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Alkaline phosphatase in seminal plasma of sperm-rich fraction of semen in fertile and subfertile dogs#

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Abstract

Twenty dogs presented for breeding soundness evaluation were selected for the present study. Based on previous six month breeding history and semen quality, these animals were grouped retrospectively into fertile (n=6) and subfertile (n=14) groups. Three semen collections were made from each of these dogs, two weeks apart. Semen evaluation parameters like volume, sperm concentration, progressive motility, viability, morphologically normal sperms, total sperm output and alkaline phosphatase (ALP) level in the sperm-rich fraction were compared between the two groups. The mean values for progressive motility, sperm concentration, total sperm output and viability were 84.17 ± 1.54 per cent, 418.33 ± 36.64 million/mL, 645.11±98.43 million and 84.92 ± 1.56 per cent, respectively, in the fertile group, while the values for these parameters were 45.71 ± 3.47 per cent, 112.07 ± 24.36 million/mL, 190.24±32.45 million and 49.54 ± 2.35 per cent, respectively in the subfertile group. The values were significantly higher in fertile group when compared to subfertile group. The mean ALP (IU/L) was recorded as 168121.67 ± 22443.25 and 49605.86 ± 12669.68 IU/L, respectively in fertile and subfertile dogs. On statistical analysis, a highly significant difference ($p<0.01$) was noticed between the groups. Though the concentration of ALP was more than 40,000 IU/L (below which is the proposed cut off value for incomplete ejaculation) in all the dogs under the present study, it was significantly higher in fertile dogs, suggesting a possible relation to fertility.

Keywords: *ALP, fertile, subfertile, dog*

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Alkaline phosphatases (ALP) are membrane-bound enzymes attached to cells by phosphatidyl inositol glycan anchors (Low and Saltiel, 1988). ALP catalyze the hydrolysis of monophosphate esters at an alkaline pH. The exact role of this enzyme in semen is not known, however, desphosphorylation reactions may be essential for normal male reproductive physiology (Bell and Lake, 1962). In dogs and rabbits, ALP is primarily secreted by epididymis (Muller, 1983; Frenette *et al.,* 1986; Buonaguidi *et al.,*1991) whereas in humans, ALP is secreted by the testis and prostate (Kavanagh and Bardsley, 1979; Lewis-Jones *et al.,*1992; Lewin *et al.,* 1993). ALP appears in very high concentrations (>10,000 IU/L) in canine epididymal fluid whereas, the prostate and testis contain very small amounts of ALP. Because of its high concentration relative to that found in serum and prostatic fluid, seminal ALP functions as a useful marker of the sperm-rich fraction of the ejaculate (Kutzler *et al.,* 2005). Unilateral or bilateral occlusion of the ductal network or the efferent epididymal deferent could lead to azoospermia and oligospermia even in dogs with normal gonadal function thereby resulting in subfertility or infertility (Meyers-Wallen, 1995). Estimation of ALP in seminal plasma of azoospermic dogs has been suggested as a diagnostic aid to determine tubular patency (Olson *et al.,*1992; Meyers-Wallen, 1995). The present study was designed with an attempt to differentiate fertile and subfertile dogs on the basis of the values of seminal ALP in sperm-rich fraction of semen.

Materials and methods

Twenty dogs aged between three to six years with body weight ranging from 15 to 60 kg, presented for breeding soundness evaluation (BSE) at the University Veterinary Hospitals, Mannuthy and Kokkalai were chosen for the study. Complete breeding history of the stud dog was collected with special emphasis on the previous six months. Semen was collected by digital manipulation (Simon, 1997). Three collection were made two weeks apart. Immediately after collection of the sperm-rich fraction of the semen, initial sperm motility was recorded. The sperm concentration was analysed using the Neubauer hemocytometer. Percentage of live sperm and sperm abnormalities were recorded by Eosin and Nigrosine staining technique.

These animals were allotted into two groups *viz*., Group I (fertile group) and Group II (subfertile group) based on the previous six months breeding history and semen quality. Breed-wise distribution of the stud dogs under the present study were as following; German Shepherd (n=8, 40%), Pug (n=5, 25%), Labrador (LB) (n=2, 10 %), Beagle (n=1, 5 %), French bull dog (n=1, 5 %), Rajapalyam (n=1, 5 %), Golden Retriever (n=1, 5 %) and St. Bernard (n=1, 5 %).

Semen from these dogs was collected by digital manipulation and volume of various fractions were recorded. The second fraction was centrifuged at 1000 × g for 15 min at room temperature and the seminal plasma was removed from the sperm pellet. The separated seminal plasma was further clarified by centrifuging at 10,000 × g for 10 min at room temperature (Strzezek *et al.,* 2015). The seminal plasma separated thus was used for estimation of ALP. It was analysed using a semi-automatic analyser (MasterT biochemistry analyser, Hospitex diagnostics, Italy) and recorded in international unit per litre (IU/L).

Results and discussion

In the present study, the mean volume of the first, second and third fractions of semen ejaculate in Groups I and II was recorded as 1.17 ± 0.07, 1.55 ± 0.13, 11.3 ± 1.22 and 1.56 ± 0.37, 1.41 ± 0.35 and 8.46 ± 0.33 mL, respectively. No significant difference ($p>0.05$) was observed in the volume of the various semen fractions between the groups (Table 1). Our observation was in accordance with those of Kustritz (2007) who reported that volume of sperm-rich fraction ranged between 0.5 to 2.0 mL. The author had further opined that although total sperm output is considered as an indicator of semen quality, the volume may not directly represent the same. However, the volume is helpful in estimating total spermatozoa output per ejaculate which of course has a binding to semen quality.

The volume of prostatic fluid or third

fraction of semen in the present study was in agreement with the findings of Tesi *et al.* (2018) and Blendinger (2007) who had reported variations in the volume of prostatic fluid collected and that it depended on how much quantity was collected (how long the manual collection procedure lasted) and the size of the dog. These authors also observed that the volume of prostatic fluid ranged between 2 to 20 mL (average 5 mL).

The mean progressive motility of sperm in Groups I (84.17 ± 1.54 %) was found to be significantly higher ($p<0.01$) than those in Group II (45.71 ± 3.47 %, Table 2). The total motile sperm in normal canine ejaculates is between 70 and 90 per cent (Johnston *et al.,* 2001; Iguer-Ouada and Verstegen, 2001), although it may be lower after prolonged periods of sexual rest. It has been proposed that fertile dogs should have at least 70 per cent of total sperm motility (Larsen 1980).

The mean sperm concentration of second fraction of semen ejaculates in Groups I and II showed a highly significant difference (418.33 ± 36.64 and 112.07 ± 24.36 million/mL, $p<0.01$, Table 2). The mean total sperm output of second fraction of semen ejaculates, calculated on the basis of volume of the sperm-rich fraction, in Groups I and II was 645.11±98.43 and 190.24±32.45 million, respectively. On statistical analysis, a highly significant difference ($p<0.01$) was noticed between the groups (Table 2).

The total sperm count in the present study is in accordance with Johnston (1991) who reported that total sperm count in normal ejaculates may range from 300 to 2000 million. Freshman (2002) reported that average sized dogs should produce at least 250 to 300 million sperm/ ejaculate. Although 12 among 14 dogs in Group II were medium to large sized dogs, the total sperm output was significantly lower than these proposed average values. Martinez (2004) opined that such lower sperm concentration requires further investigation to differentiate incomplete ejaculation or azoospermia due to testicle related issues by estimating ALP.

Table 1. Comparison of volume of various fractions of semen in fertile and subfertile groups (in mL)

Parameter	Fertile group (n=6)	Subfertile group (n=14)	t-value	p-value
F1	1.17 ± 0.07^{b}	1.56 ± 0.37^{b}	0.236^{ns}	0.816
F2	1.55 ± 0.13^{b}	1.41 ± 0.35^{b}	0.572^{ns}	0.574
F3	11.3 ± 1.22^{a}	8.46 ± 0.33^{a}	1.410^{ns}	0.175
F-value	75.363**	215.275**		
P-value	<0.001	<0.001		

*** Significant at 0.01 level (p<0.01)*

Table 2. Comparison of semen parameters between fertile and subfertile groups

Parameter	Fertile group (n=6)	Subfertile group (n=14)	t-value	p-value
Progressive motility (%)	84.17 ± 1.54	45.71 ± 3.47	10.13**	<0.001
Concentration (million/mL)	418.33 ± 36.64	112.07 ± 24.36	6.915**	< 0.001
Total Sperm Output (million)	645.11±98.43	190.24±32.45	7.133**	< 0.001
Live (%)	84.92 ± 1.56	49.54 ± 2.35	12.562**	< 0.001
Abnormality (%)	6.50 ± 0.99	11.50 ± 1.56	2.003	0.060

*** Significant at 0.01 level (P<0.01); ns non-significant (P>0.05)*

Table 3. Comparison of ALP of sperm-rich fraction between fertile and subfertile group

Parameter	Fertile group (n=6)	Subfertile group (n=14)	t-value	P-value
ALP (IU/L)	168121.67 ± 22443.25	49605.86 ± 12669.68	4.895**	< 0.001

*** Significant at 0.01 level (P<0.01); ns- non-significant (P>0.05)*

The mean total viable sperm in semen ejaculates in Groups I and II was recorded to be 84.92 ± 1.56 and 49.54 ± 2.35 per cent, respectively, which differed significantly between groups ($p<0.01$, Table 2). The mean percentage of total sperm abnormality of semen ejaculates was 6.50 ± 0.99 and 11.50 ± 1.56 per cent, respectively in in Groups I and II with no significance of difference between groups. Normal canine ejaculates should have at least 80 per cent morphologically normal and viable spermatozoa (Johnston *et al.,* 2001). When the proportion of morphologically normal spermatozoa was below 60 per cent, fertility was found to be adversely affected (Oettlé, 1993). Thus, it is evident that dogs in Group I were fertile, while those in Group II were subfertile.

Tesi *et al.* (2018) reported that progressive motility, total spermatozoa output and the percentage of morphologically normal viable spermatozoa in the semen used for artificial inseminations (AI) resulting in a pregnancy were 83.9 per cent, 627.6 $\times 10^6$ and 64.9 per cent, respectively. In case of unsuccessful AIs, these values were significantly lower and were respectively, 66.5 per cent, 389.4 $\times 10^6$ and 42 per cent, respectively ($p<0.05$). In the present study, semen parameters like sperm concentration, total sperm output, progressive motility and live sperm percentage were significantly higher in Group I than Group II.

The mean ALP (IU/L) in Groups I and II was recorded as 168121.67 ± 22443.25 and 49605.86 ± 12669.68, respectively. On statistical analysis, a highly significant difference ($p<0.01$) was noticed between the groups (Table 3).

ALP is an enzyme with dephosphorylation function present in various organs like bone, liver and intestinal tissue. Mann (1964) opined that ALP of semen had specific role in sperm glycolytic pathway and fructose formation which facilitated in providing a source of energy to spermatozoa. Kutzler (2005) reported that although the biochemical role of ALP in the seminal plasma is not fully clear, in alkaline pH it could have a role in the glycolytic pathway by hydrolysis of monophosphate esters such as fructose 1-phosphate, fructose 6-phosphate and fructose 1,6-diphosphate.

Frenette *et al.* (1986) observed that in seminal plasma of canine ALP was mainly produced by epididymis and not in the prostate. A differential diagnosis of incomplete ejaculation and azoospermia in dogs is thus possible by estimating the ALP in the canine seminal plasma wherein a reduced concentration (< 5000 IU/L) of ALP in the seminal plasma suggested bilateral occlusion of ductal network (epididymis or vas deferens) (Gobello *et al.,* 2002). Kutzler (2005) performed immuno-histochemistry in canine testis and epididymis and identified the activity of seminal ALP in the epithelial cells of the head, body and tail of the epididymis as well as in the seminiferous tubules of the testis.

In the present study, semen parameters like progressive motility, sperm concentration, total sperm output and live sperm percentage were significantly more in the Group I than Group II. Sperm rich fraction was used exclusively for estimation of the ALP. Though the concentration of ALP was more than 40,000 IU/L in all the dogs under the present study (indicating a complete ejaculation in both the groups), it was significantly higher in fertile dogs, suggesting a possible relation to fertility. Johnston *et al.* (2001) reported that ALP should be greater than 5,000 IU/L in seminal plasma and further observed that ALP in dog with gonadal causes like azoospermia ranged from 5,500 to 81,600 U/L. Bucci *et al.* (2014) opined that the ALP function is still not clear, though it may serve as a decapacitating factor. No studies could be traced out, which had compared ALP between fertile and subfertile dogs.

Conclusion

Significantly higher ALP values in sperm-rich fraction in fertile dogs when compared to subfertile dogs could potentially be of use to serve as a marker of fertility in stud dogs. Although the concentration of ALP was more than the proposed cut off value for incomplete ejaculation in all the dogs in the present study, it however suggested a possible relation to fertility. Further studies involving greater number of animals and specific and

critical analysis regarding the role of ALP on fertility in stud dogs would be required before confirming its practical utility.

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Conflict of interest

The authors declare that they have no conflict of interest.

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