UNIVERSITI PUTRA MALAYSIA

A SURVEY OF SEMEN QUALITY IN DOGS REARED UNDER A TROPICAL ENVIRONMENT

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To Mom and Dad

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ABSTRACT

Semen was collected by means of digital manipulation without a teaser from 27 dogs of various breeds. The mean age and weight of the dogs were 4.1 years and 27.7 kg. respectively. The volume of the combined first and second fractions ranged from 0.4 ml to 6.6 ml, with a mean of 3.1 ml, while that of the third fraction had a mean of 9.1 ml. The spermatozoa concentration ranged from 9.7 million per ml to 620 million per ml, the mean being 196 million per ml. The mean percentages of individual progressive motility, live spermatozoa and morphological abnormality were 76.2%, 89% and 15% respectively.

No correlation could be established between age and spermatozoa output per ejaculate or percentage morphological abnormality. Similarly, there was no correlation between body weight and spermatozoa output per ejaculate.

The most common abnormalities noted were coiled midpieces (22.1%), looped tails (20.8%), bent tails (15.0%), detached heads (14.4%) and coiled tails and midpieces (10.7%).

CONTENTS

1. INTRODUCTION

Up till presently, a considerable amount of research has been done in establishing basic parameters of canine semen such as volume, spermatozoa concentration and motility. Knowledge of these values is of utmost importance in determining the fertility and breeding soundness of potential studs. However, most of these studies have involved dogs in a temperate environment. Values of these parameters among dogs in the tropics have not been determined. Another area in which information is scarce is the type and frequency of morphological abnormalities in canine semen. Thus, the main objectives of this study were:

i) To determine the basic parameters of canine semen among dogs kept in a tropical environment;

ii) To establish the type and frequency of morphological abnormalities in canine semen;

iii) To determine a correlation, if any, between body weight and spermatozoa output per ejaculate;

iv) To determine a correlation, if any, between age and spermatozoa output per ejaculate and percentage morphological abnormality.

2. LITERATURE REVIEW

2.1 Collection Procedure

Ejaculation in the dog can be initiated by digital manipulation (2,5,7,9,18), an artificial vagina (1,8,12,16,17) or electrical stimulation (6,17). Early workers relied upon digital manipulation

but Harrop (8) abandoned this method in favour of the artificial vagina (A.V.) because he was able to collect a better semen sample with the later method. In addition, the pulsating action of the lining of the A.V. enabled him to eliminate the teaser.

The A.V., however, can be cumbersome especially in small dogs and has been noted to reduce the motility of the spermatozoa due to prolonged contact of the semen with the warm latex lining of the A.V. (5). The study also showed that by using a teaser, the quality of semen obtained by digital manipulation could be improved. Furthermore, the teaser was also useful in overcoming fear in shy dogs (5).

2.2 Semen Characteristics

2.21 Volume

Canine semen is ejaculated in three fractions (2,5,7,8,9, 11,12,17). The first fraction, which is relatively small in volume, is usually clear and contains few, if any, spermatozoa. The second fraction, also small in volume, is the thick milky sperm-rich fraction. The third fraction is the largest in volume and consists of the watery prostatic secretion (2,5,7,8,9).

Various volumes of the three fractions have been reported. Boucher et al. (5), using digital manipulation with the presence of a teaser obtained mean values of 0.8 ml, 0.6 ml and 4.0 ml for the three fractions respectively. The mean volume of the unfractionated ejaculate has been reported to be 9.5 ml (8), with smaller dogs giving smaller volumes than larger dogs (8,17). Laiblin et al. (10), however, noted that the volume of the whole ejaculate can range from 0.5 ml to 23 ml.

2.2.2. Colour and density

The colour of canine semen can vary from light gray to white, and the consistency from watery to viscous. This variation may be due, in part, to the concentration of the spermatozoa in the semen (13).

2.2.3 Spermatozoa concentration

One method to determine concentration involves the direct counting of spermatozoa in the counting chamber of a haemocytometer. Boucher et al. (5) used a dilution factor of 20 instead of 200 used by Harrop (8). Using a Kleft-Summerson colorimeter to measure the optical density of semen, Boucher et al. (5) found it to be highly correlated to actual spermatozoa number.

The average concentration of spermatozoa has been found to be 125×10^6/ml (3,8,14). It may, however, range from 4×10^6/ml to 540×10^6/ml (8). In a comparative study by Alifanov (1), dogs which attempted to mount promptly had a spermatozoa concentration 2.5 times higher than dogs which took longer than six hours to mount and 13.5 times higher than dogs which ignored the bitch completely. He did not find any correlation between semen quality and age or breed.

Spermatozoa are continuously being produced and the normal amount is restored by 48 hours (1). Studies on frequency of ejaculation showed that semen quality did not deteriorate if it was collected after a rest period of 48 hours (5,8).

2.2.4. Motility

The gross motility of canine semen appears to have a rippling movement unlike the 'wave motion' of bull semen (8). According to the type of movement, spermatozoa can be classified as those exhibiting rapid forward movement, undulating movement and oscillatory movement (5). Most dogs have more than 80% motile spermatozoa and rarely will a dog with fewer than 70% motile spermatozoa have good fertility (11,12).

Poor techniques in handling the semen sample may result in decreased motility. In bulls, low motilities have been reported in cases of testicular hypoplasia and degeneration, while an absence of motility may be seen in instances of orchitis and epididymitis (3). Similarly, scrotal dermatitis in dogs is known to cause poor motility (12). A rapid loss of motility in spermatozoa may be an indicator of poor fertility (12). Laiblin et al. (10) reported that the percentage forward motility of canine spermatozoa ranged from 60% to 95%. However, semen is considered good if 70% to 90% of the spermatozoa are actively motile (13).

2.2.5 Morphological abnormalities

Morphological abnormalities can be classified into primary abnormalities, which occur during spermatogenesis; secondary abnormalities, which arise during storage in the epididymis and transportation to the exterior; and tertiary abnormalities due to poor handling techniques (14). In bulls, primary abnormalities include pyriform, big, small, narrow and double heads. Secondary and tertiary abnormalities usually involve the midpiece and the tail (14).

It has been reported that abnormalities seen in other species are also seen in the dog (13). Total head and midpiece abnormalities in more than 40% of the spermatozoa is associated with infertility, while 20% head, acrosome and proximal cytoplasmic droplet defects may be related to decreased conception rates (15). Bent tails and loose or detached heads have been reported in dogs with scrotal dermatitis (11). Kirk (9) reported that there should not be more than 3% to 4% each of protoplasmic droplets, tail and midpiece abnormalities in any spermatozoa count. Similarly, total head abnormalities should not exceed 10% to 12% (9). The general concensus of opinions, however, appears to be that a good semen sample hould not contain more than 20% abnormal spermatozoa.

3.3 Semen Evaluation

Visual and microscopical examinations were carried out to evaluate the following characteristics of the semen sample:

Visual examination

i) Volume (ml)

ii) Colour

iii) Density (0-4 rating)

Microscopical examination

iv) General motility (0-100%)

v) Individual progressive motility (0-100%)

vi) Spermatozoa concentration (X 10^6/ml)

vii) Live spermatozoa (%)

viii) Abnormal spermatozoa (%)

The detail procedures followed in evaluating the above parameters are given in Appendix I.

The number of spermatozoa per ejaculate was calculated by multiplying the spermatozoa concentration with the volume of the sperm-rich fraction.

4. RESULTS

4.1 Animals

The 27 dogs from which semen was collected were of various breeds (Table 1). The ages ranged from 1 year to 10 years 11 months, the mean being 4 years 1 month. The mean weight was 27.7 ± 12.1 kg.

4.2 Semen Characteristics

4.2.1 Colour and density

The first fraction was clear and watery while the second fraction varied in colour and density depending upon spermatozoa

concentration. It varied from watery and translucent gray to thick and milky white. The third fraction was clear and watery.

4.2.2 Volume

The mean values of this parameter obtained for each breed are presented in Table II. The volume of the sperm-rich fraction ranged from 0.4 ml to 6.6 ml, the mean being 3.1 ml. The third fraction had a mean of 9.3 ml. The larger dogs (body weight >30 kg) had a mean total volume of 10.4 ml, while the smaller dogs (body weight < 16 kg) had a value of 1.9 ml. There was a positive correlation between body weight and the volume of sperm-rich fraction (coefficient of correlation, r = 0.56).

Table I: DISTRIBUTION OF BREED, AGE AND WEIGHT OF SAMPLE POPULATION

Breed	Number (n)	Average Age (year month)		Average Weight (kg)
Working Dogs				
German Shephard	14	5	4	36.7 ± 2.6
Labrador	3	2	10	35.3 ± 1.5
Rottweiler	1	1	9	39.0 ± 0
Pets and Mongrels				
Mongrels	5	1	9	14.2 ± 2.4
Spitz	3	2	10	11.0 ± 1.0
Miniature Pinscher	1	1	3	3.0 ± 0

Table II: MEAN SEMEN VOLUME AND SPERMATOZOA CONCENTRATION

Breed	Mean - S.D. Volume (ml)		Mean - S.D. Spermatozoa concentration	
	1st & 2nd Fractions	3rd Fraction	x 10^6/ml	10^6 per ejaculate
Working Dogs				
German Shephard	4.3±1.9	15.7±13.2	122.9±141.9	497.6±482.9
Labrador	3.0±1.1	6.8± 4.8	376.2±181.7	1009.9±136.5
Rottweiler	0.8±0	2.0± 0	400.0±0	360.0±0
Pets and Mongrels				
Mongrels	1.2±0.7	0.5± 0	327.9±227.7	312.1±176.5
Spitz	2.2±0.7		122.6± 54.2	251.5± 48.2
Miniature Pinscher	0.4±0	0.5± 0	56.0± 0	22.4± 0

.2.3 Spermatozoa concentration

Spermatozoa concentration ranged from 9.7 x 10^6/ml to 620 x 10^6/ml, the mean being 196 x 10^6/ml. The spermatozoa concentration values for each breed are given in Table II. Seventy-four per cent of the dogs had a spermatozoa output greater than 200 x 10^6 per ejaculate. However no correlation could be established between age or body weight and spermatozoa output per ejaculate.

4.2.4 Motility

Table III presents mean values for percentage general motility (GM) and percentage individual progressive motility (IM) obtained for each breed. Percentage IM ranged from 5% to 95%, with 70% of the dogs showing 80% or above. The mean percentage IM for the whole sample population was 76.3%.

4.2.5 Percentage live spermatozoa

The Spitz had the highest percentage live spermatozoa followed by the Rottweiler, Mongrels, Miniature Pinscher, Labrador and German Shephard (Table III). The mean percentage live spermatozoa for the

Table III: MEAN PERCENTAGES FOR MOTILITY, LIVE SPERMATOZOA AND ABNORMALITY

BREED	Mean ± S.D. Percentage			
	General motility	Individual motility	Live spermatozoa	Abnormal spermatozoa
Working Dogs				
German Shephard	60.0±23.0	62.1±29.7	82.8±13.0	22.5±17.6
Labrador	90.0±0	95.0±0	91.3±6.7	4.3±1.5
Tottweiler	90.0±0	95.0±0	93.0±0	3.0±0
Pets and Mongrels				
Mongrels	84.0±8.2	89.0±6.5	93.6±3.0	9.2±5.6
Spitz	86.7±5.8	93.3±2.9	99.0±0	9.0±1.7
Miniature Pinscher	80.0±0	92.0±0	92.0±0	9.0±0

able IV: PERCENTAGES OF ABNORMAL SPERMATOZOA IN CANINE SEMEN

Type of abnormality	Percentage
Head	
Detached head	15.22
Small head	0.75
Big head	0.67
Double head	0.50
Midpiece	
Bent midpiece	22.10
Coiled tail and midpiece	10.65
Thickened midpiece	0.25
ail	
Looped tail	20.80
Bent tail	16.19
Whole tail coiled	4.21
Coiled tip	3.70
Double tail	1.13
Distal protoplasmic droplet	3.04
Proximal protoplasmic droplet	0.79

sample population was 89%. Statistical analysis showed that there was a positive correlation between percentage live spermatozoa and percentage IM (r = 0.67). Ninety-four percent of the dog semen that satisfied the criteria of having percentage IM of 80% or above and a percentage abnormality of less than 20% had a live spermatozoa value of 85% or greater.

4.2.6 Percentage morphological abnormality

The abnormalities noted were of the primary, secondary and tertiary types, although there were relatively fewer of the former compared to the latter two. Table III presents the percentages of total abnormality obtained for each breed. The mean percentage of abnormality obtained for the whole sample population was 15%. The most common types of abnormality were bent midpieces followed by looped tails, bent tails and detached heads (Table IV). Approximately 88% of the detached heads counted were found to be from the working dog population. This group also had twice the number of abnormalities present compared to the pets and mongrel group. A negative correlation was computed between percentage abnormality and percentage IM (r = -0.76). However, there was no correlation between age and percentage abnormality.

5. DISCUSSION

5.1 Collection Procedure

Digital manipulation without a teaser was found to be a satisfactory method for semen collection in this study as samples were easily obtained from over 90% of the dogs at the first attempt. In cases where erection occurred before the penis could be exteriorized, digital pressure was maintained through the prepuce. In most instances, ejaculation could be initiated.

During the collection, no attempt was made to separate the first and second fractions as the thrusting movements of the dog made it difficult to do so.

5.2 Semen Characteristics

5.2.1 Volume

During ejaculation, prostatic fluid was continuously voided as long as the erection was maintained by digital pressure. In order to standardise the collection procedure, digital pressure was only removed when the dog started to show signs of restlessness and retracted the penis or when the erection subsided. This may explain the larger volume of the third fraction obtained in this study compared to others (5, 8).

In goats, testicular size and body weight have been found to be highly correlated (4). If this holds true for dogs, the larger semen volume produced by the bigger dogs in this study may be due to the corresponding larger testicular dimensions.

5.2.2 Spermatozoa concentration

The mean spermatozoa output, using digital manipulation was reported to be 314.04×10^6 (5). Total spermatozoa counts of less than 100 million per ejaculate have been associated with poor fertility and twice this number is thought to be the minimum for a dog to be sound for breeding (11,12). Similarly, for artificial insemination, the recommended dose is at least 200 million spermatozoa per insemination (2,9,13). Seventy-four per cent of the sample population had a spermatozoa output of 200 million or above per ejaculate and thus can be considered sound for breeding. However, other parameters such as percentage IM, percentage live spermatozoa and percentage and type of abnormality should also be taken into account.

It has been observed that dogs in a temperate environment showed reduced spermatozoa concentration during the hotter summer months (18). However, the mean spermatozoa concentration of the dogs in this study was higher than those reported in the temperate regions (3,8,14). Presumably, dogs constantly exposed to high ambient temperatures adapted better than those exposed seasonally. Alternatively, changes in photoperiodicity may influence spermatozoa concentration.

In evaluating spermatozoa concentration, a dilution factor of either 10 or 100 was used depending upon initial density of the semen sample. This was to ensure that sufficient numbers of spermatozoa were present in the counting chamber for more accurate counts.

5.2.3 Motility

Motility of spermatozoa was found to be markedly influenced by temperature changes. Thus, microscopic slides were warmed to body temperature on a thermostatic stage warmer prior to use. Without doing this, wave motion, percentage GM and percentage IM were reduced, and by as much as 10% to 15% in the latter two. Correlation analysis suggests that motility is positively correlated to percentage live spermatozoa ($r=0.69$) and negatively correlated to percentage abnormal spermatozoa ($r=-0.76$).

5.2.4 Percentage live spermatozoa

Good bovine semen should show a mean of 25% or less dead spermatozoa (3). No such figure, however, has been reported for canine semen. In this study, all semen samples which satisfied the criteria of having above 80% IM and less than 20% abnormality had a percentage live value of above 85%.

It is important that the Eosin-Nigrosin stained smears be immediately dried rapidly. This was to ensure that spermatozoa which

died after the smear was made, did not take up the stain. Thus, more accurate counts for percentage live spermatozoa were obtained.

5.2.5 Percentage morphological abnormality

Although the mean percentage abnormality is comparable to those reported in the literature (18), the working dog group had a much higher value than the pet and mongrel group. In the bull, prolonged storage of spermatozoa in the epididymis has been associated with increased fragility (3). This can also occur in the working dog group where all of the animals have never been used for breeding.

There is a relative lack of information on canine spermatozoa abnormality compared to that available on the other parameters. In this study, secondary and tertiary abnormalities, involving the tail and midpiece, were most frequently observed. Such abnormal spermatozoa do not exhibit forward progressive movement. Thus, if present in sufficient numbers, reduced fertility may result as a consequence of low percentage IM. Acrosomal abnormalities could not be identified in this study because the Eosin-Nigrosin stain did not demonstrate them sufficiently.

A knowledge of canine spermatozoa abnormalities is not only important for purposes of record. Certainly fertilising capacity and possibly inheritable conditions can be related to spermatozoa abnormalities. It is hoped that the results from this study will stimulate further investigations.

6. CONCLUSION

Most of the basic parameters obtained from dogs in this study were comparable to those obtained in temperate regions except for spermatozoa concentration and semen volume which were relatively higher

in this study. Thus, it can be concluded that the high ambient temperature constantly experienced by dogs in the tropics does not appear to have an adverse effect on semen quality and quantity.

The age of a dog is not an accurate estimate of semen quality in terms of spermatozoa output and percentage abnormality. Similarly, body weight cannot be used to estimate spermatozoa output. This is because there is no correlation between these parameters.

From the frequency of the types of abnormality, it can be concluded that the most common spermatozoa abnormalities of canine semen in this study are those of the secondary and tertiary types. These included bent midpieces, looped tails, bent tails, detached heads and coiled tails and midpieces.

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APPENDIX I

Procedures

1. Volume

The volume of the sperm-rich fraction and the third fraction were read directly from the graduated tubes.

. Density

This was graded on a 0-4 scale as follows:

4 - Viscous and Creamy

3 - Thick and milky

2 - Thick and transluscent grey

1 - Watery and transluscent grey

0 - Watery and clear

. General Motility

This was assessed from the type of motion exhibited and the percentage of motile sperms observed. It was graded as follows:

90% - 100%: Vigorous swirling wave motion

70% - 90%: Active swirling wave motion

50% - 70:: Slow massive movement, no swirling

30% - 50%: No wave motion, individual movement present

10% - 30%: No wave motion, very little individual movement present

. Percentage Individual Progressive Motility

A drop of warm physiological saline was added to a drop of the sperm-rich fraction and individual progressive movement was assessed at magnification x400.

The percentage progressive motility was graded according to the proportion of spermatozoa seen moving in a rapid progressive

This expressed as a percentage

Appendix I (cont.)

- Spermatozoa Concentration

The sperm-rich fraction was diluted one in ten or one in hundred, depending upon its density, with formol-saline before being counted in a haemocytometer with Neubauer ruling.

Sperm concentration per ml = $n \times \frac{25}{s} \times D \times 10^4$

n = number of sperms in five squares.

s = number of squares counted.

D = Dilution factor

10^4 = Haemocytometer chamber has a depth of 0.1mm (10^{-2}cm) and has an area of 1mm x 1mm (10^{-2} cm)

- Percentage Live and Percentage Abnormal Spermatozoa

The Eosin-Nigrosin stain was used to determine the percentage live spermatozoa. Semen was mixed with the warm stain and incubated at 37°C for 1-2 minutes. Following this, a smear was made and immediately dried on a stage warmer. A total of 100 spermatozoa were observed at random at magnification x1000 and the number alive was expressed as a percentage. The live spermatozoa were unstained while the dead spermatozoa stained pink.

The number and type of abnormal spermatozoa were also noted.

APPENDIX II

1. The Eosin-Nigrosin Stain Formula

30 gm. of Nigrosin is added to 295 ml of deionized water and together boiled on a stirring block until the Nigrosin dissolves. Following this, 5 gm. Eosin Y is added, washing it in with 5 ml of deionized water. The solution is then refluxed for half hour.

Filtering should not be necessary but is permitted upon cooling. The stain mixture is then transferred to 1 oz. Universal J 669 bottles for storate at $4^{o}C$.

2. Formal Saline

1 ml of concentrated formaline (34-38%) is added and mixed thoroughly with 500 ml of normal saline.