

Diagnosis and Treatment of Idiopathic Oligoasthenoteratozoospermia (OAT) With a Combination of Clomiphene Citrate (Clomid®) and Vitamins E, C and B in a Male Caucasian dog

Key words

oligoasthenoteratozoospermia;
canine infertility;
clomiphene citrate;
vitamins

Simon Azubuike Ubah¹, Esther Zachariya¹, Thomas Barde¹, Samson Eneojo Abalaka², Philomina Monday Omoike⁴, Ugochukwu John Egedigwe⁴, Rwang Pam Christopher⁴, Charles Amaechi Uba⁵, Bridget Mary Jessica Adah³, Edmund Chidiebere Mbegbu^{6*}, Chike Fidelis Oguejiofor⁷

¹Department of Theriogenology, ²Department of Veterinary Pathology, ³Department of Veterinary Microbiology, Faculty of Veterinary Medicine, University of Abuja, ⁴Laboratory Services Department, University of Abuja Teaching Hospital, Abuja ⁵Department of Veterinary Medicine, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, ⁶Department of Veterinary Physiology and Biochemistry, ⁷Department of Veterinary Obstetrics and Reproductive Diseases, Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Nigeria

*Corresponding author: edmund.mbegbu@unn.edu.ng

Abstract: A two-year-old Caucasian male dog weighing 55.4 kg was presented to the Veterinary Teaching Hospital with the complaint of infertility. History evaluation revealed that he had mated ten bitches in the last eight months impregnating none of them. Clinical examination, laboratory investigations and semen evaluation were performed twice, 60 days apart. Semen and sperm parameters were poor with low percentage motility and vitality, low concentration, and high percentage of sperm abnormalities. No significant bacteria were isolated from cultures of the semen and preputial swab. Scrotal ultrasonography showed no evidence of testicular degeneration. Pre-treatment serum hormonal evaluation showed normal concentrations of follicle-stimulating hormone (FSH), luteinising hormone (LH), testosterone, thyroxine (T4), triiodothyronine (T3), prolactin and resting cortisol as well as low bicarbonate level. Haematology revealed evidence of leucocytosis. Based on the history, clinical examination and laboratory findings, the case was diagnosed as idiopathic oligoasthenoteratozoospermia (OAT). Here, a possible management of idiopathic OAT in a Caucasian dog with oral Clomiphene citrate (Clomid®) together with Vitamins E, C and B supplements for 60 days is described. Altogether, there was a marked improvement in the semen and sperm parameters at 90 days from the onset of treatment. Semen volume increased, together with increased sperm motility, vitality and concentration, and decreased percentage of sperm with morphological abnormalities.

Received: 25 May 2024
Accepted: 18 June 2024

Introduction

TMale factor infertility is an important cause of pregnancy failure in canine reproduction (1). Since the quality of semen is a reflection of the health of the reproductive tissues and organs, and the general health of the dog, a thorough process of history evaluation, clinical examination, semen evaluation and laboratory investigations can assist in making appropriate diagnosis (1, 2, 3).

Oligo-astheno-teratozoospermia (OAT) is an infertility syndrome involving all three of sperm abnormalities namely; a presence of decreased number of spermatozoa (oligozoospermia), decreased motility (asthenozoospermia), and many abnormal forms on morphologic examination (teratozoospermia) (4). Testicular dysfunction is the most frequent cause of abnormal spermatogenesis that can present as OAT. This may be caused by congenital factors (e.g. testicular dysgenesis, genetic abnormalities, testicular tumours), acquired factors (trauma, orchitis, medication, irradiation, overheating, disease, surgical complication) or idiopathic (unknown) factors (4).

The endocrine system is the principal regulator of the reproductive functions. Therefore, endocrine dysfunction can interfere with the hypothalamus-pituitary axis to disrupt spermatogenesis and fertility (5, 6). Endocrine dysfunction that impact male fertility may be caused by different conditions such as hypogonadotrophic hyp-hypergonadotrophic hypogonadism, androgen excess, oestrogen excess, hyperprolactinemia or insulin disorders (5, 6).

Typically, a short-lasting testicular dysfunction will cause a decline in semen quality over a few weeks or months leading to OAT and temporary infertility, or to complete azoospermia and an irreversible loss of fertility if the condition is long-lasting and/or in the absence of treatment (5). Therefore, a number of strategies have been applied in the management of OAT depending on the underlying aetiological factors. Medical therapy for idiopathic or endocrinopathic reproductive disorders in male dogs have been attempted by using different agents including gonadotrophin-releasing hormone (GnRH) agonists, gonadotrophins and androgens (7, 8); aromatase inhibitor (9) and

antioestrogens (e.g. clomiphene citrate and tamoxifen) (10, 11), all with different results in the improvement of seminal characteristics.

Clomid® is a popular brand name for the antioestrogen-clomiphene citrate. Antioestrogen drugs work by binding to hypothalamic oestrogen receptors in a competitive manner, thereby preventing hypothalamic negative feedback of oestradiol and resulting in an upsurge in GnRH release from the hypothalamus, which drives the cascade of elevated anterior pituitary gonadotrophin (FSH and LH) secretion, and an upsurge in testicular testosterone synthesis (5, 6).

There is ample evidence of the important roles of antioxidants and vitamins in the maintenance of optimal reproductive function and fertility in males (12, 13). Several studies have reported the use of antioxidants and vitamins supplementation (including vitamins E, C and B) to improve semen and sperm parameters in infertile males, including males with OAT (14, 15).

Although antioestrogens such as clomiphene and tamoxifen have been utilised in the treatment of OAT previously (10, 11), a combination of clomiphene citrate and vitamins E, C and B in possible management of idiopathic OAT in a Caucasian dog, is explored and described herein.

Case description

History

A two-year-old Caucasian male dog weighing 55.4 kg was brought to the Veterinary Teaching Hospital, University of Abuja with the complaint of infertility. History revealed that he had mated ten bitches in the last eight months without achieving pregnancy in any of them. Deworming and vaccination records were up to date. The diet was based on a combination of commercial food (Optimax®, China) and homemade food basically made of rice and chicken.

Table 1: Physiologic parameters in a dog presented with idiopathic oligoasthenoteratozoospermia

Parameters	First presentation	Second presentation (after 60 days)	Third presentation (90 days from onset of treatment)	Reference values
Body weight (kg)	55.4	55.0	56.1	–
Rectal temperature (°C)	38.7	38.4	39.1	37.5–39.2
Pulse rate (bpm)	96	94	100	60–120
Respiratory rate (bpm)	40↑	44↑	64↑*	10–34

↑increase, ↓decrease, *marked change. Reference values: MSD Veterinary Manual (23).

Table 2: Haematological parameters in a dog presented with idiopathic oligoasthenoteratozoospermia

Parameters	First presentation	Second presentation (after 60 days)	Third presentation (90 days from onset of treatment)	Reference values
Haemoglobin (Hb; g/dL)	15.9	17.1	14.4	14.2–19.2
Packed cell volume (PCV; %)	43	48	39	29–55
Red blood cell count (RBC; $\times 10^{12}/L$)	5.9	6.28	5.1	5.5–8.5
Mean corpuscular volume (MCV; fL)	73.1	76.4	76.4	65–80
Mean corpuscular haemoglobin (MCH; pg)	27.2↑	27.2↑	28.2↑	12.2–25.4
MCH concentration (MCHC; g/dL)	37.1↑	35.6	36.9	32–36
White blood cell count (WBC; $10^9/L$)	25.3↑*	22.7↑*	18.7↑	5.9–16.6
Platelets (PLT; $10^9/L$)	234	123↓	170	170–400
Neutrophils ($10^9/L$)	21.8↑*	18.2↑*	12.9↑	2.9–12.0
Lymphocytes ($10^9/L$)	3.0	3.6	4.7	0.4–4.8
Monocytes ($10^9/L$)	0.51	0.45	0.94	0.1–1.4
Eosinophils ($10^9/L$)	0	0.45	0.19	0–1.3
Basophil ($10^9/L$)	0	0	0	0–0.14

↑increase, ↓decrease, *marked change. Reference values: MSD Veterinary Manual (23)

Clinical examination at first presentation

On clinical examination during the first presentation in March 2022, the patient had a body weight of 55.4 kg and recorded normal rectal temperature and pulse rate but a slightly increased respiratory rate, as shown in Table 1. A flesh wound approximately 2 cm long and 0.5 cm deep due to a dog fight the previous day was observed on the patient's head. Blood was collected from the cephalic vein into vials (with and without potassium-EDTA anticoagulant) for haematology and serum biochemistry, respectively. Haematology was evaluated as previously described (Cheesbrough, 2006), serum biochemistry was evaluated with an automated biochemistry analyser (Selectra PRO XL; ELITECH Group B.V, Netherlands), while serum hormones were assayed with an automated chemiluminescence immunoassay analyser (Maglumi 800, BD-R 206; Shenzhen New Ind. Biomedical Engineering Co. Ltd., Shenzhen, China). Blood and rectal faecal samples were also screened for blood parasites and gastrointestinal parasites, respectively. The length and width of the scrotal testes were measured externally with a flexible tape. Semen was collected without the presence of an estrous teaser bitch following digital manipulation and sexual stimulation of the dog, and with the use of a nylon cone and a graduated tube (Fig. 1). Thrusting and ejaculation occurred after a second attempt at semen collection, although penile erection was weak. The ejaculate had a

volume of 2.5 mL and was used for semen and sperm analysis immediately after ejaculation. A semen sample was viewed under a microscope, starting at 40× magnification for the mass activity and going up to 400× for the individual sperm motility. Sperm concentration was determined with a Neubauer haemocytometer (Hawksley, Lancing, United Kingdom). Due to the observed low sperm number, a part of the semen sample was concentrated by centrifugation at 600 × g for 10 min before staining with eosin-nigrosin (Loba Chemie, Mumbai, India) for the evaluation of sperm vitality and morphological abnormalities. Semen pH was measured with a digital pH meter. A semen sample and two preputial swab samples were also sent to the microbiology laboratory for bacterial culture and antimicrobial sensitivity test for aerobic and anaerobic organisms. Due to the observed low sperm count and poor sperm motility, the client was advised to re-present the dog after 60 days for re-evaluation.

The wound on the patient's head was properly cleaned and managed surgically by apposition with three interrupted nylon sutures, followed by topical wound care. Clinical findings showed that the right (R) and left (L) testes lengths were 5.2 cm (R) and 5.2 cm (L) while the widths were 5.0 cm (R) and 5.0 cm (L). Detailed information from the laboratory

Table 3: Serum biochemical parameters in a dog presented with idiopathic OAT

Parameters	First presentation	Second presentation (after 60 days)	Third presentation (90 days from onset of treatment)	Reference values
Sodium (mmol/L)	142	126	144	140–154
Potassium (mmol/L)	5.0	4.3	4.2	3.8–5.6
Chloride (mmol/L)	108	105	110	102–124
Bicarbonate (HCO ₃ ; mmol/L)	10↓*	10↓*	21	17–25
Urea (mmol/L)	4.6	5.6	5.3	2.9–10.0
Creatinine (μmol/L)	85	90	108	44–150
ALP (IU/L)	51	54	66	1–114
AST (IU/L)	51	49	44	8.9–49
ALT (IU/L)	31	37	32	8.2–109
Total Protein (g/L)	70	62	59	54–75
Albumin (g/L)	31	28	29	23–40
Total Cholesterols (mmol/L)	3.45	3.53	3.58	2.52–8.79
Triglycerides (mmol/L)	0.45	0.48	0.47	0.29–2.83
HDL (mmol/L)	0.90	1.37	2.60	0.94–3.01
LDL (mmol/L)	2.35	1.69	0.77↓*	1.12–4.65
Calcium (mmol/L)	NA	NA	2.40	2.18–2.95
Inorganic Phosphorus (mmol/L)	NA	NA	1.10	0.94–2.00

↑increase, ↓decrease, *marked change, NA (not available). Reference values: MSD Veterinary Manual (23).

Table 4: Serum hormonal evaluation in a dog presented with idiopathic OAT

Parameters	First presentation	Second presentation (after 60 days)	Third presentation (90 days from onset of treatment)	Reference values
FSH (ng/mL)	110.2	78.37	136.9↑	61.0–117.0
LH (ng/mL)	9.26	14.04↑	11.36	0.8–11.2
Testosterone (ng/mL)	6.74	10.44↑	6.50	0.5–9.0
T4 (nmol/L)	NA	24.19	18.13	15.0–50.0
T3 (nmol/L)	NA	1.35	1.29	1.0–2.5
Prolactin (ng/mL)	0.23	0.27	NA	0–6.0
Cortisol, resting (μg/dL)	17.01	17.22	NA	<1–18.58

↑increase, ↓decrease, *marked change, NA (not available). Reference values: FSH and LH (24,25); Testosterone (25); T4 and T3 (26); Prolactin (27); Cortisol (28); Units conversion: UnitsLab (29); WHO Technical Report (30).

Table 5: Semen and sperm evaluation in a dog presented with idiopathic OAT

Parameters	First presentation	Second presentation (after 60 days)	Third presentation (90 days from onset of treatment)	Reference values
<i>Semen</i>				
Volume (mL)	2.5	3	8	1–30
Colour	watery	milky	milky	milky/opalescent
pH	6.2	5.9	6.3	6.3–7.0
Mass activity (0–4 scale)	0↓*	1↓*	3	3–4
<i>Sperm</i>				
Motility (%)	0↓*	11↓*	80	>70%
Vitality (%)	5↓*	27↓*	85	>80%
Abnormality (%)	94↑*	90↑*	9	<10–20
Total count (× 106)	31.25↓*	45.9↓*	580	>300

↑increase, ↓decrease, *marked change. Reference values: (31–34).

examinations is presented Tables 1–5. Haematological evaluation revealed a slightly raised mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC), and a high white blood cells (WBC) count (neutrophilic leucocytosis); Table 2. Biochemical indices revealed low serum bicarbonate level; Table 3. Hormonal evaluation revealed normal serum concentrations of FSH (Maglumi 800, Snibe Diagnostic, LOT: 011220111), LH (Maglumi 800, Snibe Diagnostic, LOT: 012220111), testosterone (Maglumi 800, Snibe Diagnostic, LOT: 016230511), prolactin (Maglumi 600, Snibe Diagnostic, LOT: 013230211) and cortisol (Cortisol Diagnostic kit, Shenzhen Micro point Biotech Co. Ltd, LOT: 22001); Table 4. In general, the sperm parameters were poor (Table 5; Fig. 2 and 3) with no motility, low % vitality and concentration, and high % of sperm abnormalities. There was no significant presence of blood or gastrointestinal parasites. In addition, no significant bacteria were isolated from cultures of the semen and preputial swab. Rose Bengal test (Veterinary Laboratories Agency, LOT: SG 288) showed negative result for *Brucella* antibody screening.

Clinical examination at second presentation

On the second presentation (60 days after the first presentation), the patient weighed 55.0 kg and recorded physiologic parameters were within the normal range except for a slightly increased respiratory rate (Table 1). Blood was also collected for haematological, biochemical and hormonal evaluation, as already described. Serum levels of triiodothyronine (T3) (Maglumi 1000 Plus, Snibe Diagnostic, REF: 130203003M) and tetraiodothyronine (T4) (Maglumi 1000 Plus, Snibe Diagnostic, REF: 130203002M) were included in

the hormonal assay. The measurements of the scrotal testes were similar to the findings on the first presentation for the testis length: 5.1 cm (R) and 5.2 cm (L) and testis width: 5.1 cm (R) and 5.0 cm (L). Semen was collected again and analysed, as described earlier. Clinical observations during manual semen collection revealed normal penile erection. Initially, thrusting occurred without any ejaculate. There was ejaculation of 3 mL of semen on the second attempt, after walking the dog round the premises of the hospital. Scrotal ultrasonography (US) of the testes was performed with a sector probe (Edan, 5.0/6.5/8.0 MHz) on grey ultrasonography. A semen sample was also sent for bacterial culture and isolation.

The findings showed leucocytosis, although the WBC and neutrophil count had decreased relative to the first presentation; Table 2. Biochemical evaluation revealed low serum bicarbonate level; Table 3. Hormonal assay showed slightly elevated serum levels of LH (Maglumi 800, Snibe Diagnostic, LOT: 012220111) and testosterone (Maglumi 800, Snibe Diagnostic, LOT: 016230511), and normal levels of FSH (Maglumi 800, Snibe Diagnostic, LOT: 011220111), T4 (Snibe Diagnostic, REF: 130203002M), T3 (Snibe Diagnostic, REF: 130203003M), prolactin and resting cortisol; Table 4. Sperm parameters were still markedly deficient, with low % motility and vitality, low concentration, and high % of sperm abnormalities (Table 5; Fig. 2 and 3). No significant bacteria were isolated from the semen and preputial swab cultures. Testicular US showed no evidence of degeneration or lesions (Fig. 1 A and B). Semen culture yielded no significant bacterial growth.

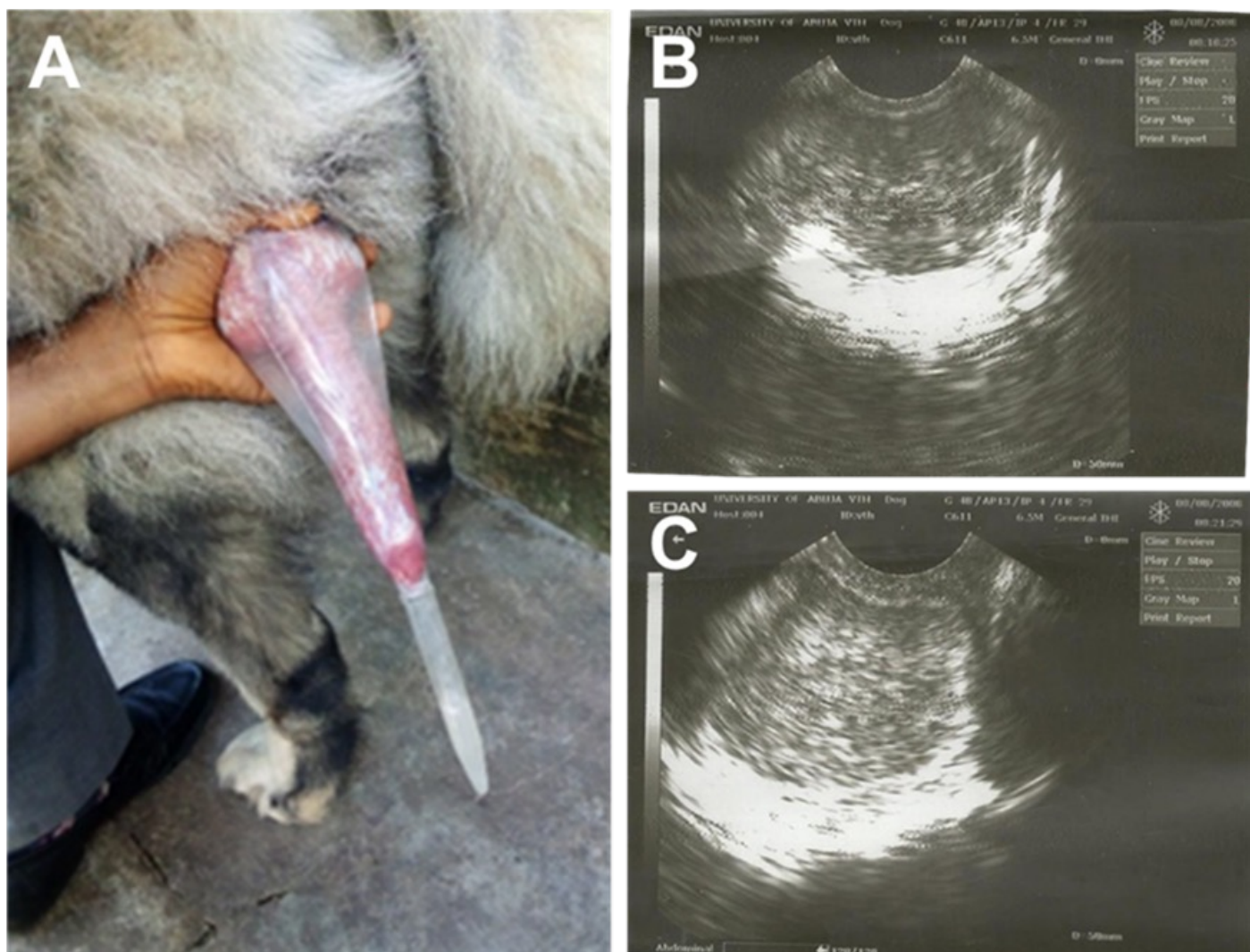


Figure 1: Semen evaluation and testicular ultrasonography in a dog presented with idiopathic oligoasthenoteratozoospermia. (A) Collection of semen from the patient through digital manipulation and with the use of a nylon cone and a graduated tube. Ultrasonography of the left (B) and right (C) testes showed no evidence of lesions or testicular degeneration

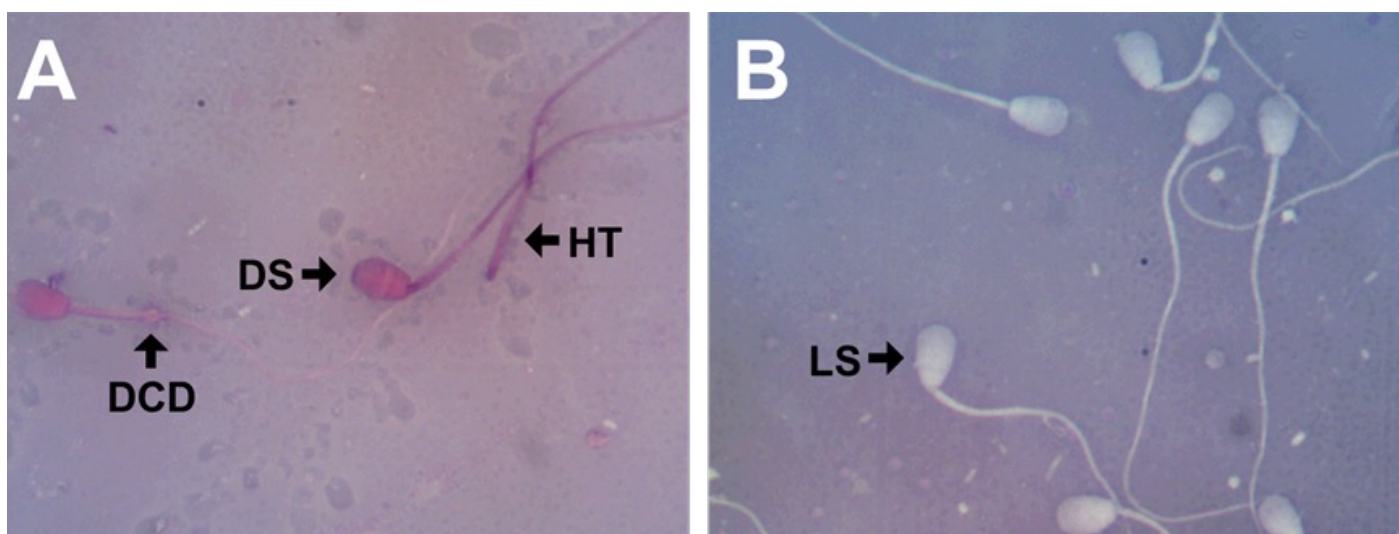


Figure 2: Evaluation of sperm vitality in a dog presented with idiopathic oligoasthenoteratozoospermia by using Eosin-Nigrosin staining; 1000× magnification. (A) shows many dead sperm (DS) and sperm with headless tail (HT) and distal cytoplasmic droplets (DCD) abnormalities in the semen of the patient before the onset of treatment. After 90 days of treatment with Clomiphene citrate, (B) shows abundance of normal and live sperm (LS) in the patient's semen sample

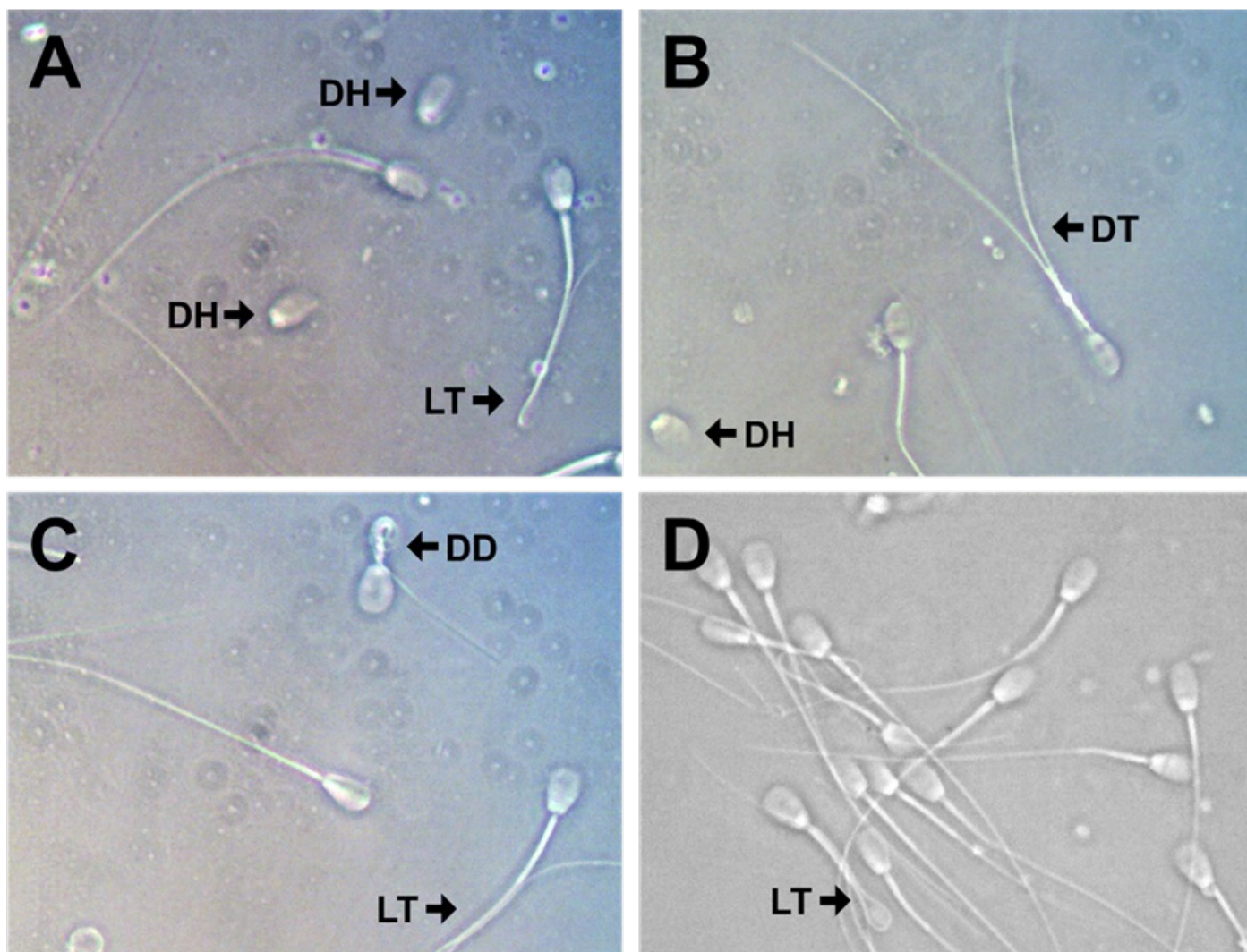


Figure 3: Evaluation of sperm morphology in a dog presented with idiopathic oligoasthenoteratozoospermia by using Phase-contrast microscopy; 1000× magnification. (A, B and C) show many sperm morphological abnormalities in the semen of the patient before the onset of treatment. Note the presence of sperm with detached head (DH), looped tail (LT), double tail (DT), and the Dag defect (DD). After 90 days of treatment with Clomiphene citrate, (D) shows abundant normal sperm cells and scanty presence of sperm abnormalities in the patient's semen sample

Based on the history, clinical evaluation and laboratory findings, the case was diagnosed as idiopathic oligoasthenoteratozoospermia (OAT). The infertile stud was treated initially with 50 mg Clomiphene citrate (Clomid®; Bruno Farmaceutica, Italy) at the dose of 0.9 mg/kg, oral route, every other day, for 60 days (9). Vitamin E, as DL-Alpha Tocopheryl Acetate (Natures field®; Bactolac Pharm. Inc., Hauppauge, NY, USA), was administered orally at 1000 IU daily for 60 days. Vitamin C (500 mg ascorbic acid), Riboflavin (15 mg), Niacin (25 mg inositol hexaniotinate) and Vitamin B12 (200 µg methylcobalamin) in a formulation (FARHAVEN Health®, USA), were administered as a daily capsule for 60 days, to augment the process of spermatogenesis. The stud was rested from any form of breeding for the period of treatment. Additional treatment was done with ciprofloxacin (May and Baker, Nigeria), administered orally at 10 mg/kg body weight, twice daily, for seven days.

Clinical examination after treatment

On the third presentation (90 days from the onset of treatment), the patient's weight was 56.1 kg with normal rectal temperature and pulse rate but an increased respiratory rate; Table 1. Blood was also collected for routine haematological, biochemical and hormonal evaluation. During manual semen collection, there was normal erection, thrusting and ejaculation. The semen had a volume of 8 mL, and was analysed as already described. Testes US was repeated, and scrotal testicular width and length were also measured. Urine was collected in the early morning hours and subjected to urinalysis by using a combo stick (CE IVD, Hanover, Germany).

The findings revealed a slightly elevated MCH following haematological evaluation; Table 2. Serum biochemical indices showed a slightly decreased low density lipoprotein (LDL) concentration; Table 3. Hormonal profile indicated only a slightly increased level of FSH and normal levels of

LH, testosterone, T4 and T3; Table 4. Adequate thyroid function was confirmed via the values recorded for repeated TSH, T3 and T4 evaluation. Urinalysis showed no abnormalities and was negative for blood, urobilinogen, bilirubin, protein, nitrite, ketones, ascorbic acid, leucocytes, and glucose. Urine pH was 5.0 while the specific gravity was 1.025. Testicular ultrasonography revealed no abnormalities. However, testes measurements revealed a slight increase from the pre-treatment values: testes lengths were 7.0 cm (R) and 7.0 cm (L) while the widths were 6.0 cm (R) and 5.0 cm (L). Altogether, there was a marked improvement in the semen and sperm parameters. Semen volume was high with increased sperm % motility and vitality, increased sperm concentration, and decreased % of sperm with morphological abnormalities; Table 5 and Fig. 2 and 3.

Therefore, at 90 days from the onset of treatment, the reproductive condition of the stud was remarkably improved from the previous poor semen and sperm quality. The semen volume and sperm motility, vitality, concentration and normal morphology, all improved significantly after therapy. To sustain the therapy, the initial treatments with Clomiphene citrate and vitamins were repeated at the same respective doses for another 60 days. The stud was rested from any form of breeding for the period of treatment. The owner was advised to maintain a suitable diet for the stud.

Discussion

Clinical evaluation and therapeutic management are important in reversing cases of infertility in male dogs (16). However, among several congenital, acquired and idiopathic factors implicated in male infertility in dogs, oligoasthenoteratozoospermia (OAT) has been insufficiently documented. Idiopathic oligoasthenozoospermia was reported as the most common cause of male infertility in humans (14). Herein, we explored a possible management of idiopathic OAT in a Caucasian dog with a combination of Clomiphene citrate (Clomid®) and vitamins E, C and B.

On initial presentation of the case, the characteristic poor sperm parameters (no motility, low % vitality and concentration, and high % of sperm abnormalities) was typical of OAT.

Idiopathic OAT is marked by abnormal spermatogenesis of unknown origin. It is usually difficult to pinpoint the specific cause by common laboratory tests. In addition to sperm abnormalities, there may be a histological picture of a mixed testicular atrophy with normal serum levels of testosterone, FSH and LH (17, 18).

In this case, endocrinopathies were excluded as causative factors because the assayed pituitary hormones (FSH, LH and prolactin) were within normal reference ranges. The normal serum testosterone concentration also provided evidence of the typical homeostatic feedback control of the

hypothalamic-pituitary-gonadal (HPG) axis in the patient (5). Haematology revealed leucocytosis and neutrophilia, which necessitated the bacterial culture and antimicrobial sensitivity testing of the semen and the preputial swab. However, the microbial growth did not yield any microorganism of reproductive importance. The observed leucocytosis, therefore, may be related to the head injury from a dog fight or to some other unknown condition. Although no other complicating systemic condition was identified, we observed a decreasing trend in the leucocyte and neutrophil counts 60 days after the first presentation, suggesting a resolving leucocytosis before the onset of therapy. However, an antibiotic intervention was made in order to protect the dog from any infection that may not have been detected in the reproductive system.

On initial presentation, the dog exhibited delayed sexual stimulation and ejaculation during semen collection. The observed normal serum concentration of prolactin rule out the possibility of a hyperprolactinemia causing azoospermia. The patient also recorded normal serum levels of testosterone together with normal penile erection. Testosterone is critical in regulating normal libido in males. Therefore, the observed delayed ejaculation prior to treatment may have been due to semen collection in an environment strange to the patient.

The low serum bicarbonate ion observed in this case indicated metabolic acidosis, although the specific cause was not clearly identified. Metabolic acidosis is known to be associated with increased heart rate which was consistent with the dog in each presentation at the clinic. Diabetes is a medical condition that can also lead to metabolic acidosis and male infertility (19). We were unable to recover urine on the second presentation but collected naturally-voided urine on the third presentation. Urinalysis did not reveal any other disorders such as urinary tract infection, kidney disease and diabetes.

Based on the diagnosis of OAT, Clomid® was utilised as management therapy. Oral clomiphene citrate has been reported to be effective in one dog for the treatment of spermatogenic dysfunction although the treated dog had normal plasma testosterone level (20).

Ultrasound imaging can be used as a diagnostic technique to confirm the diagnosis of testicular degeneration with mineralization (21). There was no ultra-sonographic evidence of testicular degeneration in this case. The measured testes showed relative incremental changes in testicular sizes, which may be an effect of the clomiphene citrate treatment on testicular function and sperm production.

Although semen oxidative stress status was not evaluated, the inclusion Vitamin E (α -tocopherol) and vitamin C (ascorbic acid) may have been beneficial due to their known antioxidant effects. Vitamin E is an important lipid-soluble antioxidant molecule. Vitamin E has been used extensively

in vivo in the treatment of a variety of diseases. The results of several *in vitro* experiments suggest that vitamin E may protect spermatozoa from oxidative damage and loss of motility, as well as enhance the sperm performance in the hamster egg penetration assay (17). On the other hand, Vitamin C (ascorbic acid) is a water-soluble ROS scavenger with high antioxidant activities. Vitamin C has been explored as an oral supplement, along with vitamin E, in the treatment of idiopathic infertility in men (22). Also, semen volume and quality, testicular blood flow, testicular volume, testosterone concentration and nitric oxide improved significantly when 15 hypofertile German shepherd dogs were fed a diet containing zinc, folic acid, Omega-3 compounds, vit B6, vit B12 and Vitamin E (13).

As no other cause of the reproductive failure could be identified in this dog, we considered his poor semen quality to be idiopathic. However, other possible causes of the fertility problem of this dog could be stress due to the head trauma causing prolonged release of cortisol and/or a concomitant, undiagnosed general condition as highlighted by his leukocytosis. Such undiagnosed condition may have persisted during the 60 days elapsing between the first and second clinical examination which might explain why little or no improvement was observed at the time of the second presentation. A significant improvement was observed in semen and sperm quality following the clomiphene therapy. However, it should be underlined that the dog was concomitantly treated with antibiotics and vitamins, both of which might have contributed to the improvement of semen quality. Nevertheless, because of the established action of anti-estrogen compounds such as clomiphene in improving canine semen quality (10), a role of clomiphene in improving semen quality in the dog of our case is postulated.

In conclusion, proper diagnosis and timely institution of specific therapy for OAT in a breeding stud are essential in managing the associated infertility. The clinical outcome of the case described here suggests that oral administration of clomiphene citrate in combination with Vitamins E, C and B, may serve as a possible treatment to improve semen quality in dogs with idiopathic OAT, and no side effect was observed throughout the 90 days of handling the case. The formulation we used of clomiphene citrate can be administered orally by clients to their dogs at home which makes it practical allowing for high level of compliance.

Acknowledgements

The authors deeply appreciate the Director and staff of the Veterinary Teaching Hospital, University of Abuja, for their assistance and cooperation. The authors declare no conflict of interest. Conceptualization, monitoring, methodology, investigation, data collection: U.S.A., Z.E., B.T., A.S.E., O.P.M., E.U.J., C.R.P., U.C.A. and A.B.M.J. Writing original draft preparation, reviewing and editing: U.S.A., M.E.C. and

O.C.F. All authors have read and agreed to the published version of the manuscript.

References

1. Lopate C. The problem stud dog. *Vet Clin North Am Small Anim Pract* 2012; 42(3): 469–88. doi: 10.1016/j.cvsm.2012.01.014
2. Oguejiofor CF. Sperm defects and infertility caused by bacterial infection of the reproductive tract in an adult male dog: a case report. *Asian Pac J Reprod* 2018; 7(5): 236–8. doi: 10.4103/2305-0500.241208
3. Kolster KA. Evaluation of canine sperm and management of semen disorders. *Vet Clin North Am Small Anim Pract* 2018; 48(4): 533–45. doi: 10.1016/j.cvsm.2018.02.003
4. Jungwirth A, Giwercman A, Tournaye H, et al. European association of urology guidelines on male infertility: the 2012 update. *Eur Urol* 2012; 62(2): 324–32. doi: 10.1016/j.eururo.2012.04.048
5. Johnston SD, Kustritz MV, Olson PS. *Canine and feline theriogenology*. 1st ed. Philadelphia: Saunders, 2001.
6. Sengupta P, Dutta S, Karkada IR, Chinni SV. Endocrinopathies and male infertility. *Life (Basel)* 2022; 12: 10. doi: 10.3390/life12010010
7. Driancourt MA, Briggs JR. Gonadotropin-releasing hormone (GnRH) agonist implants for male dog fertility suppression: a review of mode of action, efficacy, safety, and uses. *Front Vet Sci*. 2020; 7: 483. doi: 10.3389/fvets.2020.00483
8. Stempel S, Körber H, Reifarth L, Schuler G, Goericke-Pesch S. What happens in male dogs after treatment with a 4.7 mg deslorelin implant? II. recovery of testicular function after implant removal. *Animals (Basel)* 2022; 12(19): 2545. doi: 10.3390/ani12192545
9. Kawakami E, Taguchi N, Hirano T, Hori T, Tsutsui T. Therapeutic effect of aromatase inhibitor in two azoospermic dogs with high plasma estradiol -17b levels. *J Vet Med Sci* 2003; 65 (12): 1343–5. doi: 10.1292/jvms.65.1343
10. Gonzalez G, Guendulain C, Maffrand C, Gobello C. Comparison of the effect of the aromatase inhibitor, anastrozole, to the antioestrogen, tamoxifen citrate, on canine prostate and semen. *Reprod Domest Anim* 2009; 44(2): 316–9. doi: 10.1111/j.1439-0531.2009.01379.x
11. Corrada Y, Arias D, Rodríguez R, Spaini E, Fava F, Gobello C. Effect of tamoxifen citrate on reproductive parameters of male dogs. *Theriogenology* 2004; 61: 1327–41. doi: 10.1016/j.theriogenology.2003.07.020
12. Ahmadi S, Bashiri R, Ghadiri-Anari A, Nadjarzadeh A. Antioxidant supplements and semen parameters: an evidence-based review. *Int J Reprod Biomed* 2016; 14(12): 729–36.
13. Amor H, Shelko N, Mohammed M, Michael Jankowski P, Eid Hammadeh M. Role of antioxidants supplementation in the treatment of male infertility. In: Waisundara V, ed. *Antioxidants - benefits, sources, mechanisms of action*. Intech Open, 2021. doi: 10.5772/intechopen.95891
14. ElSheikh MG, Hosny MB, Elshenoufy A, Elghamrawi H, Fayad A, Abdelrahman S. Combination of vitamin E and clomiphene citrate in treating patients with idiopathic oligoasthenozoospermia: a prospective, randomized trial. *Andrology* 2015; 3(5): 864–7. doi: 10.1111/andr.12086
15. Cilio S, Rienzo M, Villano G, et al. Beneficial effects of antioxidants in male infertility management: a narrative review. *Oxygen* 2022; 2(1): 1–11. doi: 10.3390/oxygen2010001

16. Domosławska A, Zduńczyk S. Reversible infertility in male dog following prolonged treatment of Malassezia dermatitis with ketoconazole. *Acta Vet Scand* 2021; 63: 50. doi: 10.1186/s13028-021-00616-9
17. Agarwal A, Sekhon LH. Oxidative stress and antioxidants for idiopathic oligoasthenoteratospermia: is it justified? *Indian J Urol* 2011; 27(1): 74–5. doi: 10.4103/0970-1591.78437
18. Bonanomi M, Lucente G, Silvestrini B. Male fertility: core chemical structure in pharmacological research. *Contraception* 2002; 65(4): 317–20. doi: 10.1016/S0010-7824(02)00303-7
19. He Z, Yin G, Li QQ, Zeng Q, Duan J. Diabetes Mellitus Causes Male Reproductive Dysfunction: A Review of the Evidence and Mechanisms. *In Vivo* 2021; 35(5): 2503–11. doi: 10.21873/in vivo.12531
20. Kobayashi M, Hori T, Kawakami E. Therapeutic effects of oral clomiphene citrate in 2 dogs with low plasma testosterone levels and poor semen quality. *J Vet Med Sci* 2018; 80: 1233–5. doi: 10.1292/jvms.18-0108
21. Abdelnaby EA, Khalek G, Emam I. The beneficial effects of enriched diet on testicular blood flow and seminal parameters using colour pulsed Doppler ultrasound in dogs. *Bulg J Vet Med* 2023; 26(3): 410–24. doi: 10.15547/bjvm.2021-0037
22. Zduńczyk S, Domosławska A - Effects of drugs on fertility in male dogs: a review. *Reprod Domest Anim* 2022; 57(9): 949–56. doi: 10.1111/rda.14173
23. MSD Veterinary Manual 2022. <https://www.msdsvetmanual.com/special-subjects/reference-guides>. Incompletely quoted???
24. Olson PN, Mulnix JA, Nett IM. Concentrations of luteinizing hormone and follicle-stimulating hormone in the serum of sexually intact and neutered dogs. *Am J Vet Res*. 1992; 53(5): 762–6.
25. Colorado State University. Reference Values. 2022. College of Veterinary Medicine and Biomedical Sciences. Incomplete information, missing address...
26. Shiel RE, Sist M, Nachreiner RF, Ehrlich CP, Mooney CT. Assessment of criteria used by veterinary practitioners to diagnose hypothyroidism in sighthounds and investigation of serum thyroid hormone concentrations in healthy Salukis. *J Am Vet Med Assoc*. 2010; 236(3): 302–8. doi: 10.2460/javma.236.3.302
27. Corrada Y, Rimoldi I, Arreseigor S, Marecco G, Gobello C. Prolactin reference range and pulsatility in male dogs. *Theriogenology* 2006; 66(6/7): 1599–602. doi: 10.1016/j.theriogenology.2006.01.003
28. Bovens C, Tennant K, Reeve J, Murphy KF. Basal serum cortisol concentration as a screening test for hypoadrenocorticism in dogs. *J Vet Intern Med* 2014; 28(5): 1541–5. doi: 10.1111/jvim.12415
29. UNITSLAB.COM. The resource for conversion of SI units to conventional or traditional units used in laboratory and medical practice. <https://unitslab.com/node/136>. (8. 2. 2023)
30. WHO. Who expert committee on biological standardization. 26th report. Technical report series No. 565. Geneva: World Health Organization, 1975. https://iris.who.int/bitstream/handle/10665/41151/WHO_TRS_565.pdf?sequence=1 (8. 8. 2024)
31. Kustritz MVR. Clinical canine and feline reproduction: evidence based answers. Iowa: Wiley Blackwell, 2011: 75–7.
32. England G. Dog breeding, whelping and puppy care. Hoboken: Wiley-Blackwell, 2012.
33. Robert MA, Jayaprakash G, Pawshe M, Tamilmani T, Sathiyabarathi M. Collection and evaluation of canine semen- a review. *Int J Sci Environ Technol* 2016; 5(3): 1586–95.
34. Shalini I, Antoine D. Semen characteristics in German shepherd dogs. *Int J Curr Microbiol Appl Sci* 2018; 7(3): 2304–12. doi: 10.20546/ijcmas.2018.703.270

Diagnoza in zdravljenje idiopatske oligoastenoteratozoospermije (Oat) s kombinacijo klomifen citrata (Clomid®) in vitaminov E, C in B pri kavkaškem psu

S. A. Ubah, E. Zachariya, T. Barde, S. E. Abalaka, P. M. Omoike, U. J. Egedigwe, R. P. Christopher, C. A. Uba, B. M. J. Adah, E. C. Mbegbu, C. F. Oguejiofor

Izvleček: Dveletni kavkaški pes, težak 55,4 kg, je bil pripeljan v veterinarsko kliniko zaradi neplodnosti. Anamneza je pokazala, da se je v zadnjih osmih mesecih paril z desetimi psicami in nobene od njih ni oplodil. Klinični pregled, laboratorijske preiskave in ocena semena so bili opravljeni dvakrat v razmaku 60 dni. Kakovost semena je bila slaba, z nizko koncentracijo semenčic z nizkim odstotkom gibljivosti in vitalnosti ter visokim odstotkom semenčic z morfološki nepravilnostmi. Iz kultur semena in brisa prepucija niso izolirali patogenih bakterij. Ultrazvočna preiskava skrotuma ni ugotovila degeneracije mod. Hormonska ocena seruma pred zdravljenjem je pokazala normalne koncentracije folikel stimulirajočega hormona (FSH), luteinizirajočega hormona (LH), testosterona, tiroksina (T4), trijodotironina (T3), prolaktina in kortizola v mirovanju ter nizko raven bikarbonata. Pri hematološki preiskavi je bila ugotovljena levkocitoza. Na podlagi anamneze, kliničnega pregleda in laboratorijskih preiskav je bila postavljena diagnoza idiopatske oligoastenoteratozoospermije (OAT). V literaturi je opisano 60-dnevno zdravljenje idiopatske OAT pri kavkaškem psu s peroralnim klomifen citratom (Clomid®) in z dodatki vitaminov E, C in B. Opaženo je bilo izrazito izboljšanje parametrov semena v 90 dneh od začetka zdravljenja. Povečal se je volumen semena, povečale so se gibljivost, vitalnost in koncentracija semenčic, odstotek semenčic z morfološki nepravilnostmi pa se je zmanjšal.

Ključne besede: oligoastenoteratozoospermija; pasja neplodnost; klomifen citrat; vitamini