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Short Communications

ASSESSMENT OF FELINE SPERMATOZO QUALITY VIA MTT ASSAY FROM POST-ORCHIDECTOMY EPIDIDYMAL SALVAGE

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SUMMARY

This study compares two methods for salvaging epididymal spermatozoa in domestic cats - slicing and mincing. It also explores the correlation between conventional semen evaluation and MTT assay results. The parameters compared were sperm motility, number of live sperm, wave pattern, abnormal sperm, and total sperm count. Both slicing and mincing methods proved equally effective in salvaging epididymal spermatozoa. The results showed a negative tendency in the correlation between MTT absorbance and traditional semen evaluation parameters for both the left and right testes; however, these associations were not statistically significant ($p > 0.05$). This suggests that MTT absorbance values do not correlate with standard semen quality indicators, such as motility, concentration, and morphology, for either testis.

Keywords: epididymal salvage, mincing, MTT assay, slicing, domestic cat

INTRODUCTION

Cat breeding has seen a growing trend towards visually appealing breeds with pedigrees and stud lines crucial for health and genetic diversity (Byun et al., 2008). In the male reproductive system, the epididymis is a crucial location for sperm development and motility (Joram, 2016). Sperm cells within the epididymis undergo various stages of development, motility attainment and the ability to fertilise an egg. This study explores effective methods for collecting semen from deceased animals, a critical aspect of animal genetic conservation, particularly for wildlife and endangered species facing population decline (Hewwit et al., 2001). The research compares two practical techniques—slicing and mincing—for salvaging epididymal sperm, which is often one of the last viable sources of genetic material post-mortem. A notable strength of this study lies in its thorough evaluation and validation of these methods in domestic cats, serving as a model species for broader applications. By confirming that both techniques yield comparable results in semen quality assessment, the study provides valuable insights for reproductive research and conservation programs. This flexibility in method choice is especially beneficial in clinical and field settings, where access to resources, equipment, or time may be limited. The findings support adopting either approach based on situational constraints, enhancing the efficiency and practicality of genetic preservation efforts for at-risk species.

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The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5 2,5-diphenyltetrazolium bromide) assay is a colorimetric method for determining metabolic activity in living cells, including spermatozoa (Ghasemi, 2021). This study aimed to assess epididymal spermatozoa using conventional evaluation and MTT assay. There is a positive correlation between semen quality indicators (motility, concentration, and morphology) and MTT absorbance values. Higher motility and better morphology reflect greater sperm viability and metabolic activity (Aziz, 2006). This hypothesis aligns with the principle that the MTT assay measures mitochondrial activity, which is a marker of sperm cell viability and health. If sperm cells exhibit high motility, intact morphology, and good concentration, they are likely to have active mitochondria, resulting in higher MTT absorbance values.

MATERIALS AND METHODS

Testes and epididymis from 20 tomcats (mean age of 12.6 months) were retrieved via orchidectomy by a licensed Veterinarian under general anaesthesia with ketamine (10 mg/kg, intramuscularly) and xylazine (0.5 - 1 mg/kg, intramuscularly). Sample collection involved harvesting sperm cells from the vas deferens and the cauda epididymis using slicing and mincing techniques. In the slicing method, the epididymis was cut longitudinally with a sterile scalpel, placed in 5 ml of PBS, and left for 5–10 minutes to allow sperm to swim up. The upper portion of the solution was then collected and centrifuged at 10,000 rpm for 10 minutes. For the mincing method, the epididymis was suspended in 5 ml of PBS in a petri dish and minced thoroughly with clean scissors, followed by a 10-minute resting period to allow the sperm to be released.

Semen evaluation involved examining nett semen under a light microscope to observe general motility and individual motility. Live sperm percentage was verified using the eosin-nigrosin stain. Semen concentration was evaluated using a

haemocytometer, and the MTT assay was used to measure the optical density (OD) of the samples.

Data was tabulated using Google Sheets and statistical analysis was performed with IBM Statistical Package for Social Sciences (SPSS) version 27. The correlation between semen evaluation parameters and MTT assay absorbance results was determined via Spearman's correlation test, and the Wilcoxon signed-rank test was used to compare the two epididymal sperm salvage techniques.

RESULTS

The averages of the semen evaluation parameters of all 20 post-orchidectomy tomcats are shown in Table 1. There was a negative correlation between conventional semen evaluation parameters, except general motility and abnormal sperm percentage, and MTT absorbance values for left testes, but the correlations were all insignificant ($p > 0.05$). All conventional semen evaluation parameters except for abnormal sperm percentage are negatively correlated to MTT absorbance values for the right testes, but the correlation was insignificant ($p > 0.05$). There was no significant difference in spermatozoa quality and quantity between the collection methods of both epididymal sperm salvage techniques.

In studies involving mammalian sperm, higher MTT absorbance values (e.g., OD > 0.4–0.5) indicate higher sperm viability and mitochondrial activity. Lower absorbance values (e.g., OD < 0.2) suggest poor mitochondrial function, often correlating with reduced motility and viability (Kumar et al., 2018).

Table 1. Average of semen evaluation parameters collected from 20 post-orchidectomy tom cats

Parameters	Left testes	Right testes
Motility (%)	67.5 ± 20.5	64.7 ± 20.1
Wave pattern	2.7 ± 0.7	2.9 ± 0.5
Live sperm (%)	79.5 ± 10.8	82.1 ± 9.5
Abnormal Sperm (%)	17.9 ± 9.3	18.2 ± 8.1
Total sperm count ($x10^6$)	13.9 ± 5.2	14.6 ± 3.8
*MTT Absorbance	0.062 ± 0.002	0.105 ± 0.094

*MTT = 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

Table 2. Spearman's correlation test between MTT assay parameters to conventional semen evaluation parameters of the left testes of 20 post-orchidectomy tomcats.

Correlations

			Motility_L	Wavepattern_L	Livesperm_L	Abnormal_sperm_L	concentration_L	MTT_L
Spearman's rho	MTT_L	Correlation Coefficient	.061	-.021	-.409	.296	-.201	1.000
		Sig. (2-tailed)	.799	.930	.074	.206	.396	.
		N	20	20	20	20	20	20

Table 3. Spearman's correlation test between MTT assay parameters to conventional semen evaluation parameters of the right testes of 20 post-orchidectomy tomcats.

Correlations

			Motility_R	Wavepattern_R	Livesperm_R	Abnormal_sperm_R	Concentration_R	MTT_R
Spearman's rho	MTT_R	Correlation Coefficient	-.313	-.305	-.398	.396	-.370	1.000
		Sig. (2-tailed)	.179	.192	.082	.084	.108	.
		N	20	20	20	20	20	20

Table 4. Comparing semen evaluation parameters of the left testes that underwent slicing and right testes that underwent mincing epididymal sperm salvage technique of 20 post-orchidectomy tomcats.

Test Statistics[a]

	Motility_R - Motility_L	Wavepattern_R - Wavepattern_L	Livesperm_R - Livesperm_L	Abnormal_sperm_R - Abnormal_sperm_L	Concentration_R - concentration_L	MTT_R - MTT_L
Z	-.595[b]	-.577[b]	-1.281[b]	-.593[c]	-1.499[b]	-1.682[b]
Asymp. Sig. (2-tailed)	.552	.564	.200	.553	.134	.093

Table 5. Comparing semen evaluation parameters of the right testes that underwent slicing and left testes that underwent mincing epididymal sperm salvage technique of 20 post-orchidectomy tomcats.

Test Statistics[a]

	Motility_R - Motility_L	Wavepattern_R - Wavepattern_L	Livesperm_R - Livesperm_L	Abnormal_sperm_R - Abnormal_sperm_L	Concentration_R - concentration_L	MTT_R - MTT_L
Z	-1.809[b]	-1.000[b]	-.561[c]	-.665[c]	-.358[b]	-1.070[b]
Asymp. Sig. (2-tailed)	.070	.317	.575	.506	.720	.285

DISCUSSION

The MTT assay's effectiveness in assessing semen viability is questioned due to its lack of significant connections with traditional semen evaluation parameters (Aziz, 2005). Environmental factors such as temperature, storage duration, and chemical exposure can significantly affect the results of the MTT experiment for semen viability (Buranaamnuay, 2021). Sperm cells are highly sensitive to temperature fluctuations; prolonged exposure to suboptimal temperatures (either too high or too low) can cause cellular stress or damage, leading to reduced metabolic activity. If semen samples were not properly stored or cooled during processing, this could result in lower MTT values due to diminished mitochondrial activity (Buranaamnuay, 2021 & Aziz, 2005). Additionally, prolonged storage times before analysis may lead to oxidative stress, membrane damage, or ATP depletion, further lowering sperm viability and MTT values (Aziz, 2005). Genetic variances, such as mutations, polymorphisms, or differences in genes linked to sperm function, metabolism, or antioxidant defences, can also impact the metabolic activity measured by the MTT assay (Golshan, 2020).

Age and maturity levels must be considered when interpreting the results of the MTT experiment for semen viability. Younger individuals or those in their reproductive prime may have better sperm viability (Aziz, 2005). The calibre of sperm extracted from the epididymis, including its metabolic activity, motility, and overall health, can also impact the assay's outcome (Aziz, 2005). Metabolic activity of sperm can be impacted by various pre-assay procedures for handling and preparing semen, including centrifugation, washing, and dilution (Matsuura, 2017). For instance, excessive centrifugation force or prolonged centrifugation time can cause mechanical damage to sperm cells, reducing their viability and metabolic activity (Matsuura, 2017). To achieve correct results, it is crucial to maintain constant pH and temperature levels during the MTT test, as well as the quality and purity of MTT reagents, solvents, and other materials used in the experiment (Aziz, 2006; Matsuura, 2017). Similarly, exposure to inappropriate media (e.g., lacking essential nutrients or buffers) can limit the energy substrates needed for mitochondrial function, leading to lower MTT reduction rates (Buranaamnuay, 2021).

Mincing and slicing are two methods for extracting epididymal sperm from the epididymis. Mincing involves coarsely chopping tissue, allowing for a larger surface area and greater sperm extraction. However, slicing may result in a lower volume of sperm due to delayed release. Both methods are equally efficient in assessing semen properties, but mincing is more practical due to its ease of use, quicker processing, and higher observed sperm concentration. Slicing, on the other hand, takes longer and requires more equipment, making it less feasible for regular use (Joram, 2016; Dong, 2008). Variability in processing protocols, such as inconsistent resting times after slicing or mincing, might also influence sperm recovery efficiency and subsequent MTT values (Aziz, 2006; Matsuura, 2017; Buranaamnuay, 2021).

To conclude, the study found no significant difference between MTT absorbance and conventional semen evaluation parameters, and no significant difference between slicing and mincing techniques for rescuing epididymal sperm. Both methods are equally useful for assessing semen characteristics.

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