given the clear immaturity of centrosomes in cat testicular spermatozoa, it is expected that earlier sperm stages also contain immature centrosomes, as has been observed in the rabbit (Tachibana et al. 2009).

Based on studies in the porcine system, it is known that nucleation activity of the sperm centrosome influences microtubule length by attracting γ-tubulin (Sun et al. 2001). But this mechanism is complex and not well understood. Dysfunctional centrosomes in human and non-human primate testicular or ejaculated spermatozoa lead to blocked pronuclear stage formation post insemination (Hewitson et al. 1996; Palazzo et al. 2000; Nakamura et al. 2001). By contrast, centrosomal dysfunctions in cat testicular spermatozoa do not result in complete impediments as illustrated by some minimal embryo development (Comizzoli et al. 2006). For the cat, this condition appears to be more of a centrosomal immaturity (with poor nucleation capacity) rather than a true dysfunction. According to previous studies, centrosomal maturation has been defined as the change in microtubule nucleation potential occurring as cells generally pass
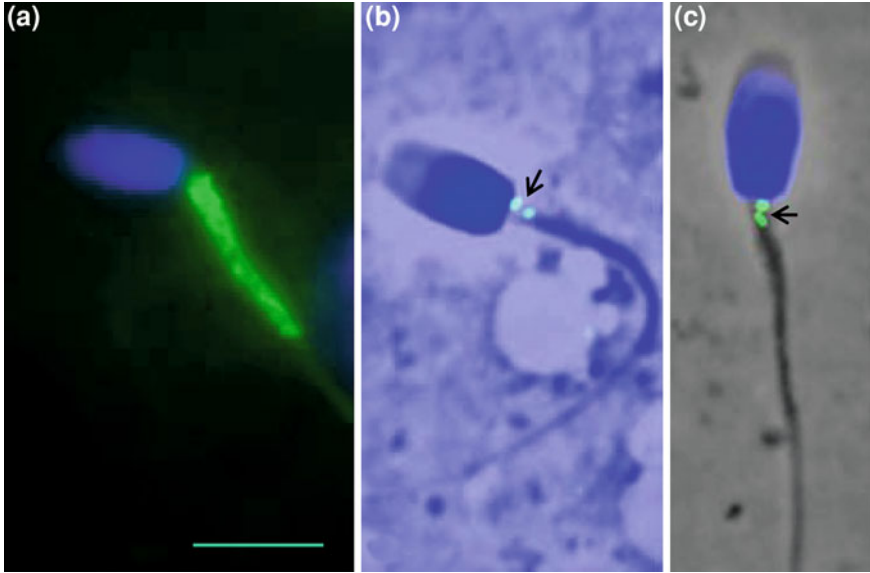
Fig. 3.1 Structure of cat spermatozoa (Sperm head DNA stained with Hoechst 33342). Mitotracker Green staining of the midpiece (**a**), centrin immunostaining (*arrow*) in testicular (**b**), and ejaculated (**c**) spermatozoa. *Bar* = 5 μm

through specific phases of the cell cycle (Palazzo et al. 2000). Although this theory remains to be tested in the cat, we suspect that sperm centrosomes in this species do not contain essential proteins, which have been argued to provoke complete maturation of this organelle in other species (Manandhar and Schatten 2000; Palazzo et al. 2000; Goto et al. 2010). Rather, our functional comparisons of spermatozoa recovered from the testis, epididymis, and ejaculate point to a full centrosomal maturation being acquired during epididymal transit. This maturational phenomenon likely is associated with the accumulation of new cytosolic proteins and/or protein phosphorylations in this region of the male reproductive tract (Axnér 2006).

3.3 Causes of Centrosomal Dysfunctions in Cat Spermatozoa

3.3.1 Impact of Teratospermia

The high proportion (>60 %) of abnormal spermatozoa in the ejaculates of certain felid species, populations, or individuals can be a significant cause of infertility through a host of functional failures, including from cells with apparently normal structure (Pukazhenthi et al. 2006). Nonetheless, Penfold et al. (2003) have demonstrated that morphologically normal cat spermatozoa recovered from a

Table 3.1 Presence of centrosome (centriole pairs detected by centrin immunostaining) and assessment of centrosomal functions (sperm aster formation after sperm injection and success of embryo development) for different types of fresh spermatozoa or after various preservation approaches

	Centrin labeling	Proportion of large sperm aster	Embryo development	Source
Epididymal spermatozoa (from cauda or ductus deferens)	+	>75 %	∼35 % blastocysts	Pers. obs.
Ejaculated spermatozoa	+	>75 %	∼35 % blastocysts	Comizzoli et al. (2006)
Testicular spermatozoa	+	0 %	Arrest at the 16-cell stage	Comizzoli et al. (2006)
Abnormal spermatozoa from teratospermic ejaculate	+	0 %	Arrest at the 16-cell stage	Pers. obs.
Normal spermatozoa from teratospermic ejaculate	+	>75 %	∼35 % blastocysts	Pers. obs.
Epididymal or ejaculated spermatozoa post-freezing	+	>75 %	∼35 % blastocysts	Comizzoli et al. (2006)
Epididymal or ejaculated spermatozoa post-drying	+	0 %	Arrest at the 16-cell stage	Pers. obs.; Ringleb et al. (2011)
Epididymal or ejaculated spermatozoa preserved in liquid	+	>75 %	Arrest at the 16-cell stage	Pers. obs.; Murakami et al. (2005)

teratospermic ejaculate can undergo normal fertilization and early embryo developmental events when microinjected into a conspecific oocyte. We confirmed these results by detecting the presence of normal sperm aster formations after injecting structurally normal spermatozoa from teratospermic ejaculates into cat oocytes (Table 3.1). Simultaneously, we observed that malformed spermatozoa from the same teratospermic semen had dysfunctional centrosomes based on the formation of small sperm asters and arrested early embryonic development (Table 3.1). This malfunction occurred even though a normally appearing centriole pair was detected in these pleiomorphic cells (Table 3.1). These observations in the cat are analogous to what occurs in some cases of human teratospermia where poor centrosomal function is due, for instance, to abnormal alignment of the head-flagellum junction (Manandhar et al. 2005; Nakamura et al. 2005). However, human centrosomal aberrations in teratospermic human ejaculate have been associated with lower expression of pericentriolar proteins, including centrin (Manandhar et al. 2005; Nakamura et al. 2005). This finding has not been apparent in the cat, although a search for alternative centrosomal proteins that might be involved in cat teratospermia is a worthy target for future study.

3.3.2 Impact of Classical and Emerging Preservation Techniques

Spermatozoa are commonly exposed to a variety of perturbations for the purposes of evaluation, processing, freezing, and practical use. Classical freeze-preservation approaches that rely on exposing these cells to cryoprotectant, osmotic, and cooling/freezing/thawing stressors are well known to adversely affect the quality of cat spermatozoa (Pukazhenthi et al. 2006). However, the use of a slow freezing (circa −1°C/min) method has been shown to retain both normal centrosomal structure and function (Comizzoli et al. 2006; Table 3.1). There has been growing interest in the scientific community in preserving male gametes via freeze-drying, desiccation, or in a liquid environment at supra-zero temperatures. While promising, these methods easily compromise sperm motility, thereby complicating the ability to select spermatozoa that have functional centrosomes. Interestingly, freeze-drying apparently is not detrimental to centrosomal functions of non-human primate (Sánchez-Partida et al. 2008) and bovine (Hara et al. 2011) spermatozoa. However, primate spermatozoa that are simply desiccated in trehalose appear to lose fertilization potential (Klooster et al. 2011). These important and contradictory findings deserve more thorough validation, especially as Ringleb et al. (2011) recently determined that the injection of freeze-dried cat spermatozoa into oocytes leads to limited early embryo development. We have also confirmed the compromised sperm aster formation using desiccated spermatozoa (dried at ambient temperature in trehalose) that were injected into conspecific oocytes (Table 3.1). We suspect that both phenomena are due to altered centrosomal functions. One study has also demonstrated loss in centrosomal capacity after storing cat spermatozoa in alcohol (Murakami et al. 2005). However, recently, we have effectively preserved cooled (4 °C) cat spermatozoa for up to 2 weeks in a 2 M trehalose solution while successfully retaining DNA integrity, centrosomal structure (presence of centrin), and function (sperm aster formation; Table 3.1).

3.4 Mitigating Centrosomal Immaturity and Dysfunctions

3.4.1 Sperm Micromanipulations and Selections

It is possible to use sonication and micromanipulation to replace an immature centrosome from a non-functioning testicular spermatozoon with a mature counterpart from an ejaculated cell. These reconstructed cat spermatozoa have biological viability, at least in capacity to develop into blastocysts in vitro (Comizzoli et al. 2006). Interestingly, such re-built spermatozoa are comprised of a head and centrosome/midpiece that are sufficiently proximate to ensure adequate interaction, pronuclear alignment, and linear migration no different from that observed after microinjecting an intact (un-reconstructed) spermatozoon (Van Blerkom and

Davis 1995). Confirmation that the sperm head remains adjacent to the centrosome/midpiece is also demonstrated by absence of arrest during the first cell cycle (Palazzo et al. 2000). These observations are important in illustrating that it is possible to replace the centrosome of one spermatozoon with that of another while ensuring the effective reorientation and elongation of the microtubule array toward the female pronucleus, which is known to be a good indicator of sperm aster quality (Navara et al. 1996). This observation naturally leads to considering the potential of this approach as a therapeutic remediation strategy in certain cases of male infertility. This prospect has already been proposed in humans with positive results (Emery et al. 2004). In the latter study, sperm heads detached from the respective flagella were co-injected into oocytes which resulted in a normal pregnancy. In our laboratory, we also envision substantial promise in centrosomal replacement across spermatozoa from different species. For example, we have studied sperm form and function in more than 25 felid species, many of which are endangered and teratospermic (Pukazhenthi et al. 2006). It would be intriguing to determine if centrosomes transferred from a normospermic species to spermatozoa from males that produce high proportions of malformed cells can be used to boost fertility or genetic management of rare individuals or populations.

Lastly, improving the sperm selection before microinjection might be another solution to avoid injecting cells with centrosomal issues. It is now possible to predict centrosomal function on the basis of midpiece morphometry as measured using a high magnification device. This process known as intracytoplasmic, morphologically selected sperm injection (IMSI) has been reported in humans (Ugajin et al. 2010) and is under development in cats.

3.4.2 Centrosomal Maturation In Vitro

Given that centrosomal maturity and normal function are acquired as testicular spermatozoa pass through the epididymis, then a priority is to learn significantly more about maturational processes within this region. Our knowledge on this subject is rudimentary for all species. For the cat, epididymal epithelial cells secrete factors (including hypotaurine or alkaline phosphatase) that impart physiological changes and could permit the sperm centrosome to complete maturation (Axnér 2006). However, a lack of information on specific mechanisms and proteins has made it impossible to artificially mature testicular spermatozoa in vitro for subsequent assisted reproduction use in any species. We are currently investigating centrosomal function and the presence of centriole maturation markers, such as cenexin (Lange BM and Gull K 1995) and speriolin (Goto et al. 2010), in spermatozoa isolated from different regions of the cat epididymis. We hope that resulting knowledge can be used to develop in vitro maturation protocols that could promote centrosomal maturation in testicular gametes and/or overcome functional deficits in spermatozoa regardless of source.

3.5 Conclusions

Clearly, the centrosome is a sperm organelle critical to both fertilization and embryogenesis and is a significant factor in male-related infertility. To date, the sperm centrosome in most species studied (including the domestic cat) share similar structural properties. Primarily, these include a pair of centrioles with centrin labeling and the distal centriole degenerating. Our studies reveal that epididymal transit is essential to normal functionality of this organelle in cats. In the absence of appropriate maturational conditions, an adequately sized sperm aster fails to develop after fertilization which leads to embryo developmental arrest. Results also reveal that the centrosome can be highly sensitive to environmental alterations. For example, some but not all methods of sperm preservation can adversely affect centrosome functionality. The cat and felids in general appear to be particular useful models for centrosomal studies due to the tendency of individuals (or species) to produce high numbers of malformed spermatozoa, as in men. Centrosomal dysfunctions are prevalent in these pleiomorphisms, thereby offering interesting models for testing remediation approaches. Based on preliminary findings, we believe it will be possible to provoke centrosomal maturation of immature (testicular) spermatozoa or overcome deficits in abnormally shaped cells using micromanipulation-cellular reconstruction and/or new in vitro culture systems. These approaches could have widespread application to animal models, endangered species, and human reproductive health.

Competing Interests
The authors declare that they have no competing interests.

Authors' Contributions
PC and DW equally contributed to the conception and writing of the review article.
Both authors read and approved the final manuscript.

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