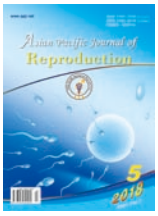

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Sperm defects and infertility caused by bacterial infection of the reproductive tract in an adult male dog: A case report

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ABSTRACT

Cases of acquired infertility in dogs often have poor prognosis and can be frustrating to clinicians in canine practice. In this case, a 3.5-year-old male Caucasian dog was presented for infertility evaluation. He had no record of successful breeding despite being mated with multiple bitches over a two-year period. Breeding soundness examination was performed including history evaluation, general physical and reproductive system examination, hematology, libido determination, and semen collection, evaluation and screening for infectious organisms. The presence of copious growth of bacteria (*Escherichia coli*) in conjunction with significant sperm abnormalities and leucospermia was applied to diagnose infertility due to a sub-chronic or chronic bacterial infection of the reproductive tract. Bacterial infection caused significant sperm abnormalities including low sperm motility, increased number of dead sperm and sperm with structural abnormalities such as detached heads, fractured necks, looped tails and coiled tails. Treatment was effected by administering ciprofloxacin orally for 4 weeks.

1. Introduction

Canine breeding is an important source of income in many parts of the world. Therefore, canine infertility is likely to cause significant economic losses in the industry. Infertility is broadly divided into congenital and acquired infertility. Congenital infertility is caused by genetic abnormalities and is present at birth whereas acquired infertility develops during the dog's lifetime[1,2]. Cases of acquired infertility in dogs can be frustrating to clinicians in canine practice. The prognosis of infertility often remains poor with fertility restored in only 10% of affected dogs after diagnosis and appropriate treatment[3].

2. Case report

In this case, a 3.5-year-old male Caucasian dog was presented for infertility evaluation. He had no record of successful breeding despite being mated with multiple bitches.

As previously recommended[2], evaluation for male infertility in this case was aimed to progress from relatively easy and inexpensive to more difficult and time consuming diagnostic tests. Breeding soundness examination was performed on the dog which included history evaluation, general physical examination, reproductive system examination, hematology, libido determination, and semen collection, evaluation and screening for infectious organisms.

Historically, upon presentation of the dog to four estrous bitches (at different times), there was normal mounting and copulation but no pregnancy in the mated bitches. Three of these bitches were bred to other males and produced litters thereby confirming their fertility. No information was available on the reproductive history of the male relatives. History of previous diet, illnesses, vaccinations and treatments were also recorded.

On physical examination, the dog appeared healthy and in good body condition (32 kg body weight). General examination of the dog revealed normal rectal temperature (37.8 ℃), pulse rate (118 bpm), respiratory rate (19 breaths/min) and pink mucous membranes of the eyes. Chest auscultation revealed normal heart and lungs. The submandibular and retropharyngeal lymph nodes were normal on palpation. Abdominal palpation did not reveal any obvious abnormalities. Examination of the limbs and foot pads did not reveal any musculoskeletal abnormalities, lesions or pain.

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Examination and palpation of the reproductive system revealed no obvious abnormalities of the scrotum and the penis, but there was moderate whitish discharge from the prepuce. The dog showed mild sign of pain on palpation of the testes and epididymides although these were of normal size and consistency and were freely movable within the scrotal sac. Upon rectal palpation of the prostate gland, there was mild sign of discomfort or pain but no obvious enlargement of the prostate.

An initial semen sample was collected using an artificial vagina and discarded. The dog was rested for 7 days before the collection of subsequent semen used for evaluation. Semen collection was performed in the absence of an estrous bitch as none was available at the time of assessment of the dog. First, the prepuce and tip of penis were cleaned with sterile saline moistened gauze. The artificial vagina was then used to collect the pre-sperm, sperm-rich and post-sperm fractions of ejaculate over a period of about 6 min. Semen was maintained between 30 ℃-37 ℃ and taken to the laboratory for further evaluation.

Gross semen evaluation was used to determine semen color, volume and pH. Microscopic semen evaluation was performed to determine sperm motility (total and progressive), sperm vitality, sperm morphology, sperm concentration, total sperm in the ejaculate and presence of white blood cells (WBC) in the semen. A sample of the semen was also sent for microbial culture and antibiotic sensitivity testing.

The results of the hematology and the gross and microscopic semen evaluation were presented in Table 1. The results were compared to reference values[4,5]. Semen colour was cloudy white, which was the same as the reference value. Micrographs of sperm vitality and morphology were also presented in Figures 1, 2, and 3. Semen culture revealed a copious growth of bacteria that was identified as *Escherichia coli* following biochemical and confirmatory tests. Antimicrobial sensitivity testing showed the isolate was most sensitive to ciprofloxacin and gentamicin but most resistant to augmentin and ampicillin.

Table 1

Haematology and semen evaluation in a case of infertility in a male.

Items	Value	Reference value
Haematology		
PCV (%)	51	37-54
Hb (g/dL)	16	13-19
Total WBC count ($\times 10^9$/L)	15	6-17
Differential WBC count (%)	L (17), M (4), N (79), E (0), B (0)	L (12-30), M (3-10), N (60-70), E (2-10), B (rare)
Semen evaluation*		
Volume (mL)	4	1-30
pH	6.1	6.3-7.0
Total motility (%)	30	70-90
Progressive motility (%)	11	⩾70
Sperm vitality (%)	42	>50-80
Sperm concentration (million/mL)	104	-
Total sperm (million/ejaculate)	416	300-2 000
Total sperm abnormalities (%)	39	<10-20
Alkaline phosphatase level (IU/L)	6 615	>5 000
Semen WBC count (/μL)	5 700	>2 000

*Reference values[4,5], PVC: packed cell volume, Hb: hemoglobin, WBC: white blood cells, L: Lymphocyte, M: monocyte; N: neutrophil, E: eosinophil, B: basophil.

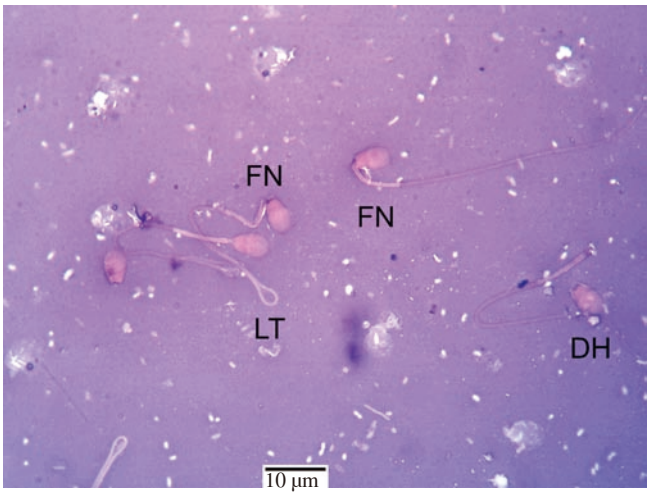

Figure 1. Micrograph of sperm vitality using eosin-nigrosin staining. Live sperm are unstained whereas dead sperm are stained red. Note theabnormal sperm with fractured neck (FN), detached heads (DH) and looped tails (LT); Magnification: × 1 000.

Figure 2. Micrograph of sperm vitality using eosin-nigrosin staining. Live sperm are unstained whereas dead sperm are stained red. Note the abnormal sperm with coiled tail (CT); Magnification: × 1 000.

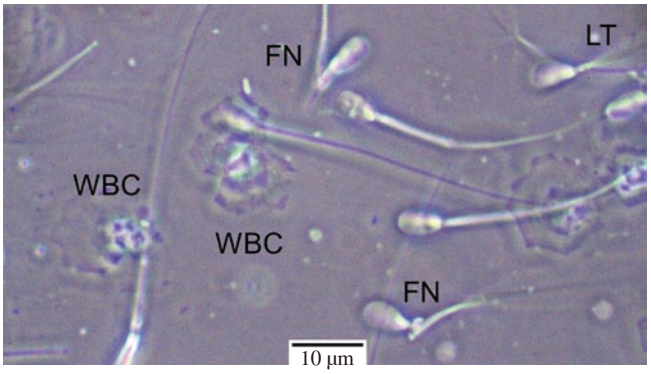

Figure 3. Phase-contrast micrograph of sperm morphology. Note the abnormal sperm with fractured neck (FN) and looped tail (LT) and the presence of WBCs in the semen; Magnification: × 1 000.

3. Discussion

Tentative diagnoses proposed included: poor libido, failure to ejaculate (aspermia), absence of sperm in ejaculate (azoospermia) and infectious disease of the genitalia. During semen collection, there was normal libido, erection and semen ejaculation in the dog (even in the absence of an estrous bitch). This eliminated infertility due to poor libido or aspermia. Grossly, the semen appeared normal although there was reduced pH possibly as a result of inflammatory activity in the reproductive tract. Following semen microscopy, the presence of sperm in the ejaculate ruled out azoospermia as the cause of infertility. However, there were significant sperm abnormalities including low progressive sperm motility (asthenozoospermia), increased number of dead sperm (necrospermia) and increased number of sperm with structural abnormalities (teratozoospermia) such as detached heads, fractured necks and looped tails. Seminal plasma alkaline phosphatase is produced in the epididymides[6]. The high level of alkaline phosphatase in the seminal plasma indicated complete ejaculation by the dog and confirmed patency of the reproductive ductal system. This also ruled out incomplete ejaculation as a cause of low motility observed in this case. Hematological results were within the normal range for healthy dogs except for increased blood neutrophils (neutrophilia) which suggested evidence of response to infection. There was also an increased WBC count in the semen (leucospermia), an evidence of active infection or inflammation in the reproductive tract.

A definitive diagnosis of infertility due to a sub-chronic or chronic bacterial infection of the reproductive tract was made based on the presence of copious growth of *Escherichia coli* bacteria in conjunction with poor sperm quality and leucospermia. Microbial infection may have occurred through local injuries, systemic blood supply, or by ascending infection of the prepuce, prostate, epididymis and testes[2]. In this case, bacterial infection of the reproductive tract caused significant damage to spermatozoa leading to infertility in affected dogs. Infertility may result from altered semen pH or sperm morphology and motility, or through direct killing of sperm cells by infectious organisms or inflammatory mediators.

Ciprofloxacin (15 mg/kg body weight) was administered orally twice daily (12 hourly). Treatment was applied for 4 weeks due to the potential long-standing nature of the infection. This is recommended to allow for adequate and sustained antibiotic levels within the reproductive tract[1]. The patient was also placed on supportive therapy including provision of adequate diet and avoidance of heat and stress. Further sexual activity was restricted for a minimum of 3 months from the end of treatment to allow for the normal length of the sperm production cycle (approximately 70 days). Three months following the completion of treatment, another semen evaluation and culture was performed. All the semen parameters were observed to have improved and were within the normal (volume: 5.5 mL, pH: 6.7, total motility: 82%, progressive motility: 75%, vitality: 89%, total sperm count: 660 million/ejaculate, total sperm abnormalities: 14%, WBC count: 750 per μL). Semen culture showed no evidence of bacterial infection. Subsequently, the dog was bred with an estrous bitch about 4.5 months following treatment. This was followed by successful conception and the whelping of four healthy puppies at the end of gestation, thereby confirming the efficacy of the diagnosis and treatment regimen. The client was advised to keep the dog and the kennel clean at all times and to avoid breeding with suspicious or unhealthy bitches.

Conflict of interest statement

The author declare that there is no conflict of interest.

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