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Quality of Tomcat Sperm Recovered by Castration after Using the FertiCult Flushing Medium

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Abstract | The study used two different cooling methods to assess the cooling capacity of tomcat epididymal spermatozoa in a brand-new stadium and after 120 hours of storage at 4°C. In this study, twenty sexually mature tomcats (*Felis catus*) were chosen based on clinical and reproductive evaluations. Their average age ranged from 12 to 18 months. Each animal underwent bilateral orchiectomy, and a significant decrease in fresh recovery sperm following cooling for VCL with Tris with Pentoxifylline was observed at 96 and 120 hours. The results demonstrate that the epididymal sperm from the tomcat in the experiment Ferticult-flushing medium showed no significant differences ($P > 0.05$) regarding four of the parameters [(motility, progressive motility, VSL (μm/s), however, STR (%)] showed a significant difference amongst colling time slots (0, 48, and 72 hr). The values of sperm parameters after varying cooling times were found to be non-significantly different between 96 and 120 hours. These finding highlight the need of optimal conditions for sperm storage and offer an improved method to preserve and yield maximum productivity.

Keywords | Castration, FertiCult flushing medium, Computer system analysis (CASA), Pentoxifylline (PX) and Cooling

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INTRODUCTION

Semen collection techniques fall into two categories including *in vitro* collection (testicular or primary epididymal semen) and *in vivo* collection (mostly ejaculated semen). In 2003, Tsutsui *et al*. have highlighted that the tomcat (*Felix catus*) may produce high-quality ejaculates *in vivo* by copulation using an artificial vagina, urethral catheterization, or electroejaculation. Given that there are many testes accessible following routine castration surgeries (Zambelli and Cunto, 2006; Pisu *et al*., 2017), the use of epididymal spermatozoa (ES) could be advantageous when needed for research purposes on assisted reproductive technologies (ARTs) (sperm freezing, artificial insemination, *in vitro* embryo production). In addition, the collection of ES is of particular importance in the event of the sudden death of valuable individuals and, more often, for the conservation of gametes of endangered species (Brunn, 2022). Epididymal mincing, *in vitro* epididymal sperm aspiration post-epididymectomy, and *in vivo* percutaneous epididymal sperm aspiration (PESA) are some of the techniques used to collect epididymal semen. After an orchiectomy or post-mortem, epididymal mincing, also known as the float-up procedure, is a reliable and non-repetitive method for collecting specimens (Tebet *et al*., 2006). After being held in a liquid (usually a semen extender), the epididymis is separated from the testicle and cut in parallel. The sperm cells can be discharged into the

media once the epididymis is incubated for a few minutes (Simons *et al*., 2019; Mohsen *et al*., 2024). Tomcats reach sexual maturity between 7 and 12 months of age, although spermatogenesis takes approximately 46.8 days and can begin as early as 5 months of age Siemieniuch and Woclawek-Potocka (2007); França and Godinho (2003). According to Tsutsui *et al*. (2004), over 80% of cats exhibit complete spermatogenesis and epididymal sperm stores beginning at 7 months. The benefit of cryopreserving semen is that it allows genetic material to be transferred between distant places, not just between living animals, ensuring that genetic variety is preserved even if the animals pass away before reproducing (Vansandt *et al*., 2021).

When animals or sperm donors passed away unexpectedly or were castrated for medical reasons, epididymal sperm were collected from the caudal or corpus areas. Kunkiti *et al*. (2016) and Chatdarong *et al*. (2016) reported suitable substitutes to preserve the priceless genetic components. Tsutsui *et al*. (2003) reported a successful pregnancy in domestic cats following the use of cryopreserved sperm cells from the caudal epididymides for unilateral intrauterine artificial insemination. Conversely, 1-(5-oxohexyl)- 3,7-dimethylxanthine is the chemical name for Pentoxifylline, a dimethylxanthine derivative. By blocking cAMP phosphodiesterase activity, the PX stops cAMP breakdown and, it is assumed, promotes sperm motility (Steiber *et al*., 2004). Furthermore, because PX scavenges ROS and subsequently lowers lipid peroxidation, it protects sperm membranes (i.e., maintaining the functional membrane integrity of the sperm tail) (Seim *et al*., 2002).

Aims of this study were to highlight the benefits of sperm cells collection from castration surgery and to establish a protocol for semen cooling tom cat., Additionally, we aim to investigate the cooling ability of semen tom cat on specific physical, parameters (sperm motility and number) and a comparative study of Pentoxifylline and Flushing medium effects on semen quality tom cat before and after cooling process different time.

MATERIALS AND METHODS

Animals

According to the University of Basrah's College of Veterinary Medicine's BCVM standards, the study was approved by an ethics committee with a number (81/37) in 2025. For this investigation, twenty sexually mature tomcats (*Felis catus*) were chosen based on their reproductive and clinical evaluations. They were 12 to 18 months old on average. A comprehensive clinical examination and a macroscopic evaluation of the reproductive organs of the research animals were performed during the preoperative phase, including palpation and inspection. We only employed clinically healthy animals that had normal testicular symmetry and consistency and showed no damage to their external reproductive organs. The study's approved cats underwent bilateral Feline castration (Orchiectomy) surgery after a 12-hour fast from food and a 6-hour fast from water (Woodruff *et al*., 2015). Antibiotic therapy with penicillin (IM), meloxicam (0.2 mg/kg IM) (Alrafas *et al*., 2023; Mohsin *et al*., 2025), 13 mg of ketamine, and 5 mg of xylazine comprised the preoperative protocol used in the study (Jassim *et al*., 2023). General anaesthesia was administered within the desired time frame, following a bilateral orchiectomy (Miller *et al*., 2018; Abduljaleel, 2024). The testis-epididymis complexes (TEC) of the animals' left and right were acquired. After recuperating from the anaesthetic, the animals were released from the College, and post-surgical therapy instructions were given to the owners. Following collection, the proximal area of the deferent duct and the epididymal cauda were used to retrieve the epididymal sperm using the slicing procedure (Figure 1). A knife was used to cut off the contours of the epididymal duct after the epididymis's surrounding connective tissue was removed (Smeak and Hazenfield, 2015). The outside of the epididymis was then cleaned with a saline solution heated to 37°C. Each complete epididymal duct was put in a petri plate that had also been heated to 37°C after the epididymis had been cleaned externally. The proximal section of the deferent duct and the epididymal cauda were ultimately sliced into tiny pieces with two scalpel blades and left in the Petri dish for a minute in order to encourage contact between the sperm

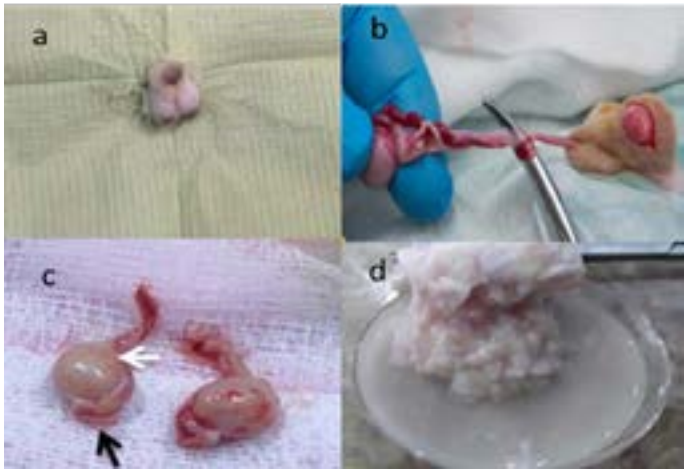

Figure 1: (A) for tomcat castration, pluck hair from the scrotum and aseptically prepare the scrotum for surgery. (B) Make cranial-to-caudal skin incisions over each testicle and separate the tunica from the testicle. Then, around the cord opposite the testicle, transect the testicle and pull the end of the cord through the wrap. Next, digitally sign the knot. (C) Testicle, Head of Epididymis (white arrows), tail of epididymis (black arrows). (D) Slicing of caudal epididymis.

and the extender. Sperm were evaluated using aliquots of the resultant diluent (semen samples were obtained from castration-tom cats and diluted with Tris-citric egg yolk-glycerol) and diluent treated with either flushing medium or Pentoxifylline (PX). In a phase contrast microscope, 10 µL of the dilution was examined using a computer system analysis (CASA) video camera. The spermatic parameters listed below were analyzed: Total and progressive motility, average path velocity(VAP), curvilinear velocity (VCL), straight line velocity (VSL), linearity index (LIN) and straightness index (STR), amplitude of lateral head displacement (ALH) and beat cross frequency (BCF) (Mota Filho *et al*., 2014). A 10µl aliquot of the (Tris-citric egg yolk-glycerol) diluter and diluent, supplemented with Pentoxifylline (PX) or flushing medium, was used to assess the total motility of the epididymal sperm.

Preparation of pentoxifylline (PX).

10 mg of PX powder (Sanofi Aventis, Egypt) was dissolved in 10 ml of PBS (0.1%) and stirred until a homogeneous solution was formed. Every day, under sterile conditions, these concentrations were prepared using a Millipore filter (0.45 µm) and UV light (Al-Dujaily *et al*., 2012).

Flushing medium (FertiCultTM contains phenol red)

HEPES, buffered medium, physiologic salts, glucose, lactate, pyruvate, bicarbonate, and human serum albumin (4.0g/l), a medicinal substance derived from human blood plasma, are all present in FertiCultTM Flushing media (Figure 2).

Figure 2: Flushing medium (FertiCultTM) contain phenol red.

Extender preparation

In 100 milliliters of distilled water, 3.028 grams of trishydroxymethyl-aminomethane, 1.78 grams of monohydrated citric acid, and 1.25 grams of fructose were dissolved to create the stock solution with a 6.6 pH (Vizuete *et al*., 2014). The three final doses of glycerol (4, 6, and 8%) were then used to create experimental treatments after 20% of the fluid was replaced with egg yolk. Tylosin (100 µg/mL), Gentamicin (500 µg/mL), and Lincomycin (300/600 µg/mL) were added as antibacterial.

Evaluation of semen quality

Physical properties

Sperm concentration: Computer-assisted semen analysis (CASA) was used to measure the sperm concentration in ejaculates (Baracaldo *et al*., 2007). Assays for motility: A computer-assisted sperm analysis (CASA) system was used to assess motion characteristics, which had been previously validated in our laboratory (Gadea *et al*., 2005). Amplitude of lateral head displacement (ALH, µm), wobble of the curvilinear trajectory (WOB, ratio of VAP/VCL%), curvilinear velocity (VCL, µm/s), straight-line velocity (VSL, µm/s), average path velocity (VAP, µm/s), linearity of the curvilinear trajectory (LIN, ratio of VSL/VCL%), straightness (STR, percentage of VSL/VAP%), and the percentage of motile spermatozoa (%motil) were the motility characteristics derived from CASA. Prior to (just ejaculated) and following sperm therapy, a 5µl drop of the sample was put on a heated (37 °C) slide and covered with a cover slip. To count spermatozoa, specific conditions must be met (Axnér *et al*., 2004). The percentage of dead sperm was calculated using the methodology of Gervasi and Visconti (2017). After the smears were dried, an examination was conducted under a light microscope at 400X. To accomplish this, 1-2 drops of the freshly drawn semen sample and 1-2 drops of eosin-nigrosine that had been warmed up were placed on a sterile slide. The semen sample and stain were then combined on the edge of the second slide, and the mixture was then dragged along the surface of the clean slide. In order to make the dead sperms turn red while the living sperms do not, nigrosine is used to stain the background and eosin to stain the dead sperms.

The stain was prepared as follows

The active ingredient of the stain Eosin (1.67 gm), Nigrosine (2-10 gm) and Sodium citrate (2.9-3 gm) were dissolved in 100 millilitres of double-distilled water, followed by heating, filtering, and combining. A heated stage was included in the study's microscope, and the Eosin-Nigrosine stain was pre-warmed at 37 °C in a water bath.

Experimental design: Using a plastic bag, semen samples were taken from castrated Tom cats. The samples were then physically examined and diluted using a Tris-citric egg yolk-glycerol diluter. Pentoxifylline (PX) or flushing medium were added to the same diluent at varying amounts. The following procedures were used to prepare twenty tom cats for chilling after they were removed from the study animals. A physical evaluation of the ejaculate was conducted, including sperm concentration, progressive motility percentage and dead sperm percentage.

- Dilution: The concentration of sperm in the ejaculates was adjusted using Tris diluent. The diluter and ejaculates were then placed in a water bath at 37°C. The diluted semen sample was divided into two equal

portions using a plastic test tube to add antioxidants. One was included with Pentoxifylline (5 mmol/mL) and 5 mL of diluent (T1 Tris) while the other one was added Flushing medium (including Phenol Red from FertiCultTM). For four hours, diluted semen with varying antioxidant concentrations was kept at the same temperature (5 °C).

- Forty epididymides were gathered and the following comparison was made between two experimental groups: Tris+pentoxyfylline (T1) was used to recover the sperm from twenty epididymides that had been chilled to 5°C at 48, 72, 96, and 120 hours; FertiCult TM Flushing medium (T2) was used to recover the sperm from twenty epididymides that had been cooled to 5°C at 0, 48, 72, 96, and 120 hours. Fresh sperm from ten epididymides were recovered using 0 hours (control contains only Tris-citric egg yolk-glycerol (TCEG). Each extension and cooling time had a total of 40 epididymides.
- Statistical analysis: SPSS (Statistical Package for the Social Sciences, Version 17, Illinois, USA) was used to collect and analyze raw data for descriptive statistics, including means and standard error of the Mean (SE). A P value of less than 0.05 was deemed statistically significant (Jasim *et al*., 2025).

RESULTS AND DISCUSSION

In the pentoxifylline experiment, recovery of epididymal sperm from in-tom cats revealed a greater non-significant mean total motility between (0 and 48;96 and 120 hr) (76.29±0.24 and 72.04±0.21); (59.78±0.31 and 56.92±0.18).In comparison to cooling at 120 hours (92.45±0.21; 32.81±0.65), the mean sperm concentration and VSL (µm/s) did not significantly change between 0, 48, 72, and 96 hours (137.94±0.31, 126.83±0.27, 109.58±0.38; 49.92±0.41, 47.18±0.25, 40.98±0.34, and 110.26±0.56; 40.3855±0.56). Fresh progressive motility and 48 hours were shown to be higher than the cool times (72, 96, and 120 hours) by computer analysis (52.07±0.66, 46.2255±0.66, and 43.15±0.42). The VAP (µm/s) of the Tris extender was significantly higher at 0 and 48 hours (46.15±0.42 and 46.03±0.36) than it was at 120 hours (40.09±0.47). However, a significant decrease in fresh recovery sperm following cooling for VCL with Tris with Pentoxifylline was observed at 96 and 120 hours (113.4130±0.07 and 119.98±0.52) as opposed to 60 and 72 hours (96.95±0.38 and102.09±0.34). Regarding Tris and Tris with Pentoxifylline, LIN(%) was lower at 0 hours (51.37±0.53) than cooling at 48 and 72 hours (44.72±0.42 and 39.30±0.54°C), and there was no significant difference between 96 and 120 hours (30.7775±0.43 and 27.68±0.90). Table 1 shows the comparison between Tris and Tris with Pentoxifylline, while the other parameters (AIH (µm), BCF(HZ), and STR(%)did not change with the various cooling durations for each extender. The study's findings on the percentage of dead sperm at various cooling times during sperm processing are presented in Tables 1 and 2.

At 0 hours, the percentage of dead sperm was at its lowest (14.21 ± 0.098%), and it was substantially different from that at 120 hours ($P < 0.05$). Meanwhile, upon cooling for 96 and 120 hours, the results were 31.71 ± 0.28 and 34.11 ± 0.27%, respectively. The maximum number of dead sperm was recorded at 96 and 120 hours(31.71 ± 0.28 % and

Table 1: Effect of Pentoxifylline diluted or Tris on the tomcat epididymal sperm parameters determined after cooling of epididymides assessed by CASA. for Mean±S.E.

Cooling time				0 hour (control contains only (Tris-citric egg yolk-glycerol (TCEG)	Parameter pentoxifylline
120 hour	96 hour	72 hour	48 hour		
34.11±0.27A	A0.28±31.71	24.05±0.08B	18.16±0.092C	14.21±0.098C	Dead sperm %
56.92±0.18C	59.78±0.31C	66.73±0.23B	72.04±0.21A	76.29±0.24A	Total motility%
92.45±0.21C	110.26±0.56B	109.58±0.38B	126.83±0.27A	137.94±0.31A	Sperm concentration
43.15±0. 42C	46.2255±0.26BC	52.07±0.66B	57.88±0.20A	61.83±0.61A	Progressive motility %
40.09±0.47C	42.3285±0.33BC	44.85±0.33B	46.03±0.36A	46.15±0.42A	VAP (µm/s)
32.81±0.65C	40.3855±0.56B	40.98±0.34B	47.18±0.25A	49.92±0.41A	VSL (µm/s)
119.98±0.52A	113.4130±0.07A	102.09±0.34B	88.54±0.47C	96.95±0. 38B	V CL (µm/s)
3.12±0.12A	3.1620±0.09A	2.38±0.06B	2.47±0.082B	1.25±0.05C	AIH (µm)
14.25±0.07A	12.4090±0.06A	10.09±0.01A	3.43±0.073B	2.22±0.06B	BCF (HZ)
27.68±0.90D	30.7775±0.43D	39.30±0.54C	44.72±0.42B	51.37±0.53A	LIN(%)
82.47±0.53B	85.2550±1.02AB	88.28±0.65A	89.34±0.67A	91.80±0.70A	STR(%)

The amplitude of lateral head displacement (ALH), beat cross frequency (BCF), linearity index (LIN), straightness index (STR), average path velocity (VAP), curvilinear velocity (VCL), and straight line velocity (VSL). Statistically significant variations between extenders for the various cooling durations are indicated by different capital letters within a row ($P < 0.05$).

Table 2: Effect of ferticult flushing medium or Tris on the tomcat epididymal sperm parameters determined after cooling of epididymides assessed by CASA for Mean±S.E.

Cooling time				0 hour(control contains only (Tris-citric egg yolk-glycerol (TCEG)	Parameter Ferticult flushing medium
120 hour	96 hour	72 hour	48hour		
15.69±0.071A	13.05±0.041A	10.18±0.053B	9.15±0.063B	7.97±0. 049C	Dead sperm %
75.63±0.29C	77.28±0.21B	80.29±0.37A	81.47±0.18A	82.09±0.24A	Total motility%
180.02±0.45C	186.91±0.24C	191.58±0.43C	207.72±0.19B	235.36± 0.26A	Sperm concentration
64.12±0.28B	66.83±0.42B	69.53±0.38A	69.97±0.30A	71.47±0.53A	Progressive motility %
38.84±0.45BC	40.60±0.60B	42.30±0.07B	46.24±0.29A	47.94±0.37A	VAP(µm/s)
31.26±0.56B	39.91±0.49A	39.10±0.63A	41.10±0.28A	45.92±0.29A	VSL(µm/s)
138.97±0.31A	138.80±0.47A	136.29±0.34A	128.75±0.46B	131.02±0.56B	V CL(µm/s)
2.55±0.07A	3.60±0.08A	3.33±0.07A	2.67±0.05B	1.94±0.09C	AIH(µm)
17.30±0.07A	15.37±0.08A	13.39±0.06A	7.45±0.07B	3.25±0.06C	BCF(HZ)
21.85±1.03C	29.27±0.42B	30.11± 0.53AB	32.56±0.67A	34.29±0.07A	LIN(%)
83.61±0.73B	91.24±0.54A	93.57±0.53A	93.99±0.49A	94.08±0.51A	STR(%)

The amplitude of lateral head displacement (ALH), beat cross frequency (BCF), linearity index (LIN), straightness index (STR), average path velocity (VAP), curvilinear velocity (VCL), and straight line velocity (VSL). Statistically significant variations between extenders for the various cooling durations are indicated by different capital letters within a row (P < 0.05).

34.11±0.27%), which was substantially different from the 0-hour value in the Pentoxifylline experimental group. Pentoxifylline (PX) has been shown to enhance testicular sperm motility (Sato and Ishikawa, 2004), as well as hyperactivation and the acrosome reaction after activation (Abid, 2005; Al-Dujaily *et al*., 2007). When spermatozoa were washed and activated *in vitro* using FertiCult medium TM, active sperm motility was observed. This may be related to the ingredients in FertiCult flushing medium, which comprise a mixture of human serum albumin (4.00 g/L), glucose, lactate, physiologic salts, bicarbonate, and HEPES (Dickey *et al*., 2009). As a buffering system, HEPES, when present in the media alone, enhanced the sperm's buffering ability. The statistics in this study, however, indicate that the cooling process causes the percentage of dead sperm to rise. "PTX can be used as a potent antioxidant substance against oxidative stress and subsequent effects to reduce abnormal sperm and dead count in tom cats," according to Cooper (2010). The longer the storage period, the higher the abnormality rate, which is attributed to cold shock and an imbalance in osmotic pressure resulting from metabolic processes during storage at 5˚C, according to Yani and Nuryadi (2001). The results showed that the lowest significant value (p < 0.05) occurred at 0 hours, while the greatest non-significant value (p < 0.05) occurred at 96 and 120 hours (13.05 ± 0.041% and 15.69 ± 0.071%, respectively) in the ferticult flushing medium experiment. The results showed a significant (P < 0.05) increase in dead sperm. Table 2 and Figure 3 show the percentage of tomcat spermatozoa after cooling at 120 hours (15.69±0.071%) compared to after cooling at 0 hours (7.97±0.049%). When a high genetic value male is castrated (electively or in an emergency), the epididymis includes a valuable source of sperm that may be recovered. As sperm from a testis can only be obtained once, the most effective method for maximizing the recovery, preservation (germplasm banking), and utilization of gametes through assisted reproductive technologies must be employed. In small and laboratory animals, the only method for collecting epididymal spermatozoa is through the ductus deferens, which has a smaller diameter (Ali *et al*., 2021). Our study examined the effects of cool storage after 48, 72, 96, and 120 hours of storage. The initial evaluation was conducted at day 5 (120 hours) using a difficult-to-flush medium; however, our findings suggest that motility may not have decreased even before 72 hours of incubation. Previous research has examined the impact of cool storage on the quality of tomcat sperm (Angrimani *et al*., 2018).

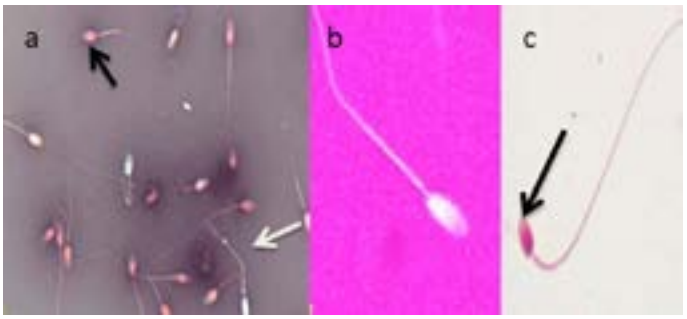

Figure 3: Normal tomcat spermatozoon (white arrows) A and B, dead spermatozoon (black arrows) at C (eosin nigrosin stain, x100).

The results of our study also showed that the epididymal sperm from the tomcat in the experiment ferticult-flushing medium analyzed showed no significant differences (P >0.05) regarding four of the parameters

(motility, progressive motility, VSL (µm/s), and STR (%). It also showed a non-significant difference between 0 hr (81.09±0.24, 71.47±0.53, 45.92±0.29, and 94.08±0.51), 48 hr (81.47±0.18, 69.97±0.30, 41.10±0.28, and 93.99±0.49), and 72 hr (80.29±0.37, 69.53±0.38, 39.10±0.63, and 93.57±0.53) of cooling time. The assessed groups listed in Table 2 did not differ in these values (P > 0.05), including Mean (± S.E.) sperm concentration, progressive motility, cooling sample VCL (µm/s), AIH (µm), and BCF (Hz). The values of sperm parameters after varying cooling times were found to be non-significantly different between 96 (186.91±0.24; 66.83±0.42; 138.80±0.47; 3.60±0.08 and 15.37±0.08) and 120 hours (180.02±0.45; 64.12±0.28; 138.97±0.31; 2.55±0.07 and 17.30±0.07). Our study's results verified that the epididymal sperm from the tom cat in the experiment's ferticult-flushing medium analysis demonstrated that the chilling procedure had no discernible impact on the sperm characteristics, and the ferticult-flushing medium was the same for all cooling times. These studies show a gradual deterioration in semen quality (measured by sperm parameters). For seven days, male cat semen was stored at 5°C in cold storage for this study. The findings of this investigation align with those of earlier research. Perez-Marin *et al.* (2017) kept the tomcat epididymides at 4 °C for 48 hours. At the time of evaluation, the scientists found that the acrosomal integrity of epididymal spermatozoa was unaffected by the cool storage. During the 72-hour storage period, the acrosome integrity of tomcat epididymal spermatozoa was not significantly impacted by cool storage at 5°C, according to Angrimani *et al.* (2018). For tomcat spermatozoa, a similar study by Gañán *et al.* (2009) found no discernible impact of cool storage at the same temperature (5°C) over 72 hours. In contrast to earlier studies, Woodruff *et al.* (2015) examined the impact of cool storage on the characteristics of tomcat spermatozoa. However, the sperm samples were kept at 4°C for ten days. This study was the first to use ferticult-flushing media to recover the epididymal sperm after cooling the epididymis of domestic cats for up to 120 hours. The substantial improvement in sperm viability after the chilling time was most likely due to the components of the difficult-flushing medium, in particular. In addition, the samples obtained using the ferticult-flushing media exhibited greater motility values and progressive motility than the slices. Even though the sperm were not impacted by the various chilling times caused by the castration collection methods, similar outcomes were noted in Tom Cats, where samples obtained by castration demonstrated 82.09±0.24% higher motility and 71.47±0.53% higher viability (Turri *et al.*, 2012). However, the samples in this investigation showed increased Mean (±S.E.) sperm concentration, Progressive motility, V CL (µm/s), AIH (µm), and BCF (Hz) in the cooling samples. In line with the findings of Simons *et al.* (2019), who noted higher VAP and ALH in cooling-collected samples, a comparison of sperm parameter levels after varying cooling times revealed no significant difference between 96 and 120 hours. Because the freeze/thaw process greatly reduces sperm activity, fresh spermatozoa are of higher quality than frozen samples (Clark and Swain, 2014; Terrell *et al.*, 2012). The composition of FertiCult flushing medium includes a mixture of human serum albumin (4.00 g/L), glucose, lactate, physiologic salts, bicarbonate, and HEPES (Dickey *et al.*, 2009). When used as a buffering system, HEPES alone in the media enhanced the buffering capacity and pH stability between 7.2 and 7.6. To prevent the pH from falling below 7.0, no CO_2 incubation is necessary, as this enables the media to withstand pH variations caused by changes in cellular metabolism more effectively (Clark and Swain, 2014). According to Al-Sultani *et al.* (2013), the current study found that FertiCult sperm washing medium TM has a higher impact on particular male sperm function metrics than other sperm washing media.

CONCLUSIONS AND RECOMMENDATIONS

The study was the first to use a ferticult-flushing medium to recover epididymal sperm and cool the epididymis of domestic cats for up to 120 hours. The significant increase in sperm viability following the chilling period was probably caused by the components of the ferticult-flushing solution, in particular. In our investigation, we found that incubation for 120 hours did not affect total motility. However, the percentage of progressive motile cells was not significantly reduced in the group incubated for 120 hours before cooling with ferticult-flushing medium when compared to cool samples with Pentoxifylline. These data indicate that when transporting the heritable material of a freshly dead sample (e.g., a castrated individual) to the laboratory. For future, we recommend making straws, freezing them, and then thawing them in a Ferticult flushing medium. We recommend studying the deformities after the cooling, freezing and thawing process of castrated tomcats. Also, we suggest the integrity of the sperm membrane using a fluorescent microscope.

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NOVELTY STATEMENT

The novelty of our work entitled (Quality of tom cat sperm recovered by castration after using the FertiCult flushing

medium) lies in its investigation of a novel method for sperm recovery in tom cats. Specifically, we explore the efficacy of using FertiCult flushing medium during castration to optimize the retrieval of viable sperm. This approach presents a significant departure from traditional sperm collection methods in felines, potentially offering a more efficient and less invasive alternative for genetic resource banking and assisted reproduction in this species.

AUTHOR'S CONTRIBUTION

All authors equally contributed.

Conflict of interest

The authors have declared no conflict of interest.

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