Buletin Veteriner Udayana **Vol.1 No.2. :77-81**
ISSN : 2085-2495 **Agustus 2009**

PERCENTAGE OF VIABLE SPERMATOZOA COLLECTED FROM THE EPIDIDYMES OF DEATH LOCAL DOG

(Persentase Spermatozoa hidup yang Dikoleksi dengan Waktu Berbeda dari Epididimis Anjing Lokal yang Telah Mati)

I Nyoman Sulabda and I Ketut Puja

Faculty of Veterinary Medicine, Udayana University

Bukit Jimbaran, Badung, Telp.0361701808.

Email address : asubali@hotmail.com

ABSTRACT

The purpose of this study to determine the effectof post mortem time on percentage of life epididymessperm from postmortem dog caudae epididymides. A total of 9 dog were used and divided into three group. T0 was control group, T1, 3 hours postmortem and T2, 6 hours postmortem. This way, samples were obtained at different times postmortem. Sperm were extracted from the caudae epididymes by means of cuts.

The result showed that the percentage of life sperm were 67,16 ± 5.67(T0), 46.33 ± 5.60 (T1) and 24.00 ± 4.35 respectively. We could appreciate that percentage of life was affected by postmortem time. There was significant decrease life sperm recovered from epididymes postmortem (P<0.01). In conclusion, epididymes sperm from dog undergo decrease of percentage of life, but it could stay acceptable within many hours postmortem. We intepreted these data to indicate that it may still be possible to obtain viable spermatozoa many hours later.

Keywords : dog, epididymes spermatozoa, post mortem

ABSTRAK

Penelitian ini bertujuan untuk mengetahui pengaruh lama waktu kematian terhadap persentase hidup spermatozoa yang diambil dari epididimis anjing local.Sebanyak 9 ekor anjing lokal digunakan dalam penelitian ini. Sampel dibagi secara acak menjadi tiga kelompok, yaitu Kelompok I (T0), sebagai kontrol, kelompok II (T1), tiga jam postmortem, danKelompok III (T2), enam jam postmortem. Dengan demikian, pengambilan spermatozoa dilakukan pada waktu kematian yang berbeda. Spermatozoa diambil dengan cara menoreh kauda epididimis dengan pisau bedah.

Hasil penelitian menunjukkan bahwa persentase hidup spermatozoa yang diperoleh dari pengambilan pada ekor epididimis anjing dengan lama waktu kematian berbeda berturut turut adalah 67,16 ± 5.67(T0), 46.33 ± 5.60 (T1) dan 24.00 ± 4.35 (T2). Jadi, terdapat penurunan daya hidup spermatozoa dengan sangat nyata (P<0,01) yang diambil dari ekor epididimis anjing pada waktu kematian yang berbeda Hasil penelitian ini menunjukkan bahwa masih dimungkinkan untuk mendapatkan spermatozoa hidup pada anjing beberapa jam setelah kematian.

Kata kunci: Anjing, Spermatozoa epididimis, postmortem

INTRODUCTION

Over the last 20 years, artificial breeding techniques have been established in our domestic animal species. Initially developed our domestic dairy cattle breeds. Artificial has extended to include beef cattle, sheep, goats, deer, horses and even the family ofdog. The successful utilization of these new procedures has resulted in significant gains in fertility and a definite increasing in offspring. These techniques have included the now common procedures of semen collection, semen freezing, artificial insemination, in vitro fertilization and the transfer of embryo.Only in the last few years have these techniques been successfully applied in basic research in canids (Farstad,2000).

Several methods of semen collection have been well described. Semencan be collected from testes of dogs that either alive and die accidentally or through illness. These tissues can be preserved by freezing and utilized in future breeding programs by extracting sperm for *in vitro* maturation, fertilization and culture. It has been demonstrated in many species that sperm in cauda epididymis exhibited fertility in in vitro fertilization and artificial insemination (Blash et al.,2000.; Marks et al.,1994). Blash *et al*.(2000) reported that epididymes sperm obtained at necropsy from goats have fertilization ability. Hori *et al*.(2004) also carried out an experiment where they found that frozen epididymes sperm in Beagle dog can make conception. As reported by Yu and Leibo (2002), even after storage for 192 h (8 days) at 4^0C, motile spermatozoa could be recovered from dog epididymes, and such refrigerated spermatozoa were capable of binding to zona pellucidae. Marks *et a*l. (1994) reported that the spermatozoa recovered from post-mortem dog epididymes, can make conception, although the semen was stored for 3.5 month at -197^0 C in liquid nitrogen.

There are implications for wildlife conservation programs, as epididymes l sperm is a good source of germplasm. If valuable animals die and it is not possible to process their sperm immediately, it may still be possible to obtain viable spermatozoa many hours later.

The recent study was to determine the effect of postmortem time (PT) in percentage of live of epididymal sperm salvaged from dog.

MATERIALS AND METHODS

The purpose of this study was to determine the effect of postmortem time on percentage of life epididymes l sperm from postmortem dog caudaeepididymes. A total of 9 dog were used and divided into three group. T0 was control group, T1, 3 hours postmortem and T2, 6 hours postmortem. This way, samples were obtained at different times postmortem.

Sperm were extracted from the caudae epididymes by means of cuts.

The testes was excised in the caudal part of epididymes by surgical blade. Spermatozoa were removed from the caudal part of epididymes by flushing with physiologic saline solution. Percentage of live spermatozoa were evaluated under amicroscope utilizing a drop of semen between a warmed glass slide and coverslip, both at a temperature of 38^0 C. The ratio of live to dead sperm is most easily estimated using nigrosin/eosin stain. Seven drops of stain (pH 6.8 to 7.4) are added to one drop of semen and a smear is prepared. Dead sperms are stained by the eosin. The percentage of live and dead spermatozoa were examined by counting 100 spermatozoa using the classification of Christiansen (1984).

Data analysis was performed using SPSS for Windows Version 6.0 computer program. The percentage live or dead sperm were analysis by ANOVA.

RESULTS AND DISSCUSION

The results (Table 1) showed that the percentage of life spermatozoa were 67,16 ± 5,67; 46,33 ± 5,60 and 24,00 ± 4,35%. respectively. There was a significant decrease in the number of live spermatozoa recovered from epididymes with various times ($P<0.01$). We could appreciate that the percentage of live was affected by postmortem time. and it was clear that after 3 hours found only low quality samples The decrease of percentage of live epididymes sperm possibly due to tissue decomposition (Martinez-Pastor et al.,2004**).** The decrease in percentage of live spermatozoa is likely due to the diminishfunction, energy utilization, synthesis, and growth. However, these characteristics occur at different rates and disappear at different times in different cells (Cheville,1976).

In death, the essential characteristics of life-maintenance of structure, function, energy utilization, synthesis, growth, and work are absent. But in the dying, these characteristics diminish at different rates and disappear at different times in different cells. In dying, cells in many organs could be survive for various period of time, depending on the cell type, humidity, animal condition, environment and temperature (Hill and Lavia, 1980).

The percentage of live sperm extracted from caudae epididymes under the percentage of live sperm per ejaculation. The percentage of live spermatozoa per ejaculation at room temperature varies from 85% in second fraction to 80% in the total ejaculate.

Table 1. The percentage of live sperm extracted from caude epididymes depending on post mortem time

Treatment	Percentage of live spermatozoa (%)
T0 (collection immediately after death	67,16 ± 5,67
T1 (Collection after 3 hours postmortem	46,33 ± 5,60
T2 (collection after 6 hours postmortem	24,00 ± 4,35

Even after salvaged 6 days, live spermatozoa could be recovered from epididymes. Thus, when interpreted these data, it is indicated that it might be possible for us to recover functional spermatozoa within many hours postmortem.

In this study, the percentage of live of caudal epididymal sperm was lower than that of ejaculated sperm. reported by Christiansen (1984) that the percentage of dead spermatozoa per ejaculation at room temperature varies from 15% in the second fraction to 20% in the total ejaculate, possibly due to the effect processing semen and the methods of spermatozoa retrieve. It was found that during processing semen has some factors that may affect the viability of dog spermatozoa (England and Allen,2002). Root-Kustritz (1998) revealed that staining or preparation technique may alter the morphology of dog spermatozoa. As reported by Yu and Leibo (2002), even after storage for 192 h (8 days) at 4^0C, motile spermatozoa could be recovered from dog epididymes, and such refrigerated spermatozoa were capable of binding to zona pellucidae. Marks *et a*l. (1994) reported that the spermatozoa recovered from post-mortem dog epididymes, can make conception, although the semen was stored for 3.5 month at 197^0C in liquid nitrogen

CONCLUSIONS

In conclusion, epididymes sperm from dog undergo a decrease of quality with postmortem time, but it could stay acceptable within many hours postmortem. Interpretation of these data may lead us to an indication that it might be possible for us to recover functional spermatozoa from post-mortem specimen of domestic canids and the possibility of usingepididymes spermatozoa recovered from postmortem dog as good source of germplasm.. If valuable animals die and it is not possible to process their sperm immediately, it may still be possible to obtain viable spermatozoa many hours later.

REFERENCES

Axner, E., C.L. Forsberg and Einarsson. 1999. Morphology and motility of spermatozoa from different region of the epididymal duct in the domestic cat. Theriogenology 45:767-777.

Blash, S., D. Melican and W.Gavin.2000. Cryopreservation of epididymal sperm obtained at necropsy from goats. Theriogenology 54:899-905.

Cheville, N.F. 1976. Cell Pathology. The Iowa State University Press.Ames

Christiansen, I.B.J.1984. Reproduction in the Dog & Cat. Bailliere Tindal.

England, G.C., W.E. Allen and D.J. Middleton.1990. An investigation into the origin of the first fraction of the dog ejaculate. Res Vet Sci. 49:66-70.

Farstad W,2000. Assisted reproduction technology in canid species. Theriogenology. 53:175-186.

Hafez, E.S.E. 2003. Reproduction In Farm Animal.7th Ed. Lea Febiger, Philadelphia.

Hewwit, D.A., R. Leahy, I.M. Sheldon and G.C. England. 2001. Cryopreservationof epididymal dog sperm. Anim Reprod Sci.67:101-11.

Hill, R.B.N. and M.F.Lavia. 1980. Principle of Patobiologic. Ed.3rd .Oxford University Press.

Hori, T., M.Ichikawa, E. Kawakami and T.Tsutsui. 2004. Artificial insemination of frozen epididymal sperm in Beagle dogs. J Vet Med Sci 66:37-41.

Johnston, S.D.1991. Performing a complete dog semen evaluation in small animal hospital. Vet Clin North Am Small Anim Pract. 21:545-551.

Kibble, R.M.1969. Artificial insemination in dogs. Aus Vet.J. 45:194-199.

Mark, S.L., J.Dupuis, W.D. Mickelsen, M.A. Menon and C.C. Plattz. 1944. Conception by use post mortem epididimal semen extraxtion in the dog. J Am.Vet Med.Assoc.204:1639-1640

Pangestu, M.1997. Potensi spermatozoa epididimis yang diperoleh setelah kematian sebagai sumber sel gamet. Forum Komunikasi Reproduksi. Denpasar.

Rootkustritz, M.V., P.M. Olson and S.D. Johnston.1998. The efects of stains and investigators on assessment ofmorphology of dog spermatozoa. J Am Anim Hosp Assoc.34:348-52.

Salisbury, G.W., and N.L. Demark.1985. Fisiologi Reproduksi dan Inseminasi Buatan Pada Sapi. Terjemahan R Djanuar. Fakultas Peternakan Universitas Gajah Mada. Gajah Mada Press.

Smith, T.P.D., G.J. Sotwick, C.A. Yates, A.O. Trounson and D.M. de Krester.1985. Human pregnancy by in vitro fertilization (IVF) using sperm aspirated from the epididymis . J In Vitro Ferti.Embryo Transfer.2:119-112.

Watkins, W., H. Bourne, F. Nieto, M. Gronov andG. Baker. 1996. Testicular aspiration of sperm for intracytoplasmic sper injection : a novel treatment for ejaculatory failure on the day of oocyte retrievel. Fertil Steril 66:660-661

Watkins, W., F. Nieto, H. Bourne, B.Wutthipham, A.Speirs and H.W.Baker. 1997. Testicular and epididymal sperm in a microinjection program : methods of retrievel andresults. Fertil Steril 67:527-535.

Yu, I. and S.P. Leibo. 2002 Recovery of motile, membrane-intact spermatozoa from dog epididymides stored for 8 days at 4^{0} C. Theriogenology.57:1179-1190.