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The Semen Property and Preservation in Shih Tzu Dogs

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ABSTRACT

This study was carried out to investigate the general characteristics of semen such as semen volume, pH, sperm motility and sperm concentration of the semen collected from Shih Tzu dogs (age of 24 to 48 months, weight of 4 to 8 kg) by using the method of digital manipulation of the penis. The effect of preservation temperature and time on motility of fresh semen was also investigated in the present study. Semen was collected for 16 times from 4 male Shih Tzu dogs by multiple ejaculations (four times ejaculation per dog). The average of semen volume, semen pH, sperm motility and sperm concentration of the second fraction containing small volume of the initial third fraction per ejaculation were 2.11 ± 0.31 ml, 6.25 ± 0.07, $97.59 \pm 1.03\%$ and $2.05 \pm 0.14 \times 10^8$ cells/ml, respectively. Average semen volume per ejaculate, semen pH, sperm motility and sperm concentration of the first fraction from the ejaculation were 1.12 ± 0.15 ml, 5.99 ± 0.14, $16.09 \pm 6.18\%$ and $5.16 \pm 2.03 \times 10^5$ cells/ml, respectively. Those of second fraction were 2.07 ± 0.29 ml, 6.36 ± 0.13, $97.31 \pm 1.36\%$ and $2.15 \pm 0.30 \times 10^8$ cells/ml, respectively. Those of third fraction were 2.60 ± 0.29 ml, 6.63 ± 0.08, $95.72 \pm 1.61\%$ and $6.03 \pm 1.83 \times 10^7$ cells/ml, respectively. Sperm motility was significantly higher at 17℃ preservation temperature than at 5℃ or 36℃ during preservation period except 1 h preservation ($P<0.05$). When preservation temperature was 17℃, sperm motility was $96.69 \pm 1.49\%$ at 1 h, $91.38 \pm 1.90\%$ at 6 h, $88.38 \pm 2.34\%$ at 12 h, $78.13 \pm 4.58\%$ at 18 h, $58.44 \pm 8.57\%$ at 24 h and $29.56 \pm 5.06\%$ at 30 h, respectively.

(Key words : Shih Tzu dog, semen, sperm motility, digital manipulation, preservation)

INTRODUCTION

The dog, which was the first domesticated animal, has been the most widely kept working, hunting and pet animal in human history. It has been improved to a number of new varieties by a variety of purposes over a long period of time (Tanabe *et al*., 1991). Moreover, the research understanding physiological characteristic may need in dog because dog have vary in breed from small breed to large breed and have much more variation by reproductive physiological characteristic between breed (Boucher *et al*., 1958). In dog, the research for reproductive physiology and improvement of artificial insemination technic is important for not only commercial advantage but also the ability to increase reproductive efficiency. Furthermore, improvement of cryopreservation technic of semen may contribute that thoroughbred lineage as well as outstanding descent lineage can be stored permanently as a form of germ cell, that dog reproduction can be used conveniently without restriction on time and place through artificial insemination. This indicates that it can be contributed to the academic and industrial.

Harrop (1954), who introduced the study on dog semen for the first time in the world, have reported that semen collection via artificial vagina method has been shown much more collection volume of semen than digital manipulation of the penis when the method as digital manipulation of the penis and artificial vagina was compared as a method of semen collection in dog. Furthermore, studies on semen collection, preservation and artificial insemination have been reported by Seager and Flecher (1972). Gunzel (1986) have reported that semen volume obtained from ejaculation is completely depended upon dog weight. It has been reported that semen volume as well as sperm concentration have showed seasonal variation by Takagi (2001). Also, study on seminal fluid of Beagle dog has been reported by Kawakami *et al*. (2007). On the other hand, studies on artificial insemination and short term preservation

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of semen in dog have been reported by several groups. For example, fresh semen after collection was introduced to artificial insemination (Pinto *et al*., 1998; Farstad, 2000) and the diluted semen, which is stored under low temperature, were also used for artificial insemination (Seager and Fleccher, 1973). The method of semen cryopreservation as well as general characteristics of post frozen-thawing by supplement has been well established (Ji *et al*., 2005; Hori et al., 2006). Nevertheless, very diverse research about general characteristics of semen in dog has not recently been reported even though dog was widely breeding as a pet. Moreover, the research activity on general reproductive physiology was lower than livestock species including cattle, pig and horse.

In the present study, we investigated general characteristics of semen including semen volume, pH, sperm motility and sperm concentration as well as effect of preservation temperature and time on motility of fresh semen collected from Shih Tzu dog.

MATERIALS AND METHODS

Unless otherwise state, all chemicals used in the present study were purchased from Sigma-Aldrich Chemical Co (USA).

1. Animals

Four healthy male Shih Tzu dogs, which is 24 to 48 months old and 4 to 8 kg weight, were used for this study. Any of morphometric and physiological disease was not detected during the study. Shih Tzu dogs were introduced to this study after completing anthelmintics and vaccination, and supplying basic care for 4 weeks. 30 g/kg per day for feed was given twice daily; pelleted feed contained at least 25% crude protein and 15% crude fat. Dogs also exercised twice daily for 30 min each. These were used to the study without checking whether or not it had natural mating experience.

2. Semen Collection

Semen was collected between April and June to reduce seasonal variation. Interval of semen collection was once a week per dog indicating total 4 times ejaculation. Semen collection was carried out between 9:00 AM and 11:00 AM by digital manipulation method using a warm 20 ml sterile plastic tube (30～36℃) since the penis was washed with 20～25℃ normal saline solution. Finally, semen was collected for 16 times from 4 male Shih Tzu dogs.

3. Examination and Preservation of Semen

The collected fresh semen was used to examine general characteristic after transferring to laboratory. To determine the effect of preservation temperature and time on motility, semen were stored and examined at 5℃, 17℃ and 36℃ for 1, 6, 12, 18 and 24 hours, respectively. A 10 ul pipette was introduced to measure semen volume in this study. The pH were measured by BTB pH measurement paper (TOYO, Japan) having 5.8～8.2 range. Sperm motility was determined by using slide and cover glass on 36℃ warm plate under the stereo microscope. Magnification was 400×. Sperm concentration was determined using the same protocol as described in the previous study (Salisbury and Vandemark, 1961).

Fresh semen was stored without dilution in 10 ml sterile plastic tube at 5℃, 17℃ and 36℃, respectively. For 36℃ storage, fresh semen were transferred to 36℃ water bath. For 5℃ and 17℃ storage, temperature was decreased until the proposed temperature by 0.5℃ per min speed.

4. Statistical Analysis

All data were obtained from four replicated experiments. The results were expressed as mean±SD. Means were analyzed using a repeated measurement model of ANOVA, followed by Duncan's post hoc test to determine significant differences using the IBM SPSS statistics program (version 19.0; IBM®, USA). Differences with values of $P<0.05$ were considered to be statistically significant.

RESULTS

1. General Characteristics of Semen

The process of ejaculation in dog is consist of the first, second and third fraction. Most of semen for artificial insemination and cryopreservation were produced using semen obtained from the second fraction containing small volume of the initial third fraction besides semen obtained from the first fraction and the third fraction. Because it has been known that semen obtained from the second fraction showed higher quality and sperm concentration. In Table 1, we investigated general characteristics of semen. Results indicated that the average of semen volume of the second fraction containing small volume of the initial third fraction, semen pH, sperm motility and sperm concentration per ejaculation were 2.11±0.31 ml, 6.25±0.07, 97.59±1.03% and 2.05±0.14×10^8 cells/ml, respectively.

Table 1. Semen characteristics of Shih Tzu dogs*

Volume (ml)	pH	Motility (%)	Concentration of sperm ($\times 10^8$ cells/ml)
2.11±0.31**	6.25±0.07**	97.59±1.03**	2.05±0.14**

* Semen was the 2nd fraction and small volume of the 3rd fraction. Method of semen collection was digital manipulation of penis.
** Values were mean±SD for 16 samples in 4 Shih Tzu dogs.

2. General Characteristic of Semen in Each Fraction

In Table 2, we investigated general characteristics of semen including semen volume, pH, sperm motility and sperm concentration in each fraction. The average of semen volume, semen pH, sperm motility and sperm concentration from the first fraction were 1.12±0.15 ml, 5.99±0.14, 16.09±6.18% and $5.16\pm2.03\times10^5$ cells/ml, respectively. Average of semen volume, semen pH, sperm motility and sperm concentration from the second fraction were 2.07±0.29 ml, 6.36±0.13, 97.31±1.36% and $2.15\pm0.30\times10^8$ cells/ml, respectively. Average of semen volume, semen pH, sperm motility and sperm concentration from the third fraction were 2.60±0.29 ml, 6.63±0.08, 95.72±1.61% and $6.03\pm1.83\times10^7$ cells/ml, respectively. Especially, sperm motility was dramatically increased in the second fraction and the third fraction than in the first fraction.

3. Short Term Preservation of Fresh Semen

In Table 3, we investigated the effect of preservation temperature and time on motility of fresh semen. Semen were stored and examined at 5℃, 17℃ and 36℃ for 1, 6, 12, 18 and 24 hours, respectively. Sperm motility was significantly higher at 17℃ preservation than at 5℃ or 36℃ preservation. When preservation temperature was 17℃, sperm motility was 96.69±1.49% at 1 h, 91.38±1.90% at 6h, 88.38±2.34% at 12 h, 78.13±4.58% at 18 h, 58.44±8.57% at 24h and 29.56±5.06% at 30 h, respectively. When preservation temperature was 17℃ and 36℃, sperm motility was 95.94±1.35% to 7.63±3.89% and 93.38±2.23% to 0%. Results indicated that sperm motility can be stable at 17℃ even though there is no significantly difference between 17℃ and 5℃ preservation temperature at 1 h.

Table 2. Semen characteristics of Shih Tzu dogs by the fractional collection*

Semen fraction	Volume (ml)	pH	Motility (%)	Concentration of sperm (cells/ml)
The 1st fraction	1.12 ± 0.15^b	5.99 ± 0.14^c	16.09 ± 6.18^b	$5.16\pm2.03\times10^{5,c}$
The 2nd fraction	2.07 ± 0.29^a	6.36 ± 0.13^b	97.31 ± 1.36^a	$2.15\pm0.30\times10^{8,a}$
The 3rd fraction	2.60 ± 0.29^a	6.63 ± 0.08^a	95.72 ± 1.61^a	$6.03\pm1.83\times10^{7,b}$

* Values were mean±S.D. for 16 samples in 4 Shih Tzu dogs.
[a~c] Means in the same column with different superscripts differ significantly($P<0.05$).

Table 3. Sperm motility according to preservation time and temperature[1]

Treatment (℃)	Preservation time (hours)					
	1	6	12	18	24	30
5	$95.94^a\pm1.35$	$76.75^b\pm4.45$	$60.75^b\pm6.76$	$54.44^b\pm6.17$	$33.44^b\pm8.62$	$7.63^b\pm3.89$
17	$96.69^a\pm1.49$	$91.38^a\pm1.90$	$88.38^a\pm2.34$	$78.13^a\pm4.58$	$58.44^a\pm8.57$	$29.56^a\pm5.06$
36	$93.38^a\pm2.23$	$32.13^c\pm6.99$	$16.81^c\pm5.56$	$5.81^c\pm3.47$	0	0

[1] Values were mean±SD for 16 samples in 4 Shih Tzu dogs.
[a~c] Means in the same column with different superscripts differ significantly($P<0.05$).

DISCUSSION

From the results of general characteristic of semen on Shih Tzu dog, the average of semen volume, semen pH, sperm motility and sperm concentration of the second fraction containing small volume of the third fraction per ejaculation were 2.11±0.31 ml, 6.25±0.07, 97.59±1.03% and 2.05±0.14×10^8 cells/ml, respectively. Interestingly, these results were similar with the previous result which has been reported that the average of semen volume from one time ejaculation was 3.47 ml (Roychoudhury and Dubay, 1974). However, the average of semen volume was 5.4 ml in the dog having less than weight 20 kg, and 12.8 ml in the dog having more than weight 20 kg (Gunzel, 1986). In the present study, average of semen volume was significantly lower than previous report. Because the dog, which is weight 4 to 8 kg, were used for this study. This indicated that there is no experimental error. General semen pH range is 5.8～6.7 or 6.1～7.0, which is slightly acidic (Cho *et al*., 1990). We also obtained same range of semen pH in this study. For sperm motility, adult dog has been shown 60～100% and semen having 70～90% sperm motility can be used favorably for artificial insemination (Gunzel, 1986). The results in this study are considered to be fair. Sperm concentration result was analogous to Harrop (1954) who reported the sperm concentration to be 1.25×10^8 cells/ml. However, the concentration obtained from the present study was somewhat higher than previous report by Roychoudhury and Dubay (1974) which indicated that semen concentration from second fraction was 0.7×10^8 cells/ml as well as by Takagi (2001) which indicated that semen concentration from second fraction was 0.92×10^8 cells/ml.

In the study on semen volume of each fraction, it has been reported that semen volume of the first, second and third fraction was 0.3～2.0 ml, 0.5～4.0 ml and 3.0～25 ml, respectively (Maule, 1960). It has also been reported that semen volume of the first, second and third fraction was 0.3～5.0 ml, 0.5～3.5 ml and 2.0～30 ml, respectively (Arthur, 1975). The reason for such results is estimated to be an increase in variation of semen volume, due to differences in species, age, nutrition status, collection method and collection condition during semen collection. Harrop (1954) reported previously that semen pH in each fraction would be the first fraction 6.37, second fraction 6.10 and third fraction 7.20 with an average pH of 6.75. This conforms to the results obtained from the present study.

The result of this study concerning short term preservation of fresh semen depending on the preservation temperature and time showed highest preservation ability at 17℃. Nonetheless, sperm showed 76.8% motility when preserved at 5℃ for 1～6 h, which means that the fresh semen preserved at 5℃ still showed sperm motility for 6 h which can be used for artificial insemination. Also, this result conforms to Arthur (1975), where he reported that the sperm preserved at 35～37℃ still showed sperm motility after 20 h.

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