International Journal of Veterinary Sciences and Animal Husbandry

**ISSN:** 2456-2912
VET 2024; 9(2): 885-888

www.veterinarypaper.com
Received: 01-12-2023
Accepted: 06-02-2024

**G Shalini**
Ph.D., Scholar, Department of Animal Reproduction, Gynaecology and Obstetrics, CVAS, Mannuthy, Rajiv Gandhi Institute of Veterinary Education and Research, Puducherry, India

**D Antoine**
Professor and Head, Department of Veterinary Gynaecology and Obstetrics, (RIVER), Rajiv Gandhi Institute of Veterinary Education and Research– Puducherry, India

**S Kantharaj**
Professor and Head, Department of Veterinary Gynaecology and Obstetrics, (RIVER), Rajiv Gandhi Institute of Veterinary Education and Research, Puducherry, India

**K Murugavel**
Professor, Department of Veterinary Gynaecology and Obstetrics, (RIVER), Rajiv Gandhi Institute of Veterinary Education and Research, Puducherry, India

**Corresponding Author:**
**G Shalini**
Ph.D., Scholar, Department of Animal Reproduction, Gynaecology and Obstetrics, CVAS, Mannuthy, Rajiv Gandhi Institute of Veterinary Education and Research, Puducherry, India

# Biochemical characterization of canine (*Canis familiaris*) ejaculate

**G Shalini, D Antoine, S Kantharaj and K Murugavel**

**Abstract**
Biochemical evaluation of seminal plasma is important aspect of semen evaluation. Spermatozoa's capacity to fertilize is regulated by seminal plasma, a complex mixture of secretions derived from the testis, epididymis, and accessory sex glands. Seminal plasma proteins support capacitation, spermatozoa adhering to the oocyte's zona pellucida and spermatozoa survival and viability in the female reproductive system. The canine seminal plasma proteins also function as signaling molecules for the female immune System, influencing the female genital tract's ability to reject or tolerate spermatozoa. A total of 22 dogs aged between 2-6 years were selected for semen collection and evaluate the biochemical parameters of canine ejaculate. Semen was collected by digital manipulation at weekly interval for six consecutive weeks. A total of 115 ejaculates from eight different breeds of dogs. The colorimetric method was used to estimate the total protein, albumin, globulin, ALP, and ACP. The biochemical attributes of semen for fertile and infertile dogs were characterized and compared. The ALP and ACP level in the seminal plasma were significantly low in infertile dogs compared to fertile dogs. The observed notable variations in ALP and ACP levels among the groups may be attributed to dysfunction in the prostate gland and epididymis, as these biochemical markers indicate the functional integrity of the gland and epididymis, respectively. The percentage of fertile and infertile dogs in the present study was 59% and 41% respectively.

**Keywords:** Alkaline phosphatase (ALP), acid phosphatase (ACP), total protein, albumin, dogs

## 1. Introduction
The most popular technique for collecting semen from dogs is digital manipulation (Farstad, 2010) [4]. There are three components to the canine ejaculate. The first portion, known as pre-sperm, is modest in volume, mostly composed of secretions from the prostate gland and contains few to no sperm. Prostatic fluid is scarce in the second (sperm-rich) portion, which is primarily of epididymal origin. Prostatic fluid makes up the majority of the third (post-sperm) fraction, which upon ejaculation gives the biggest volume to the seminal plasma (Johnston *et al*., 2001) [6]. A dog's ejaculate can range in volume from 1-30 ml (England and Heimendhal, 2010; Ettinger and Feldman, 2010) [2, 3]. An additional crucial component of evaluating semen is the biochemical analysis of seminal plasma. Semen volume is greatly increased by seminal plasma. Prostatic secretion is the source of the majority of the seminal plasma components that are linked to sperm activity (Cheema *et al*., 2011) [1]. The seminal plasma proteins facilitate fertilization and sperm contact with the oviduct epithelium. The primary sources of the ground breaking plasma enzyme alkaline phosphatase are the testicles and the epididymis. An alkaline phosphatase level is frequently used to diagnose inadequate ejaculation, azoospermia and oligozoospermia (Schafer-Somi *et al*., 2013) [13]. The seminal plasma exhibited 100 times higher levels of acid and alkaline phosphatase activity in comparison to the sperm fractions. Prostate activity most likely influences phosphotase concentrations. For this reason, measuring acid phosphatase in dog semen could be a helpful indicator of prostate function. Seminal plasma alkaline phosphatase activity (AP) can be used to diagnose epididymis lesions that cause inadequate ejaculation and azoospermia in dogs. AP aids in processes crucial to fertilization by catalyzing the entry of phosphate groups into spermatozoa (Olson, 1991) [11]. Reduced concentration of alkaline phosphatase in the seminal plasma suggests bilateral obstruction of the vas deferens or epididymis (Gobello *et al*., 2002) [5].

Another seminal plasma enzyme, acid phosphatase is a biochemical marker, primarily related to the metabolic functions of spermatozoa and prostate diseases in dogs.

## 2. Materials and Methods

Puducherry dog breeders and pet owners look after 22 various types of seemingly healthy dogs that range in age from 2 to 6 years. Every dog appeared to be in good health, and they were provided with commercial dry dog food twice a day along with unlimited access to water. Dog semen was extracted using digital processing and stimulation of the Bulbus Glandis (Linde-Forsbrg, 1991) [8]. A sterile graduated measuring plastic tube was used to collect the entire ejaculate, including the pre-sperm, sperm rich, and post-sperm. The collected semen was transported right away in a shipping container to the laboratory. Enough care was given to guarantee that the semen was not subjected to unfavourable circumstances both during and after collection.

### 2.1 Canine seminal plasma's biochemical composition

The collected semen was centrifuged for 15 minutes at room temperature at 1000x g. After being separated, the seminal plasma was stored at -80°C after being centrifuged at 10,000x g for 10 min at ambient temperature (Strzezek *et al*., 2015) [14]. The biochemical components were estimated using the seminal plasma that had been isolated.

#### 2.1.1 Estimation of total Protein, albumin and globulin

A colorimetric approach was used to measure the amount of albumin and total protein in the seminal plasma. The $A_{536}$nm for total protein and $A_{630}$nm for total albumin were used in the Beacon diagnostic kits to estimate the concentration of total protein and albumin expressed in mg/ml. The code numbers for the total protein and albumin are Z-17, Z-17, and Z-07, Z-07A, respectively. Based on the distinction between seminal plasma albumin and total protein, the concentration of total globulin (G) was computed.

#### 2.1.2 Estimation of Alkaline and Acid phosphatase

The diagnostic kits were utilized to measure the quantity of alkaline phosphatase and acid phosphatase enzymes using the colorimetric technique $A_{405}$nm.

## 3. Results and Discussion

A total of 22 dogs aged between 2-6 years from eight different breeds maintained for breeding purpose were selected for breeding soundness examination. First semen collection was used as test trial and not included for the analysis of data. Subsequently the semen was collected at weekly interval for six consecutive weeks.

### 3.1 Biochemical components of fertile dogs' seminal plasma

#### 3.1.1 Total protein

The total protein level in seminal plasma was between 1.21 ± 0.18 to 2.98 ± 0.23 g/dl for in fertile dogs (Table 4). The total protein level was 2.98 ± 0.23 g/dl (2.1-4 g/dl) for GSD, 2.48 ± 0.46 g/dl (0.9-3.7 g/dl) for Pug, 1.86 ± 0.28 g/dl (0.7-2.5 g/dl) for Rottweiler, 1.65 ± 0.28 g/dl (1.2-3.5 g/dl) for Dachshund, 1.61 ± 0.59 g/dl (0.6-4.5 g/dl) for Cocker spaniel and 1.21 ± 0.18 g/dl (0.8-1.6 g/dl) for Labrador. The total protein level in the seminal plasma was high in GSD and it was followed by pug, Rottweiler, dachshund, cocker spaniel and Labrador dogs (Table 1). The concentration of total proteins in the canine seminal plasma of mixed breeds (GSD and Rottweiler) was ranged between 1.88g/dl to 2.3g/dl (Motheo, 2014) [10].

**Table 1:** Seminal plasma biochemical parameters in different breeds of fertile dogs

| SI. No | Parameters | No. of ejaculate in dogs | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | GSD n=36 | Labrador n=12 | Rottweiler n=6 | Pug n=6 | Dachshund n=12 | Cocker Spaniel n=6 | Mean ± SE n=78 |
| 1 | Total Protein (g/dl) | 2.98 ± 0.23$^{a}$ | 1.21 ± 0.18$^{b}$ | 1.86 ± 0.28$^{ab}$ | 2.48 ± 0.46$^{ab}$ | 1.65 ± 0.28$^{bc}$ | 1.61 ± 0.59$^{ab}$ | 2.27 ± 0.15 |
| 2 | Albumin (g/dl) | 0.35 ± 0.04 | 0.23 ± 0.07 | 0.20 ± 0.03 | 0.28 ± 0.07 | 0.19 ± 0.02 | 0.17 ± 0.06 | 0.27 ± 0.02 |
| 3 | Globulin (g/dl) | 2.66 ± 0.22$^{a}$ | 0.98 ± 0.19$^{b}$ | 1.66 ± 0.29$^{ab}$ | 2.20 ± 0.50$^{ab}$ | 1.46 ± 0.28$^{bc}$ | 1.44 ± 0.55$^{ab}$ | 2.01 ± 0.14 |
| 4 | ALP (IU/L) | 7422 ± 520 | 7062 ± 1214 | 8767 ± 1045 | 5758 ± 275 | 6568 ± 561 | 6184 ± 359 | 7115 ± 330.5 |
| 5 | ACP (IU/L) | 1264 ± 33.0$^{a}$ | 1268 ± 36.60$^{ac}$ | 1367 ± 0.00$^{ad}$ | 1135 ± 89.82$^{ab}$ | 871 ± 84.9$^{b}$ | 1287 ± 46.7$^{ae}$ | 1204 ± 27.53 |

Mean value having different super script within same row differ significantly ($p$<0.05)

#### 3.1.2 Albumin

The albumin level in seminal plasma was between 0.17 ± 0.06 to 0.35 ± 0.04 g/dl fertile dogs (Table 4). The albumin level was 0.35 ± 0.04 g/dl (0.16-0.66 g/dl) for GSD, 0.28 ± 0.07 g/dl (0.1-0.5 g/dl) for Pug, 0.23 ± 0.07 g/dl (0.1-0.4 g/dl) for Labrador, 0.20 ± 0.03 g/dl (0.1-0.3 g/dl) for Rottweiler and 0.19 ± 0.02 g/dl (0.18-0.2 g/dl) for Dachshund. There was no significant difference between breeds in the level of albumin in the seminal plasma (Table 1). Rui xiang Feng *et al*. (2015) [12] reported 2.87±0.51 g/l of albumin level in the seminal plasma of infertile men.

#### 3.1.3 Globulin

The globulin level in seminal plasma was between 0.98 ± 0.19 to 2.66 ± 0.22 g/dl in fertile dogs. The globulin level 2.66 ± 0.22 g/dl (1.9-3.4 g/dl) for GSD, 2.20 ± 0.50 g/dl (0.5-3.6 g/dl) for Pug, 1.66 ± 0.29 g/dl (0.4-2.3 g/dl) for Rottweiler, 1.46 ± 0.28 g/dl (1.1-1.7g/dl) for Dachshund, 1.44 ± 0.55 g/dl (0.5-4.1 g/dl) for Cocker spaniel and 0.98 ± 0.19 g/dl (0.6-1.3 g/dl) for Labrador. The globulin level was significantly high in the seminal plasma of GSD and it was followed by pug, Rottweiler, dachshund, cocker spaniel and Labrador (Table 1). Khan *et al*. (2015) [7] reported 1.08±0.23 to 1.18±0.31 of globulin level in bull semen.

### 3.2 Enzyme constituents in seminal plasma of fertile dogs

#### 3.2.1 Alkaline phosphatase (ALP)

The alkaline phosphatase level in seminal plasma was between 5758 ± 275 to 8767 ± 1045 IU/L in fertile dogs. The ALP level was 8767 ± 1045 IU/L (5410-11523 IU/L) for Rottweiler, 7422 ± 520 IU/L (6361-11778 IU/L) for GSD, 7062 ± 1214 IU/L (6405-7720 IU/L) for Labrador, 6568 ± 561 IU/L (5309-7836 IU/L) for Dachshund, 6184 ± 359 IU/L (5124-7372 IU/L) for Cocker spaniel and 5758 ± 275 IU/L (4788-6529 IU/L) for Pug. The alkaline phosphatase level in seminal plasma was high for Rottweiler followed by GSD, Labrador, dachshund, cocker spaniel and pug.

### 3.2.2 Acid phosphatase (ACP)

The acid phosphatase level in seminal plasma ranged between 871.4 ± 84.9 to 1367 ± 0.00 IU/L in fertile dogs (Table 4). The ACP level was 1367 ± 0.00 IU/L for Rottweiler, 1287 ± 46.7 IU/L for Cocker spaniel, 1268 ± 36.60 IU/L for Labrador, 1264 ± 33.0 IU/L for GSD, 1135 ± 89.82 IU/L for Pug and 871.4 ± 84.9 IU/L for dachshund. The level of acid phosphatase in the seminal plasma was high for Rottweiler followed by cocker spaniel, Labrador, GSD, pug and dachshund. The level of ACP in the seminal plasma of dachshund was significantly low ($p$<0.05) compared to other breeds in dog. According to Miteva *et al*. (2010) [9], seminal plasma acid phosphatase levels were 1031.9±536.5 IU/l in humans, 11.5±3.6 IU/l in jacks, and 18.7±1.7 IU/l in rams.

**3.3 Biochemical seminal attributes between fertile and infertile dogs:** The Total protein, albumin and globulin level in the seminal plasma of infertile dogs were 2.47 ± 0.29 g/dl, 0.28 ± 0.05 g/dl and 1.96 ± 0.24 g/dl, respectively and there was no significant difference between groups (Table 2). Infertile dogs had seminal plasma levels of alkaline and acid phosphatase of 1904 ± 329 IU/L and 925 ± 79.0 IU/L, respectively. Compared to fertile dogs, infertile dogs had much lower levels of ALP and ACP in their seminal plasma (Table 2). In the current study, the percentage of dogs that were infertile and fertile among those who had their semen evaluated biochemically was 41% and 59%, respectively. Infertile dogs had a 32 percent Aspermia incidence rate; Fig. 2 shows the incidence of Aspermia in several breeds. This study observed and found a number of poor semen quality indicators in infertile dogs, including poor membrane integrity of spermatozoa, as well as Aspermia, Azoospermia, Oligo, and hypokinozoospermia. The ALP level in the seminal plasma, which is a good indicator of the epididymal secretary functions, was significantly lower in the semen of all infertile dogs than in the semen of fertile dogs (Fig. 1).

**Table 2:** Biochemical seminal attributes between fertile and infertile dogs

| SI. No | Parameters | Fertile dogs (n=54) | Infertile dogs (n=31) | P value |
|---|---|---|---|---|
| | **Biochemical constituents** | | | |
| 1 | Total protein (g/dl) | 2.53 ± 0.19 | 2.47 ± 0.29 | 0.8627$^{ns}$ |
| 2 | Albumin (g/dl) | 0.31 ± 0.03 | 0.28 ± 0.05 | 0.6416$^{ns}$ |
| 3 | Globulin (g/dl) | 2.23 ± 0.18 | 1.96 ± 0.24 | 0.3754$^{ns}$ |
| 4 | Alkaline phosphatase (IU/L) | 7157 ± 439 | 1904 ± 329 | 0.0001*** |
| 5 | Acid phosphatase (IU/L) | 1250 ± 25.6 | 925 ± 79.0 | 0.0001*** |

Results are in Mean ± SE; ** Significant ($p$≤0.01); * Significant ($p$≤0.05); $^{ns}$: Non-significant

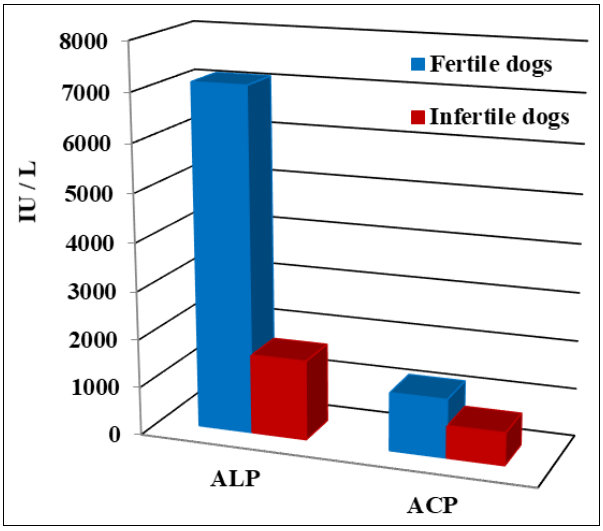


**Fig 1:** Alkaline and Acid phosphatase level in the seminal plasma of fertile and infertile dogs

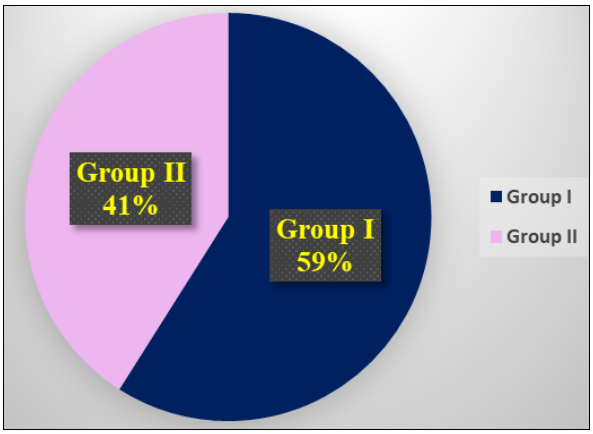


**Fig 2:** Percentage of occurrence of fertile (Group I) and infertile dogs (Group II)

## 4. Conclusion

Among the 22 dogs subjected to biochemical constituent of semen ejaculates, there are 9 dogs (41%) were infertile and 13 dogs (59%) were fertile. Among the infertile dogs testicular degeneration, aspermia, oligozoospermia, hypokinozoospermia, poor membrane integrity, poor secretary functions of the epididymis and prostate gland were commonly observed

## 5. References

1. Cheema RS, Bhakri G, Gandotra VK, Dhanju CK. Characterization of mongrel dog seminal plasma proteins and their correlation with semen characteristics. J Reprod Stem Cell Biotechnology. 2011;2:55-63.
2. England G, Heimendahl A. BSAVA Manual of Canine and Feline Reproduction and Neonatology In: Book BSAVA Manual of Canine and Feline Reproduction and Neonatology Editor (2nd Edn). 2010;18-22, 70-78.
3. Ettinger SJ, Feldman EC. Textbook of Veterinary Internal Medicine: Diseases of the dog and the cat (7th Edn) St. Louis MO, Elsevier Saunders; c2010.
4. Farstad WK. Artificial Insemination in dogs. In: BSAVA manual of Canine and Feline Reproduction and Neonatology, 2nd ed. England G. and Von Heimedable A (Eds) British Small Animal Veterinary Association (BSAVA), Gloucester, UK; c2010.
5. Gobello C, Castex G, Corrada Y. Serum and seminal markers in the diagnosis of disorders of the genital tract of the dog. Theriogenology. 2002;57:1285-1291.
6. Johnston SD, Root Kustritz MV, Olson PN. Clinical approach to infertility in the male dog. Canine and Feline Theriogenology. Philadelphia, WB Saunders; c2001. p. 370 -387.
7. Khan A, Yasinzai MM, Kakar MA. Biochemical analysis of bovine (Bos. Indicus) seminal plasma. International

journal of Advanced Biological and Biomedical Research. 2015;3(4):361-369.
8. Linde-Forsberg C. Achieving Canine Pregnancy using frozen or chilled extended semen. Veterinary Clinics of North Am Small Anim Pract. 1991;21:467-485.
9. Miteva R, Zapryanov D, Fasulkov, Yotov S, Mircheva, T. Investigations on acid phosphatase activity in the seminal plasma of humans and animals. Trakia Journal of Sciences. 2010;8:20-23.
10. Motheo TF. Semen parameters and seminal plasma protein and biochemical profiles of dogs with benign prostatic hyperplasia after botulinum toxin type A intraprostatic injection. Ciência Rural. 2014;44(6):1113-1118.
11. Olson PN. Clinical approach for evaluating dogs with azoospermia or aspermia. Vet Clin North Am. 1991;21:591-608.
12. Rui-Xiang Feng, Jin-Chun LU, Hong-Ye Zhang, Nian-Qing Lu. A Pilot Comparative Study of 26 Biochemical Markers in Seminal Plasma and Serum in Infertile Men. BioMed Research International Volume 2015. Article ID 805328, 7 pages.
13. Schafer-Somi S, Frohlich T, Schwendenwein, I. Measurement of alkaline phosphatase in canine seminal plasma. Reprod Domest Anim. 2013;48:10-12.
14. Strzeżek R, Koziorowska-Gilun M, Kiełczewski K, Kordan W. Effect of dialysis of dog semen on sperm characteristics and some biochemical components of seminal plasma. J Vet Sci. 2015;18:447-448.