ABAH BIOFLUX
Animal Biology & Animal Husbandry
International Journal of the Bioflux Society

# Short term preservation of chilled tomcat (*Felis catus* L.) spermatozoa, obtained by urethral catheterization after medetomidine administration, diluted with a laboratory prepared extender

Constantin Pavli, Irina O. Tănase

Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine "Ion Ionescu de la Brad", Iasi, Romania. Corresponding author: C. Pavli, pavli_constantin@yahoo.com

**Abstract.** Semen collection in tomcat represents the first step to establish a breeding protocol. Therefore we used a method of harvesting that involves little difficulties and discomfort for the test male. The method was described previously by Zambelli (2006) and is represented by semen collection by a tomcat catheter, after medetomidine administration. In a few cases, when was possible, semen was also collected by electroejaculation, the males were housed in individual cages and collection protocols were repeated twice weekly, two days apart. In order to preserve and manipulate the reduced volume of semen we used a triss glucose extender supplemented with egg yolk. The authors used a protocol originally designed for dog semen, respectively Uppsala. The semen sample was centrifuged and the sperm pellet suspended in Uppsala Extender 1, later chilled to 4°C and assessed from the point of motility parameters periodically.
**Key Words:** Urethral catheterization, medetomidine, tris egg yolk glucose extender.

**Introduction**. In Romania and also all over the world there are several endangered wild felines that need help in order to prevent their extinction. One method to ameliorate the species demographic number is to keep some specimens in captivity in order to manipulate their reproduction and their numbers grow. The second approach assumes interference in theirs reproduction using assisted reproduction biotechnologies on site, or in a controlled environment (Hay & Goodrowe 1993).

Assisted reproduction is used to widen the gene pool. Preserved feline semen, to allow transport, is required; therefore a tris egg yolk buffer laboratory prepared extender was used in tom cat spermatozoa preservation, obtained by urethral catheterization after medetomidine administration. The tomcat (*Felis catus* L.) represents a good experimental model for wild species.

The aim of the study was to assess extender qualities in order to allow semen facile manipulation, evaluation and short term storage for immediate or later usage.

## Material and Method

***Sperm collection and evaluation***. We were unable to predict fertilizing capacity of the preserved spermatozoa; we assessed the extended semen from the point of motility, plasma membrane integrity and acrosome integrity.

Must be noticed the fact that there are differences between tom cats used in experimental protocol, differences of age, physiologic condition therefore both collection methods were used. Electroejaculation was used only to assess differences between harvesting methods (Dooley et al 1991).

All subjects presented similar periods of abstinence from sexual activity and the preliminary collection was done to select males suitable for experimental protocol, rest period between collections was 4 days.

From a number of 32 privately owned adult, European breed male cats between 1-4 years of age enrolled in this study only a number of 14 were used in the experimental protocol. Only the clinically healthy cats were used, the rest of them were used to increase the amount of experience and data on studied topic (semen harvested and male neutered).

The collection method provides a practical noninvasive and repeatable procedure (Zambelli et al 2006, 2004); we used a tomcat catheter marked for volume approximation.

The main sperm parameters were assessed on pre-warmed glass slides, concentration was measured by means of a Thoma counting chamber, the morphology and membrane integrity on diff-quick stained slides, the motility by using subjective assessment of the percentage motile and progressively motile spermatozoa, assessment realized by phase contrast microscopy (x200-x400) (Sojka et al 1970).

Percentages of morphologically normal spermatozoa were determined on phase contrast microscope (x1000).

For spermocytogram we used simplified Harris-Shorr Staining.

***Extender used- Uppsala Extender 1, content***. Tris 2.4 g, citric acid 1.4 g, glucose 0.8 g, Na-Benzylpencillin 0.06 g, Streptomycin sulfate 0.1 g, egg yolk 20 ml, glycerol 3 ml, Distilled Water up to 100 ml (Axner & Linde-Forsberg 2002).

All xtender constituents are produced by Sigma Aldrich. A larger quantity was produced and stored at -20 $^{\circ}$C.

Considering the small volume of semen in Tomcat we removed seminal plasma only in cases where it was possible. Usually the dilution rate was 18 µl of extender to a semen aliquot of 2 µL (Scott 1970; Howard et al 1990).

Cooling rate was 0.5 $^{\circ}$C/minute and storage temperature was 4.5 $^{\circ}$C (all readings were done with an electronic thermometer with data recording).

Semen evaluations were performed on five occasions (considering low volume): after collection, after cooling (after semen reaches 5 $^{\circ}$C), and at 24 h, 48 h and 72 h. On each occasion the motility, vigor and progressive movements were determined.

**Results and Discussion**. We analyzed semen prior and after dilution considering total motility, forward progressive motility, finally carried out a morphological study.
The morphological investigation purpose was to identify the agglutination percentage and relationship with semen quality.

With a volume of 10 ± 4 µL and concentration of 1700 ± 600x$10^6$/mL semen can be considered with a lower volume and a higher concentration than samples collected by electroejaculation.

The most important impediment for the sperm evaluation in the tomcat is the very low volume of the ejaculate, which limits the number of evaluations drastically. It is considered that semen harvested by urethral catheterization has a much diminished quantity of seminal plasma due to a reduced participation of accessory glands.

In the assessed period motility and vigor drop was less than 5 - 7 % per time interval.

All investigated males presented less than 10 % agglutinated semen (Figure 1-2), percentage diminished with extending process to 1 - 3 % (depending initial percentage), it can be observed a higher percentage in agglutinated spermatozoa during hot season (observation based on ours previously experience in semen evaluation during other seasons).

In this study were included all available males, motility rates did not represent an election characteristic; we used males with less than 50 % forward progressive motility (Table 1).

Table 1

Total motility (tm), forward progressive motility (fpm)

| *Semen* | *Prior extending* | *Extended and chilled day 1* | *Extended day 2* | *Extended day 3* |
|---|---|---|---|---|
| tm | 72 – 89 % | 69 – 78 % | 58 – 68 % | 45 – 55 % |
| fpm | 40 – 58 % | 52 – 57 % | 41 % | 28 % |

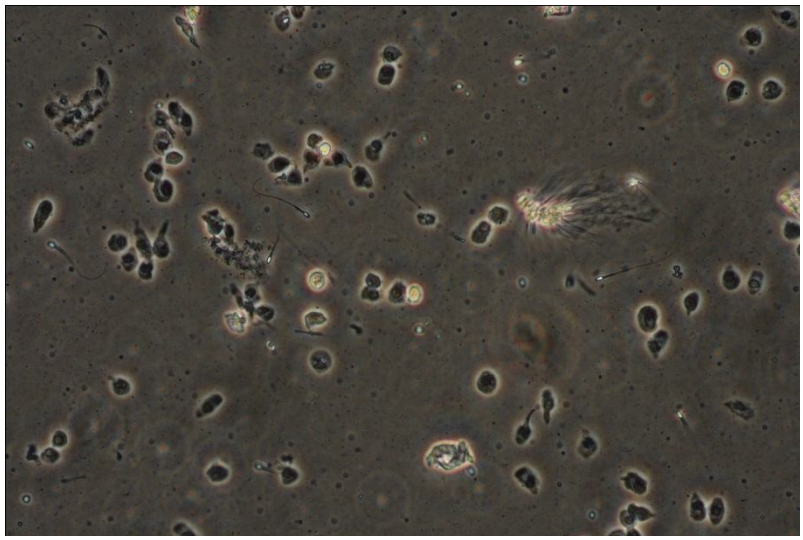

Figure 1. Phase contrast microscopy, objective 40X, agglutinates and epithelial cells in an ectopic tom cat, semen collected by urethral catheterization.

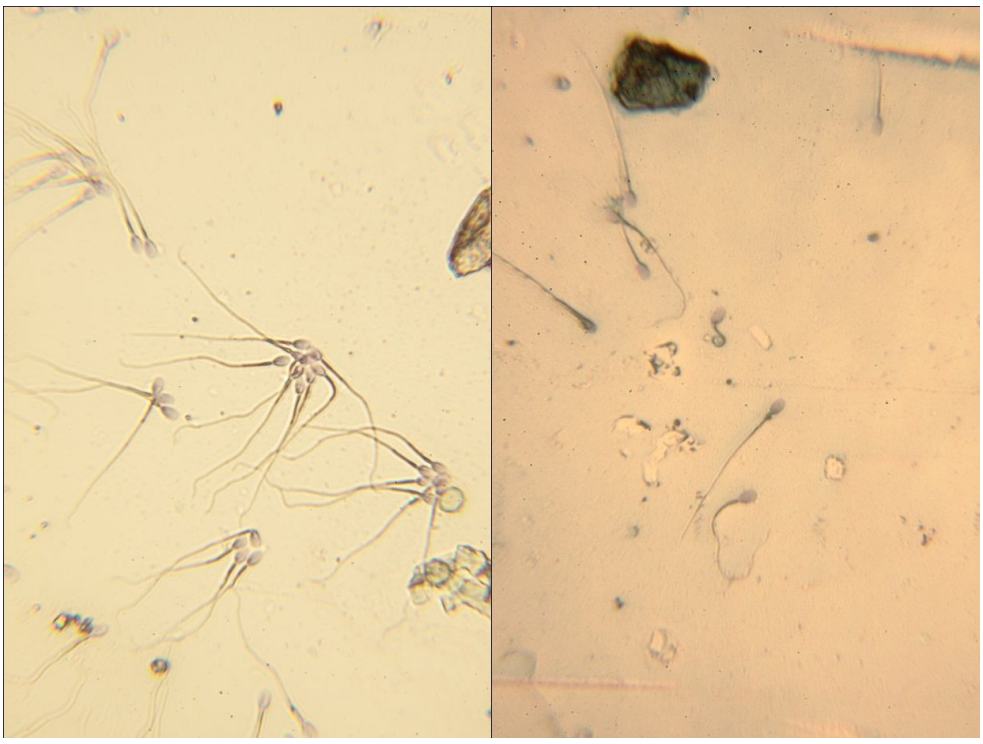

Figure 2. Tail abnormalities and agglutinates. Morphology, Harris Shore stained slides, objective 100X.

It must be presented the fact that undiluted semen is sensitive to environment without immediate dilution, so dissimilarities between research teams can be the result of technique, sperm preparation protocols and evaluation procedures.

Collecting technique proves to be useful to ours needs considering no expensive equipment for on field semen harvesting. It is believed to be safer and no animal training is necessary (Zambelli et al 2006).

**Conclusions**. The semen collected by urethral catheterization is characterized by a lower volume and a higher concentration than samples collected by other means, therefore in order to assess its characteristics dilution was needed.

Lower volume can be linked to the reduced contribution to the ejaculate from the accessory glands, same reason seem to be the origin of the lower pH.

The collection method provides practical advantages and is appropriate to practice because no special equipment is needed.

The main sperm cell damage occurred within first 24 hours with a decrease in morphologically normal sperm due to increase in bent sperm tail and coiled tail.

At 4.5 $^{\circ}$C egg yolk extender protects sperm motility parameters providing optimal results for artificial insemination at three days from collection, spermatozoa with motility being present up to six days from harvesting.

All investigated males presented less than 10 % agglutinated semen, percentage diminished during extending process to 1 - 3 %.

Semen quality depends on male's quality (reproductive condition), differences between tom cats being easily noticeable.

**Acknowledgements**. Paper realized and published under guidance of Postdoctoral School in Agriculture and Veterinary Medicine, contract no. POSDRU/89/1.5/S/62371.

## References

Axner E., Linde-Forsberg C., 2002 Semen collection and assessment, and artificial insemination in the cat. In: Recent advances in small animal reproduction. Concannon P. W., England G., Verstegen J., Linde-Forsberg C. (eds.), International Veterinary Information Service (www.ivis.org), Ithaca, New York, USA.

Scott P., 1970 Cats. In: Reproduction and breeding techniques for laboratory animals. Hafez E. S. (ed), p. 205, Lea and Febiger, Philadelphia.

Howard J. G., Brown J. L., Bush M., Wildt D. E., 1990 Teratospermic and normospermic domestic cats: ejaculate traits, pituitary-gonadal hormones, and improvement of spermatozoa motility and morphology after swim up processing. J Androl 11:204–15.

Hay M. A., Goodrowe K. L., 1993 Comparative cryopreseration and capacitation of spermatozoa from epididymides and vasa deferentia of the domestic cat. J Reprod Fertil Suppl 47:297-305.

Sojka N. J., Jemings L. L., Hamner C. E., 1970 Artificial insemination in the cat (*Felis catus* L.). Lab Anim Care 20:198–204.

Dooley M. P., Pineda M. H., Hopper J. G., Hsu W. H., 1991 Retrograde flow of spermatozoa into urinary bladder of cat during electroejaculation, collection of semen with an artificial vagina, and mating. Am J Vet Res 52:687–91.

Zambelli D., Prati F., Merlo B., Cunto M., 2006 Collection of semen by urethral catheterization after pharmacologically induced spermatozoa releasing in the domestic cat. In: 5th biannual congress, European Veterinary Society for Small Animal Reproduction (EVSSAR), p. 300.

Zambelli D., Baietti B., Prati F., Belluzzi S., 2004 The effect of ketamine and medetomidine on quality of domestic cat sperm after electroejaculation. In: EVSSAR IV European congress on reproduction in companion, exotic and laboratory animals; pp. 311–312.

Received: 18 March 2013. Accepted: 15 April 2013. Published online: 19 August 2013.
Authors:
Constantin Pavli, University of Agricultural Sciences and Veterinary Medicine, Faculty of Veterinary Medicine, Romania, Iasi, Mihail Sadoveanu Avenue, No. 8, pavli_constantin@yahoo.com
Irina Oana Tănase, University of Agricultural Sciences and Veterinary Medicine, Faculty of Veterinary Medicine, Romania, Iasi, Mihail Sadoveanu Avenue, No. 8, tanase_oana@yahoo.com

How to cite this article:
Pavli C., Tănase I. O., 2013 Short term preservation of chilled tomcat (*Felis catus* L.) spermatozoa, obtained by urethral catheterization after medetomidine administration, diluted with a laboratory prepared extender. ABAH Bioflux 5(2):132-136.