

## ***In VIVO* EFFECT of AMPICILLIN, ENROFLOXACIN, COLISTIN, and SULFONAMIDES on SPERM PARAMETERS in BREEDING ROOSTERS**

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### ABSTRACT

The present study aimed to investigate the effect of commonly prescribed antibiotics on sperm parameters in breeding roosters. Twenty adult subjects were divided on the basis of their response to the dorso-abdominal massage into four experimental groups (G1: 30 mg/kg of ampicillin, G2: 10 mg/kg of enrofloxacin, G3: 2.5 mg/kg of colistin, and G4: 140 mg/kg of a sulfonamides association (sulphaquinolaxine sodium 150 mg, sulphamethazine sodium 70 mg, sulphadiazine sodium 70 mg). The measurements were performed at (T0) (before treatment), (T3 and T9) (representing 3 and 9 days of treatment) by Computer Aided Sperm Analysis (CASA). All antibiotics did not affect ejaculate color, pH, seminal fluid viscosity, and agglutination. Ampicillin increased slightly sperm volume at T3 (0.31±0.19) with no significant change at T9 (0.30±0.15). No significant effect was observed at T3 on sperm count (224.05±41.9), viability (94±2.08), and total motility (TM) (57%). However, progressive motility (PM) decreased slightly on T3 (36.92%). Enrofloxacin and colistin induced a significant decrease in sperm volume (0.40±0.10, 0.16±0.08), count (2.13±0.27, 0.65±0.33), viability (81.33±5.45, 37.33±18.67), TM (54.95%, 46.91%), and PM (31.24%, 23%) on T3. On T9 some parameters were improved to be closer to those observed at T0. Ampicillin, colistin, and enrofloxacin decreased significantly all (CASA) kinematic parameters. The highest impact was observed on the third day of treatment (T3). Sulfonamides decreased sperm volume and gametes concentration but enhanced significantly sperm viability, total motility TM, progressive motility PM, and all kinematic parameters.

**Keywords:** Antibiotics, Breeders, Computer-Aided Sperm Analysis, Kinematics Parameters, Poultry, Reproduction, Sperm toxicity

### INTRODUCTION

The reproduction and health status of the breeding rooster are important factors in the success of the broiler production industry, and it is mandatory to monitor semen quality routinely to evaluate male fertility performances. In this respect, it is well known that sperm concentration and motility are key factors concerning male fertility outputs; insufficient sperm concentrations and decreased motility could seriously affect fertilizing ability (Aly and Khafagy, 2014). According to Sun *et al.* (2019), in the poultry industry, around 5 to 12 % are eliminated from the breeding program because of low sperm motility (MOT). Other parameters such as curvilinear velocity (VCL), straight-line velocity (VSL), amplitude lateral head displacement (ALH), average path velocity (VAP), viability, sperm concentration, and percentage of deformed or dead sperm, are some of the crucial parameters strongly related with individual male Fertility (Tefay *et al.*, 2020). These parameters are usually used in the selection of roosters in artificial insemination programs.

The male reproductive system is highly susceptible to some stress conditions, various technological factors (temperature, humidity, density, light intensity, and nutrition) in addition to noxious influences, such as oxidative stress, diseases, and even drugs (Tudorache *et al.*, 2018; Martins *et al.*, 2021). Antimicrobials, which are particularly misused, are one of the potentially harmful factors that could affect fertility, in addition to bio-resistance and antibiotic residues that can have adverse impacts on human health (Ronquillo and Hernandez, 2017). Antibiotics are generally used in poultry breeding to prevent and treat bacterial infections in the birds. These various bacterial infections can cause multiple diseases inducing a high mortality rates and decrease the overall productivity of the flock resulting in massive economic losses for farmers. Previous studies have demonstrated that antibiotics, including Tetracycline derivatives, Fluoroquinolones,

Aminoglycosides, Beta-lactams and more, used in different species have detrimental effects on fertility (Nudell *et al.*, 2002; Brezina *et al.*, 2012; Millsop *et al.*, 2013; El-Maddawy and Bogzil, 2015; Samplaski and Nangia, 2015; Semet *et al.*, 2017). They can affect spermatogenesis and sperm parameters in humans and animals (Khaki, 2015). Currently, their use as growth promoters and as a preventive antimicrobial measure is a widespread strategy to increase broiler breeder performances in Algeria and many other countries, despite its prohibition (Ashour *et al.*, 2020).

In the last decade, antibiotic therapy has attracted much attention from research teams and great effort has been devoted to studying their relation to male infertility. Serious negative effects of some Quinolone members have been reported in animal species such as bucks with a significant alteration in sperm count, viability, gametes motility and testicular activity (Yücel *et al.*, 2021). Testis apoptosis, with a significant decrease in testicular weight and testosterone level, is particularly reported (Abd-Allah *et al.*, 2000; Khaki *et al.*, 2008; Zobeiri *et al.*, 2013). Similarly, Aral *et al.* (2008) reported that enrofloxacin increases sperm cell abnormalities with a disruption of spermatogenesis and sperm motility in mice. Aminoglycosides (gentamycin, neomycin, and streptomycin) stand as a mainstay of antibacterial therapy against serious Gram-negative and Gram-positive bacterial infections. Previous studies investigated their effects on sperm parameters and male reproductive tissue. Gentamycin can induce oxidative stress in the male reproductive tract causing spermatogenesis damage. In rats, at high doses, it decreases sperm motility and increases sperm abnormality (Zahedi *et al.*, 2010, 2012). Similarly, previous observations have demonstrated that tetracycline and doxycycline induce reproductive toxicity in male rats (Hargreaves *et al.*, 1998; Farombi *et al.*, 2008; Elzeinová *et al.*, 2013). Administration of tetracycline irreversibly reduces viability and sperm motility *in vitro* and *in vivo*. It causes a decrease in testicular and epididymal weight, sperm counts, and an increase in

abnormal sperm morphology, as well as the induction of oxidative stress. Elzeinová et al. (2013) argue that in addition to the well-known immediate effect of tetracycline on the reproductive system in mammals, some deleterious effects may persist long after antibiotic exposure.

Concerning avian species, to the best of our knowledge, no previous studies are reporting the antibiotics' potential effects on living rooster sperm parameters to understand the harmful impacts on the reproductive system. Even if roosters represent only 10% of the breeding herd, they contribute to 50% of the genetic load, progeny and fertility outputs. Hence, this current work aimed to investigate *in vivo* effects of commonly prescribed antibiotics, ampicillin, enrofloxacin, colistin, and sulfonamide on breeding rooster sperm quality using Computer Aided Sperm Analysis (CASA) system, known to generate objective parameters.

**MATERIAL AND METHODS**

**Drugs**

The tested antibiotics are: ampicillin at 30 mg/kg (Neoampicline P® 20%, VETOPHARM PRO, Algeria), enrofloxacin 10 mg/kg (Baytril®, MED VET, Algeria), colistin 2.5 mg/kg (Colistin ACTi coli®, VETOPHARM PRO, Algeria), and sulfonamides (sulphaquinoxaline sodium 150 mg, sulphamethazine sodium 70 mg, sulphadiazine sodium 70 mg) at 140 mg/kg added to the drinking water daily (Coccidiopan®, AVICO ANIMAL HEALTH, Algeria). The drug selection was based on the most popular antibiotics used in poultry production. Animals received treatments in drinking water for nine consecutive days (9 days). The treatments were prepared every day just before administration. Ampicillin, enrofloxacin, colistin, and sulfonamides were provided to groups (G1, G2, G3, and G4) respectively.

**Animals and breeding conditions**

Twenty COBB 500 reproductive roosters (n=20) weighing 5-6 kg were used in the current study. The sexually mature males (45 weeks old) were provided by S.A.R.L Group SALEM Avicole, Biskra Algeria. Animals were housed in cleaned, disinfected, and equipped large cages (180cm length x 80cm width) under a 16 L/8 D photoperiod (light intensity = 60 Lux) with adequate ventilation and humidity. A standard commercial breeder diet, that met nutrient requirements (corn 55 %, soybean meal 30 %, wheat bran 10 %, calcium 2 %, phosphorus 1.2 %, soya oil 0.5 %, salt 0.25 %, sodium bicarbonate 0.15 %, Vitamin and Mineral complex 1 %) was provided at 140 g/day/animal, and water was allowed *ad libitum*.

The present study is consistent with the international guidelines of animal care and use in research and teaching (NRC, 2011). Animals were carefully manipulated during the sperm collection, carried out by abdominal massage.

**In Vivo experiment**

Animals were randomly divided into four groups each consisting of five roosters that were selected and identified according to their response to the dorso-abdominal massage. Semen donors were maintained at a strictly controlled temperature (20±1 °C). Each group was subjected to three semen collections carried out by the same operators and under the same conditions to minimize animal stress, just before treatment (T0), after 3 days (T3), and after 9 days of treatment (T9).

**Fresh Semen Evaluation**

Sperm was collected by abdominal massage as described by (Burrows and Quinn, 1937). The conventional semen quality traits were performed immediately (volume, pH, color, and viscosity); the volume was measured by direct assessment in a graduated vial; a tape test was used to analyse sperm pH according to the color scale; viscosity and color were subjectively observed; an optical microscope was used to estimate agglutination; sperm viability was determined by eosin-nigrosine staining (5% eosin, 10% nigrosine).

**Kinematic Parameters Assessment**

Various sperm motility parameters were assessed by a Computer-Aided Sperm Analyser (CASA) (Sperm class analyser, SCA Microptic, S.L., Version 3.2.0, Barcelona, Spain) at a 10X field using a phase-contrast microscope. Aliquots were diluted (1/16 ratio) to facilitate image capture and to avoid overlapping of spermatozoa cells, 3 video fields were considered at each analysis.

The measured parameters were: sperm concentration, total motility (TM%), progressive motility (PM%), curvilinear velocity (VCL µm/s), straight-line velocity (VSL µm/s), average path velocity (VAP µm/s), the amplitude of the lateral head displacement (ALH µm), linearity (%) [LIN = (VSL / VCL) x 100] and frequency to which the sperm head crosses the mean trajectory (beat-cross frequency [BCF]/Hz).

**Statistical analysis**

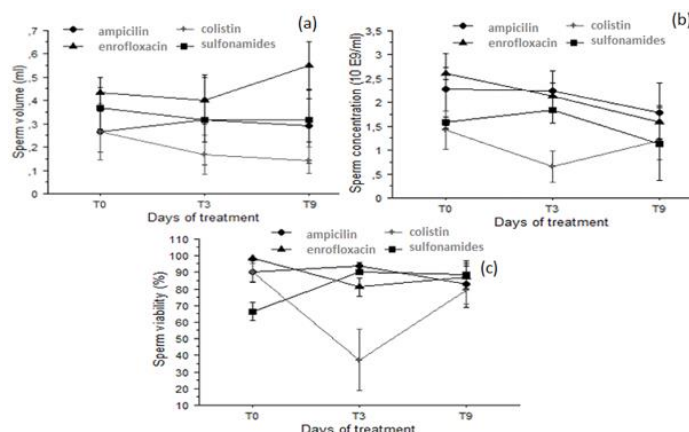
The results are expressed as mean ± SEM. The data were analyzed using Statview 4.02 software (Abacus Concepts Inc., Berkeley, CA, USA). Differences in parameters were determined using a one-way ANOVA, followed by Fisher's test. Values were considered significant when P < 0.05.

**RESULTS**

**Fresh Semen Traits**

There was no significant difference (P < 0.05) between the pre-treatment samples and those of T3 and T9 concerning sperm color, pH, seminal fluid viscosity and agglutination.

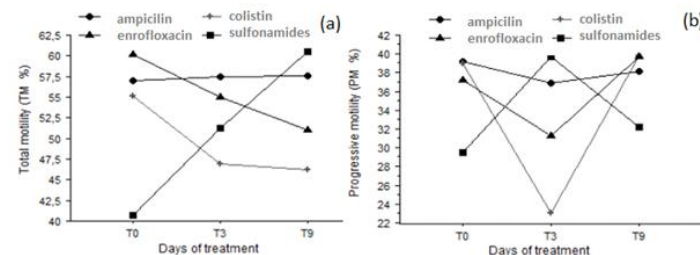
As shown in Figure 1a, after (T3), colistin, enrofloxacin and sulfonamides decreased significantly (P < 0.05) sperm volume (0.167±0.08 ml, 0.400±0.10 ml, 0.317±0.09 ml respectively) when compared to (T0) (0.267±0.12 ml, 0.433±0.06 ml, 0.367±0.08 ml). Except for sulfonamides, the values lightly increased at T9, especially for enrofloxacin (0.533±0.06 ml). Ampicillin showed no significant effect (P < 0.05) on sperm volume, which remained relatively stable during the study period. Figure 1b shows that sperm concentration was adversely affected by all antibiotic treatments. Values were significantly (P < 0.05) lower at T9 compared to T0 except for colistin, where after an initial decrease at T3, values raised at T9 to be closer to those of T0. Colistin and enrofloxacin reduced significantly (P < 0.05) gametes viability after 3 days of treatment with enrofloxacin affecting dramatically this parameter; on T9, gametes viability raised again to be similar to T0. Interestingly, as for sperm concentration, sulfonamides enhanced significantly spermatozoa viability to reach the highest values at T3 and T9 (Figure 1c).



**Figure 1** Effects of ampicillin, colistin, enrofloxacin, and sulfonamides on rooster sperm volume (1a), sperm concentration in billion (10<sup>9</sup>/ml) (1b), and viability (1c) on days 0 (T0), 3 (T3), and 9 (T9) of treatment. Values are presented as Mean (±SEM).

**Sperm Motility Parameters**

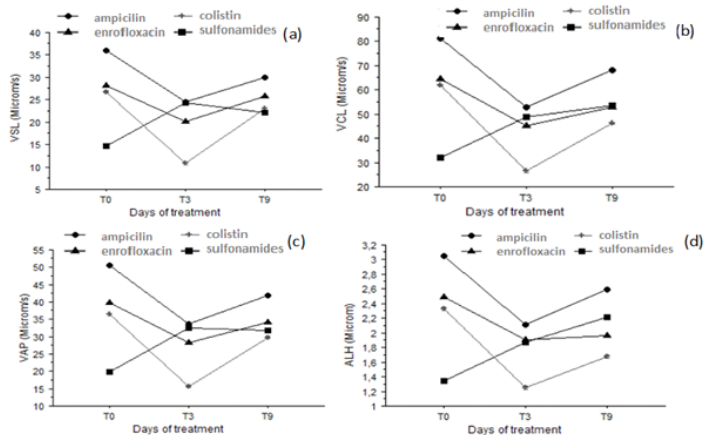
The results showed that the tested antibiotics presented varying effects on sperm motility (Figure 2). Total motility (TM %) expressing the percentage of total moving spermatozoa regardless of the quality of the movement (Figure 2a) showed no significant effect with ampicillin (57% on T3 compared to 56.95% on T0), whereas progressive motility (PM %), representing forward spermatozoa (Figure 2b), was slightly affected on T3 and T9 (36.92%, 38.17% respectively compared to 39.15% on T0). The lowest percentages for TM and PM were observed on T3 in colistin (46.91 % and 23 %, respectively) and enrofloxacin (54.95 %, 31.24 %, respectively) when compared to T0. Sulfonamides enhanced total sperm motility with TM% increasing regularly during the study period.



**Figure 2** Effects of ampicillin, colistin, enrofloxacin, and sulfonamides on total sperm motility (TM %) (2a) and progressive motility (PM %) (2b) at days 0 (T0) 3 (T3) and 9 (T9) of treatment. Values are presented as Mean (±SEM).

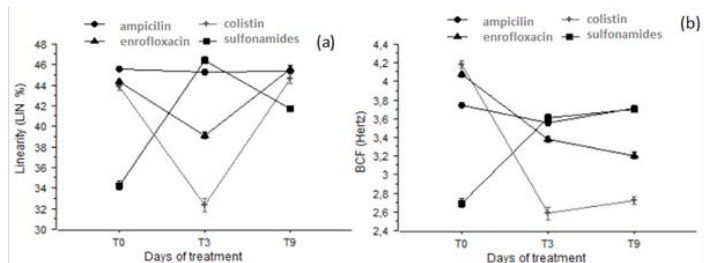
Sperm Kinematic Parameters

The effects of tested antibiotics on sperm kinematic parameters are represented in Figures 3 and 4. The results indicated that ampicillin, colistin, and enrofloxacin decreased significantly ( $P < 0.05$ ) all CASA motile variables including VSL (Figure 3a), VCL (Figure 3b), VAP (Figure 3c), ALH (Figure 3d), LIN (Figure 4a), and BCF (Figure 4b), with colistin showing the most important adverse effects. The highest impact was observed on (T3); and on T9, values increased but remained lower than T0.



**Figure 3** Effects of ampicillin, colistin, enrofloxacin, and sulfonamides on straight-line velocity (VSL) (3a), curvilinear velocity (VCL) (3b), average path velocity (VAP) (3c) and amplitude of the lateral head displacement (ALH) (3d) on days 0 (T0), 3 (T3), and 9 (T9) of treatment. Values are presented as Mean ( $\pm$ SEM).

No significant difference was observed in the group treated with ampicillin concerning LIN (Figure 4a) and BCF (Figure 4b). However, the most spectacular amelioration was expressed in roosters treated by sulfonamides with a significant enhancement ( $P < 0.05$ ) of all kinematic parameters including velocities (VSL, VCL, and VAP), ALH, and BCF on T3 and T9 of treatment compared to T0. Except for LIN that was increased significantly ( $P < 0.05$ ) on T3 ( $46.41 \pm 0.32$ ) compared to T0 ( $34.26 \pm 0.45$ ) and slightly decreased on T9 ( $41.67 \pm 0.26$ ).



**Figure 4** Effects of ampicillin, colistin, enrofloxacin, and sulfonamides on linearity (LIN) (4a) and beat-cross frequency (BCF) (4b) on days 0 (T0) 3 (T3) and 9 (T9) of treatment. Values are presented as Mean ( $\pm$ SEM)

DISCUSSION

Extensive and inappropriate use of antibiotics leads to many health problems worldwide. Particularly, previous research reveals that antibiotics have harmful effects on males impairing negatively sperm parameters during the treatment period (Ahmadi et al., 2016). This exposure to different antibiotics could decline male fertility both in humans and animals with effects on spermatogenesis or sperm function. This has been demonstrated in different species including rams, peccaries, mice, and rats when investigating how antibiotics could affect sperm quality (Tanyildizi and Bozkurt, 2003a, b; El Harouny et al., 2010; Santos et al., 2021). However, the spermatotoxicity of antibiotics in living breeding roosters is still unknown.

In the current investigation, we tested the effects of ampicillin, enrofloxacin, colistin, and both sulfaquinolone, sulfamethazine belonging respectively to beta-lactams, fluoroquinolones, polypeptides, and sulfonamides antibiotic families on rooster sperm. These drugs are indicated for the prevention and treatment of bacterial infections caused by susceptible gram-positive and gram-negative germs.

The present study showed that remarkable variations were found between four groups of antibiotics. The average volume was slightly increased on the 3<sup>rd</sup> day of ampicillin treatment. The opposite effect was observed with enrofloxacin and colistin, which decreased sperm volume, particularly after 3 days of treatment. As the ejaculate volume for a large part is determined by the production of seminal fluid, this heterogeneity could be related to variable antibiotics effects on reproductive secretory glands (Vicari et al., 2016).

Three days after the antibiotic therapy, enrofloxacin and colistin showed a significant reduction in sperm count, viability and sperm motility. The results are in agreement with those reported by Elsayy et al. (2018) who showed that the administration of enrofloxacin (18 mg/kg of body weight) to mature male rats causes a decrease in sperm motility and count; it increases total sperm abnormalities with some histopathological alterations in the reproductive organs. This is also reported by Aral et al. (2008) who suggest that the administration of enrofloxacin to male mice at 150 mg/kg would lead to a significant decrease both in sperm count and sperm motility with increased abnormal spermatozoa rate. Similar results are reported with fluoroquinolones revealing negative effects on testis tissue, a significant decrease in sperm count, viability, motility, and increased apoptotic cells in male rats (Ebadimanis et al., 2018).

Regarding Polypeptide antibiotics, it is showed previously that colistin causes a variety of adverse effects, particularly in rats with decreased sperm motility and increased sperm abnormality, overexpression of oxidative stress, apoptosis, and autophagy expression in testis (Aksu et al., 2018, 2020; Bouchicha et al., 2022). These negative effects associated with some antibiotics could be related to a decline in anti-oxidant enzymes through the production of reactive-oxygen-species, disorder in proliferation cells in the tubules, a toxic effect on sperm cell membranes and reproductive hormones (Drobnis and Nangia, 2017; Manas and Najafi, 2017). However, Qadeer et al. (2013) suggest that colistin in combination with penicillin does not deteriorate semen quality.

The ampicillin treated group showed a significant decrease in PM and gametes velocities and sperm count after 3 days of treatment. However, no significant effect was observed concerning sperm viability, TM, and LIN. Ampicillin presents fewer negative effects on sperm motility parameters in comparison with enrofloxacin and colistin. The effects of ampicillin were previously reported by Gupta et al. (2013), suggesting reversible infertility in male rats. Raji et al. (2006) reported that ampicillin caused a significant decrease in sperm counts, motility, and gametes viability. Additionally, there is a significant reduction ( $p < 0.05$ ) in the weight of the testes, epididymis, seminal vesicles, and prostate glands and a significant decrease in testosterone levels. The same results were found by Elsayy et al. (2018) who suggest that rats injected with ampicillin at a dose of 40 mg/kg express reduced testes, epididymis, and accessory sex organs weights, with a negative change in sperm characters (sperm count and motility), and increased sperm abnormalities. According to Gupta et al. (2013), the reduction of sperm motility, count, and weight of sexual organs could be attributed to decreased fructose and protein levels that affect glycoproteins secreted by the epididymis.

A wide variety of sulfonamides is used to treat bacterial infections and to prevent coccidiosis (Foote and Salisbuby, 1948). Several sulfonamides are combined with trimethoprim to potentiate their effect and exhibit a broader antibacterial spectrum (Plumlee, 2004). In our study, sulfonamides affected positively sperm parameters. All sperm velocities have expressed the same tendency with a significant increase during the study period. A positive effect on TM, PM, and viability was also observed. However, a marked reduction in sperm volume and sperm count on the 9<sup>th</sup> day of treatment was observed. This antibiotic was able to enhance sperm motility probably through the elevation of testosterone levels as previously suggested by Tanyildizi and Bozkurt (2003b) who used a therapeutic dose of trimethoprim/sulfamethoxazole in rams, and showed that the percentage of motile sperm is higher compared to the control group. Nevertheless, sperm concentration and total sperm count are significantly decreased similar to the current results. In humans, sulfamethoxazole and trimethoprim association improve men's semen quality with confirmed prostatitis (Drobnis and Nangia, 2017). However, Salarkia et al. (2017) reported that rats treated with a combination of sulfamethoxazole and trimethoprim showed a significant decrease in the percentage of sperm number, motility, viability and testes structural abnormalities at high doses. Other reports showed that some sulfonamide derivatives harm ejaculate volume, sperm concentration, total sperm count, semen viscosity, and sperm motility in humans (Helstrom and Sikka, 2009). Similar results are reported in rats by Schlegel et al. (1991) about a spermatotoxic effect of sulfasalazine on sperm quality. They showed particularly an overexpression of oxidative stress, which in turn might act as a possible mechanism of male-induced infertility (Alonso et al., 2009).

CONCLUSION

The results of the current study provide the first information on the potential effects of antibiotics on vital rooster sperm parameters. They showed that ampicillin presented fewer negative effects when compared to enrofloxacin and colistin. These two antibiotics caused a significant decrease in sperm volume, count, viability, TM and PM as well as all CASA motile parameters after 3 days of treatment. The results highlighted the effectiveness of sulfonamides to enhance sperm motility but have a negative impact on sperm volume and concentration. There is a lot to know about the interaction between sperm quality and different classes of antibiotics. The scarcity of information regarding the subject prompts us to carry out further investigations particularly concerning the effect on fertility outputs when using antibiotics in poultry breeding farms.

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