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Short Communication

ARTIFICIAL INSEMINATION IN SHE DOGS WITH LIQUID SEMEN

MALATI NAIK*, SANTOSH SARANGAMATH[1], D. JAHANGIRBASHA[2] and D.S. SUPRITH
Department of Veterinary Gynaecology and Obstetrics, [1]Department of Veterinary Medicine,
[2]Department of Veterinary Surgery and Radiology, Veterinary College, Gadag–582 101, India
Karnataka Veterinary, Animal and Fisheries Sciences University, Bidar

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SUMMARY

Semen collection, semen evaluation and artificial insemination are essential skills for small animal practitioners. Semen collection was performed using digital manipulation technique among all the different breeds of dogs and several tests including macroscopic (semen volume, semen colour, hydrogen ion concentration) and microscopic tests (mass motility, progressive motility, sperm concentration, live and dead sperms by eosin nigrosin staining) were performed to estimate the fertility of the male. Even exfoliative vaginal cytology (EVC) was also performed to detect the proper phase of oestrous cycle. She dogs which had more than 90% cornification index, were selected for AI and evaluation of conception was done. Finally concluded that digital manipulation of semen collection is a cost-effective method and AI should be conducted for those bitches which had issues with natural mating.

Keywords: Artificial insemination, Canines, EVC, Semen collection, Semen evaluation

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Canine breeding is a rapidly growing industry and there is influx of exotic breeds of dogs into India for breeding purposes (Singh *et al.*, 2019). Among different assisted reproductive techniques, artificial insemination (AI) is one that involves collection of semen from a stud male and introducing it into genital passage of a female or female reproductive tract, so that fertilization can occur in the absence of natural mating (Mason, 2018). In animals and humans, AI is one of the earliest techniques employed for assisted reproduction. It took longer period to be implemented and standardised in pet dogs due to species specific particularities. Abbe Spallanzani performed first successful AI in dogs in 1780. By 1950's, the use of stored dog semen for AI was practiced. Later by 1990's, the technology of frozen semen was developed for breeding practice in dogs (Foote, 2002; England and Millar, 2008). Currently, there has been a huge development in reproductive biology and biotechnology. However, initial efforts to improve AI failed, mainly due to the two limitations i.e., unexplained reproductive physiology of dog and unresponsiveness of dog sperm to freezing as opined by Hermansson and Forsberg (2006). The most common reasons attributed for AI in dogs are that the male or female may be shy, inexperienced or too aggressive. There may be presence of obstructions in the genital passage such as vaginal band, small hard mass at vaginal floor. Other possible reasons may be that both male and female would have grown together and know each other to aid in controlling the risk of sexually transmitted diseases on either gender (Johnston *et al.*, 2001).

Case presentation and observation: Seven female dogs were presented to Veterinary Clinical Complex, Veterinary College, Gadag with a history of vaginal bleeding since six to ten days and unwillingness to mate naturally. The details are given in Table 1. Further, history revealed that natural mating was not possible in four out of seven dogs during previous two to three oestrous cycles. On clinical examination, all physiological parameters were found to be normal. It was observed that two females were too aggressive and three were inexperienced. In one female, the height was relatively shorter. On per-vaginal examination, it was observed that two female dogs had a small hard mass of 3 cm diameter which was not occupied fully on the vaginal floor and able to pass finger. Vaginal smear was collected by cotton swab technique and study of vaginal cells (EVC) was done as recommended by Antonov (2017). Those females having more than 90% cornification index (more of anuclear keratinized and superficial cells) upon EVC were selected for AI (Fig. 3). EVC was performed using cotton swab technique as recommended by Aydin *et al.* (2011). The smears were stained using Giemsa staining and observed under 100x.

Semen collection: The most commonly employed methods of AI are artificial vagina, manual collection and electrical stimulation (Payan-Carreira *et al.*, 2011). In the present study, the semen collection was performed using manual collection. For semen collection, male was placed in quite, non-stressful environment and adequately spaced, non slippery surface for footing. Semen collection was performed using digital pressure and massage technique as recommended by Jahangirbasha *et al.* (2018). In those dogs which did not show sexual interest, semen collection using digital manipulation was followed using the she dog in estrus being positioned infront of male dog and aiding it to mount the female. When the male dog exhibited sexual interest, using a gloved hand, the preputial skin was pushed caudally, exposing the glans penis. The base of the penis behind the bulbous glandis was grasped through prepuce and firm and constant pressure was applied by the palm and fingers. Pressure was continuously applied with backward and forward movements until erection was brought (Fig. 1). The ejaculated semen was collected in a sterile test tube for further evaluation.

*Corresponding author: malatinaik91@gmail.com

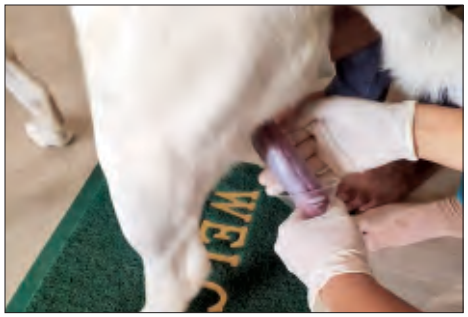

Fig. 1. Semen Collection

Fig. 2. Artificial Insemination

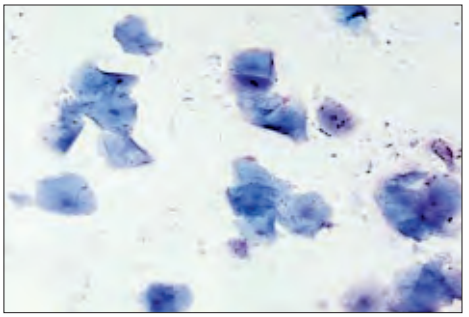

Fig. 3. Anuclear keratinized and superficial cells under EVC

Table 1

Age and parity (Mean± SE)

S.No.	Age (yrs)		Parity
	Male	Female	Female
1	2	2	0
2	3	2	0
3	3	3	1
4	2	3	2
5	4	2	0
6	2	4	1
7	3	3	0
Mean± SE	2.71±0.28	2.714 ±0.28	0.57±0.29

Procedure of Artificial Insemination: Female was placed in quite, non-stressful environment and adequately spaced non-slippery footing surface. In order to facilitate easy insemination, the she dog was positioned as per the method described by Jahangirbasha *et al.* (2018). The hind quarters of the she dog was elevated to an angle 45 to 60 degree from the surface of the examination table with the help of an assistant (Fig. 2). Perineal area of the vulval lips was cleaned with antiseptic solution and vaginal speculum was inserted aseptically. Sterile AI sheath was then introduced as much as possible to the level of external os of the cervix. Another assistant was directed to connect the free end of the AI sheath with the syringe pre-loaded with the semen. The semen was then injected slowly to the level of external os. The she dog was maintained in the required position for 10 minutes to prevent the back flow of the semen. The procedure was performed once in all the cases.

Semen Evaluation: The evaluation of collected semen was performed as per the procedures described by Roberts *et al.* (2016). Macroscopic and microscopic tests were carried out to evaluate the effectiveness of semen for AI.

Macroscopic tests:

1. Semen volume: The volume of ejaculate was assessed using graduated or calibrated test tube. The mean ± SE of volume was 11.07 ± 0.51 ml (Table 2).
2. Semen colour: Semen colour was milky-white.
3. Hydrogen ion concentration (pH): the mean pH of dog semen was 6.24 ± 003 (Table 2).

Microscopic tests:

1. **Progressive motility:** Normal progressive motility test was performed immediately after semen collection in order to assess the forward motion and rapid progression of the motile spermatozoa to correlate with plasma membrane integrity as recommended by Roberts *et al.* (2016). A drop of semen was diluted with normal saline and placed in a grease-free pre-warmed glass slide and was covered with coverslip (Roberts *et al.* (2016). The mean progressively motile spermatozoa was 86.42 ± 0.92 percent (Table 2).
2. **Sperm concentration:** Sperm concentration was estimated following the gold standard technique using haemocytometer as described by Payan-Carreira *et al.* (2011). The mean sperm concentration in the present study was 1037 ± 149.99 million/ml (Table 2).
3. **Live and dead staining:** Live and dead spermatozoa were estimated using Eosin-nigrosin staining. The mean viable spermatozoa in current study was 85.42 ± 0.53 percent (Table 2).

Table 2

Microscopic and macroscopic evaluation of canine semen (Mean±SE)

S.NO.	Parameters				
	Semen Volume (ml)	Sperm Concentration (million/ml)	Hydrogen ion concentration	Progressive Motility (percent)	Live sperms (percent)
1	10.5	1500	6.3	85	85.0
2	9.5	1680	6.2	90	86.5
3	11.0	850	6.4	85	85.0
4	12.5	700	6.2	85	85.0
5	11.5	835	6.3	90	88.0
6	13.0	680	6.2	85	83.5
7	9.5	1020	6.1	85	85.0
Mean± SE	11.07 ± 0.51	1037±149.99	6.24±0.03	86.42±0.92	85.42 ± 0.53

Pregnancy diagnosis: On 35-40 days post insemination, pregnancy diagnosis was carried out ultrasonographically.

In the present study, the AI was carried out in seven she dogs using the fresh semen collected from male counterpart using manual manipulation technique where constant application of pressure behind the bulbous glandis was prime important in maintaining a proper pressure continuously in a pulsating manner for collecting sufficient quantity of semen. These findings were similar to those observed by Shukla (2011) and Jahangirbasha *et al.* (2018).

The findings of current study varied in normal range. The mean volume of semen was 11.07 ± 0.51 ml which was similar to observation made by Payan-Carreira *et al.* (2011). The volume of semen depends upon age, breed of dog, the frequency of semen collection, size of prostate gland, size of the dog and third fraction of the ejaculate and is not a factor for semen quality assessment in dogs as opined by Payan-Carreira *et al.* (2011). They opined that the decrease in volume of ejaculate could be due to prostatic cysts, benign prostatic hyperplasia, inflammatory lesions on prostate and epididymis.

Normal colour of dog semen is greyish-white. The volume of the third fraction of the ejaculate is reflective of the colour of semen. The appearance of semen colour in pathological condition varies depending upon the contaminant present i.e., red in erythrocyte contamination, yellow in urine contamination, greyish in presence of pus in semen and more of fat droplets in semen indicates azoospermia as observed by Johnston *et al.* (2001), hemospermia in prostatic disease and penile injury (Root Kustritz, 2007).

The mean pH of semen reported in current study was 6.24±0.03. Similar observation was made by Threlfall (2003) who recommended that the pH evaluation should be performed immediately after semen collection. However, the prostatic fluid has a normal pH range of 6.5 to 6.8 (Roberts *et al.*, 2016).

In the current study, the mean progressive motility was 86.42±0.92 percent. Roberts *et al.* (2016) reported that progressive motile spermatozoa should have at-least 70 percent motility. Contamination of water, urine, blood, prolonged sexual rest, systemic or infectious causes/ diseases contribute decline in percent motile spermatozoa (Payan-Carreira *et al.*, 2011).

In the present study, the mean live spermatozoa was 85.42±0.53 percent. Similarly, Johnston *et al.* (2001) recommended that a good semen should have a minimum of 80% morphologically viable spermatozoa. Upon Eosin-nigrosin staining technique, dead spermatozoa with disintegrated plasma membrane, take pink colour and live spermatozoa appear transparent (Payan-Carreira *et al.,* 2011). The mean sperm concentration was 1037 ± 149.99 million/ml, whereas, Roberts *et al.* (2016) reported normal sperm concentration as 300-2000 million/ml, they further opined that an inverse correlation exist between sperm concentration and volume of semen.

Upon transabdominal ultrasonography, all the cases were confirmed pregnant with the presence of foetal parts in foetal sacs. Normal whelping was recorded among six she dogs at 62 to 65 days of gestation. However, in one she dog, the puppies had to be delivered with professional assistance on 68th day of gestation.

The present study showed that digital manipulation is cost-effective technique for semen collection in dogs. Semen evaluation and artificial insemination is a valuable provision that can readily be performed in females and/or male dogs having issues with natural mating. The success of AI depends on proper timing of the insemination, proper skill and expertise with regard to evaluation of EVC in order to identify the stage of oestrus. Good quality semen collected from healthy sire with a mean progressive motility 86.42±0.92 percent, mean live spermatozoa 85.42 ± 0.53 percent and mean volume of 11.07 ± 0.51 ml semen along with hygienic collection and handling are very essential. In the present study single insemination was sufficient for successful conception.

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REFERENCES

Antonov, A.L. (2017). Application of exfoliative vaginal cytology in clinical canine reproduction-a review. *Bulg. J. Vet. Med.* **20(3)**: 193-203.

Aydin, I., Sur E., Ozaydin. T. and Dinc. D. (2011). Determination of the stages of the sexual cycle of the bitch by direct examination. *J. Anim. Vet. Adv.* **10**: 1962–1967.

England, G.C. and Millar, K.M. (2008). The ethics and role of AI with fresh and frozen semen indaily production and output of spermatozoa and extragonadal spermatozoal reserves of the dog. *Biol. Reprod.* **29**: 1114–1120.

Foote, R.H. (2002). The history of artificial insemination: Selected notes and notables. *J. Anim. Sci.* **80**: E22–E32.

Hermansson, U. and Forsberg, C.L.(2006). Freezing of stored, chilled dog spermatozoa. *Theriogenology.* **65(3)**: 584-593.

Jahangirbasha, D., Usturge, S.M. and Tandle, M.K. (2018). Semen collection by digital manipulation technique in dogs. *Intas Polivet.* **19(1)**: 129-130.

Johnston, S.D., Kustritz, M.V.R. and Olson, P.N.S. (2001). Semen collection, evaluation, and preservation. In: Canine and feline theriogenology (1st Edn.), W.B. Saunders, Philadelphia. pp. 287-306.

Mason, S.J. (2018). Current review of artificial insemination in dogs. *Vet. Clin. Small Anim. Pract.* **48(4)**: 567-580.

Payan-Carreira, R., Miranda, S. and Nizanski, W. (2011). Artificial insemination in dogs. In: Manofi, M. (Edt.), Artificial insemination in farm animals. InTech. Rijeka. pp. 51-78.

Roberts, M.A., Jayaprakash, G., Pawshe, M., Tamilmani, T. and Sathiyabarathi, M. (2016). Collection and evaluation of canine semen-A review. *Int. J. Sci.* **1586(5)**: 1586-1595.

Root Kustritz, M.V. (2007). The value of canine semen evaluation for practitioners. *Theriogenology.* **68**: 329–337.

Shukla, M.K. (2011). Applied veterinary andrology and frozen semen technology. New India Publishing Agency. New Delhi, p. 50.

Singh, G., Dutt, R., Kumar, S., Kumari, S. and Chandolia, R.K. (2019). Gynaecological problems in she dogs. *Haryana Vet.* **58**: 8-15.

Threlfall, W.R. (2003). Semen collection and evaluation. In: Root Kustritz M.V. (Edt.), The practical veterinarian: Small Animal Theriogenology. St. Louis: Butterworth-Heineman, pp. 97-123.