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Spermatological and Bacteriological Evaluation of the Semen of Breeding Dogs

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Abstract:

Semen evaluation is an indispensable concept for prediction of fertility in animals. Here, we used 105 stud dogs of different breeds (German shepherd, Rottweiler and pit-bull) to investigate the spermatological characteristics (sperm motility, concentration, morphology and viability) and microbiological studies of dogs' ejaculates. The results of spermatological characteristics revealed that sperm motility decreased to 25%, 40% and 30% and sperm cell concentration clearly decreased to 18 x 10^6, 27 x 10^6 and 50 x 10^6 in the abnormal semen samples of German shepherd, Rottweiler and pit-bull dogs, respectively, while the sperm abnormalities increased in the abnormal ejaculates of different breeds of dogs. Moreover, percentages of sperm viability were 45%, 30% and 50% in German shepherd, Rottweiler and pit-bull dogs, respectively. Bacteria were isolated from abnormal semen samples either in single or mixed infections. *S. aureus* was the dominant bacteria causing single infection followed by *E. coli*. In mixed infections, *S. aureus+ E. coli* were the highest, followed by *S. aureus* + *Streptococcus* spp. Sensitivity of antibiotics against isolated bacteria revealed that levofloxacin was the most effective antibiotic against microorganisms, followed by ciprofloxacin. In conclusion, sperm patterns of abnormal semen deteriorated in motility, concentration, morphology, and viability. *S. aureus* is the predominate microbe contaminating dogs' ejaculates. Levofloxacin and ciprofloxacin were the ideal antibiotics to be used against the bacteria in semen samples of stud dogs. The Comet assay could be used to assess dog sperm oxidative damage and, subsequently, evaluate semen quality.

INTRODUCTION

Canine breeding in many parts of the world has recently been considered an important source of revenue, so canine infertility causes major economic losses (Oguejiofor, 2018). The fertility of dog semen is not typically deteriorated by impurity with the physiological flora hosting the penis, pre-putial sheath and/or distal urethra. Therefore, in case of normal semen from highly fertile dogs, contaminating microorganisms like *Escherichia coli*, *Staphylococcus spp*., *Streptococcus spp., Pseudomonas spp., Pasteurella multocida* and *mycoplasma* could be detected (Johnston, 1991; Kustritz *et al.,* 2005). Semen of fertile dogs could be contaminated by *mycoplasma* including *M. canis,* these unique microorganisms are categorized as a part of normal flora of the mucus membranes (Zoldag *et al.,* 1993). Even though, *M*. *canis* was conveyed in triggering urogenital infection and infertility in dogs including posthitis, balanitis, urethritis, scrotal dermatitis, orchitis, epididymitis (Kustritz, 2007). Various microorganisms such as *E. coli, Ureaplasma urealyticum, S. aureus,* and *Mycoplasma hominis* are known to interact with sperm (Golshani *et al.,* 2006**)**. In particular, *E. coli* rapidly adheres to and agglutinates sperm, there by immobilization of sperm is associated with tight adhesion between *E. coli* and sperm resulting in agglutination of sperm (Diemer *et al.,* 2003).

Semen quality is a constant concern for dog owners. Unfortunately, many factors can adversely affect semen quality in the dog including age, infections, diet, medications, environmental factors and unrelated health conditions. Aspects related to sperm quality like morphology, motility, semen volume, sperm cell concentration and oxidative stress have not been extensively investigated in a huge group of stud dogs (Hesser *et al.,* 2017). Numerous studies have described dog sperm morphology, motility and oxidative status, however these studies have used a small number (4-6) of stud dogs (Kim *et al.,* 2007; Linde-Forsberg *et al.,* 1999; Nizanski, 2006; Thomassen *et al.,* 2001). Regarding human male infertility, the cause remains unknown in 70% to 74 % of cases (Johnston *et al.,* 2001). In human medicine, when the semen quality is poor, assisted reproduction technologies are applied like intra-cytoplasmic sperm injection (ICSI) or in vitro fertilization (IVF). Such techniques are not commonly available to be applied in dogs, nonetheless it might be used in the future (Fulton *et al.,* 1998; England *et al.,* 2001); so, the prognosis of infertility problems still deprived. Practitioners of veterinary field usually make semen analysis as an essential step to comprehensive breeding soundness inspection, in order to assess fitness of semen sample either for preservation by chilling or freezing, to perform AI, or to examine infertility or subfertility. Unfortunately, there is a very little data linking the measured parameters through semen evaluation to the real matters that veterinarians need to estimate (Kustritz, 2007**).** Therefore, the aim of the present study is to investigate the morphometric and bacteriological patterns of semen of different breeds of breeding dogs.

MATERIALS AND METHODS

Ethical statement

The present study was approved by institutional Animal Care and Welfare Committee Ethics (approval No. 135/2014) Animal Reproduction Research Institute.

Animals

A total of 105 stud dogs from different breeds; German shepherd (50), Rottweiler (30) and pit-bull (25) with age ranged from 3 - 6 years and body weight ranged from 18 to 35 kg according to the breed were used. The animals were clinically healthy and their genital organs were normal and free from any abnormalities. The dogs were fed on a commercial dog feed and water was provided ad-libitum.

Collection of semen

Semen samples were collected from dogs by manual massage into sterile glass vial as previously mentioned by (Kutzler, 2005; Pesch *et al.,* 2006). The contact between the penile mucous membrane and the collecting glass was

avoided to minimize the risk of contamination of the sample from the preputial flora. All dogs were examined and showed no signs of illness and none of the dogs received antibiotics before or at admission for semen collection. Full erection was observed in all dogs and ejaculation reflexes were followed completely, indicating a normal reflex course.

Analysis of semen

Sperm individual motility

The motility of the sperm cells was determined by using a sterile glass stirring rod to place a small droplet of fresh semen which diluted with sodium citrate 2.9% on a warm slide and a cover slide was placed over the semen droplet then the percent of motile spermatozoa was microscopically estimated under the microscope at (×10) and (×40) magnification and scored objectively using the scoring pattern as shown in table (1).

Table 1. Scores and description for progressive motility (Hafez and Hafez, 2013).

Scores (%)	Description
10-19	No motion
40-49	Shift non-progressive motionless
50-59	Very active non-progressive motionless
60-69	Shift continuous progressive motility
70-79	Good, continuous progressive motility
89-90	Very good, continuous regressive motility and rotary
90-100	Very good, continuous progressive motility

Sperm concentration (sperm count, 10^9/ml)

Sperm cells were counted using a Neubauer haemocytometer after 1:400 dilution of semen in a 0.5% eosin solution to determine sperm concentration. An aqueous solution of 1% formaldehyde was used as diluent that kills sperm, so that counting can be accomplished. The number of sperms in five squares was multiplied by 10.000 to obtain the number per ml as shown by Evans and Maxwell (1987).

Sperm viability

To determine sperm vitality, 40 µl of freshly liquefied semen was thoroughly mixed with 10 µl of eosin-nigrosin (Merck, Germany), and 1 drop of this mixture was transferred to a clean slide. At least 100 sperms were counted at a magnification of ×100 (Olympus, Japan). Sperms that were stained pink or red were considered dead, and those unstained were considered viable as shown by Raji et al. (2003).

Percentage of abnormal spermatozoa

The smears were prepared by placing a drop from semen sample and one or two drops of previously warmed (37°C) methyl violet stain at one of clean slide and another side (spreader) was brought towards the mixture until touched it. The air-dried smears were examined using high power (100X) microscope oil immersion objective. 100 sperm cells from different fields were examined and the number of abnormal ones was calculated as percentage (Hafez and Hafez, 2013).

COMET Assay

Abnormal semen samples, that tested by ordinary semen analysis were applied to COMET assay. They were 18 German shepherd, 10 Rottweiler and 6 pit-bull. In addition, 3 normal semen samples by ordinary semen analysis from the 3 breeds were used as a control for COMET assay. This method was done as described by Simon et al. (2011). Spermatozoa analyzed for COMET were visualized under an epifluorescence microscope. Whole sperm heads, without a COMET, were considered not damaged, whereas spermatozoa with fragmented DNA that migrated from the sperm head, causing a "COMET" pattern, were considered damaged.

A total of 10^5 sperm cells per slide were assessed for COMET. The COMET was

captured with a still camera connected to the fluorescent microscope and the images were evaluated for percentage of tail DNA.

Microbiological examinations

Bacteriological examination

Collected semen samples were cultured on Mannitol salt agar, Edward's medium, MacConkey agar plates, Blood agar media then incubated at 37°C for 24 hrs. Identification of the bacteria was performed to suspected colonies according to colony morphology; gram staining catalase test and coagulase test (Quinn *et al.,* 2002).

Antibiotic susceptibility test

Isolated strains of *S. aureus*, Coagulase negative *Staphylococcus* (CNS), *Streptococcus* spp. and *E. coli* either in single or mixed infection were subjected to 8 commercially available antibiotics discs [Ciprofloxacin; CIP (5µg), Norfloxacin; NOR (5µg), Levofloxacin; LEV(5µg), Amoxicillin-clavulanic acid; AMC (30µg), Gentamycin; CN (10µg), Amikacin; AK(30µg), Ampicillin and Sulbctam; SAM (30 µg) and Cefotaxime; CTX (30 µg)] and the inhibition zones were recorded as sensitive and resistant according to the (NCCLS, 2008) recommendations.

Statistical analysis

Graph Pad Prism 5 software (GraphPad Software Inc., La Jolla, CA, USA) was used for statistical analysis. Statistical significance between two groups was measured by Student's *t* test for two groups. One-way ANOVA followed by Bonferroni's multiple comparison test was used to detect differences between more than two groups. Results were considered to be statistically significant at $p < 0.05$.

RESULTS

Percentage of infection of semen samples for different breeds of dogs

The percentage of infected semen samples was varied between the different breeds of stud dogs, as it was (36%), (34%) and (24%) in German shepherd, Rottweiler and pit-bull, respectively from the examined samples for each breed as shown in table (2).

Table 2. Percentage of infection of semen samples of different breeding dogs. Superscript letters within the same column are significant at $p < 0.05$.

Breed	Total samples		Normal		Abnormal	
	No	%	No	%	No.	%
German shepherd	50	47.62	32	64	18	36[a]
Rottweiler	30	28.57	20	66	10	34[a]
pit bull	25	23.81	19	76	6	24[b]
Total	105	100	71	67.62	34	32.38

Effect of semen sample (normal $_{Vs}$ abnormal) on different sperm parameters

The data in table 3 showed that there were great differences in results between normal and abnormal samples as motility decreased, respectively in German shepherd, Rottweiler and pit-bull to 25%, 40% and 30%. In addition, sperm cell concentration clearly decreased to 18 x 10^6, 27 x 10^6 and 50 x 10^6 in German shepherd, Rottweiler and pit-bull, respectively. Moreover, the sperm abnormalities were increased than normal levels as in German shepherd (15 major, 11 minor), in Rottweiler (38 major, 7 minor) and in pit-bull (19 major, 16 minor). Also percents of live dead sperm were 45%, 30% and 50% in German shepherd, Rottweiler and pit-bull, respectively.

Table 3. Sperm patterns of normal and abnormal ejaculates of different breeding dogs. Superscript letters within the same column are significant at $p < 0.05$.

Breed	Motility %		Concentration		Abnormalities				Live sperm%	
	Normal	Abnormal	Normal x 10^6	Abnormal x 10^6	Normal		Abnormal		Normal	Abnormal
					Major	Minor	Major	minor		
German shepherd	85	25[a]	650	18[a]	3	6	15[a]	11	85	45[b]
Rottweiler	80	40[b]	450	27 [b]	5	5	38[b]	7	90	30[a]
pit bull	85	30[a]	740	50 [c]	2	4	19[a]	16	85	50[b]

Bacteriological examination of abnormal semen samples of different breeds of dogs

As shown in table (4) bacteria were isolated from abnormal semen samples either in single or mixed infection from 3 different dog breeds. *S. aureus* was the most bacteria causing single infection as it was isolated from 29.41% followed by *E.coli* 14.70% then *CNS* and *Streptococcus* spp. were isolated as single infection from 5.88% of examined samples. In mixed infection, *S. aureus* + *E. coli* were representing 20.6% followed by *S. aureus* + *Streptococcus* spp. 17.65 % and finally *CNS* + *E. coli* by percentage 5.88%.

Table 4. Bacteriological examination of abnormal semen samples.

Bacteria	German shepherd 18		Rottweiler 10		pit bull 6		Total 34	
Single infection	No	%	No	%	No	%	No	%
S. aureus	5	27.8	3	30	2	33.3	10	29.41[c]
CNS	2	11.1	0	0	0	0	2	5.88[a]
E.coli	3	16.7	1	10	1	16.7	5	14.7[b]
***Streptococcus* spp.**	1	5.5	1	10	0	0	2	5.88[a]
Mixed infection	No	%	No	%	No	%	No	%
***S. aureus* + *Streptococcus* spp.**	3	16.7	2	20	1	16.7	6	17.65[b]
S. aureus* + *E. coli	3	16.7	2	20	2	33.3	7	20.6[b]
CNS + *E.coli*	1	5.5	1	10	0	0	2	5.88[a]

Antibiotics sensitivity tests against isolated bacteria from semen of dogs

Sensitivity of antibiotics against isolated bacteria was demonstrated in table (5) which revealed that levofloxacin was the most effective antibiotic against *Streptococcus* spp. (100%), CNS (100%), *S. aureus* (90%) then *E. coli* (80%) followed by ciprofloxacin, as *Streptococcus* spp. was sensitive by percentage (100%), CNS (100%), *E. coli* (80%) and *S. aureus* (80%). In mixed infection levofloxacin was the most effective antibiotic against (CNS +*E.coli*), (*S. aureus* + *E. coli)* and (*S. aureus* + *Streptococcus* spp.) by percentages (100%, 85.71% and 83.33%), respectively.

COMET Assay

The results showed that group of German Shepheard presented higher levels of sperm oxidative DNA damage % (49.8 ± 22.7) followed by Rottweiler (36.9 ± 15.9) while pit-bull were lower (28.4 ± 13.7) in comparison to control groups with mean percent DNA damage (12.6 ± 3.2; 10.4 ± 2.2 and 7.4 ± 1.3) respectively. Mean tail moment shown 5.7±1.3 for German shepherd, 3.8±0.9 for Rottweiler and 2.1±0.3 for pit bull in comparison to control groups (2.42 ± 0.71; 1.35 ± 0.2 and 1.02 ± 0.08), respectively. The results of comet assay shown mean tail length for German shepherd breed 54.7 ± 21.6 , for Rottweiler 43.6 ± 18.4 and Pit bull 38.4 ± 16.3 while the control groups were (17.8 ± 4.3; 12.6 ± 2.7 and 9.8 ± 1.5), respectively as demonstrated in table (6).

Table 5. Antibiotic sensitivity against isolated bacteria.

Antibiotics	Bacteria													
	Single infection								Mixed infection					
	S.aureus (10)		CNS (2)		*E.coli* (5)		*Streptococcus* spp. (2)		*S.aureus +Streptococcus* (6)		*S.aureus +E.coli* (7)		CNS + *E.coli* (2)	
	Sensitive	Resistance	Sensitive	resistance	Sensitive	Resistance	Sensitive	Resistance	Sensitive	Resistance	Sensitive	Resistance	Sensitive	Resistance
Ciprofloxacin	8 (80)	2 (20)	2 (100)	0 (0)	4 (80)	1 (20)	2 (100)	0 (0)	5 (83.33)	1 (16.67)	5 (71.43)	2 (28.57)	1 (50)	1 (50)
Norfloxacin	7 (70)	3 (30)	1 (50)	1 (50)	2 (40)	3 (60)	2 (100)	0 (0)	4 (66.67)	2 (33.33)	4 (57.14)	3 (42.86)	1 (50)	1 (50)
Levofloxacin	9 (90)	1 (10)	2 (100)	0 (0)	4 (80)	1 (20)	2 (100)	0 (1)	5 (83.33)	1 (16.67)	6 (85.71)	1 (14.29)	2 (100)	0 (0)
Gentamycin	5 (50)	5 (50)	0	2 (100)	2 (40)	3 (60)	0 (0)	2 (100)	2 (33.33)	4 (66.67)	2 (28.57)	5 (71.43)	0 (0)	2 (100)
Amikacin	6 (60)	4 (40)	1 (50)	1 (50)	1 (20)	4 (80)	1 (50)	1 (50)	3 (50)	3 (50)	3 (42.86)	4 (57.14)	1 (50)	1 (50)
Ampicillin/ sulbctam	4 (40)	6 (60)	1 (50)	1 (50)	2 (40)	3 (60)	1 (50)	1 (50)	3 (50)	3 (50)	2 (28.57)	5 (71.43)	1 (50)	1 (50)
Amoxicillin /Clavulenic acid	7 (70)	3 (30)	2 (100)	0 (0)	3 (60)	2 (40)	2 (100)	0 (0)	4 (66.67)	2 (33.33)	4 (57.14)	3 (42.86)	0 (0)	2 (100)
Cefotaxime	4 (40)	6 (60)	0 (0)	2 (100)	3 (60)	2 (40)	2 (100)	0 (0)	1 (16.67)	5 (83.33)	3 (42.86)	4 (57.14)	0 (0)	2 (100)

Table 6. Comet assay results. Results are represented as mean ± SE, significant to control ($P < 0.05$**)**. * Tail moment= tail length* % DNA in the tail. This calculated automatically by computer software system as an average for the 50 cells selected for measurement. Different superscript letters are significant at $p < 0.05$ for different breeds and its control.

Samples	Mean %DNA Damage in tail	Mean Tail Moment	Mean Tail Length
Control German Shepherd	$12.6^{a} \pm 3.2$	$2.4^{a} \pm 0.71$	$17.8^{a} \pm 4.3$
German shepherd	$49.8^{b} \pm 22.7$	$5.7^{b} \pm 1.3$	$54.7^{b} \pm 21.6$
Control Rottweiler	$10.4^{a} \pm 2.2$	$1.35^{a} \pm 0.2$	$12.6^{a} \pm 2.7$
Rottweiler	$36.9^{b} \pm 15.9$	$3.8^{b} \pm 0.9$	$43.6^{b} \pm 18.4$
Control pit bull	$7.4^{a} \pm 1.3$	$1.02^{a} \pm 0.08$	$9.8^{a} \pm 1.5$
pit-bull	$28.4^{b} \pm 13.7$	$2.1^{b} \pm 0.3$	$38.4^{b} \pm 16.3$

DISCUSSION

Reproduction plays an important role in ensuring the efficiency of animal production (Woelders *et al.,* 2012), maintaining their biodiversity, and supporting the conservation programs of vulnerable or threatened species (Costa and Martins, 2008).

Prediction of the fertilizing capacity of a semen sample is the definitive goal of semen evaluation. Usually, males with best fertility produce semen with high number of actively motile, viable and morphologically normal spermatozoa. Till date, light microscopy is usually used to evaluate the main criteria of semen (concentration, motility and morphology).

Moreover, sperm cell concentration is frequently determined using a Neubauer counting chamber and motility is assessed subjectively on a pre-warmed glass slide (Johnston, 1991; Iguer-Ouada and Verstegen, 2001), while morphological defects are assessed with various staining techniques (Oettle, 1993).

Motility is an indicator of mechanical and functional capability of sperm; thus, progressive motile sperm are frequently associated with standard morphology (Ellington *et al.,* 1993).The present data revealed that motility of normal semen samples were 85%, 80% and 85% while motility of abnormal samples decreased to 25%, 40% and 30% for in German shepherd, Rottweiler and pit-bull, respectively. Similarly, it was shown that the percentage of total sperm motility in normal dog semen ranged from 70 to 90% (Johnston *et al.,* 2001; Iguer-Ouada and Verstegen, 2001). Sperm concentration has a limited value as a pointer of semen quality. The normal sperm cell concentration in dog semen is greater than 300 - 2000 million (Johnston, 1991). Concentration is reversely related to semen volume, total sperm number is reliant on testicular size and it may diminish with repeated semen collection, due to depletion of epididymal reserves (England, 1999).Here, the sperm cell concentration in normal ejaculates of dogs were 650 x 10^6, 450 x 10^6 and 740 x 10^6 in German shepherd, Rottweiler and pit-bull, respectively. In the current study, the sperm abnormalities in abnormal semen samples were (15 major, 11 minor) in German shepherd, (38 major, 7 minor) in Rottweiler and (19 major, 16 minor) in pit-bull. Moreover, it was shown that normal semen must contain <10% primary abnormalities, <20% secondary abnormalities and over all abnormalities should be <10-20% (Feldman and Nelson, 1996; Freshman, 2001).In the present study, the percentage of alive sperm in normal semen of dogs were 85%, 90% and 85% in German shepherd, Rottweiler and pit-bull, respectively. In harmony with the current data, good canine semen sample should contain at least 80% morphologically normal and viable spermatozoa (Johnston *et al.,* 2001). When the proportion of normal spermatozoa was below 60 %, fertility was found to be adversely affected (Oettle, 1993).

To our knowledge, this is the first study evaluating the results of bacteriological examination of fractionated canine ejaculates in relation to semen quality. The presence of contaminant bacteria in semen affect spermatological characters as it associated with a decrease in sperm motility and viability (Bussalleu *et al.,* 2011).

Recent studies have shown that the simple presence of bacteria in semen samples may compromise the sperm quality and proposed to be significant etiological factors for infertility. The present data yielded that bacteria were isolated from abnormal semen samples either in single or mixed infection, where *S. aureus* was the most bacteria causing single infection, followed by *E. coli* then CNS and *Streptococcus* spp. our results revealed that *S.aureus* was isolated from 29.41% followed by *E.coli* 14.70% nearly similar results were recorded by Enwuru et al. (2016) who recorded that *S. aureus* (29.6%) and *E. coli* (10.5%) had the highest occurrence of facultative bacteria in semen.

In mixed infection, *S. aureus* and *E. coli* were the highest, followed by *S. aureus* + *Streptococcus* spp. and finally *CNS* + *E. coli*. In agreement with current results semen of dogs might be associated with bacteria like *E. coli*, *Staphylococcus spp*., (Johnston, 1991; Kustritz *et al.,* 2005). In addition, Goericke-Pesch et al. (2011) reported that pathogenic bacteria isolated from semen were β-haemolytic *Streptococcus* spp. and *E.coli*. Moreover, Oguejiofor (2018) added that definitive diagnosis of infertility due to a sub-chronic or chronic bacterial infection of the reproductive tract was made based on the presence of copious growth of *E.coli* bacteria in conjunction with poor sperm quality and leucospermia. It was published that fertile dogs, fertile dogs with benign hypertrophy of prostate gland and infertile dogs revealed 53.6 %, 66.6 % and 85.7 % positive bacterial growth of the ejaculates, respectively (Schafer-somi *et al.,* 2009).

It should be known that bacterial infection constitute a significant threat of dog fertility and should be treated by using accurate and suitable antibiotics. The antibiotic sensitivity tests in the

current study showed that levofloxacin was the most effective antibiotic in both single and mixed infection against *Streptococcus* spp*., CNS*, *S. aureus* then *E. coli* followed by ciprofloxacin. Oguejiofor (2018) recommended using of Ciprofloxacin (15 mg/kg body weight) was administered orally twice daily (12 hourly). Treatment was applied for 4 weeks due to the potential long-standing nature of the infection.Ten days post treatment; ejaculate should be examined by bacterial culture. Additional 8 weeks of sexual rest are required after eradication of all microbes for due time of spermatogenesis and thence recovery of ideal semen quality. Moreover, feeding 500 mg of vitamin E daily for 10 weeks was advised for germinal epithelium regeneration (Hatamoto *et al.,* 2006).

Contents Sperm DNA integrity is a fundamental prerequisite in fertilization and embryo development. Among DNA integrity tests, the COMET assay is an accurate and sensitive test for the detection of sperm oxidative damage (Pereira *et al.,* 2017).

The Comet assay has mainly remained an assay of academic and scientific interest until quite recently. Currently, the Comet assay has however the potential to be used as a tool in DNA damage testing and regulatory submissions for new chemicals and mixtures (Tice *et al.,* 2000; Hartmann *et al.,* 2003; Kumaravel and Jha, 2006). Comet Assay data and interpretation depend on good and optimum slide staining, adoption of robust image analysis practices and use of reliable and meaningful Comet assay measurement (e.g. % Tail DNA, Tail moment). Many researches indicated that one of important reasons for get success pregnancies is sperm DNA integrity (Haidl *et al.,* 2015). The current data revealed that German shepherd group preserved significantly the tail moment and tail length (49.8, 5.7 and 54.74, respectively) compared with other species. Meanwhile, group pit bull drastically affected the DNA integrity tail moment and tail length (7.4, 1.02 and 9.8, respectively). The results of this study revealed that bacterial infection drastically affected the DNA integrity in all dog breeds compared with the non-infected control. This may be attributed to bacterial infection affect by stimulation of inflammatory mediators which effect on DNA integrity of dogs.

CONCLUSION

Our results manifested the requisite for spermatological and microbiological evaluations of the ejaculates of stud dogs. Sperm parameters of abnormal semen sample are deteriorated in motility, concentration, morphology, viability and DNA damage. *S. aureus* are the predominated microbes contaminating dogs' ejaculates. Levofloxacin is the ideal antibiotic to be used against bacteria of semen sample of stud dogs.

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CONFLICT OF INTEREST

Authors declare have no conflicts of interest.

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