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Collection and evaluation of dog semen: A mini review

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Abstract
The sophisticated and highly specialized spermatozoa is designed to carry the male genome to the female genital canal and fertilize the oocyte. Different tests are required since a simple test cannot provide sufficient information on a sample's fertilizing capacity due to its complexity. Semen was collected in dogs using digital manipulation technique. Macroscopic (Color, homogeneity, total ejaculate volume, pH) and microscopic (Mass motility, individual motility, sperm concentration, percentage of viable sperm, abnormal sperm and membrane integrity) sperm Laboratory tests were performed on all fresh sperm.

Keywords: Digital manipulation, macroscopic evaluation tests, macroscopic evaluation tests

Introduction
Predicting a semen sample's ability to fertilise is the ultimate goal of semen evaluation. In general, males that are fertile create a lot of progressively motile, viable, and anatomically normal spermatozoa in their semen. Semen analysis can be used to determine a male dog's fertility. Light microscopy has until now been the primary method used to assess the concentration, motility, and morphology of semen. Concentration is usually measured with a Neubauer counting chamber. Motility was assessed as needed on preheated glass, while morphological defects were assessed using multiple staining methods (Johnston, 1992; Iguer-ouada & Verstegen, 2001) [34, 12]. However, sperm samples such as motility, sperm concentration, percentage of viable sperm and sperm morphology are not sufficient to predict fertility and detect infertile dogs (Gadea *et al*., 2004) [7]. Spermatozoa analysis, sperm infiltration, timely and appropriate capacitation, etc. supported by experiments designed to assess the ability to adapt to changes. The sperm should have a well-functioning acrosome and a plasma membrane that can be detected using the hypotonic swelling test (HOST) (Kumi-Diaka, 1993) [16], phase-positive microscopy (gelatin digestion test) (Arabi, 2006) [1] or Light. Microscopy of sperm staining (Oettlé, 1986) [19].

Semen collection techniques in dogs
Anatomy of dog's penis
The bulbus glandis, often known as a bulb or knot in canines, is an erectile tissue structure, according to Miller's Anatomy of the Dog, Fourth Edition (2013). Before ejaculation occurs during mating, the tissues enlarge, locking (tying) the male's penis inside the female. Circular muscles located just inside the female vagina-known as "the knot"-complete the locking process by tightening, preventing the male from withdrawing. The erratic contraction of the circular muscles also promotes sperm ejaculation, which is followed by prostatic fluid, as well as prolonging the penis's enlargement and, as a result, the knot. The erratic contraction of the circular muscles also promotes sperm ejaculation, which is followed by prostatic fluid, as well as prolonging the penis's enlargement and, as a result, the knot.

Spermatogenic cycle of dog
Spermatogenesis is a finely controlled process of germ cell multiplication and differentiation that results in spermatozoa production in the seminiferous tubules (ST). Spermatocytogenesis is the proliferative stage in which primitive germ cells (Spermatogonia) are multiplied by a series of mitotic divisions to produce the primary spermatocytes. The duration to complete spermatogenesis is of 61 days with one spermatogenic cycle of 14 days approx. and total 4.3 number of cycles are required to complete spermatogenesis. Spermatocytogenesis consists of spermatocytogenesis formation of spermatids from spermatogonia and spermiogenesis, differentiation of spermatids in to spermatozoa.

The least differentiated germ cell in the testis is the spermatogonium.

Digital manipulation technique in dogs for semen collection

Lambert and Mackenzie (1940) [17] were the first to advise using digital manipulation techniques on dogs to gather sperm. It was suggested that the bulbus glandis be rhythmically crushed while applying moderate pressure behind it. The dog was less distracted by background noises and adapted more easily to semen collecting by digital manipulation. Various workers (Rota *et al*., 2006a; Sridevi, 2007; Pramod, 2009; Kurien *et al*. 2012) [5, 36, 37, 38]; Gharajelar *et al.* (2016) [8] Zorinkimi *et al.* (2017) [33] Ray *et al.* (2019) [24] Arunmozhi *et al.* (2021) [2]. Srinivas Rao *et al*. (2022) [31] in their study used the method of digital manipulation in semen collection in dogs.

Fig 1: Semen collection (Manual massage)

Fig 2: Semen collection (Rotation of penis and collection of sperm rich fraction)

Some parameters related to reproductive behaviour were also recorded during semen collection in dogs, such as reaction time, ejaculation time, and other actions like pelvic thrusting or jumping on the dog's controller, rate of breathing during the course, and response to positioning of erect penis reverse during the phase of ejaculation of III fraction, just as tie or natural locking stance during coitus. A stopwatch was used to reaction and ejaculation times.

Reaction time

It can be described as the time length from the start of digital manipulation on the bulbous glandis of the penis to the start of dribbling of the I portion of ejaculation by artificial means of semen collection.

Ejaculation time

It is the time duration that begins with the dribbling of the first drop of the I fraction of ejaculation and ends with the last drop of the III fraction of ejaculation.

Semen evaluation

Macroscopic Evaluation of Semen

Volume

According to Canine and Feline Theriogenology (2001) [4] Dog Semen ejaculate consists of three fractions, pre-sperm, sperm rich, post sperm fractions. First or pre-sperm fraction, probably originates in the prostate. It is usually small in volume, although occasional dogs may ejaculate as much as 5 ml or more; the pre-sperm fraction is clear and a cellular. This fraction usually is ejaculated during rapid thrusting by the male. Second or sperm-rich fraction originates in the tail of the epididymis where spermatozoa are stored. It is variable in volume (typically 1 to 4 ml) and opalescent in color. The sperm-rich fraction may be ejaculated either during vigorous thrusting or immediately thereafter. The third or prostatic, fraction of the semen usually is a large volume of clear fluid in the normal dog. It is variable in volume (typically 1 to 80 ml) and clear in color. Zorinkimi *et al*. (2017) [33] study was to characterize mongrel dog semen of Mizoram. Specific semen characteristics obtained (mean±S.E.) were ejaculate volume 2.95±0.41ml. Shalini and Antoine (2018) [30] studied on six German shepherd dogs. The volume of semen was 8.68±0.47 ml. Ray *et al*. (2019) [24] study were undertaken with six numbers of German Shepherd dogs, a total of 36 ejaculates were collected. The volume of semen was 5.516±0166 ml. Arunmozhi *et al*. (2021) [2] collected semen from six dogs of different breeds aged between 2 to 6 years. The mean volume of sperm rich fraction of was 2.54±0.22 ml.

Fig 3: Three fractions of dog semen1. Pre-sperm fraction 2. Sperm rich fraction 3. Post sperm fraction

Fig 4: Sperm rich fraction of dog semen

Colour and consistency

Zorinkimi *et al*. (2017) [33] study to characterize mongrel dog semen of Mizoram. Milky white coloured semen was observed. Shalini and Antoine (2018) [30] observed that colour of semen in German shepherd dogs was uniformly opalescent. Pignataro *et al*. (2020) [21] evaluated ejaculates was an opalescent white colour with a consistency ranging from aqueous to milky. Khye *et al*. (2021) [14] collected semen from four Doberman Pinschers dogs by massage method. They observed that colour and consistency of sperm rich fraction was Greyish-white and Watery, respectively.

pH

Zorinkimi *et al*. (2017) [33] studied on mongrel dog semen of Mizoram, pH of ejaculate found 6.59±0.76. Shalini and Antoine (2018) [30] studied on six German shepherd dogs, pH of ejaculate found 6.25±0.04. Ray *et al*. (2019) [24] study was undertaken with six numbers of German shepherd dogs, pH of ejaculate found 6.383±0.031. Pignataro *et al*. (2020) [21] reported that the mean pH values observed in these dogs were around 6.6±0.2.

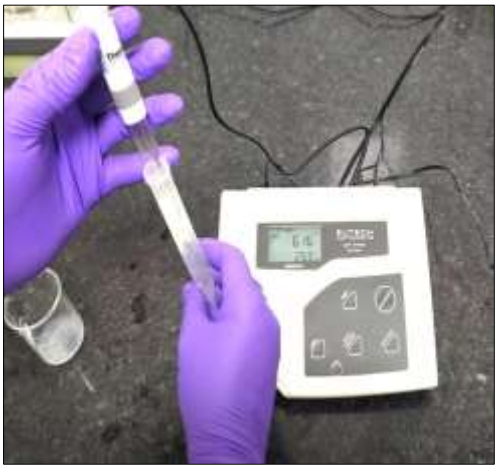

Fig 5: pH of dog semen

Evaluation of Semen

Mass Motility

Raut (2009) [39] observed that the average mass activity of sperm rich second fraction was 3.32±1.07 and 3.5±0.98 in Group I (Younger) and in Group II (Older) German shepherd dogs respectively. Shalini and Antoine (2018) [30] observed the mean mass motility of dog spermatozoa as 3.16±0.06.

Individual motility

Shalini and Antoine (2018) [30] studied Initial motility in German shepherd dog semen 83.3±0.79%. Ray *et al*. (2019) [24] conducted study on six German shepherd dogs, mean individual motility percentage were 84.166±1.990. Pignataro *et al*. (2020) [21] evaluated the Fresh semen had motility of 86.4±9.8%. Khye *et al*. (2021) [14] observed that the mean percentage of motile spermatozoa in the sperm-rich fractions was in the normal range, which was 86.67±2.50%.

Sperm Concentration

Shalini and Antoine (2018) [30] studied, the biophysical and biochemical attributes of semen for GSD was characterized and compared with standard reference value for fertile dog semen. The sperm concentration of GSD semen was 376±13.6 million/ml. Pignataro *et al*. (2020) [21] conducted experiment on six male German Shepherds and eighteen ejaculates were collected by manual masturbation. Immediately after the semen was collected sperm concentration were assessed. Which was 175±103.1 x 10^6sperm/ ml. Khye *et al*. (2021) [14] conducted the experiment on four Doberman Pinschers dogs. The mean spermatozoa concentration of the sperm-rich fractions was 155.83±65.61 million sperm/ml.

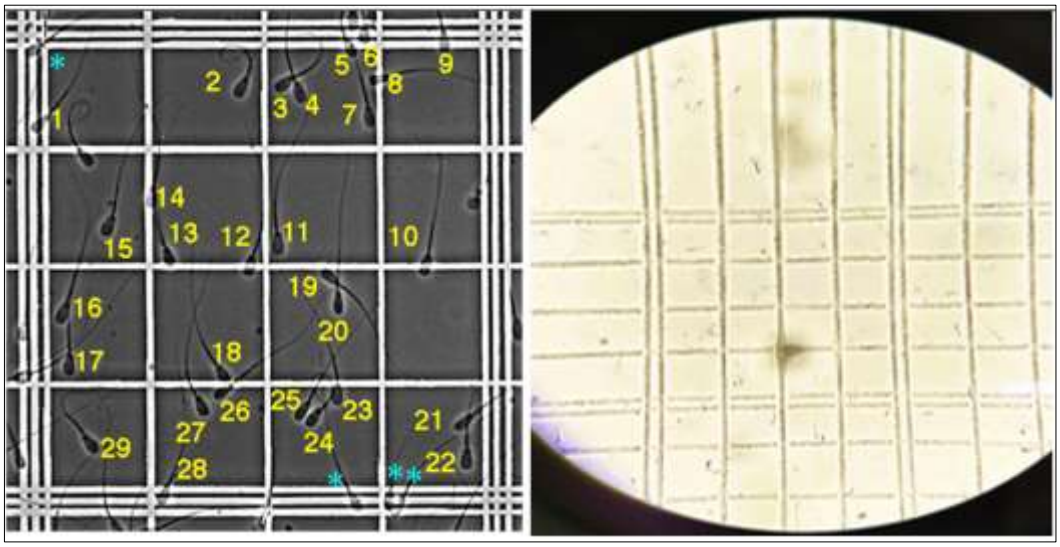

Fig 6: Number of sperm (million/ml) = Number of sperm counted in 5 chambers x 10 million

Live and dead count

Zorinkimi *et al*. (2017) [33] observed the overall mean percentage of live spermatozoa in mongrel dog semen was 87.84±1.8. Shalini and Antoine (2018) [30] were found that mean live spermatozoa count in German shepherd dogs was 86.3±0.78%. Ray *et al*. (2019) [24] evaluated semen sample of six German shepherd dogs. Live percentage of spermatozoa was 84.166±1.90%. Khye *et al*. (2021) [14] observed Viability (%) was 89.98±4.89 in sperm-rich fractions from Doberman Pinschersean.

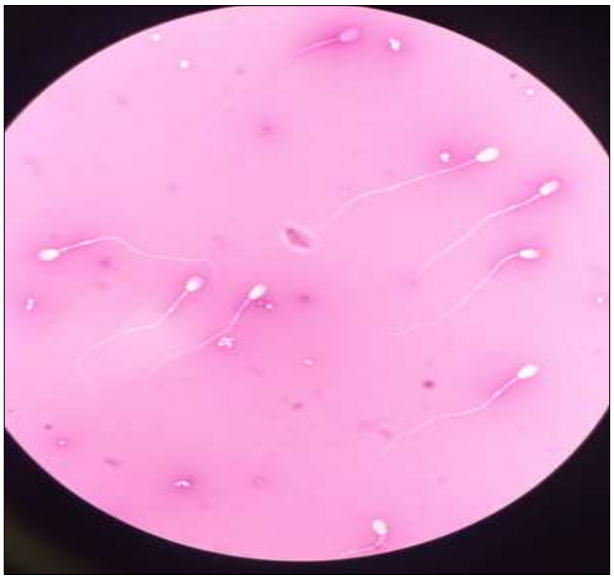

Fig 7: Dead sperms–Pink colour Live sperms – White colour

Morphological abnormalities of spermatozoa

Ray *et al*. (2019) [24] evaluated semen sample of six German shepherd dogs. Total sperm abnormality percentage was 14.830±2.386. Pignataro *et al*. (2020) [21] conducted experiment on six male German Shepherds and eighteen ejaculates were collected. They were found 68.1± 21.6% of the spermatozoa were considered normal, 10.0±6.3% had minor defects, and 21.7±18.2% had major defects.

Fig 8: Coiled tail

Hypo-Osmotic Swelling Test (HOST)

The major site of freezing injury is thought to be the plasma membrane, and the sort of damage depends on the freezing condition. When exposed to the hypo-osmotic solution during Hypo-Osmotic Swelling Test, spermatozoa with a biochemically active plasma membrane would expand and experience a rise in volume due to an intracellular water inflow, which is a marker of membrane integrity and typical functional activity of spermatozoa. The HOS test may involve the capacity to freeze canine semen using various cryoprotectants since fertilization will not take place if the sperm membrane is physically intact but biochemically inactive. It is thought to be a more reliable way to assess membrane integrity. With frozen-thawed spermatozoa, an aqueous solution of fructose and sodium citrate produced the best results (Rota *et al*., 2006a) [35]. Ray *et al*. (2019) [] study mean value of hypo-osmotic swelling test (HOST) were 80.166±2.522. Pignataro *et al*. (2020) [21] conducted experiment on six male German Shepherds. Mean value of membrane integrity was showed 70.4±27.9%. Arunmozhi *et al*. (2021) [2] evaluated mean percentage of spermatozoa showing plasma membrane integrity in fresh semen was 91.92±1.07.

Fig 9: HOST positive- Coiled tail

Conclusion

Digital manipulation was used to collect the semen from dogs in a sterile and hygienic manner, and at least one week apart. The average volume, colour, consistency and pH were 2.17±0.16, milky white, Thick, and 6.35±0.03 respectively. The Mass motility, Individual motility, live sperm percentage, percentage of morphologically abnormal spermatozoa and total sperm concentration were 3.91±0.15, 86.45±0.97%, 87.37±0.98%, 9.08±0.25% and 353.89±11.1 millions/ml respectively. The sperm having intact plasma membrane percentage judged by HOST were 89±0.94% with a range of 80 – 95% in the fresh dog semen.

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Conflict of Interests

There is no conflict of Interest.

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