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Effect of the breed on the properties and chemistry of semen in dogs

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A B S T R A C T

This work was planned to study the effect of dog breeds on the physical and chemical properties of semen. A total number of 53 dogs of Caucasian (n=3), German shepherd (n=37), Malinois (n=8) and Rottweiler (n=5) breeds and mixed ages were used for semen collection along a year from 2016 to 2017. Semen was collected using an artificial vagina and examined for color, density, volume, pH, sperm individual motility, livability rate, cell concentration (SCC) and morphology. Seminal plasma was harvested after centrifugation and examined for testosterone hormone levels, aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) and total anti-oxidant capacity. Results indicated that dog breeds markedly ($p<0.05$) affect pH and SCC values. Seminal plasma testosterone, ALT and ALP significantly ($p< 0.001$, 0.001 and 0.05, respectively) differed between dog breeds. Semen pH and ALT values of Caucasian breed was the lowest among the examined breeds. SCC were substantially ($p<0.05$) higher in Malinois breed than in Rottweiler breed. Testosterone hormone was higher in Caucasian and Malinois than German Shepherd and Rottweiler breeds. The ALP enzyme activity was higher in Rottweiler than German shepherd and Malinois breeds. It conclusion, dog breeds markedly affect the fertility potential of semen though its influence on testosterone-dependent libido intensity, sperm count per ejaculate and seminal plasma enzymatic activity.

Key words: *Breed, Chemical properties, Dog, Physical properties, Semen*

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1. INTRODUCTION

The first commensal association between human beings and the ancestor of the dog has evolved into a form of mutualism (Marinelli, 2007). The number of registered dogs in Egypt is about 35000 dogs (Sonia Haron, 2012). Dogs perform many benefits for human being such as hunting, pulling loads protection, assisting police, military work, companionship and more recently aiding handicapped individuals.

Caucasian dog is a large breed of dog originated from Russia, and has a harmonious built, large, strong body with plenty of bone and powerful muscular system. The Caucasian dog is generally healthy and long-lived, averaging a life span of 10-12 years.

German Shepherd dog is a medium to large-sized breed originated in Germany. German Shepherds were bred specifically for their strength, intelligence, trainability, and obedience. They often the preferred breed for many types of work, including disability assistance, search-and-rescue, police and military roles, and even acting. Their life span is 9-13 years

Malinois dog is a medium-to-large and square-proportioned dog originated from Belgium in Germany. It has a square build in comparison to the German Shepherd. Malinois is dog used primarily as a working dog for tasks including detection of odors such as explosives, accelerants (for arson investigation), and narcotics; tracking humans for suspect apprehension in police work; and search and rescue missions and used for personal protection and sport works. The average life span is 12-15 kg.

Rottweiler is a medium-to-large breed of domestic dog originated from Germany. Rottweiler dog is now used as search and rescue dogs, as guard dogs and police dogs. The mean life span is 8-10 years.

Normal male dog attains puberty at approximately 6- 8 months of age, while, sexual maturity is generally attained at 18-30 months (Dunbar, 1999). Males may successfully breed bitches prior to sexual maturity but they will not attain maximal fertility or daily sperm output until mature. The reproductive organs of male dog are analogous to any other animal species. However, in contrast to humans and many animal species, the prostate is the only accessory sex gland in the dog (Wheaton, 1979). The prostatic secretory fluid (rich in proteins and zinc ions) is practically the only component of the seminal plasma (represents 95% of the ejaculate) in this species (Mann and Lutwak-Mann, 1981) helps in feeding and maintenance of spermatozoa during their passage into the female dog (Iguer-ouada and Verstegen, 2001).

Ejaculated canine semen consists of the pre-sperm fraction, sperm-rich fraction and post-sperm fraction (Kutzler, 2005). The second fraction contains spermatozoa and is easily differentiated from the others because of it is milky and dense (England and Allen, 1992). The potential fertility of semen sample depends on the presence of sufficient number of normal, viable, functionally competent spermatozoa able to accomplish normal fertilization of all the ovulated oocytes, and contribute to embryo development (Peña Martinez, 2004). There is a close relationship between sperm quantity and quality as the fertility increases when the numbers of viable sperm inseminated increase up to a threshold level (Salisbury and VanDemark, 1961).

The variations in the fertility rate among the breeding males were not addressed by the routine semen evaluation parameters, such as sperm cell concentration, motility, viability and morphology (Larson and Miller, 2000). Besides, semen evaluation is influenced by sample collection technique and timing, concentration of spermatozoa in the sample, amount of time from sample collection to evaluation, temperature at which the sample was held, equipment used, and many other factors (Rui et al., 1986; Root Kustritz, 2007). Subsequently, attention is now being directed towards the assessment of other aspects of semen quality as predictors of fertility. Therefore, the presented study aimed spot the light on the variations in the fertility potential among the breeding males through assessing the influence of dog breed (Caucasian, German she pherd, Malinois and Rottweiler) on the physical and chemical properties of semen.

2. MATERIALS AND METHODS

2.1. Animals

The present study was conducted on a total number of 53 dogs belonged to different breeds (Caucasian (n=3), German shepherd (n=37), Malinois (n=8), and Rottweiler (n=5)) (Fig. 1) and of mixed ages during the period from Jan. 2016 to Jan. 2017. Dogs were belonged to public owners (n=28) came to Animal Reproduction Research Institute at El Haram, Giza for andrological examination, or belonged to Transportation Police (n=25), Ministry of Interior Affairs, Al- Abbasia.

Fig. 1 Representative photographs for the dogs of different breeds under this study

2.2. Semen collection and evaluation

Ejaculated semen was collected from all dogs using an artificial vagina according to the method described by Allen (1991). Briefly, dog's penis was vigorously massaged through the prepuce at the level of the bulbus glandis until a partial erection develops. Thereafter, dog's penis was directed toward the collection cup and the prepuce was quickly retracted caudally. A firm constant pressure was applied to the penis behind the bulbus glandis by squeezing the penis between index finger to stimulate pelvic thrusting during the development of full erection. Ejaculation began immediately following the placement of pressure behind the bulbus glandis. The semen and the prostatic fluid are expelled by peristaltic contractions in the muscles surrounding the urethra. Soon after collection, semen was evaluated to avoid the deleterious effects of delay from collection to evaluation, temperature, light on semen characteristics and biochemical constituents.

2.3. Physical parameters of semen

The semen was evaluated physically through measuring the volume, pH, Individual motility, Sperm livability rate, Sperm cell concentration and Sperm morphology.

2.4. Biochemical parameters of semen

Seminal plasma was harvested after centrifugation of the semen at 3000 rpm for 10 min and stored at -80 °C until being analyzed for the chemical parameters.

Seminal plasma testosterone was determined by according to the method described by Furuyama et al. (1972) using commercial Testosterone Immunoassay Kit.

The activity of aspartate transaminase (AST) and alanine transaminase enzyme (ALT) in seminal plasma were determined calorimetrically at 505 nm described by Reitman and Frankel (1957) using commercial kit. Seminal plasma alkaline phosphatase (ALP) activities were determined by colometric method according

to the method described by EL-Aaser et al. (1972) using commercial kit.
Seminal plasma total anti-oxidant capacity (TAC) was estimated spectrophotometry at 505 nm wavelength according to method adopted by Koracevic et al. (2001) using commercial kit.

2.5. Statistical analysis
Data were tabulated and subjected to statistical analysis, including the calculation of the mean, standard error of the mean (SEM) and one-way ANOVA at a confidence limit of 95%. Statistical analyses were conducted according to the method of Armitage (1971) using practicing statistical analysis program (SPSS) Ver. 21 (2017). Duncan's multiple range test was used for comparison between means of groups at p<0.05 (Duncan, 1955).

3. RESULTS

3.1 Physical properties of semen
Semen samples from all animals belonged to the different breeds examined appeared whitish in color with variable density from watery to milky.
Physical parameters meaningfully (p<0.05) varied between dog breeds with special emphasis to pH and sperm cell concentration (Table 1). Nevertheless, the variation in semen volume, and spermatozoa motility, livability and abnormality rates between dog breeds were not reached to statistically significant level.
The semen pH values from Caucasian breed was lower than that from other breeds examined. The mean values of sperm cell concentration were higher in Malinois breed than that of the Rottweiler breed.

3.2. Biochemical constituents of seminal plasma
Seminal plasma testosterone, ALT and alkaline phosphatase significantly differed between dog breeds (Table 2). The variation in seminal plasma (AST) and TAC between dog breeds were not statistically verified.
The Caucasian and Malinois breeds showed higher values of testosterone hormone than German Shepherd and Rottweiler breeds. Caucasian breed showed the lowest ALT enzyme activity among the examined breeds. The activity of ALP enzyme was higher in Rottweiler dogs than German shepherd and Malinois breeds.

Table 1 Effect of dog breeds on physical properties of the semen

Item	Caucasian (n=3)	German Shepherd (n=37)	Malinois (n=5)	Rottweiler (n=8)	P value
Volume (ml)	7.67±1.45	7.25±0.61	4.88±0.69	6.38±1.95	0.31
pH	$6.13±0.07^{b}$	$6.46±0.04^{a}$	$6.58±0.01^{a}$	$6.43±0.15^{a}$	0.05
Motility (%)	80.00±2.89	70.27±1.70	73.13±3.13	73.00±3.74	0.38
Livability (%)	81.67±3.38	75.70±1.82	76.88±4.19	84.80±3.06	0.31
SCC ($\times10^{6}$/ml)	$72.33±6.23^{ab}$	$107.32±7.26^{ab}$	$113.75±16.04^{a}$	$59.80±3.68^{b}$	0.05
Abnormalities (%)	19.33±4.63	19.27±1.30	15.50±1.97	18.00±4.43	0.66

Values (mean ± SE) with different superscripts ($^{a, b}$) within the same row were significantly different at p<0.05.

Table 2 Effect of dog breeds on the chemical components of the semen

Parameter	Caucasian (n=3)	German Shepherd (n=37)	Malinois (n=5)	Rottweiler (n=8)	P value
Testosterone (ng/ml)	$3.42±0.81^{a}$	$0.39±0.08^{b}$	$2.41±0.22^{a}$	$0.53±0.02^{b}$	0.001
AST (U/ml)	34.82±2.49	38.17±1.97	37.91±4.02	35.86±2.26	0.92
ALT (U/ml)	$9.55±0.40^{b}$	$29.49±1.48^{a}$	$23.04±1.46^{a}$	$26.52±2.55^{a}$	0.001
ALP (U/L)	$24972±667^{ab}$	$14742±1912^{b}$	$13007±4373^{b}$	$28394±3486^{a}$	<0.05
TAC (mmol/L)	33.04±2.39	33.78±2.63	39.47±0.76	33.36±0.63	0.87

Values (mean ± SE) with different superscripts ($^{a, b}$) within the same row were significantly different.

4. DISCUSION

The optimal fertility of male is based on the production of semen with high number of motile, viable, morphologically normal spermatozoa. However, serval external and internal factors implicate with male reproductive function (Albrizio, 2013). In the current study we found that the breed factor markedly impacted the physical (pH and sperm cell concentration) and biochemical (testosterone, ALT and ALP) characteristics of semen in dogs.

Regarding the seminogram of dogs, the current study verified a marked difference between the semen from dog breeds in terms of pH value of semen and sperm cell concentration, and absence of statistical variations between breeds in terms of spermatozoa motility, viability and abnormalities rate. Physiologically, high or low pH of semen is related to the shared part by prostatic fluid in seminal plasma as it was mentioned by Kurien (2012). Zhou et al. (2015) reported that the pH can influence the metabolic rate and the motility of sperm, and consequently alters the vitality of sperm and fertility., tended to vary between dog breeds, where the highest values were recorded in Malinois breeds, while the lowest was recorded in the Rottweiler breeds. The differences between breeds in sperm cell concentration found in the current study perhaps reflect the difference in testicular size. These findings are in accordance with Olar (1983) and Lopate (2016), who showed that the semen concentration and daily sperm output are directly related to testicular size volume. Peña Martinez (2004) reported that the total number of spermatozoa in the ejaculate might increase in association with the presence of sufficient sexual stimulation or teaser bitch but decrease in the presence of stress from pain or other environmental factors. There absence of significant differences in volume between breeds in our study possibly because all animals were sexually active, and they were used for breeding purposes. The invariance of spermatozoa motility rate between dog breeds perhaps due to the high impact of other factors, being stronger than breed factor, such as dog age or number of sperm collections (Rizzoto, 2016).

Seminal plasma, a complex mixture of secretions originates from the testis, epididymis and accessory sex glands, modulates the fertilizing ability of spermatozoa (Russell et al., 1984; Strzeżek et al., 2005). In dogs, most of the seminal plasma components, which are implicated in the sperm function, originate in the prostatic secretion (Cheema et al., 2011). These components may break down at variable rates after semen collection due to metabolism by spermatozoa and enzyme degradation, and may vary with interval from previous ejaculation, degree of sexual excitement, and health status of accessory sex glands (Olar et al., 1983).

Regarding the influence of breed on chemical constituents of seminal plasma, the present findings assured that testosterone, ALT and ALP significantly affected by breed, while the variation in AST and TAC between dogs were not statistically verified. Former studies mentioned that the level of testosterone is affected by physiological conditions such as age and season (DePalatis, 1978; Creel et al., 1997; Monfort et al., 1997; Martins-Bessa et al., 2006) and pathological conditions such as testicular atrophy and oligospermia (Feldman and Nelson, 1987; Fontbonne, 2011). The increase in transaminase enzymes concentration in the extracellular fluid is associated with an increase in sperm membrane damage and leakage of enzymes from spermatozoa (Gündoğan, 2006). Transaminase activities (AST and ALT) in semen is considered a good indicator of sperm membrane stability and consequently semen quality (Corteel, 1980). Alkaline phosphatase is a useful indicator of the presence of the sperm-rich (2nd) fraction in the canine ejaculate (Kutzler et al, 2003). Low ALP indicates ductal blockage, incomplete ejaculation or bilateral obstruction of the epididymis or of vasa deferens (Cuasnicú et al., 2002; Romagnoli, 2002). High levels of ALP activity in seminal plasma throughout the year are indicate of normal epididymal function (Bartlett, 1962; Strzeżek et al., 2015). There are several factors could contribute in the variation of semen antioxidants, including nutrition, age, infection, hormones, individuality and even between ejaculates (Miller and Rice-Evans, 1997). There are no former studies clarified the level of TAC in dogs' semen. The absence of significant variation in TAC between dog breeds assured the equality of oxidative stress defense mechanisms in the examined breeds. These mechanisms responsible for preserving the male fertility and conservation of high semen quality in dog breeds. In human, the determination of seminal plasma TAC has proven to be relevant for fertility assessment because its decrease is associated with infertility and abnormal semen parameters (Eroglu et al., 2014; Khosravi et al., 2014; N'Guessan et al., 2016). Former studies in bulls (Saini et al., 2016) and stallions (Kandiel and El Khawagah, 2018) indicated the strong relation between seminal TAC and high male fertility. Seminal plasma TAC expresses the defensing mechanism of semen against oxidative stress (Kanďár et al., 2011), and its decrease could participate in the etiology of impaired sperm functions (Pahune et al., 2013) and subsequently infertility (Eroglu et al., 2014).

5. CONCULOSIONS

The physical and chemical properties of dog semen is markedly affected by breed. Although dogs of different breeds seem equally fertile under the present study conditions, variations between breed could exist due to the differences in testosterone-dependent libido intensity, sperm count per ejaculate and seminal plasma enzymatic activity.

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