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Study of the Antibiotics Effect on Sperm Parameters in the Breeding Rooster

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In front of the Jury committee composed of:

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Abstract

Antibiotics have become an integral part of modern medical treatments in humans and animals, but their potential effects on male reproductive health remain an area of concern. The present thesis aims to investigate and elucidate the impact of commonly prescribed antibiotics on sperm parameters in breeding roosters, that to our knowledge, no similar studies have been conducted before; focusing on both *in-vitro* and *in-vivo* settings. This study sheds light on the effects of oxytetracycline, enrofloxacin, erythromycin, ampicillin, colistin, tylosin, and sulfonamides on sperm movement characteristics and viability. Semen from forty Cobb 500 ($n = 40$) reproductive roosters, 45-week-old, weighing 5-6 kg was collected (using Burrows and Quin method) and evaluated for different parameters. Neat semen samples were handled immediately after collection. The sperm volume and PH were evaluated by graduating tube and PH Test paper strips respectively. Whereas sperm count, viability, and all motile parameters (total and progressive motility (TM% and PM%) in addition to all sperm cells kinematics including velocities. [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] were assessed by CASA system (Computer-Aided Sperm Analyzer) after dilution. In the *in-vitro* phase of the study, only ejaculates with good quality (volume, mass motility, sperm concentration) were sampled and used. The semen donors were collected at a 3-day interval. The pooled sperm was immediately diluted 1/4 ratio (1 part of semen and 3 parts of physiological solution NaCl 0.9%) to protect and preserve the fresh semen quality and the motility of spermatozoa, then transferred to the laboratory where it was diluted again (1/16 ratio) and divided into eight groups. A control group without antibiotics diluted using 0.9% NaCl and treated groups using varying concentrations of oxytetracycline, erythromycin, tylosin, ampicillin, enrofloxacin, colistin, and sulfonamide solutions (30 µg/ml, 15 µg/ml, 100 µg/ml, 30 µg/ml, 5 µg/ml, 10 µg/ml and 300 µg /ml of 0.9% NaCl respectively). Motility parameters were assessed through advanced analyses after 0, 1, 2, 3, 4, 5, 6, and 24 hours of incubation at 37ºC. Moving to the *in-vivo* investigation and based on the treatment, the breeding roosters were divided into seven groups with five (n=5) individuals each. They were respectively received therapeutic doses of oxytetracycline (40 mg/kg/bwt), erythromycin (20 mg/kg/bwt), tylosin (20 mg/kg/bwt), ampicillin (30 mg/kg/bwt), enrofloxacin (10 mg/kg/bwt), colistin (2.5 mg/kg/bwt) and sulfonamides (140 mg/kg/bwt) for 9 consecutive days via drinking water that was prepared each day just before its administration. Each group was subjected to 3 semen collections: before treatment (control T0), 3rd day (T3), and 9th day (T9) of treatment. Our in-vitro results revealed that spermatozoa were particularly sensitive to almost all antibiotics except for enrofloxacin, which showed a positive relationship with sperm kinematics. In the in-vivo study, enrofloxacin, colistin, tylosin, and oxytetracycline caused a significant negative effect on motile parameters including velocities compared to ampicillin-treated samples. Consequently, *in-vitro*, most antibiotics appear not suitable in cryopreservation extenders contrary to enrofloxacin. The drug was highly tolerated by the rooster's sperm cells by enhancing their motility and prolonging their survival by up to 6 hours compared to the other antibiotics. The negative effect of tylosin on sperm motile parameters was greater than erythromycin although both drugs are macrolides. The results highlighted the *in-vivo* effectiveness of sulfonamides and erythromycin on sperm movement characteristics. Both antibiotics considerably improve total and progressive motility, viability as well as all kinematics.

Key words: Antibiotics, Semen, Kinematic Parameters, Breeding Rooster

Résumé

Les antibiotiques sont devenus une partie intégrante des traitements médicaux modernes pour les humains et les animaux, mais leurs effets potentiels sur la santé reproductive masculine restent une préoccupation. La présente thèse vise à étudier et élucider l'impact des antibiotiques couramment prescrits sur les paramètres spermatiques chez les coqs reproducteurs, pour lesquels, à notre connaissance, aucune étude similaire n'a été réalisée auparavant ; en se concentrant à la fois sur les paramètres in vitro et in vivo. Cette étude met en lumière les effets de l'oxytetracycline, de l'enrofloxacin, de l'érythromycine, de l'ampicilline, de la colistine, de la tylosine et des sulfamides sur les caractéristiques du mouvement des spermatozoïdes et leur viabilité. Les échantillons de sperme de quarante coqs reproducteurs Cobb 500 (n = 40), âgés de 45 semaines et pesant entre 5 et 6 kg, ont été recueillis (à l'aide de la méthode de Burrows et Quin) et évalués pour différents paramètres. Les échantillons de sperme non dilués ont été manipulés immédiatement après la collecte. Le volume de sperme et le pH ont été évalués à l'aide d'un tube gradué et de bandelettes de papier indicateur de pH, respectivement. Tandis que le nombre de spermatozoïdes, leur viabilité et tous les paramètres de motilité (motilité totale et progressive (TM% et PM%), ainsi que toutes les cinématiques des cellules spermatiques, y compris les vitesses (vitesse curviligne (VCL µm/s), vitesse en ligne droite (VSL µm/s), vitesse moyenne du trajet (VAP µm/s), l'amplitude du déplacement latéral de la tête (ALH µm), linéarité (LIN% = (VSL / VCL) x 100) et la fréquence à laquelle la tête du spermatozoïde croise la trajectoire moyenne (fréquence de croisement du battement [BCF]/Hz]), ont été évaluées à l'aide du système CASA (analyseur de spermatozoïdes assistée par ordinateur) après dilution. Dans la phase in vitro de l'étude, seuls les éjaculats de bonne qualité (volume, mobilité en masse, concentration en spermatozoïdes) ont été échantillonnés et utilisés. Les donneurs de sperme ont été prélevés à un intervalle de 3 jours. Le sperme combiné a été immédiatement dilué dans un rapport de 1/4 (1 part de sperme et 3 parts de solution physiologique NaCl à 0,9 %) pour protéger et préserver la qualité du sperme frais et la mobilité des spermatozoïdes, puis transféré au laboratoire où il a été dilué à nouveau (rapport de 1/16) et divisé en huit groupes. Un groupe témoin sans antibiotiques a été dilué à l'aide de NaCl à 0,9 % et des groupes traités ont été traités avec des concentrations variables de solutions d'oxytetracycline, d'érythromycine, de tylosine, d'ampicilline, d'enrofloxacin, de colistine et de sulfamides (respectivement 30 µg/ml, 15 µg/ml, 100 µg/ml, 30 µg/ml, 5 µg/ml, 10 µg/ml et 300 µg/ml de NaCl à 0,9 %). Les paramètres de motilité ont été évalués par des analyses avancées après 0, 1, 2, 3, 4, 5, 6 et 24 heures d'incubation à 37°C. Passant à l'investigation in vivo et en fonction du traitement, les coqs reproducteurs ont été divisés en sept groupes, comprenant chacun cinq individus (n = 5). Ils ont respectivement reçu des doses thérapeutiques d'oxytetracycline (40 mg/kg/poids corporel), d'érythromycine (20 mg/kg/poids corporel), de tylosine (20 mg/kg/poids corporel), d'ampicilline (30 mg/kg/poids corporel), d'enrofloxacin (10 mg/kg/poids corporel), de colistine (2,5 mg/kg/poids corporel) et de sulfamides (140 mg/kg/poids corporel) pendant 9 jours consécutifs via l'eau de boisson, qui était préparée chaque jour juste avant son administration. Chaque groupe a été soumis à 3 prélèvements de sperme : avant le traitement (témoin T0), le 3eme jour (T3) et le 9eme jour (T9) du traitement. Nos résultats in vitro ont révélé que les spermatozoïdes étaient particulièrement sensibles à presque tous les antibiotiques, à l'exception de l'enrofloxacin, qui a montré une relation positive avec la cinématique des spermatozoïdes. Dans l'étude in vivo, l'enrofloxacin, la colistine, la tylosine et l'oxytetracycline ont eu un effet négatif significatif sur les paramètres de motilité, y compris les vitesses, par rapport aux échantillons traités à l'ampicilline. Par conséquent, in vitro, la plupart des antibiotiques semblent peu adaptés aux diluants de cryopreservation, contrairement à l'enrofloxacin. Le médicament a été très bien toléré par les cellules spermatiques du coq en améliorant leur motilité et en prolongeant leur survie jusqu'à 6 heures par rapport aux autres antibiotiques. L'effet négatif de la tylosin sur les paramètres de motilité des spermatozoïdes était plus important que celui de l'érythromycine, bien que les deux médicaments soient des macrolides. Les résultats ont mis en évidence l'efficacité in vivo des sulfamides et de l'érythromycine sur les caractéristiques du mouvement des spermatozoïdes. Les deux antibiotiques améliorent considérablement la motilité totale et progressive.

Mots clés : Antibiotiques, Sperme, Paramètres Cinématiques, Coq Reproducteur

ملخص الاطروحة

المضادات الحيوية أصبحت جزءًا لا يتجزأ من العلاجات الطبية الحديثة في البشر والحيوانات، ولكن آثارها المحتملة على صحة الإنجاب لدى الذكور لا تزال مجالًا للقلق. تهدف أطروحة الدكتوراه الحالية إلى التحقيق وتوضيح تأثير المضادات الحيوية الموصوفة بشكل شائع على معايير الحيوانات المنوية في الديوك التكاثرية، والتي لا توجد دراسات مماثلة لها قد تم إجراؤها من قبل؛ مع التركيز على كل من الإعدادات المختبرية والحيوية. ألقت هذه الدراسة الضوء على تأثيرات الأوكسيتتراسيكلين، والإينروفلوكساسين، والإريثروميسين، والأمبيسلين، والكولستين، والتيلوسين والسلفونااميدات على خصائص حركة الحيوانات المنوية وحيويتها.

تم جمع السائل المنوي من أربعين ديكًا تكاثريًا من(n = 40) Cobb 500 ، عمرها 45 أسبوعًا، وتزن 5-6 كجم باستخدام طريقة Burrows وQuin وتقييمها من أجل معلمات مختلفة. تمت معالجة عينات السائل المنوي النقية فور جمعها. تم تقييم حجم الحيوانات المنوية ودرجة الحموضة بواسطة أنبوب التدرج وشرائط اختبار درجة الحموضة على التوالي. في حين تم تقييم عدد الحيوانات المنوية وحيويتها وجميع معلمات الحركة (الحركة الكلية والتقدمية TM ٪ وPM ٪) بالإضافة إلى جميع حركية الخلايا المنوية بما في ذلك السرعات. السرعة الخطية VCL (µm/s) وسرعة الخط المستقيم(VSL µm/s) ، ومتوسط سرعة المسار(VAP µm/s) ، وسعة الإزاحة الجانبية للرأس(ALH µm) ، والخطية LIN٪ (VSL / VCL) x 100) =والتردد الذي يعبر فيه رأس الحيوانات المنوية عن المسار المتوسط (تردد عبور الضربة [BCF]/Hz) تم تقييمها بواسطة نظام CASA محلل الحيوانات المنوية بمساعدة الكمبيوتر بعد التخفيف.

في المرحلة المختبرية من الدراسة، تم أخذ عينات من القذف ذي الجودة الجيدة (الحجم، والحركة الجماعية، تركيز الحيوانات المنوية). تم جمع مانحي السائل المنوي بفاصل 3 أيام. تم تخفيف الحيوانات المنوية المجمعة على الفور بنسبة (4/1) جزء واحد من السائل المنوي و 3 أجزاء من المحلول الفسيولوجيNaCl 0.9 ٪ (لحماية والحفاظ على جودة السائل المنوي وقدرة الحيوانات المنوية على الحركة، ثم نقلها إلى المختبر حيث تم تخفيفها مرة أخرى (نسبة 16/1) وتقسيمها إلى ثماني مجموعات. مجموعة تحكم بدون مضادات حيوية مخففة باستخدام NaCl ٪0.9 ومجموعات معالجة باستخدام تركيزات مختلفة من محاليل الأوكسيتتراسيكلين، والإريثروميسين، والتيلوسين، والأمبيسلين، والإينروفلوكساسين، والكولستين والسلفونامايد (30 ميكروغرام/مل، 15 ميكروغرام/مل، 100 ميكروغرام/مل، 30 ميكروغرام/مل، 5 ميكروغرام/مل، 10 ميكروغرام/مل و 300 ميكروغرام/مل من 0.9٪ NaClعلى التوالي. تم تقييم معلمات الحركة من خلال التحليلات المتقدمة بعد 0، 1، 2، 3، 4، 5، 6 و 24 ساعة من الحفظ عند 37 درجة مئوية.

بالانتقال إلى التحقيق الحيوي ووفقًا للعلاج، تم تقسيم الديوك التكاثرية إلى سبع مجموعات تضم خمسة (n = 5) أفراد لكل منها. تلقوا على التوالي جرعات علاجية من الأوكسيتتراسيكلين (40 ملغ/كغ/وزن الجسم)، والإريثروميسين (20 ملغ/كغ/وزن الجسم)، والتيلوسين (20 ملغ/كغ/وزن الجسم)، والأمبيسلين (30 ملغ/كغ/وزن الجسم)، وإنروفلوكساسين (10ملغ/كغ/وزن الجسم)، والكوليستين (2.5 ملغ/كغ/وزن الجسم) والسلفونااميدات (140 ملغ/كغ/وزن الجسم) لمدة 9 أيام متتالية عن طريق مياه الشرب التي يتم تحضيرها كل يوم قبل تناولها مباشرة. تم إخضاع كل مجموعة لثلاث تجميعات من السائل المنوي: قبل العلاج (التحكم T0)، اليوم الثالث (T3) واليوم التاسع (T9) من العلاج. كشفت نتائجنا في المختبر أن الحيوانات المنوية كانت حساسة بشكل خاص لجميع المضادات الحيوية تقريبًا باستثناء الإنروفلوكساسين الذي أظهر علاقة إيجابية مع حركيات الحيوانات المنوية. في الدراسة داخل الجسم الحي، تسبب الإنروفلوكساسين والكوليستين والتيلوسين والأوكسيتتراسيكلين في تأثير سلبي كبير على المعلمات الحركية بما في ذلك السرعات مقارنة بالعينات المعالجة بالأمبيسيلين. ونتيجة لذلك، يبدو أن معظم المضادات الحيوية في المختبر غير مناسبة في موسعات الحفظ بالتبريد على عكس الإنروفلوكساسين. وقد تم تحمل الدواء بشكل كبير من قبل الخلايا المنوية للديك من خلال تعزيز حركتها وإطالة بقائها لمدة تصل إلى 6 ساعات مقارنة بالمضادات الحيوية الأخرى. كان التأثير السلبي للتيلوسين على مؤشرات حركة الحيوانات المنوية أكبر من الإريثروميسين على الرغم من أن كلا الدواءين عبارة عن ماكروليدات. أبرزت النتائج فعالية السلفونااميدات والإريثروميسين داخل الجسم الحي على خصائص حركة الحيوانات المنوية. يعمل كلا المضادين الحيويين على تحسين الحركة الكلية والتقدمية بشكل كبير.

الكلمات المفتاحية: المضادات الحيوية، السائل المنوي، المعلمات الحركية، الديوك التكاثرية

Publications

1. **Mohammedi, L**., Messai, A., Touazi, L., & Iguer-Ouada, M. (2023). In Vitro and In Vivo Effect of Oxytetracycline on Sperm Parameters in Breeding Rooster. Cryoletters, 44(5), 291-298. https://doi.org/10.54680/fr23510110412

2. MOHAMMEDI, L., MESSAÏ, A., OUAMANE, H., BENCHARIF, S., TOUAZI, L., & IGUER-OUADA, M. (2024). In VIVO EFFECT of AMPICILLIN, ENROFLOXACIN, COLISTIN, and SULFONAMIDES on SPERM PARAMETERS in BREEDING ROOSTERS. *Journal of microbiology, biotechnology and food sciences*, e9607-e9607. https://doi.org/10.55251/jmbfs.9607

LIST OF ABBREVIATIONS:

CASA: Computer Aided Sperm Analysis

MOT: Sperm Motility

TM: Total Motility

PM: Progressive Motility

VSL: Straight-line Velocity

VCL: Curvilinear Velocity

VAP: Average Path Velocity

ALH: Amplitude of the Lateral Head Displacement

LIN: Linearity

BCF: Beat-Cross Frequency

STR: Straightness

AI: Artificial Insemination

ART: Assisted Reproduction Technology

ARB: Antibiotic Resistance in Bacteria

ARGs: Antibiotic Resistance Genes

DNA: Deoxyribonucleic Acid

RNA: Ribonucleic Acid

IM: Intramuscular Injection

IV: Intravenously Injection

OTC: Oxytetracycline

GTLS: Antibiotics Combination (Gentamycin Sulphate, Tylosin tartrate, Lincomycin Hydrochloride, Spectinomycin Hydrochloride)

ZZ (homozygous): the sex chromosomes in male birds

ZW (heterozygous): the sex chromosomes in female birds

GnRH: Gonadotropin-Releasing Hormone

FSH: Follicle-Stimulating Hormone

LH: Luteinizing Hormone

SST: Sperm Storage Tube

US: the United States

EU: the European Union

FAO: Food and Agriculture Organization

FDA: Food and Drug Administration

USDA: United States Department of Agriculture

OECD: Organization for Economic Cooperation and Development

WHO: World Health Organization

Lux (lx): Unit of illuminance (luminous flux per unit area)

NaCl 0.9%: Sodium chloride 0.9%

Mg/kg: Milligram per kilograms

μm/s: micrometer per second (unit for speed)

μg/ml: microgram per milliliter (unit of mass concentration)

Hz: Hertz (the standard unit of frequency)

Kcal: Kilocalorie

°C: degree Celsius

Mt: Metric ton (unit of weight)

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Introduction

The poultry sector represents a very important production system worldwide; it plays a crucial role in the socio-economic development and nutritional requirements of rural and peri-urban households, and it provides additional sources of income for millions of people in most countries (Conan *et al.* 2012; Rahman *et al.* 2021). Commercial Poultry production has become one of the world's leading and fastest growing industries in the last decades due to the high demand for poultry meat and eggs (Muhammad *et al.* 2020). There has been a markedly increased consumer interest in poultry meat, which constitutes a major source of animal protein. Along with chickens, some other poultry, such as turkey, duck, geese, quail, and pigeon, also gain popularity day by day (Rahman *et al.* 2021). Relatively, low and competitive prices compared to other meats, absence of cultural or religious obstacles in addition to nutritional qualities are the main factors explaining poultry products' attractiveness (Magdelaine *et al*. 2008).

One of the bases for this production is the reproductive efficiency of today's poultry. This, in turn, is due to their inherent reproductive physiology, intensive genetic selection, and advances in husbandry management (Scanes *et al.* 2020).

The object of any breeding program is to provide increased feed efficiency, faster growth, and ameliorate the genetic make-up of progeny by preserving genetic variance, for increasing productive performance along with descent appearance. The reproduction and health status of the breeding rooster is one of the most important factors in the success of the broiler production industry. The first days of life are key for the good development of the bird since good early growth promotes a better initial uniformity, which has a strong influence on fertility. Consequently, the chicks must leave according to the standards with a balanced quality of diet in the first six weeks. In 2011, Khan reported that deficiencies in some micronutrients essential for the proper functioning of metabolic processes within the testes decrease both sperm production and sperm quality. Raising roosters in the best conditions of well-being, with housing density are also of vital importance as well. Rigorous selection of the males is a significant component of a breeding program (Beranger; 2007).

The flock will get a good fertilization rate only when the breeder cocks have the correct skeleton development, testicular development, and evenness at various stages of age. The weight of the male is a critical factor that has the greatest correlation with the fertility of the flock (Gonçalves *et al.* 2015). Therefore, it is mandatory to ensure the proportion and the

appropriate degree of obesity of the cocks to effectively mate throughout the entire laying period. Fertility declines also with age; it represents one of many epigenetic factors that affect semen quality (Tabatabaei *et al.* 2010). Lagares *et al.* (2017) confirmed that in addition to the weight increase with age, testicular atrophy and impairment of sperm production seemed to be the main reason for the decrease in the rooster's fertility at 50 weeks of age. Moreover, temperature, photoperiod, and nutritional deficiencies are factors that can negatively affect semen production and thus must be controlled (Gonçalves *et al.* 2015).

A producer needs far fewer males than females to be retained for breeding stock. Although fertility rates are dependent on both sexes, this characteristic is most often related to male performance. Even roosters represent only 10% of the breeding herd; they represent 50% of the genetic load, progeny, and fertility outputs (Triques *et al.* 2019). On average, one male for 10 to 12 females for broiler breeders is optimum for good fertility, depending on light or heavy breeds.

There is evidence that male' fertility is a trait of major interest in the broiler industry; it is largely dependent on the genetics and selection of birds as an inherent trait. Already in 1999, Amann reported that selection for reproductive traits received low or zero priority. It was limited to culling males who were difficult to collect, or that provided "aqueous" semen. He suggested that with little or no selection for reproductive capacity or fertilizing potential of sperm, the number of offspring produced per elite male (or his progeny) probably would decline. According to Gonçalves *et al.* (2015), subjective visual selection is often used for the diagnosis of roosters as being unfit for reproduction, with those deemed as overweight or underweight, or those with too small a comb and/or too slightly pigmented, being removed. Reliance on visual diagnosis often results in the discarding of roosters suitable for reproduction but with unfavorable visual characters.

Therefore, the infertility diagnosis in poultry would be more accurate if sperm-quality information was evaluated. Thus, it should be an integral part of rooster management. Similarly, Novikova *et al* (2019) reported that enterprises maintain a high percentage of rooster rotation about 25-50%. In some herds, it can reach 100% of the livestock, which leads to a decrease in the profitability of production. Nowadays, in the modern poultry industry, in which artificial insemination (AI) is practiced, analysis of various sperm motility parameters is a basic part of selecting males with outstanding fertility rates and culling out males which have poor fertility performance (Donoghue, 1999). According to Sun *et al.* (2019), around 5

to 12% of males are eliminated from the breeding program because of low sperm motility (MOT). Other parameters such as sperm velocities, sperm count, viability, and spermatozoa morphology also have a great contribution to fertility rate (Tesfay *et al.* 2020).

As we have said before, a single male is responsible for fertilizing dozens of female birds. Thus, the production of high-quality semen is of major importance; especially the motile parameters that are probably the most common laboratory assays performed and one of the most important semen quality traits highly related to male fertility (Mocé and Graham 2008). Semen quality patterns have been analyzed by different methods; the most common one is the microscopic observation of the motility semen samples. However, recently computer-assisted sperm analysis (CASA) has allowed an objective and accurate approach for sperm motility assessment. It has been demonstrated to be a useful tool to assess the kinematic properties of individual spermatozoa in an ejaculate. This powerful tool represented a revolution in the production of seminal doses and the knowledge of reproductive biology. In addition to the use of CASA technology, the estimation of kinematics, including curvilinear velocity (VCL), straight-line velocity (VSL), average path velocity (VAP), linearity (LIN), straightness (STR), wobble (WOB), amplitude of lateral head displacement (ALH), and beat cross frequency (BCF), have been incorporated as novel parameters for reproducibility. They represent a crucial issue in the assessment of fertility in poultry and the potential selection of roosters for artificial insemination programs. (Valverde *et al.* 2020; Vincent *et al,* 2021).

Based on the information documented in (Fragoso *et al.* 2013), male chicken infertility has been associated with a decline in testicular weight, spermatogenesis impairment, and alteration of hormonal levels. It could be due to the high susceptibility of the male reproductive system to some stress conditions and noxious influences such as inflammations, diseases, oxidative stress, and even drugs (Martins *et al.* 2021). In the last decade, antibiotic therapy has attracted much attention from research teams and great efforts have been devoted to study their potential risks to both human and animal health.

Antibiotics are commonly prescribed as therapeutic agents, pre-emptive antimicrobial measures. Moreover, despite their prohibition, they are routinely used as growth promoters to increase broiler performances in several countries (Ashour *et al.* 2020). They represent a growing number of environmental contaminants leading to public health concerns. Because they are not completely metabolized in the body tissues of chicken; they get deposited in meat as parent compounds, and ultimately excreted through poultry droppings into the

environment, which unfortunately creates an antibiotic resistance in bacteria (ARB) via activation of antibiotic resistance genes (ARGs) leading to huge physical and economic losses (Muhammad *et al.* 2020).

In addition to the bio-resistance and antibiotic residues, they can affect sperm parameters and spermatogenesis by directly damaging germ cells in the testis or by inhibiting the function of the supporting Sertoli cells (Khaki 2015; Nudell *et al.* 2002). Previous research reported the evidence that reproductive drug toxicity is possible and considerable attention has been paid to their effects on male reproductive tract, exactly on sperm motility (Khaki *et al.* 2008; Connor *et al.* 2014). Millsop *et al.* (2013) have announced that antimicrobial agents, which are particularly misused, are one of the potentially harmful factors that may impair semen parameters, leading to temporary or persistent infertility. It depends on the dose and treatment duration (El-Harouny *et al.* 2010). It seems that the mechanism underlying their spermatotoxic effects has not yet been fully understood, as pointed out by Samplaski and Nangia (2015).

It is known that some of these commonly prescribed 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; Khaki, 2015; Samplaski and Nangia, 2015; Semet *et al.* 2017; Yücel *et al.* 2021). Previous studies investigated the tetracyclines' effects on sperm parameters and male reproductive tissue. Adelakun *et al* (2018) showed that this molecule could cause fertility damage to humans and is suspected to induce testicular toxicity in animals. In 1974, Timmermans' results reported that male rats' spermatogenesis was adversely affected by oxytetracycline at doses adjusted to human posology after 8 days of treatment. Minocycline, one of the tetracyclines' derivatives, is toxic to sperm at any concentration (Schlegel *et al.* 1991).

Similarly, the *in-vitro* study of Hargreaves *et al* (1998), which was designed to investigate the effects of commonly prescribed antibiotics on normozoospermic sperm parameters, demonstrated that tetracycline at concentrations as low as 2.5 µg/ml led to a significant dose-dependent inhibition in percent rapid-moving spermatozoa, curvilinear velocity (VCL), mean path velocity (VAP), and straight-line velocity (VSL). However, at 50 µg/ml, all spermatozoa were static. Nudell (2002), in his review, revealed that tetracyclines potentially affect motility via binding to mature spermatozoa. Furthermore, in 2008, Farombi

et al found that the administration of tetracycline to male rats caused a reduction in the sperm count, epididymal sperm motility, viability, and an increase in abnormal sperm morphology, as well as induction of adverse histopathologic changes in the testes through induction of oxidative stress. Elzeinová *et al.* (2013) argue that the treatment of adult outbred mice strain (CD1) by tetracycline and doxycycline led to long-lasting effects on reproductive organs and spermatozoa. In contrast, low-level leukocytospermia patients treated by doxycycline did not show statistically significant differences in the semen parameters (Hamada *et al*. 2011). However, they revealed a substantial improvement in sperm volume, sperm motility and concentration in addition to the number of normal sperm morphology (Skau and Folstad, 2003). Recently, oxytetracycline has been reported to be restricted in Saanen bucks during the reproduction period and antibiotics with fewer side effects on sperm should be preferred (Yücel *et al*. 2021).

Serious negative effects of some quinolone members have been reported in different animal species. A significant alteration in sperm count, gametes motility, viability, testicular activity, and an increase in sperm with damaged DNA was observed in Saanen bucks by Yücel *et al.* (2021). Testis apoptosis, with a significant decrease in testicular weight and testosterone level, is particularly reported by (Abd-Allah *et al*., 2000; Khaki *et al.*, 2008; Zobeiri *et al.*, 2013). The adverse effects of enrofloxacin on the reproductive system were mainly investigated in males to assess the impact of this antibiotic on fertility parameters. Aral *et al.* (2008) revealed that enrofloxacin increases sperm cell abnormalities with a disruption of spermatogenesis and sperm motility in mice. Similarly, it produces dose-dependent testicular toxicity in rats through dysregulation of spermatogenesis and the presence of necrotic debris in seminiferous tubules (Rungsung *et al*. 2016). Ciprofloxacin, one of the fluoroquinolones, can adversely influence fertility parameters in male rat. It significantly reduces sperm motility and concentration. It also increased the level of DNA fragmentation significantly with a clear reduction in sperm chromatin quality (Abd *et al.* 2018). On the opposite, a study carried out in 2008 by Mohammed Al-Nazawi, suggested that enrofloxacin at a therapeutical dose does not affect the sperm parameters in male chickens after slaughter including sperm motility, testes' weight, and the testicular concentration of testosterone.

Beta-lactams, stand as a mainstay of antibacterial therapy against serious bacterial infections. Previous studies investigated their effects on sperm parameters and male reproductive tissues (Karaman *et al.* 2019). Ampicillin, a broad-spectrum antibiotic belonging

to the penicillin group, was found to be impairing the fertility of males when used at the dose of 40 mg/kg (Raji *et al.* 2006). It reduced serum testosterone levels at a dose of 4mg/kg (Adesanya and Awobajo 2006). Gupta *et al* (2013) investigated the toxicity of ampicillin and sulphasalazine which are generally used as a combination in certain pathological conditions. They found that the drugs reversibly reduced the reproductive activity of rats separately, and even in combination. A similar kind of experiment was done in 2018 by EL-Sawy *et al.* who evaluated some pharmacodynamic effects of enrofloxacin and /or ampicillin. The obtained results showed a significant reduction in sex male organs weight, a decrease in sperm count, motility, and an increase in total sperm abnormalities. There were also a variety of side effects such as biochemical and histo-pathological alterations on reproduction organs. Furthermore, Hui *et al.* (2022) in their review suggested that penicillins including ampicillin were associated with an impairment of sperm viability at higher doses. It could adversely affect spermatogenesis or inhibit the fertilizing capacity of the spermatozoa (Darussalam 2007).

A wide variety of sulfonamides is used to treat bacterial infections and prevent coccidiosis (Foote and Salisbuby 1948). Many studies confirmed the potential risk of these drugs on males' fertility. Sulphasalazine, which has both anti-inflammatory and antibiotic properties, has been shown to have spermatotoxic effects. It decreased sperm counts, motility, and sperm morphology, as well as reduced serum testosterone levels (Schlegel *et al.* 1991). Salazopyrin (trade name for sulphasalazine) appeared to have a reversible toxic effect on the maturation of sperm cells, resulting in male infertility (Steeno 1984). Sulphapyridine (one of the sulpha drugs) caused a reversible reduction in male rats' fertility at doses between 125 and 450mg/kg. Fertility was decreased to 25.9% at the high dose after 5 weeks of treatment (Pholpramool *et al.* 1991); this effect was rapidly recovered by 3 weeks after drug withdrawal. Several sulfonamides are combined with trimethoprim to potentiate their effect and exhibit a broader antibacterial spectrum (Plumlee 2004). Samplaski and Nangia in their review (2015) reported that the trimethoprim–sulphamethoxazole combination reduced sperm motility by 34% in humans. By contrast, they announced that Merino and Carranza found no change in sperm quality parameters following 1 month of treatment. Drobnis and Nangia (2017) found that this association improves men's semen quality with confirmed prostatitis. However, Salarkia *et al.* (2017) reported that rats treated with sulfamethoxazole and trimethoprim showed a significant decrease in the percentage of sperm number, motility, viability, and testes structural abnormalities at high doses. According to Tumkiratiwong and Lerkchundhakriat (2011), the combined administration of pyrimethamine and sulfanilamide

potentiated temporal infertility effect in male Wistar rats. 5 days of treatment with tamsulosin induced a negative effect on sperm concentration, total sperm count, sperm motility, and semen viscosity in healthy men (Hellstrom and Sikka 2009). Similar results are reported in rats, 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).

Regarding Polypeptide antibiotics, previous studies showed that colistin could induce a variety of reproductive side effects, particularly in rats. It increased oxidative stress, sperm abnormality, apoptosis, and autophagy expression levels in the testis after 7 days of treatment. However, it decreased the sperm motile parameters (Aksu *et al.* 2018; Aksu *et al.* 2021). On the opposite, Qadeer *et al.* (2013) suggest that colistin in combination with penicillin does not deteriorate semen quality. In an *in vitro* study from 2008, subtilosin was established as a general spermicidal agent of human spermatozoa. All of the subtilosin concentrations tested reduced motility compared to the control samples. It impairs sperm forward progression in a dose-dependent manner (Sutyak *et al.* 2008).

Besides B-lactams, tetracyclines, quinolones, sulfonamides and polymyxines' spermatotoxicity, Macrolides also have been linked to male reproductive issues. They could impair spermatogenesis and sperm function in some manner (Sikka and Wang 2008). According to research results in various species, erythromycin and tylosin can have effects on male fertility that are generally reversible. They decreased the frequency of mitotic division in rat testes (Schlegel *et al.* 1991; Stearns and Turek 2013). Erythromycin had a significant decline in sperm viability, rapid moving spermatozoa, VAP, VSL, and VCL at concentrations >100µg/ml (Hargreaves *et al.* 1998). Similar results had been observed in an earlier study that showed that both erythromycin and tylosin depressed the motility of frozen thawed bovine spermatozoa stored at 5 C° with up to 1000 µg/ml of extender (Berndtson and Foote 1976). The same concentration reduced the motile parameters of koala's spermatozoa as it was suggested by Bodetti *et al*. in 2003.

On the opposite, a double-blind controlled trial in 78 men with asthenospermia demonstrated that erythromycin treatment had no significant effect on semen quality (Baker *et al.* 1984). Little published evidence about tylosin kinematic effects; it is usually used for cryopreservation of semen to control bacterial growth as an additive antibiotics combination (GTLS), containing gentamycin sulphate, tylosin tartrate, lincomycin hydrochloride, and spectinomycin hydrochloride. There it deteriorated the motility of *Camelus dromedarius*'

cooled stored semen (Ghoneim et al. 2022). However, it could be considered effective and sperm-safe for equine frozen semen and preserved the spermatozoal quality of extended buffalo bull semen for 3 days at 5°C (Akhter *et al.* 2008; Brito *et al.* 2022). Moreover, in a previous study, Gloria *et al.* (2014) showed that the combination of ceftiofur/tylosin had a negligible effect on cryopreserved bulls' spermatozoa. Furthermore, progressive motility (PM) was significantly higher compared to samples without antibiotics.

Aim of Study

Based on the information documented, fertility is a complex phenomenon, involving the interaction of two individuals and their gametes. In the broiler production industry, although fertility rates are dependent on both sexes, male and female, reproductive efficiency is most often related to male performance. Generally, one single bird is responsible for fertilizing dozen of females; therefore, the production of high semen quality is of great importance. It is well known that motile parameters are probably one of the most important sperm quality traits highly related to male fertility. Furthermore, sperm concentration, live/dead sperm, and morphology represent key factors concerning male fertility outputs.

The fertilizing ability of the semen can be affected by drugs through various mechanisms. They may directly and indirectly induce sexual dysfunction and spermatogenesis impairment in humans and animals. Although antibiotic treatment has been widely advocated to preserve or restore normal sperm parameters in urogenital infections, some of these commonly prescribed antibiotics have long been suspected of contributing to male infertility; they are found to have spermatotoxic effects and affect semen quality and quantity adversely. In this document, the antibiotics to be discussed are only antibacterial agents; the terms antibiotic and antimicrobial will be used interchangeably.

Concerning avian species, to the best of our knowledge, no previous studies have reported the potential effects of antibiotics on live roosters' sperm parameters. There is no data on its mechanism of action on the cocks' reproductive system. Therefore, the aim of this research was to emulate the situation in rooster breeding populations by investigating and comparing the *in-vivo* and the *in-vitro* effects of ampicillin, enrofloxacin, oxytetracycline, colistin, tylosin, erythromycin and sulfonamides on sperm kinematic parameters, motility, viability, and sperm count. The mechanism underlying the effects of these antibiotics has not been entirely understood. Further investigation is needed into the relative toxicity of antibiotics and the mechanisms by which antibiotics affect motility, spermatogenesis, and spermatozoal function.

First Section :
Literature Review

Chapter I: Poultry Sector

I.1. Background

Why poultry products are important?

The poultry market is positioned as one of the most important sectors due to its participation in the world's food security. Since 2017, poultry has overtaken pork as the most produced type of meat worldwide (Shahbandeh 2022). During the last several decades, it has played a vital role in fulfilling the daily protein requirements of humans through meat and eggs consumption that have become mass consumer products throughout the world. Their consumption has risen in virtually all countries and regions because of their increasingly preference in the human diet for a variety of reasons. Both products are very suitable for quick and easy home cooking and have several desirable nutritional and organoleptic properties. They rank high in terms of protein quality and vitamins with lower fat content.

Poultry meat, compared to red meats, contains less cholesterol and lower calories. Furthermore, it contains all the essential amino acids presently known to be required by consumers. Therefore, this meat represents a good foodstuff for a weight-control diet, especially for people who are physically less active. These qualities are the main factors explaining its attractiveness and consequently sophisticated professional poultry industry has become the order of the day (Prabakaran 2003). Moreover, Chicken egg is a favorite food of human daily consumption. It serves as a high quality nutrient resource and represent one of the best food option to assist with weight management (75 kcal per egg). While the yolk provides essential vitamins, minerals, antioxidants, and many vital healthy fats, the egg white contains multiple proteins that are essential for the strength and recovery of our muscles and tissues.

I.2. Poultry products production and consumption

Chicken meat showed the largest growth in absolute and relative terms since 2000. It was the most produced type of meat in 2020 with almost 40% of the global meat production (FAO 2021). In 2021-2022, the United States (US), the leader of poultry production in the world; topped the world ranking with 20.5 million metric tons. During the same year, China, Brazil, and the European countries (EU) represent also the largest broiler meat producers worldwide with 14.7, 14.5, and 10.8 million metric tons respectively (Roth *et al.* 2019; FAO 2019; Shahbandeh 2022). According to Statista estimates, the world's broiler meat production amounted to about 100.9 million metric tons in 2022 and it is predicted to increase by 2030

(Shahbandeh 2022). According to the official website of the United States government, poultry has become the most consumed livestock commodity in both the developed and developing world (Dohlman *et al*. USDA 2022). These robust growth rates in poultry consumption reflect its significant role in the diets of several populous countries, including China, India, and Pakistan…etc (OECD/FAO 2022). Shahbandeh (2022) projected the global consumption of poultry meat to amount to 153.85 metric Kilotons by 2031 up from 133.35 metric Kilotons in 2021. According to the same source, Chicken is incredibly popular in the United States (US). Americans consume the greatest amount of chicken every year almost 15.000 metric tons; followed by China and the European countries (EU) with approximately 12.000 and 11.000 metric tons respectively. The per capita consumption of poultry meat in the United States (US) has increased every year since 2010 to reach about 96 pounds in 2021, and it is forecast to increase slightly to 96.9 pounds by 2022. As demand for poultry products grew during the period from 2000 to 2021, global imports and exports increased. It is expected that poultry remain the world's largest imported and exported livestock commodity by volume over the next ten years (Miller *et al.* USDA 2022). Over the past two decades, global poultry imports rose an average of 4% a year; reaching 14.2 million metric tons in 2021, when Japan topped the list, and projected to amount to approximately 17.5 million metric tons by 2031 (Dohlman *et al*. USDA 2022). However, Brazil and the United States are the two top exporters of poultry meat in the world, at about 3.9 million metric tons, and 3.3 million metric tons respectively; followed by the European Union (EU) at 1.3 million metric tons.

According to Food and Agriculture Organization's data FAO (2022), global egg production has witnessed impressive growth steadily over the last decade; it reached 87 million tonnes in 2020. China ranked as the largest producing country With 30 million tonnes (38 percent of the world total). However, the United States of America (US), India, Japan, Indonesia, Brazil, Mexico, and Russia vary between 3 and 8 percent of the global hen egg production. Asia is considered the largest egg-producing region, with more than 64% of global output. Concerning global egg consumption, a large variation between countries was declared. According to the world Population Review (2022), Japan is the country that consumes more eggs than just about any other place on the planet. The average person in Japan consumes approximately 320 eggs per year, the equivalent of one egg per day using them in a wide variety of dishes such as rice, noodles, and pastries. The United States (US) is considered as one of the top consumers of eggs per year but not in terms of eggs eaten per

person. Paraguay is a surprising country that consumes many eggs every year, where the average person eats about 310 eggs per year.

In Algeria, with the changing eating habits and the global population growth, poultry meat production rose 1% year on year since 2014, to reach 290.863 metric tons by 2019. According to FAO's report (2019), the country ranked number 47 worldwide. It is overtaken by Ecuador at 312.386 metric tons and followed by Bangladesh at 259.912 metric tons and more poultry meat is thought to be produced in the future. Concerning poultry meat consumption according to 2019' FAO statistics, it reached 6.67kg per capita. Algeria has been ranked 119th among 161 countries in terms of interest rate on poultry meat consumption per capita.

What makes the broiler poultry industry grow so fast ?

According to Feed and Additive magazine' report in 2021, the poultry sector represent the fastest growing agricultural sub-sector, especially in developing countries (Yildiz *et al.* 2021). Chicken is the main breeding area; it represents more than 90% of the poultry sector. Other species including ducks, turkeys, and geese are also known as eminent species after chicken breeding. Population growth, urbanization, and income level growth are the main factors contributing to poultry sector grow and development. As we have said before, the industrialization of poultry is growing rapidly every year, and the innovative technological development used in poultry breeding makes farms more intelligent. Over recent decades, the FAO's programs have focused on animal genetics, food and feed efficiency, animal health and welfare, animal husbandry, disease control…etc to sustainable livestock production and guarantee sufficient food supplies. These factors have played a pivotal role for such a vast growth of this industry. Moreover, they have contributed to the large increase in bird size over the last 50 years (Agyare *et al.* 2018; Chowdhury and Morey 2019).

The term 'broiler' is mostly used for meat-producing poultry birds, especially chickens. Wei Zhai and Jessica Wells (2019) argued that the only purpose of broiler industries is to produce a big bird and a large amount of meat in a short amount of time. It will cost less to produce, which in turn creates an inexpensive product for the consumer. Algerian citizens as well as most of the planet's inhabitants often ask, "Why chicken are drastically bigger than those grown years ago?"; "Why chicken grow faster in poultry broiler industries?". The genetic selection and improved nutrition are practically the main reasons. In addition to many other factors that can have some positive effect on growth. For example, better environmental

control helps to lessen stress on birds through the grow-out phase. Overall, better management practices with automated housing and lighting programs contribute to the maximum growth of the modern broiler.

I.3. Control of avian pathogens

In general, national poultry health programs should be followed to prevent and control avian pathogens such as Mycoplasma gallisepticum, Mycoplasma synoviae, Salmonella enteritidis, Salmonella gallinarum, Salmonella pullorum, Salmonella typhimurium, Newcastle disease, and avian influenza. These programs contain biosecurity standards and key strategies for the prevention, monitoring, and control of these pathogens.

I.3.1. Vaccination

It is very important to consult a veterinarian and recommend a vaccination program that should be specific to the area where the chickens are raised. This program must respond to the health challenges of the region and use available vaccines, but attention must be paid to the methods used to reduce stress and promote animal welfare.

I.3.2. Medication

Prevention is still the best and most economical method of withstanding livestock diseases. In particular, it is the equivalent of a good biosecurity and vaccination programs. However, farms can be affected by many diseases. In this case, the use of a good drug at the right time can be crucial to combat these diseases. Sometimes the choice of drug, dose, and duration of treatment may be inadequate; therefore, laboratory-level sensitivity testing may be undertaken to assess antibiotics that will be effective against the pathogen.

However, in reality, poor vaccination management, lack of biosecurity on farms, self-medication, and unsubstantiated use of antibiotics by farmers is a wake-up call for all poultry sectors. Professionals in the field, including veterinarians, researchers, technicians, and breeders, must propose solutions to establish health barriers to prevent the occurrence of infectious diseases in the future. This cannot be done without strengthening this sector through a battery of organizational and legislative measures.

Chapter II: Antibiotic therapy

II.1. Antibiotics history, classification, and mechanism of action

Antibiotics are chemical substances that possess bactericidal or bacteriostatic properties. They have had a major effect on society by changing morbidity and mortality. Antimicrobials are generally produced by microorganism species to kill other microorganisms without being toxic to the person, animal, or plant being treated. They are extensively used to treat and prevent the disease in human and animal medicine, as well as to increase feed efficiency and improve the growth rate in livestock and poultry industries (Gao *et al.* 2012). During the subsequent two decades, several new drugs were discovered and developed one after another, providing clinicians with more therapeutic options for previously life-threatening diseases, leading to a golden age of antimicrobial chemotherapy. However, their wide use has introduced a new era in which clinicians have to face the emergence of drug-resistant pathogens (Saga and Yamaguchi 2009; Zaffiri *et al.* 2012).

Indeed, antibiotics can be categorized into distinct classes based on their molecular structures, spectrum of activity, and mode of action but rarely on their route of administration (injectable, oral, and topical) (Calderon and Sabundayo 2007). The beta-lactams, macrolides, tetracyclines, sulphonamides, glycopeptides, quinolones, and aminoglycosides are the most common classes of antibiotics based on chemical structure classification (Jafari Ozumchelouei *et al.* 2020). Generally, antibiotics within the same class show similar patterns of effectiveness, toxicity and allergic potential side effects (Etebu and Arikekpar 2016).

II.1.1. Tetracyclines

Tetracyclines group have been useful as therapeutic agents since their discovery in the last mid-century. They were first reported in the scientific literature in 1948 as a class of broad-spectrum antibiotics. Exhibiting activity against a wide range of both aerobic and anaerobic gram-positive and gram-negative bacteria, mycoplasmas, rickettsia, chlamydiae, and even some protozoa (Klein and Cunha 1995; Onal 2011). They are considered as bacteriostatic antimicrobials, they inhibit protein synthesis by preventing the attachment of aminoacyl-tRNA to the ribosomal acceptor (A) site (Chopra and Roberts 2001; Mahajan and Balachandran 2012). The initial first-generation natural products (chlortetracycline, oxytetracycline, and tetracycline), which all possess a chemically unstable C6 hydroxyl group in conjunction with a C11 carbonyl ketone; led the way to semisynthetic derivatives with better bioactivity profiles.

By the mid-1950s there were now three tetracyclines used clinically, their chemical names became in order of their discovery, chlortetracycline (aureomycin), oxytetracycline (terramycin), and tetracycline. Chlortetracycline was the first four-ringed compound discovered by the mycologist Benjamin Minge Duggar at Lederle laboratories under the supervision of Yellapragada Subbarow from Streptomyces aureofaciens, a golden yellow bacterium found in soil, this is why it was named aureomycin. It is bacteriostatic with a wide range of activity, including gram-positive bacteria, gram-negative bacteria, intracellular organisms, and protozoan parasites (Bennett *et al.* 2020). The drug was approved by the FDA for clinical use and was an immediate success in the clinic, saving countless lives against a broad spectrum of infectious diseases (Janser 2016). In veterinary medicine, it is commonly indicated for the treatment of bacterial gastrointestinal and respiratory infections in poultry (Mycoplasmosis CRD, non-specific enteritis, hexamitiasis, infectious sinusitis, and for the protection of animals in all cases of stress). Furthermore, it is used to treat conjunctivitis and infected wounds in different species (Nelson and Levy 2011).

Within a short time, other chemical companies were announcing their discoveries of new bio-prospected antibiotics. By 1950, Alexander Finlay and colleagues at Charles Pfizer laboratory had isolated the soil bacterium Streptomyces rimosus, which produced a compound with similarity in color to aureomycin, but it was slightly more water soluble and had better bioactivity, giving it a medical and competitive edge over aureomycin in the treatment of infectious diseases. The compound was named terramycin about terra, Latin for earth (Nelson and Levy 2011). Oxytetracycline (terramycin) is widely used as an additive to livestock feed because it stimulates weight gain in some domestic animals (Speer *et al*. 1992). The third compound is a broad-spectrum polyketide antibiotic produced by the Streptomyces genus of actinobacteria. The drug was obtained by Lloyd Conover who chemically modified aureomycin by treating it with hydrogen in the presence of a palladium metal catalyst (catalytic hydrogenation) and then synthesized the C-7 deschloro derivative named tetracycline. This new compound displayed higher potency, better solubility, and more favorable pharmacology than the other molecules in its class, leading to its FDA approval in 1954 (Janser 2016).

Years following, new derivatives were discovered while attempting to develop various tetracycline analogs for use with multi-drug resistant organisms. The ability to modify the side chains attached to the tetracyclic core to produce new compounds was the driving force behind the development of the second-generation semisynthetic tetracyclines, including

doxycycline, lymecycline, and minocycline. The increasing resistance mechanisms, led to the development of tigecycline in the early 2000s. It was the first of the third-generation tetracyclines. This glycylcyclines agent has activity against many tetracycline-resistant organisms such as Streptococcus pneumoniae, Staphylococcus aureus Enterobacteriaceae, and Clostridioides (Clostridium)… it shows the continued evolution of the tetracycline scaffold toward derivatives that has an increased potency with an improved pharmacokinetic and chemical properties (Nelson and Levy 2011; Heaney *et al.* 2019). In 2018, 2 new tetracyclines were approved: eravacycline and omadacycline. They were approved for the treatment of complicated intra-abdominal infections, bacterial pneumonia, and skin structure infections respectively (Heaney *et al.* 2019).

II.1.2. Quinolones

Quinolones and fluoroquinolones are classes of synthetic broad-spectrum antibiotics. They appear to be quite effective against both Gram-negative and Gram-positive bacteria especially Escherichia coli (E. coli) and Pseudomonas aeruginosa by stopping their ability to grow and infect the body's cells. These compounds work against two different enzymes (topoisomerase IV and DNA gyrase) that interfere with the synthesis of DNA replication. Fluoroquinolones, which were derived from quinolones by modifying their structure with fluorine molecule at the 6-position of the basic quinolone nucleus, are of value in certain infections. They are widely used in the treatment of infections in human medicine and in preventive and therapeutic treatment of farm animals (ruminants, pigs, and birds) (San Martin *et al.* 2010). Generally, they are used to treat respiratory, renal, and digestive diseases in poultry when there is a concern for multidrug resistance from other antibiotics (Martinez *et al.* 2006).

Enrofloxacin, the first fluoroquinolone approved for use in animals, was launched by the laboratory Bayer in 1991 under the trade name Baytril®. It has proven to be highly effective against a wide range of bacterial types (Martinez *et al*. 2006). It has a bactericidal effect by inhibiting the DNA gyrase activity in bacterial cells. According to Ebrahim and Afra (2011), the studies carried out on its pharmacological safety in laboratory animals such as rats and mice showed that enrofloxacin at therapeutic or even excessive doses does not exert significant effects on vital parameters: (blood composition, blood coagulation, renal function and central nervous system). However, previous studies found that enrofloxacin caused

various biochemical and physiological changes in sperm (Abd-Allah *et al*., 2000; Khaki *et al.,* 2008; Aral *et al*., 2008; Zobeiri *et al.*, 2013; Yücel *et al.,* 2021).

II.1.3. Beta-lactams

B-lactams (penicillin, penicillin derivatives, cephalosporines, cephamycins, carbapenems, monobactams, and monocarbams), one of the most relevant drug classes of antibacterial agents worldwide, have a long history in the treatment of infectious diseases; they retain a central place in the antimicrobial armamentarium against Gram-negative and Gram-positive microorganisms. Their discovery and development represent one of the most powerful achievements of modern science and technology (Demain *et al*. 1999; Poole, 2004). Members of this class contain a 3-carbon and 1-nitrogen ring that is highly reactive (Etebu and Arikekpar 2016). They are bactericidal agents that interrupt bacterial cell wall formation by inhibiting the synthesis of the peptidoglycan resulting in lysis and cell death (Etebu and Arikekpar 2016). Since their initial discovery, many of them have become available for clinical use worldwide.

Penicillin, derived from the mold/fungi Penicillium notatum, was discovered by the English bacteriologist Alexander Fleming in 1928 and was first used in clinical trials in 1942 (Mohr 2016). It can be divided into two groups, namely natural and semisynthetic penicillins. Modifications of the original molecule have led to new compounds with a greater antimicrobial spectrum and activity. The addition of an amino group to the benzylpenicillin molecule resulted in the creation of ampicillin (C16H18N3NaO4S), a broad-spectrum antibiotic. It has bactericidal activity against a wide range of common gram-positive and gram-negative organisms (Raynor, 1997. Dumancas *et al*. 2014). Ampicillin acts as a competitive inhibitor of the enzyme transpeptidase, which is needed by bacteria to make their cell walls. It inhibits the third and final stage of bacterial cell wall synthesis in binary fission, which ultimately leads to cell lysis (Sharma *et al.* 2013). Since 1961, it has been used extensively to prevent and treat several bacterial infections. In veterinary medicine, it comes in several forms: ampicillin trihydrate and ampicillin sodium, which can be administered orally, added to the drinking water for the flock, or used via intramuscular injection (IM); ampicillin/sulbactam combination that represents an inhibitor of bacterial beta-lactamase. It needs to be administered via intramuscular (IM) or intravenously (IV) injections because it has poor absorption when given orally. The introduction of a hydroxyl group at the para-position of the phenyl ring of ampicillin has resulted in the discovery of α-amino-p-hydroxy-

benzylpenicillin named amoxicillin, which has an identical spectrum of activity to ampicillin (Lima *et al.* 2020).

The cephalosporins, used to manage a wide range of infections from Gram-positive and Gram-negative bacteria, are a large family of b-lactam antimicrobial drugs with the dihydrothiazine ring fused to the b-lactam nucleus, originating the cephem scaffold. They are usually classified into "generations" (1^{st}-5^{th}) by their target organism. The first cephalosporins were isolated in Cephalosporium acremonium cultures. A natural antibiotic that showed some activity against penicillin resistance (Marshall and Blair 1999; Lima *et al.* 2020).

According to Christensen (2021), the carbapenems' spectrum of activity is broader than that of penicillins and cephalosporins. They possess the greatest potency against Gram-positive and Gram-negative bacteria, this is why they are classified as antibiotics of last resort (Etebu and Arikekpar 2016). The first isolated carbapenem was olivanic acid isolated from the broth of Streptomyces clavuligerus. Later, different molecules (thienamycin, imipenem, panipenem, biapenem, meropenem, ertapenem, and doripenem) were approved as drugs. Sadly, the emergence of bacterial pathogens resistant to this class of antibiotics has become an international concern (Papp-Wallace *et al.* 2011).

Each new class of b-lactams has been developed either to improve the spectrum of activity or to counteract the bacterial mechanisms of resistance. The monobactams group is the last discovered type of b-lactams (Bush and Bradford 2016). Their discovery was first reported by Skyes and co-workers. The antibiotic was obtained from the bacterium Chromobacterium violaceum with no second ring fused to the b-lactam ring in contrast to most other b-lactams, which have at least two rings. They are effective only against aerobic Gram-negative bacteria by inhibiting the peptidoglycan synthesis process leading to cell death (Etebu and Arikekpar 2016).

II.1.4. Sulfonamides

Similar to the b-lactams, sulfonamides (A-SO2NHR) and their structurally related derivatives (sulfamates and sulfamides) have broad-spectrum activity against both Gram-positive and Gram-negative bacteria such as E. coli, Shigella, Klebsiella, Salmonella, Enterobacter and some Protozoa (Etebu and Arikekpar 2016; Supuran 2017). These bacteriostatic antimetabolite drugs induce the inhibition of folate synthesis. Studies have shown that sulphonamides may become bactericidal if their concentration is sufficiently high

or if their use is accompanied by other environmental conditions unfavorable to bacteria such as poor cultural conditions, adverse temperature, antibodies, etc (Henry 1943). Through Domagk's efforts (1895–1964), the first sulfa antibiotic was introduced in 1932 to be commercialized as Prontosil in 1935. Then many thousands of derivatives with sulfanilamide structures were created in the following years. These drugs were synthetic compounds. They have been used for almost a century and reached particular popularity in the United States despite their limitations in terms of safety and efficacy (Zaffiri *et al.* 2012). Generally, sulfa drugs are mainly given in combination with trimethoprim or pirymethoxazole.

II.1.5. Polypeptide antibiotics

Colistin, also known as polymyxin E, belongs to the polymyxin class of antibiotics, which also includes polymyxin B. This first Japanese-origin antibiotic was discovered by Dr. Yasuo Koyama in 1947 from Paenibacillus polymyxa subspecies colistinus cultures. These molecules were initially considered “miracle” antibiotics when they were first commercialized in the 1950s, with significant bactericidal activity against aerobic Gram-negative pathogens, notably Pseudomonas aeruginosa (Kumazawa and Yagisawa 2002; Durand *et al*. 2019; Hamel *et al.* 2021). By binding to lipopolysaccharides, displacing both magnesium and calcium ions, which leads to disruption of the outer cell membrane and bacterial death. This polycationic peptide is considered a crucial last-resort option for the treatment of multidrug-resistant bacterial infections despite its toxicity (Kempf *et al*. 2016; Hamel *et al*. 2021).

II.1.6. Macrolides

Macrolides, an old class of antibiotics, was discovered by James M. McGuire who isolated the first compound (erythromycin) in 1952 from the soil Bacterium Streptomyces Erythreus. In the 1970s and 1980s, synthetic derivatives of erythromycin (clarithromycin and azithromycin) were developed (Lohsen and Stephens 2019). Macrolide antibiotics (erythromycin, spiramycin, tylosin, midecamycin acetate, oleandomycin, and troleandomycin) are so named because of their large (12 to 16 atoms) lactone ring structure. They are generally used to manage and treat various bacterial infections especially lung ones (pneumonia) (Mazzei *et al.* 1993; Zhanel *et al*. 2001). They act as bacteriostatic by binding to the 50S subunit of the bacterial ribosome, specifically the 23s rRNA, preventing the translocase and therefore inhibiting the protein synthesis (Schlegel *et al.* 1991; Mohr 2016), mainly against Gram-positive pathogens such as Staphylococci and Streptococci. However, their utility

against Gram-negative bacteria is extremely limited (Myers and Clark 2021). Erythromycin and tylosin antibiotics are widely recommended and used in chicken livestock. Routinely, they are prescribed by veterinarians as an aid in the treatment of chronic respiratory disease (associated with Mycoplasma gallisepticum CRD), and for the treatment of infections due to their sensitive bacteria in poultry (ex: infectious synovitis and sinusitis) (Garmyn *et al.* 2019; Huang *et al.* 2021). Ketolides (cethromycin and telithromycin), are a new class of 14-membered-ring macrolides. They have recently been developed as an attempt to address the increasingly prevalent problems of macrolide-resistant and multiresistant organisms. The erythromycin derivatives were produced by substituting the l-cladinose sugar with a 3-keto group and their mechanism of action is similar to that of macrolides (Zhanel *et al.* 2001).

II.2. Antibiotics use in poultry production

Antimicrobial agents in general are active against bacteria, protozoa, viruses, and even fungi. Some antibiotics, either of natural or synthetic origin, referred to as broad-spectrum drugs; treat a wide range of infections. Others, called narrow-spectrum molecules, are effective against only a few types of bacteria. Antibiotics are generally administered to the entire flock in all stages of animal production for the treatment of disease (therapy), disease prevention (metaphylaxis), and growth promotion (Poole and Sheffield 2013). According to Roth *et al.* (2019), their use as preventive agents is permitted in all large poultry-producing countries. However, antibiotic growth promoters were banned due to the global threat of antibiotic resistance in the European Union (EU) in 2006, and in the United States (US) in 2017 and are currently allowed in Brazil and China. Antibiotics are one of many tools veterinarians and farmers rely upon to protect animal health. Their use in poultry livestock generally improved chicken performance but at the same time, the prevalence of antibiotic resistance strains of pathogenic and non-pathogenic organisms leads to serious consequences on public health (Agyare *et al.* 2018).

II.3. Antibiotic resistance

Poultry products are one of the highest consumed products worldwide, but the overuse and misuse of many antibiotics in poultry livestock threatened the safety of such products in several countries through antimicrobial residues and the increased development and spread of multi-drug resistance bacteria such as Campylobacter and Salmonella (Donoghue 2003). Antibiotic resistance means that the bacteria develop the ability to defeat the drugs designed

to kill it and continue to grow. The emergence of antibiotic-resistance genes in bacteria has enormous human and economic consequences (Muhammad *et al.* 2020). Those resistant organisms may be transferred to human beings in various ways. Either by direct contact with animals or via consumption of contaminated food and water (Tenhagen *et al.* 2018). The World Health Organization (WHO) identified antibiotic resistance as one major global human health challenge (WHO 2015). As it was argued by Xie *et al.* (2018), about 7,000,000 human deaths per year have been associated with antibiotic resistance. If antibiotics lose their effectiveness, illnesses that were once easy to treat become more difficult to cure and more expensive to treat, then we lose the ability to treat infections and control these public health threats. Bacteria can acquire resistance in two ways: either through a new genetic change that helps the bacterium survive, or by getting DNA from a bacterium that is already resistant. When a simple DNA changes, it ensures bacterial protection from antibiotics. It can prevent an antibiotic from getting into the cell, or prevent the antibiotic from working once it is inside. The genetic change can spread through the bacterial population via reproduction or DNA transfer.

This serious issue that both human and animal health experts are working to address it. Restrictions were applied by the US Food and Drug Administration (FDA) on the antibiotics that are used in livestock and at the same time used in humans. Their use in humans should be correct and appropriate. If they are needed, they should be taken exactly as prescribed (Muhammad *et al.* 2020). It is very important to take the entire course of medication and the directed amount each day. Doing so helps prevention bacterial antibiotic resistance development. Glasgow et al. in their study on antibiotic use in poultry production in Grenada surveyed 30 poultry farmers each having 500 or more chickens grown for commercial purposes. He reported that the majority of farmers, especially in low-income countries, were not very knowledgeable about the prudent use of antibiotics in poultry production, the development of antimicrobial resistance, and risks from the presence of antibiotic residue in poultry products. Many of them do not respect the mandatory withdrawal period and do not seek veterinary advice for disease diagnosis or an antibiotic prescription but, instead, they generally rely on personal experience or advice from other farmers (Glasgow *et al.* 2019)

Chapter III: Reproductive System of Roosters

III.1. Main objectives of breeding farms

The main objective of any broiler breeding is to maximize the genetic potential and to obtain fertile eggs that can be brooded and which, once hatched, correspond to the demand for good quality chicks while keeping their reproductive potential intact. What is obvious, in broiler breeding programs; is a good food efficiency, and rapid growth with excellent quality of meat are always desired.

III.2. Management of Cobb 500 livestock

The Cobb rooster brings a unique balance between the performance characteristics of the breeders and the broiler chicken. This rooster has excellent feed conversion, viability, and fertility, while the offspring of broiler chickens demonstrate improved viability and feed conversion ratio.

The control of reproduction is generally the consequence of a research strategy in which zootechnics, physiology, genetics, and nutrition programs are involved. In all countries where Cobb 500 males and females are bred, an effective and constantly monitored breeding management program is used to achieve good production results. The success of such breeding chickens has led to a considerable enrichment of knowledge of the strain, and this within many different environments (hot climate, cold climate, open building, etc.). In these types of breeding, a very rigorous monitoring of the growth of the animals is set up and is most often dictated by poultry guides (www.cobb-vantress.com).

According to the breeder management guides, the life of broiler breeders consists of two stages: rearing (breeding) and mating (reproduction).

III.2.1. The rearing phase of roosters: (20 to 22 weeks)

Good management of the breeding phase and especially of the start-up is essential to obtain an excellent lot of breeding stock to have subsequently uniformity in production. A homogeneous batch with correct synchronization will have a better receptivity of the hens and a greater effectiveness of the ticking. Everything is correlated to the planning, organization, and monitoring of key performance indicators; in particular the average weight, the batch homogeneity, the consumption index, and finally the morbidity and mortality rates to react quickly to any problems.

During this phase, chicks (1 day old) are raised until they reach the desired sexual development, uniformity, and fleshing (development of the breastbone). Often and for better results, males are reared separately from females (separate management of males and females) due to their different growth rates and nutritional needs. This method offers the advantage of implementing a rationing program and controlling the live weight of each sex before finally placing them together in breeding poultry houses. However, mixed-sex management is rarely used in the rearing phase of breeding animals. Generally, when the live weight of males exceeds that of females by 40%, both sexes are mixed based on the weight of the females regarding the amount of feed distributed.

Fathers account for half of the chicks genetic heritage. Therefore, it is important to ensure good management of the males so that they remain productive throughout the entire reproductive period. Their fertility performance is closely correlated with light stimulation (light duration and intensity) from the outset. The latter is related to the homogeneity of weight, age, and well-being of the animals. A good lighting program ensures that birds can find food and water more easily.

III.2.2. The reproduction phase of roosters: (22 to 63 weeks)

Transferring animals to production facilities is a very stressful act. Therefore, birds handling should be done carefully and with caution; every effort should be made to ensure that this passage is as comfortable as possible. A good environment (lighting program, temperature, ventilation, and humidity) with a controlled feeding program will result in good fertility in general.

According to the Cobb 500 breeder management guide, males are selected and transferred 2 to 3 days before females; this allows for better adaptation of males to their feeding equipment and promotes better weight control. At the time of transfer, the batch should be accompanied by a copy of the development curves during rearing with all the information concerning live weight, feeding time, number of animals transferred, health problems, vaccination program, treatments, and any other necessary information. The ratio between males and females, already sexually mature, varies depending on the breed being raised. For light breeds, one rooster is required for 10 to 12 hens. On the other hand, one rooster is sufficient for 6 hens for heavy breeds. In the poultry house, the roosters check the hens naturally and without any intervention from technicians.

III.3. Lot homogeneity

Maintaining a homogeneous flock is extremely important in the management of breeding roosters from a young age. Birds that experience stagnation or weight loss in the first 15 weeks of life may lead to a loss of reproductive potential. Generally, roosters and hens that are similar in weight are mixed. The heaviest roosters without any skeletal or leg defects are used for recharging. Whereas, stunted or underdeveloped roosters are removed outright from the population. At 30 weeks of age, when sexual activity is at its peak, the weight differential can be 12 to 16%, depending on the Cobb hen used. The best results in terms of homogeneity and fertility are obtained from lots sorted at 25, 35, 45, and 55 weeks.

III.4. The recharge

Refilling or recharging is the addition of young roosters to an older flock to compensate for the decline in fertility that usually occurs after 45 weeks of age. Only one recharge during the life of the batch should be sufficient. Lots recharged twice within an interval of 8 to 10 weeks also show good results. After 55 weeks of age, recharge is not economical. This technique can be the solution either to loss of interest in mating, low ticking efficiency (roosters with weight, leg, or foot problems), reduced sperm quality, or much from excessive mortality of males. The greatest risk with recharge is the possibility of introducing unwanted diseases or parasites into the refilling lots. Sometimes, exchanging 25 to 30% of the roosters between buildings on the same farm can stimulate fertility and mating activity, especially if done at 40 and 48 weeks of age. This is an internal recharge. It is an easy, inexpensive technique with less risk (Coq Cobb MV- cobb- vantress).

III.5. Male reproductive system

The male reproductive system has some unique features in birds. The sex chromosomes in male birds are ZZ (homozygous) compared to ZW (heterozygous) in females. They do not have a scrotum, but instead have two internal testicles that are bean-shaped. These two glands are located in the abdominal cavity at the base of the lungs, near the kidneys, and close to the spine (Scanes *et al.* 2020). Their size and weight vary notably depending on the species, individuals, and season (breeding or non-breeding). In roosters, they also vary according to their genetic origin. Although the two testicles are positioned symmetrically, they are often different in size. The left testicle is often larger than the right, and both increase in size after puberty (sexual maturity) depending on the photoperiod. They produce male gametes

(spermatozoa) and secrete sex hormones that influence mating and male behavior (Kharayat *et al.* 2016).

The testicular development of roosters occurs in different phases. It starts at birth with a slow growth phase, called pre-puberty, the duration of which varies between species. This phase is followed by puberty, during which there is a considerable increase in testicular weight and size, production of the first spermatozoa, and the onset of sexual activity. Sexual maturity is the final phase of testicular development, with sperm production reaching maximum levels in adult animals.

III.6. Reproduction Process

The reproductive system of birds is different from that of mammals. Both roosters and hens do not have external genital organs, and their sex is often determined by secondary sexual characteristics such as the presence of a wattle and comb, size, feather color, etc. The two partners reproduce by using the cloaca (a common opening for the genital, urinary, and intestinal cavities). Copulation involves the brief contact of the male's cloaca with that of the female, lasting only a few seconds, during which the sperm is deposited. The male gametes then migrate along the female's reproductive tract until they reach the oviduct, where fertilization takes place with the ovum. After fertilization, the zygote, which is the first cell of the future individual, is formed. It will divide, organize itself, and develop into a chick inside an egg.

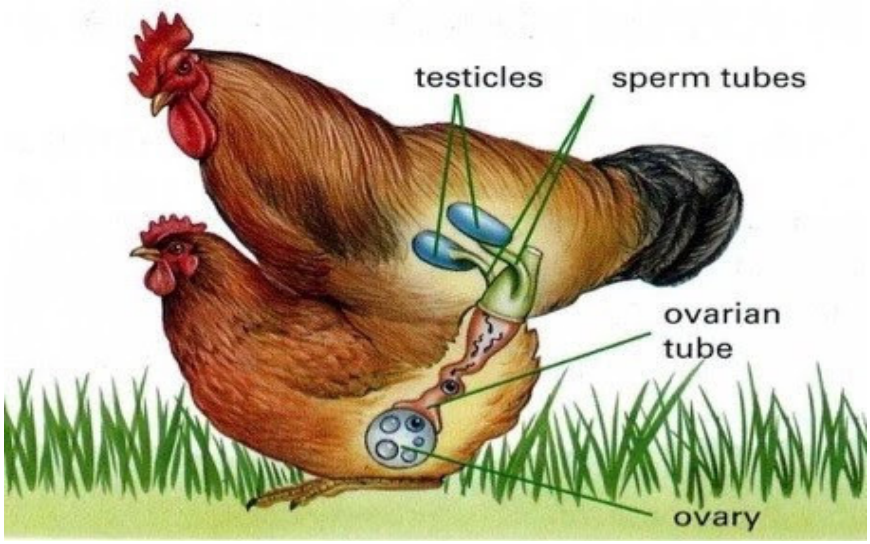


Figure 1: *Reproduction process*. (www.Vedantu.com)

III.7. Physiology of roosters reproduction

There is evidence that the reproductive physiology of roosters is influenced by the photoperiod (duration of light exposure). Lengthening daylight hours stimulates the hypothalamus to release GnRH, initiating the reproductive processes. In commercial poultry production, artificial lighting is often used to manipulate the photoperiod and optimize reproduction (Scanes *et al.* 2020).

Hormones play a crucial role in regulating the reproductive processes in roosters. Only small amounts of gonadotropin-releasing hormone (GnRH) are released from the hypothalamus in the brain, they function as neuroendocrine signals that stimulate the anterior lobe of the pituitary gland which responds by releasing the adequate amount of follicle-stimulating hormone (FSH) and luteinizing hormone (LH). These interstitial Cell Stimulating Hormones appear to be part of a functional axis, which controls the development of the gonads. Their levels (LH and FSH) in the peripheral circulation are directly related to testicular activity and the production of spermatozoa. FSH stimulates the Sertoli cells in the testes, which support sperm development by producing nutrients for the maturing spermatozoa. LH stimulates the Leydig cells in the testes to produce different steroid hormones. Levels of LH and testicular steroids including testosterone in plasma are maintained in a state of dynamic equilibrium. This is achieved by the negative feedback effects of testicular steroids on LH release (Sharp and Gow 1983; Dunn *et al.* 2009; Getachew 2016; Stephens and Johnson 2020).

Testosterone, the primary male sex hormone, is essential for the development and maintenance of male reproductive organs, secondary sexual characteristics, and spermatogenesis. It promotes the growth and maturation of the testes, stimulates sperm production, and influences mating behavior. Testosterone levels in roosters increase during puberty and reach peak levels in sexually mature adults (Riters and Alger 2011; Scanes *et al.* 2020).

III.8. Spermatogenesis

Spermatogenesis is a complicated process tightly regulated by neuroendocrine and endocrine mechanisms (Scanes *et al* 2020). It refers to the division, differentiation, and finally the production of functional spermatozoa (sperm cells) in the seminiferous tubules inside the testes. The spermatogenesis in roosters consists of three main phases, spermatocytogenesis,

meiosis, and spermiogenesis (Getachew 2016). During spermatocytogenesis that refers to the first phase, diploid germ cells called spermatogonia differentiate into primary spermatocytes (spermatocytes I), which undergo DNA replication to form secondary spermatocytes (spermatocytes II). This phase takes place continuously throughout the life of the male. The second phase, meiosis, is divided into two rounds of cell division. During the first meiotic division, secondary spermatocytes undergo a reduction division to form haploid cells called spermatids. During the second meiotic division, these spermatids undergo further cell division to form four haploid cells, each with half the number of chromosomes as the original cell. These haploid cells are the mature sperm cells or spermatozoa. The final phase, spermiogenesis, involves the maturation of the sperm cells. During this phase, the haploid cells undergo a series of morphological changes, including the formation of the acrosome, tail, and midpiece. The acrosome is a specialized organelle that contains enzymes needed to penetrate the egg during fertilization. The tail and midpiece provide motility to the sperm cell. Once spermatozoa enter the vagina of the oviduct, they are stored in the infundibular sperm storage tubules. It can retain their ability to fertilize the oocyte for several weeks (Scanes *et al* 2020). It is known that the size and shape of the mature sperm vary considerably among different species.

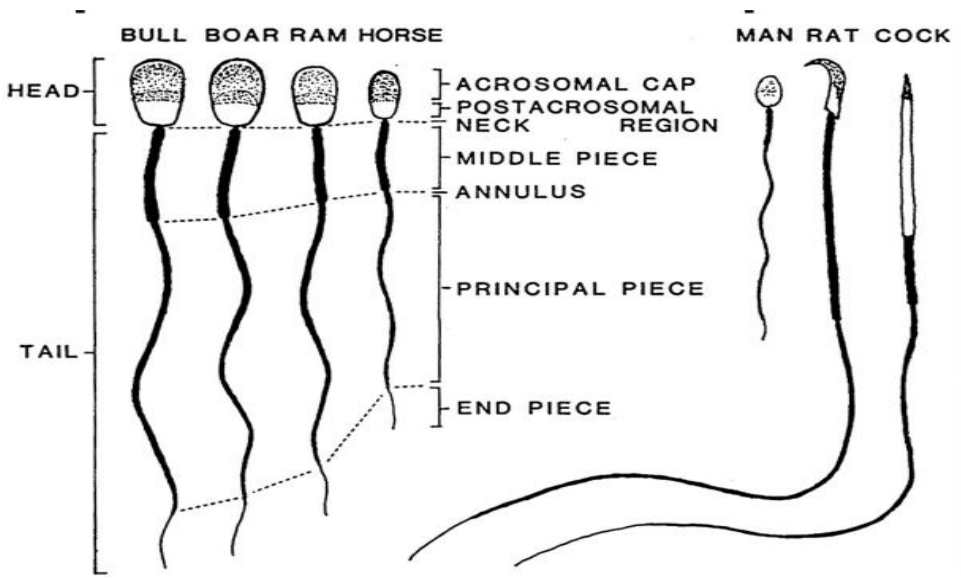


Figure 2: *Sperm from different species.* (www.slideserve.com)

These variations are related to the specific reproductive strategies and challenges faced by different species. Factors such as the environment in which fertilization occurs, the presence of competition from other males, the type of reproductive tract or organ in females,

and the need to traverse barriers to fertilization can all influence sperm morphology. Bird sperm often have a characteristic "hook" shape at the tip, which assists in penetrating the protective layers of the egg.

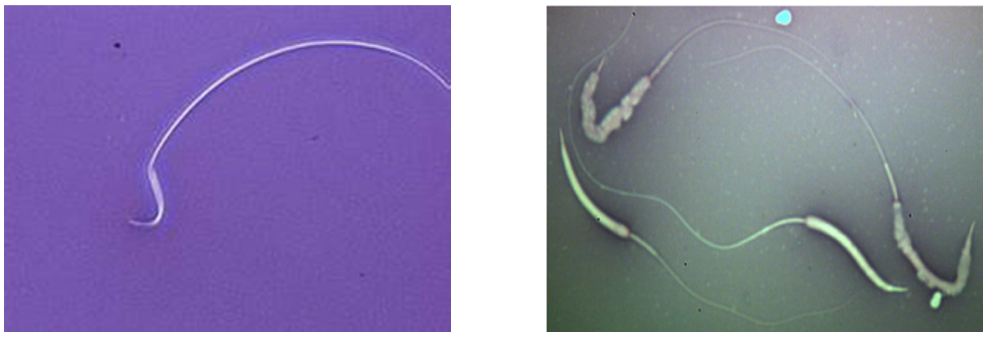


***Figure 3**: The shape of the roosters spermatozoa.*(www.um.es)

The roosters' semen contains sperm cells suspended in a fluid called seminal plasma. Seminal plasma provides nutrients and protection to the sperm cells during storage and transport. Generally, the rooster produces approximately 35,000 spermatozoa every second of his life, and his semen contains about 40 times more spermatozoa than that of a man. However, quantity does not always equal quality. The quality of roosters' sperm depends on many factors such as nutrition, genetics, and environment.

The volume, concentration, motility, and morphology of the sperm cells are important factors for fertility and successful artificial insemination.

III.9. Artificial insemination

Artificial Insemination (AI), the manual deposition of semen into a female' vagina, is considered the most important Assisted Reproduction Technology (ART) that contributes to increasing poultry production and facilitating the genetic improvement of livestock (Kharayat *et al.* 2016). This valuable tool has had a major impact on the structure of breeding programs by enabling the rapid dissemination of genetic material from fewer superior males to a high number of females compared to natural mating. One ejaculate can cover up to 20 to 25 female birds. AI is commonly practiced in North America and Europe where it is exclusively used for the production of hatching eggs (Kharayat *et al.* 2016). Furthermore, it minimizes managemental costs by reducing the number of males and holding the service of genetically superior males even after their death (Dhama *et al*. 2014, Mohan *et al*. 2018). The AI procedure required superior quality semen that should be evaluated in terms of volume,

concentration, viability, and motility. To achieve the best fertility in chicken, fresh semen should be used immediately, within half an hour of its collection. Otherwise, the sperm must be diluted, and possibly cooled for several hours or days.

For the management of breeding programs and the application of reproductive technologies, two important factors are indispensable: the ability to collect semen and the evaluation of its quality and quantity (Kanatiyanont *et al.* 2012).

III.10. Semen collection technique

Semen collection is considered as the first essential factor for successful AIs where a clean semen sample of sufficient volume is regularly required (Mohan *et al.* (2018). According to Castillo *et al.* (2021), the collection method largely influences the ejaculate's quality. It is fundamental to avoid semen contamination and to minimize handling stress on the bird during the collection. More than 80 years ago, in 1937, Burrows and Quinn developed the abdominal massage technique for collecting semen from chickens and turkeys. This technique helped to get rid of the drastic old method, which consist of killing hens after a natural mating and then collecting semen surgically from their oviduct (Mohan *et al.* 2018). Burrows and Quinn's technique involves two collectors, the first one restraining the male and stroking the back of the bird from behind the wings to the tail with firm rapid stroks. The phallus will enlarge after 3–6 such massages depending upon variation between males. At this time, the second operator gently squeezes the cloaca and directly semen is aspirated into a graduating tube. Another technique for avian semen collection is the low-voltage electro-ejaculate technique. It may be the alternative method for untamed and aggressive male birds. This technique is rarely used for routine semen collection in avian species, especially in chicken (Kanatiyanont *et al.* 2012). To ensure high-quality semen, ideal semen collection frequency is very important. Previous studies reported that three collections per week (alternate days) were shown to yield better results; periods of sexual rest aided in the maintenance of high semen quality and quantity (McDaniel and Sexton 1977; Mohan *et al.* 2018; Pimprasert *et al.* 2023).

III.11. Sperm Analysis (Spermiogram)

A spermiogram, also known as semen analysis, is a diagnostic test performed on male poultry to assess the quality and fertility of their semen. It provides valuable information about the reproductive health and breeding potential of roosters.

The following are key aspects typically evaluated during a spermiogram in poultry:

- Sperm concentration refers to the number of sperm per unit volume (milliliter) of semen. However, the sperm count is the total number of spermatozoa in one ejaculate, which can be calculated by multiplying sperm concentration by semen volume.
- Sperm viability is defined as the percentage of live sperm found in a semen sample.
- Sperm morphology refers to the shape, size, and appearance of spermatozoa.
- Total motility (TM %) expresses the percentage of total moving spermatozoa regardless of the quality of the movement.
- Progressive motility (PM %), representing forward spermatozoa.
- Straight-line velocity (VSL μm/s) is the time average velocity of a sperm head along the straight line between its first and last detected positions.
- Curvilinear velocity (VCL μm/s) is the average velocity measured over the actual point-to-point track followed by the cell.
- Average path velocity (VAP μm/s) refers to the speed sperm are moving.
- The amplitude of lateral head displacement (ALH μm) is the maximum lateral displacement of a sperm head as it moves along its average trajectory.
- Linearity (LIN %) is the linearity of a curvilinear path.
- Beat cross frequency (BCF Hz) refers to the average rate at which the curvilinear path crosses the average path.
- Straightness linearity of the average path (STR).

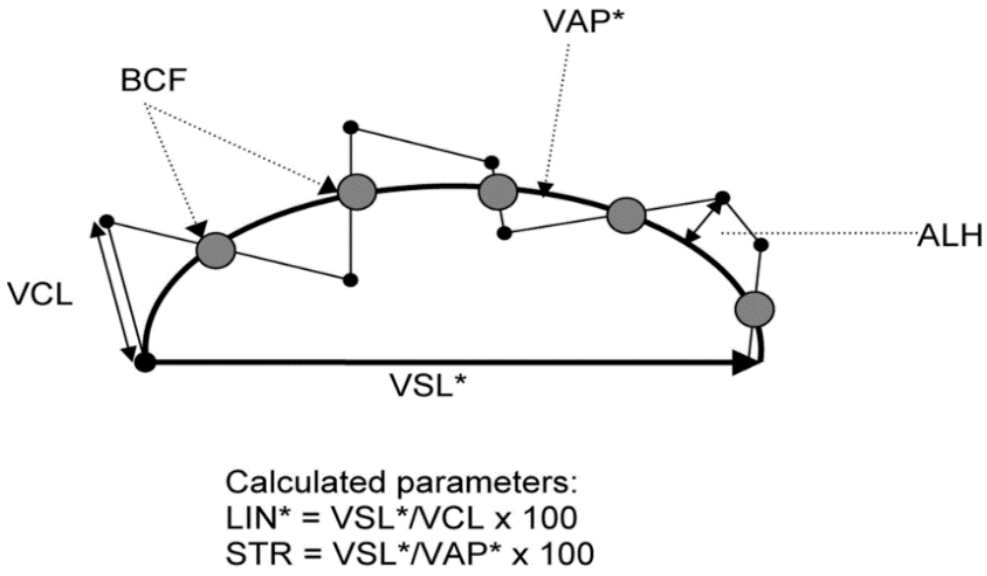


Figure 4: *Sperm kinematic parameters.*

Spermatozoa are capable of ascending through the vagina to reach the hen's Sperm Storage Tube (SST) (Getachew 2016). To ensure sperm viability, dilution of semen immediately after collection is necessary (Chankitisakul *et al.* 2022). Two to three-fold dilutions are typically Semen quality evaluation

Wishart in his chapter, from the Biology of Breeding Poultry book, declared that sperm quality assays represent an excellent indicator of poultry birds' reproductive potential, fertility, and subsequent hatchability of eggs. They are generally used to select the best breeders from a flock of chickens when artificial insemination is employed (Wishart, 2009). To evaluate semen quality traits, assessment of sperm volume, concentration, motility, viability and morphology of spermatozoa is of great importance.

A semen sample of good quality is generally thick and pearly white. This trait could serve as an indicator of semen contamination by feces, urine, or blood because of excessive force during the collection process. In addition, it may determine the presence of any lesion or infection in the male's genital tract (Tarif *et al.* 2013). Poultry semen has exceptionally high sperm concentration (up to 6-7 billion spermatozoa/ml) (Getachew 2016; Mohan *et al*. 2018). Sexually mature males usually produce, in rapid spermatogenesis, a high number of spermatozoa in a small volume of semen. The average volume generally ranged from 0.01 ml to 1 ml per ejaculation, depending on cockerel species, strain, and individual (Bernard Sauveur 1988; Getachew 2016). According to Tarif *et al*. (2013), in their study, the ejaculate

volume might be also influenced by body weight. Generally, heavier cocks with larger testes produce more semen (up to 1.5 ml per ejaculation). Immediately after collection, ejaculate volume may be determined either by weighing the sample and calculating the volume using a known density or by using a graduated cylinder. The PH of the seminal fluid may play a significant role not only in maintaining the viability and quality of the sperm but also in ensuring fertilization. It affects spermatozoa capacitation and thus sperm motility (Zhou *et al.* 2015). Semen acidity varies between different bird species. The optimum semen pH usually ranges between 7.0 and 7.4 (Getachew 2016). Isidahomen (2016) also found the semen pH of five strains of cocks to be slightly alkaline; it ranges between 7.5 and 8. Among the several factors that influence the semen quality, sperm morphology and the percentage of live spermatozoa that is positively related to sperm motility. Only morphologically normal is required for chicken semen (Mohan *et al.* 2018). Sperm motility measures the overall swimming performance of a population of sperm. According to Froman and Feltmann (2000), Froman and Kirby (2005), mobility ejaculates tend to contain a greater proportion of sperm cells with straight-line velocity (VSL) higher than 30 μm/s compared to lower mobility ejaculates.

Sperm motility, the ability of spermatozoa to move properly through the female reproductive tract to reach the egg, is the most obvious function of spermatozoa that has been assessed using a variety of qualitative and quantitative assessments (Wishart, 2009). It represents a key factor tightly related to male fertility. Their decrease could seriously affect fertilizing ability and thus conception (Aly and Khafagy, 2014). According to Sun *et al.* (2019), in the poultry industry, around 5 to 12% of males are eliminated from the breeding program because of low sperm motility (MOT). Recently, kinematics such as curvilinear velocity (VCL), straight-line velocity (VSL), amplitude lateral head displacement (ALH), and average path velocity (VAP), are some of the crucial parameters usually used in the assessment of fertility in poultry and the potential selection of roosters for artificial insemination programs. These parameters are generally determined by the computer-assisted semen analysis system.

III.12. Computer-assisted semen analyzer (CASA)

This powerful tool represented a revolution in reproductive biology and the knowledge of fertility by providing precise and accurate information on different sperm motion characteristics (Kathiravan *et al.* 2011). Computerized motility analysis provides several objective measures of sperm motion characteristics taken from tracks of large numbers of sperm. The microscope has a high-resolution video camera attached. The video camera feeds data into the computer where it undergoes analysis by software. It gives many more parameters that are useful to the specialists of fertility. As the percentage of total motile sperm (motility of any form), percentage of progressively motile sperm (rapid and linear movement), average path velocity in micrometers per second, curvilinear velocity in micrometers per second, and amplitude of lateral head displacement during forward movement (Brinsko *et al.* 2011; Lu *et al*. 2014; Valverde *et al.* 2020; Vincent *et al,* 2021).

III.13. Antibiotics' effects on male fertility

Antibiotics have the potential to influence male fertility in various species, including humans, mice, rats, and livestock animals. While some antibiotics have been linked to adverse effects on sperm quality and reproductive parameters, the extent of these effects can vary depending on the specific antibiotic, dosage, and duration of treatment.

Many experimental studies record the negative effect of certain antibiotics on the testicular tissue and spermatogenesis through a change in the level of hormones of the hypothalamic-pituitary-gonadal axis or through a direct effect on the testicle itself (Kadyrov *et al.* 2021).

Table 1: Summary of antibiotics' effects on male fertility in different animal species.

Study	Authors	Medication	Species	Side effects
Effects of co-trimoxazol, erythromycin, amoxycillin, tetracycline, and chloroquine on sperm function *IN-VITRO*	Hargreaves *et al*. **1998**	Co-trimoxazol, erythromycin, amoxycillin, tetracycline and chloroquine	Human	-This *IN-VITRO* study showed that amoxycillin did not affect sperm movement parameters. However, it reduces the percentage of viable sperm. -Chloroquine at higher concentrations adversely affects rapid motility of spermatozoa; the same parameter was enhanced at low concentrations. -Erythromycin significantly declines rapidly moving spermatozoa, VAP, VSL, VCL, and ALH but it enhances these parameters at high concentrations. -Spermatozoa were particularly sensitive to tetracycline, the drug decrease rapid sperm movement, VAP, VSL and VCL. At high doses, all spermatozoa were static. -Only high concentrations of co-trimoxazol impair rapid sperm movement. However, it significantly increases ALH at low concentrations. -The effects of these antibiotics were mostly irreversible.
Tetracycline-induced reproductive toxicity in male rats: Effects of vitamin C and N-acetylcysteine	Farombi *et al*. **2008**	Tetracycline	Rats	- Significant decrease ($P<0.05$) in the relative weights of testis, epididymis, and seminal vesicles. - Reduction in the epididymal sperm motility, sperm count, percentage of live spermatozoa, and an increase in abnormal sperm morphology.
Adverse effects of ciprofloxacin on testis apoptosis and sperm parameters in rats	Khaki *et al.* **2008**	Ciprofloxacin	Rats	- Significant decrease ($P<0.05$) in the testes, epididymis, and seminal vesicle weights. - Significant decrease in sperm concentration, motility ($P<0.05$) and viability ($P<0.001$). - Significant decrease in the number of spermatogenic cells (spermatogonia, spermatocyte, spermatid, and sperm) in the seminiferous tubules. - Significant increase of the apoptosis germ cells per seminiferous tubules.

Table 1: Summary of antibiotics' effects on male fertility in different animal species (following).

Study	Authors	Medication	Species	Side effects
Effects of enrofloxacin and marbofloxacin administration on some fertility parameters of male chicken.	Al-Nazawi **2008**	Enrofloxacin and marbofloxacin	Chicken	-There was no change in the testes, wattles and combs weight. -No significant change in sperm motility, testosterone level, ascorbic acid, total protein and cholesterol concentration.
Long term ofloxacin testicular toxicity	El-Harouny *et al.* **2010**	Ofloxacin	Rats	-Ofloxacin had a detrimental effect on the testis of rats and the long-term use of the drug causes more damage.
Empirical treatment of low-level leukocytospermia with doxycycline in male infertility patients	Hamada *et al.* **2011**	Doxycycline	Human	-Doxycycline significantly improved the pregnancy rate among low-level leukocytospermia patients ($P = 0.04$), but it did not show statistically significant differences in the semen parameters.
The adverse effect of tetracycline and doxycycline on testicular tissue and sperm parameters in CD1 Outbred mice	Elzeinová *et al*. **2013**	Tetracycline and doxycycline	Mice	-Both antibiotics led to long-lasting effects on reproductive organs and spermatozoa in adult mice. -Significant decrease in anogenital distance and thickness of the seminiferous epithelium. -The lowest doses of both antibiotics significantly reduced the testis weight. -Pathological changes in the testes had an impact on sperm quality.
The effects of levofloxacin on testis tissue and spermatogenesis in rat	Ahmadi *et al.* **2016**	Levofloxacin	Rats	-Levofloxacin did not affect testosterone levels; however, the FSH and LH concentrations were significantly ($P<0.01$) increased. -Sperm concentration decreased linearly as levofloxacin was increased ($P<0.05$). -The drug has obvious histopathology effects on the spermatocyte cells ($P<0.05$).

Table 1: Summary of antibiotics' effects on male fertility in different animal species (following).

Study	Authors	Medication	Species	Side effects
Effect of levofloxacin treatment on semen hyperviscosity in chronic bacterial prostatitis patients	Vicari *et al.* **2016**	Levofloxacin	Human	-In this study, fertile patients (control group) were compared to infertile patients with chronic bacterial prostatitis (CBP) who received levofloxacin as treatment. After Antibiotherapy, the CBP group was divided into two subgroups based on two different microbiological outcomes: responders and poor responders. -Levofloxacin significantly ($P<0.01$) improves the percentage of sperm with progressive motility, the seminal fluid viscosity, and the liquefaction time in the responder subgroup, and significantly decreases the semen leucocyte concentration.
Protective effects of royal jelly on the histomorphologic, oxidative stress, and sperm parameters in ofloxacin-treated rat	Manas and Najafi, **2017**	Ofloxacin	rats	-Significant decrease ($P<0.05$) in sperm count, sperm viability, FSH, LH, testosterone, and total antioxidant capacity. -Significant increase ($P<0.05$) of immature sperm levels, DNA-impaired sperm, malondialdehyde and nitric oxide.
Amoxicillin-clavulanic acid-induced sperm abnormalities and histopathological changes in mice	Fahmy *et al.* **2017**	Amoxicillin-clavulanic acid	Mice	- Reduction of spermatogenesis, atrophy of seminiferous tubules, and significant percentages of sperm morphological abnormalities were recorded.
Senecio biafrae defeated tetracycline-induced testicular toxicity in adult male Sprague Dawley rats	Adelakun *et al*. **2018**	Tetracyclines	Rats	-Significant decrease ($P<0.05$) of the testis, epididymis, and seminal vesicle weight. -Significant decrease ($P<0.05$) in mean sperm count, viability, and motility. -Significant increase ($P<0.05$) in the proportion of abnormal sperm. -Significant decrease ($P<0.05$) in the mean testosterone, FSH and LH levels. -The drug impaired fertility of males (90% of the female rats with confirmed copulation were unable to get pregnant).

Table 1: Summary of antibiotics' effects on male fertility in different animal species (following).

Study	Authors	Medication	Species	Side effects
Improvement in colistin-induced reproductive damage, apoptosis, and autophagy in testes via reducing oxidative stress by chrysin	Aksu *et al.* **2018**	Colistin	Rats	-Colistin-induced reproductive toxicity via decreasing sperm motility and viability significantly (P<0.05). - Sperm abnormality rates were significantly (P < 0.05) increased. -The drug caused an increase (P<0.001) in oxidative stress (OS) in the testis.
Some pharmacodynamic studies on ampicillin and enrofloxacin in male rats	EL-Sawy *et al.* **2018**	Ampicillin and enrofloxacin	Rats	-Administration of ampicillin and/or enrofloxacin each alone and their concomitant induces a reduction of testes, epididymis, and accessory sex organs weight, change in sperm characters, decreases sperm count and motility, and increases the sperm abnormalities.
Amoxicillin and gentamicin antibiotics treatment adversely influence fertility and morphology by decreasing the Dazl gene expression level and increasing the oxidative stress	Karamen *et al*. **2019**	Amoxicillin, cefazolin and gentamicin	Mice	- The drugs did not significantly affect the body, testes, and cauda epididymis weights. However, they cause spermatogenesis failure by decreasing the Dazl gene expression level. -The drugs significantly decreased sperm motility. -Gentamicin significantly elevated sperm anomalies (morphology and structure of the sperm were destroyed).
Testosterones disruptor effect and gut microbiome perturbation in mice: Early life exposure to doxycycline	Hou *et al.* **2019**	Doxycycline	Mice	-The drug-induced steroidogenesis disturbances via mitochondrial dysfunction in Leydig cells, decreasing testosterone levels, and ultimately affecting sperm quality.

Table 1: Summary of antibiotics' effects on male fertility in different animal species (following).

Study	Authors	Medication	Species	Side effects
Influence of antibiotics on bacterial load and sperm parameters during short-term preservation of collared peccary semen	Santos *et al.* **2021**	Streptomycin-penicillin and gentamycin	Peccaries	-Compared to the streptomycin-penicillin association, gentamycin at 70 µg/ml maintained the sperm parameters for longer during short-term storage of peccary semen.
Adverse effects of oxytetracycline and enrofloxacin on the fertility of Saanen bucks	Yücel *et al.* **2021**	Oxytetracycline and enrofloxacin	Saanen bucks	-There were no changes in sperm volume, viability and abnormal sperm rate. - Sperm motility, sperm density, mass activity, and testosterone levels were decreased significantly. However, an increase in sperm DNA damage was detected. -The greater damage was in the enrofloxacin group treatment.
Effect of fluoroquinolones treatment on certain sperm function parameters and sperm DNA denaturation in rats	Al-Dujaily *et al.* **2021**	Ciprofloxacin and levofloxacin	Rats	-Ciprofloxacin and levofloxacin did not affect sperm parameters when they were administered orally for 14 days. However, the two medicines significantly reduced sperm concentration, percentage of morphologically normal sperm, and sperm motility after 28 days of treatment. -Both antibiotics significantly increase the level of DNA fragmentation with a significant reduction in chromatin quality after 28 days of treatment.
Study of antibiotics and symbiotic effects on sperm quality using the CASA system	Bouchicha *et al.* **2022**	Colistin and co-trimoxazol	Goats	-Drugs negatively affect the sperm cell by decreasing sperm motility and altering linearity.

Second Section :
Experimental section

Chapter IV: Material and Methods

The present experiment (*in vitro* and *in-vivo*) was conducted between October 2020 and April 2021 to investigate the effects of commonly prescribed antibiotics on sperm movement characteristics and viability. The study was approved and supported by the Ecosystem Diversity and Dynamics of Agricultural Production Systems in Arid Zones Laboratory (DEDSPAZA), University of Biskra. Laboratory of Promotion of Innovation of Agriculture in Arid Regions (PIARA), University of Biskra. Laboratory in Marine and Aquaculture Ecosystems, Faculty of Nature and Life Sciences, University of Bejaia, and medical laboratory analysis Ouamane of Biskra.

IV.1. Drugs

The drug selection was based on the commonly used antibiotics in poultry production. The treatments were daily added to the drinking water just before its administration. These Chemicals were:

- Ampicillin at 30 mg/kg (Neoampiclline P® 20%, VETOPHARM PRO, Algeria),
- Enrofloxacin at 10 mg/kg (Baytril®, MED VET, Algeria),
- Colistin at 2.5 mg/kg (Colistin ACTi coli®, VETOPHARM PRO, Algeria),
- Sulfonamides (sulphaquinoxaline sodium 150 mg, sulphamethazine sodium 70 mg, sulphadiazine sodium 70 mg) at 140 mg/kg (Coccidiopan®, AVICO ANIMAL HEALTH, Algeria),
- Oxytetracycline at 40 mg/kg (Limoxin-400, INTERCHEMIE WERKEN “De Adelaar” BV),
- Tylosine at 20 mg/kg (Tylolide, VETOPHARM PRO, Algeria),
- Erythromycin at 20 mg/kg (Erythro POS 20%, VETOPHARM PRO, Algeria),

IV.2. Experimental birds and husbandry

In total, forty Cobb 500 reproductive roosters, 45-week-old, weighing 5-6 kg, provided by S.A.R.L Group SALEM Avicole, Biskra, Algeria were used. The sexually mature males were reared in large, cleaned, and disinfected cages (180cm length x 80cm width), each containing 5 roosters, under standard conditions with a regular day-night cycle (16L/8D), light intensity = 60 lux, at a controlled temperature (20 ± 1°C), 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 complex1 %) was provided at 140 g/day/animal, and water was allowed ad libitum.

IV.3. Semen collection technique

The animals had no experience of semen collection. They were trained for the dorso-abdominal massage technique as described by Burrows and Quinn for 4 consecutive weeks. The technique involves restraining the male and gently stroking the back of the bird from behind the wings towards the tail with firm rapid strokes. The operator gently squeezes the cloaca extracting semen through the external papillae of the duct us deferens collect the semen into a container (Getachew 2016). To maximize semen quality and avoid rooster stress, the collections were carried out by the same operators and under the same conditions.

IV.4. Experimental design

A-In-vitro experimentation

Only ejaculates with good quality (volume, mass motility, sperm concentration) were sampled and used. The best semen donors were collected at a 3-day interval. The pooled sperm was immediately diluted 1/4 ratio (1 part of semen and 3 parts of physiological solution NaCl 0.9%) to protect and preserve the fresh semen quality and the motility of spermatozoa, then transferred to the laboratory where it was diluted again (1/16 ratio) and divided into eight groups. A control group without antibiotics diluted using 0.9% NaCl and treated groups using oxytetracycline, erythromycin, tylosin, ampicillin, enrofloxacin, colistin, and sulfonamide solutions (30 µg/ml, 15 µg/ml, 100 µg/ml, 30 µg/ml, 5 µg/ml, 10 µg/ml and 300 µg /ml of 0.9% NaCl respectively). Subsequently, analysis of total and progressive motility in addiction to all kinematic parameters was carried out at different time intervals 0, 1, 2, 3, 4, 5, and 6 hours of incubation (37°C) using a Computer Aided Sperm Analyzer (Sperm class analyzer, SCA Microptic, S.L., Version 3.2.0, Barcelona, Spain).

Kinematics analysis was based on the 4-5 consecutive digitalized images obtained from a single field of view obtained using a 10x negative-phase contrast objective.

B-In-vivo experimentation

Based on the treatment, the breeding roosters were divided into seven groups with five (n=5) individuals each. They respectively received therapeutic doses of oxytetracycline,

erythromycin, tylosin, ampicillin, enrofloxacin, colistin, and sulfonamides for 9 consecutive days via drinking water that was prepared each day just before its administration. Each group was subjected to 3 semen collections: before treatment (control T0), 3rd day (T3), and 9th day (T9) of treatment.

IV.5. Fresh semen evaluation

The undiluted semen was evaluated immediately for ejaculate volume and PH using a graduated collection tube and Test paper strips respectively. Color and viscosity were assessed subjectively whereas an optical microscope was used to estimate sperm agglutination.

IV.6. Sperm parameters assessment

Sperm count, viability, total (TM %) and progressive motility (PM %) in addition to all kinematic parameters; [curvilinear velocity (VCL μm/s), straight-line velocity (VSL μm/s), average path velocity (VAP μm/s), amplitude of lateral movement of the head (ALH μm), frequency to which the sperm head crosses the mean trajectory (beat-cross frequency [BCF]/Hz), and movement linearity (LIN %)] were assessed by CASA system. 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.

Chapter V: Results and Discussion

Previous research highlighted the medication effects on the male reproductive system in different species (humans and animals) (Tanyildızi and Bozkurt, 2003a, b; El Harouny *et al.,* 2010; Santos *et al.,* 2021). However, the spermato-toxicity of antimicrobials in living breeding roosters is still unknown. In the current study, which represents the first *in-vitro* and *in-vivo* investigation, we tested the effects of the most extensively used antibiotics, which belonging to tetracyclines, beta-lactams, fluoroquinolones, macrolides, polymyxines, and sulfonamides families, on the breeding rooster' sperm motile parameters. The following results showed that remarkable variations were found between these antibiotics.

V.1. *IN-VITRO* experimental results

It is evident that in normal conditions, spermatozoal motility diminished after prolonged incubation of diluted semen. Beigi *et al*. (2022) in their study, where they assessed the sperm parameters in addition to other characteristics in samples of normozoospermic men *in-vitro* at different time intervals (0, 1, 1.5, and 2 hours) after incubation at 37°C, argued that time could affect sperm quality as one of many factors. Therefore, in the present study, different effects on roosters' sperm motility patterns (TM, PM, VCL, VSL, VAP, ALH, BCF, and LIN were investigated and compared regularly to 0 hours and the control after 24 hours of incubation period at 37°C in the presence of different types and concentrations of antibiotics (figures 1 and 2).

V.1.1. Tested antibiotics' effect on sperm total and progressive motility

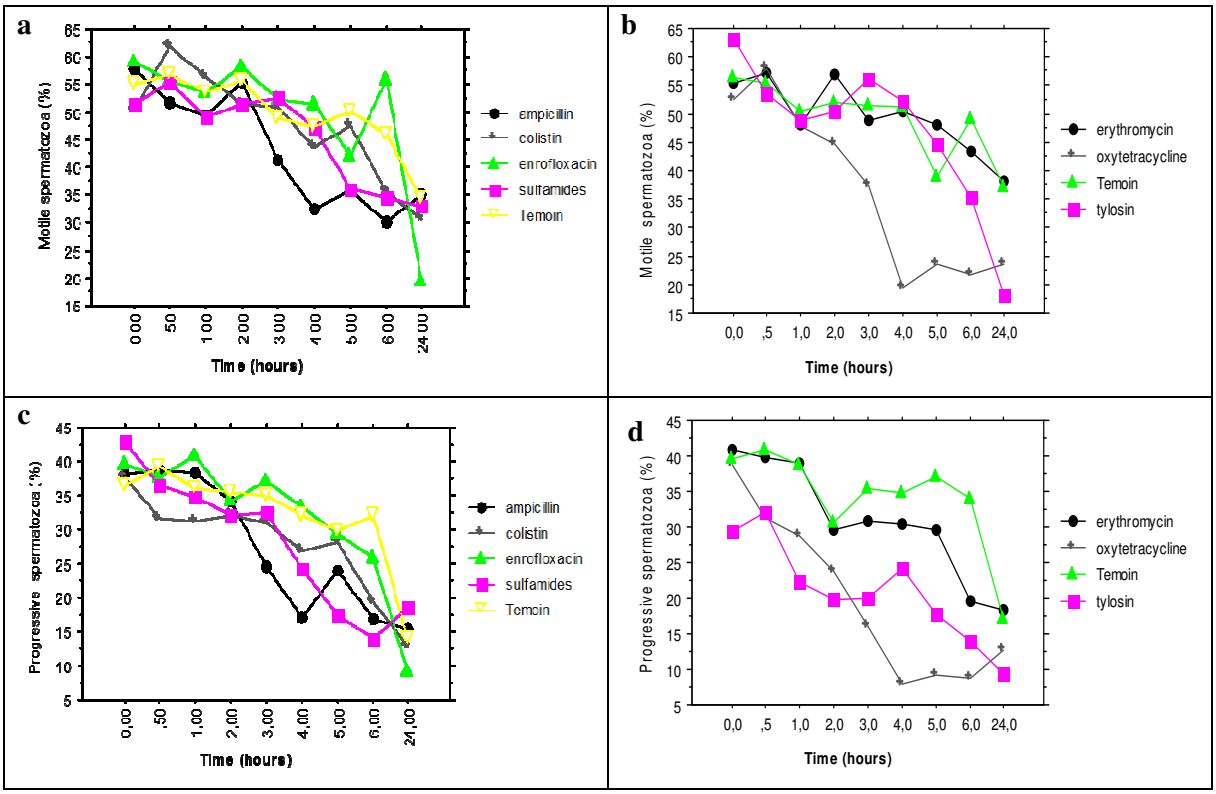


Figure 5. *In-vitro effects of oxytetracycline, ampicillin, colistin, tylosin, erythromycin, enrofloxacin, and sulfonamides on rooster' sperm total motility TM%* ***(a and b)*** *and progressive motility PM%* ***(c and d)*** *after 0, 0.5, 1, 2, 3, 4, 5, 6 and 24 h of incubation at 37°C. Values are presented as mean (± S.E.M).*

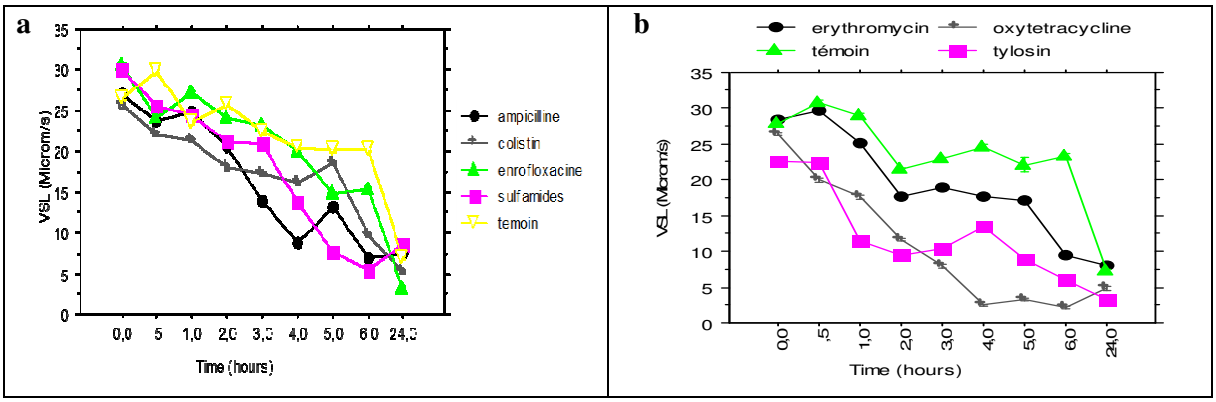
The findings of the present study revealed that spermatozoa were particularly sensitive to almost all antibiotics. The percentage of total moving spermatozoa (TM%) decreased significantly (P<0.05) after 1 to 4 hours of *in-vitro* culture in the presence of most antibiotics, (oxytetracycline, colistin, and ampicillin respectively), to reach the lowest values after 6 hours compared to the control (figure 1a and b). The irregular decrease was observed in sulfonamides, tylosin, and erythromycin groups, contrary to enrofloxacin in comparison to the control, it slightly decreased TM% within the first hour of contact to significantly increased ($P < 0.05$), especially after 5 hours of incubation, the same tendency was observed in progressive sperm motility (PM%). Only 30 minutes after the incubation, it showed a significant decline except for enrofloxacin which was significantly decreased just after 5 hours compared to the control (figure 1c and d). Similar results were found by Beigi *et al*. in

2022, they demonstrated that after 2 hours, both progressive motility and normal morphology decreased when the percentage of non-motile sperm increased compared to 0 hours. Similarly, just after the thawing of ram's samples, no significant differences were observed either for TM or for PM percentages among the different antibiotic treatments. However, after 2 hours of incubation at 37°C, all the samples showed a significant decrease from 0 hours (Anel-Lopez *et al*. 2021).

Previous reports showed that antimicrobials could induce reversible or irreversible effects on sperm motility. The ability of drugs such as tetracycline to chelate calcium is probably the main factor in this impairment (Hargreaves *et al*., 1998; Khaki, 2015). Conversely, King *et al*. (1997) observed no significant differences in sperm motility as well as kinematics in samples treated with six different antibiotics.

Although both drugs belong to the same antibiotic family, the negative effect of tylosin on sperm motility was greater than erythromycin in a time-dependent manner. However, a strong and positive relationship between enrofloxacin and sperm motility was observed. It means that the drug was tolerated by the rooster spermatozoa at a concentration of 5 µg/ml of 0.9% NaCl by prolonging their survival and enhancing their motility for up to 6 hours just like in the control group and comparing to the other antibiotics.

V.1.2. Tested antibiotics' effect on the kinematic parameters



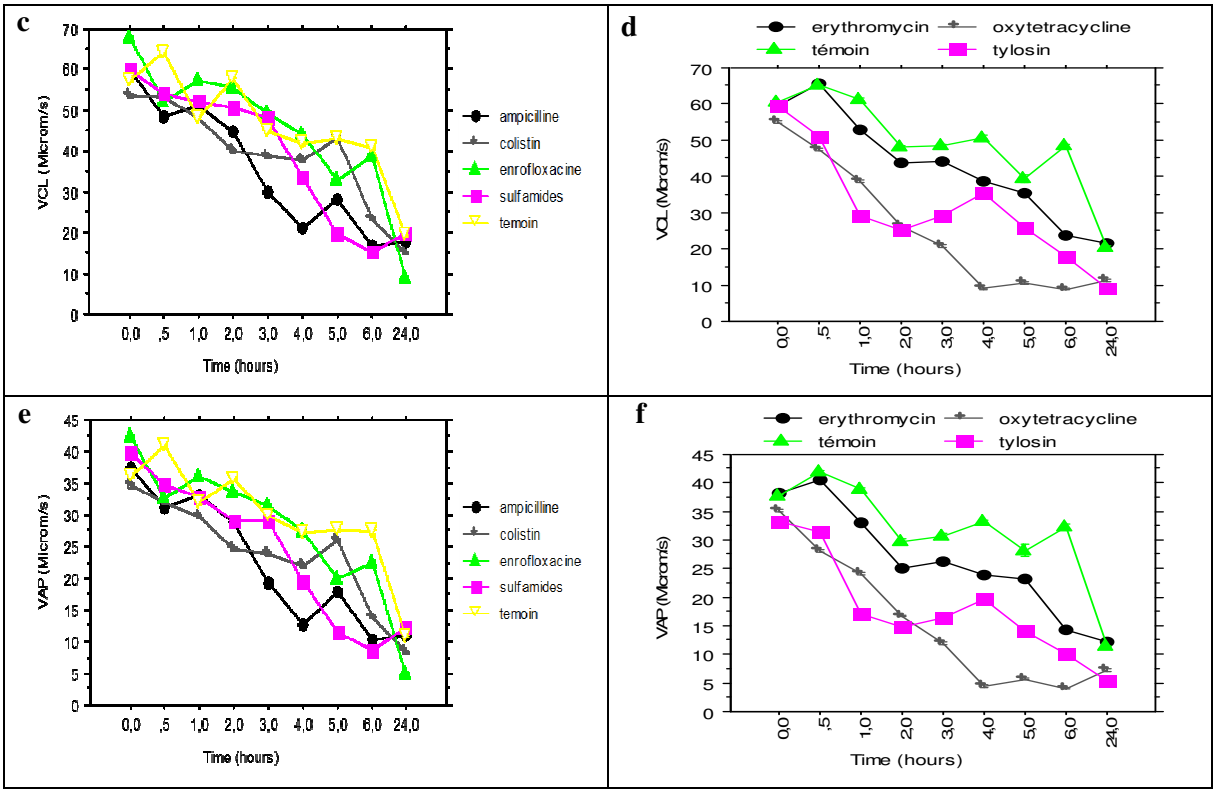


Figure 6. *In-vitro effects of oxytetracycline, ampicillin, colistin, tylosin, erythromycin, enrofloxacin and sulfonamides on rooster' sperm straight linear velocity (VSL)* ***(a and b)****, curvilinear velocity (VCL)* ***(c and d)*** *and average path velocity (VAP)* ***(e and f)*** *after 0, 0.5, 1, 2, 3, 4, 5, 6 and 24 h of incubation at 37°C. Values are presented as mean (± S.E.M).*

Analysis of straight-line velocity (VSL), curvilinear velocity (VCL) and average path velocity (VAP) (figures 2 a, b, c, d, e, and f) showed a regular decrease in oxytetracycline group. The highest effect was recorded after 4 hours of incubation. After 6 hours, all spermatozoa were static. Also, a significant decline ($P < 0.05$) was observed in the other treated groups, except for enrofloxacin which was highly tolerated by spermatozoa within the first 4 hours of contact. In contrast, a slight improvement was noticed after 3 to 4 hours in ampicillin, tylosin, and colistin groups to reach the lowest values after 5 hours. At 24 hours, all spermatozoa in the treated samples as well as in the control were immotile. Our results are relatively consistent with Millsop's and Semet's reviews that reported the negative effects of antibiotics on male fertility via inducing direct and indirect sexual dysfunction and spermatogenesis impairment (Millsop *et al*. 2013; Semet *et al*. 2017).

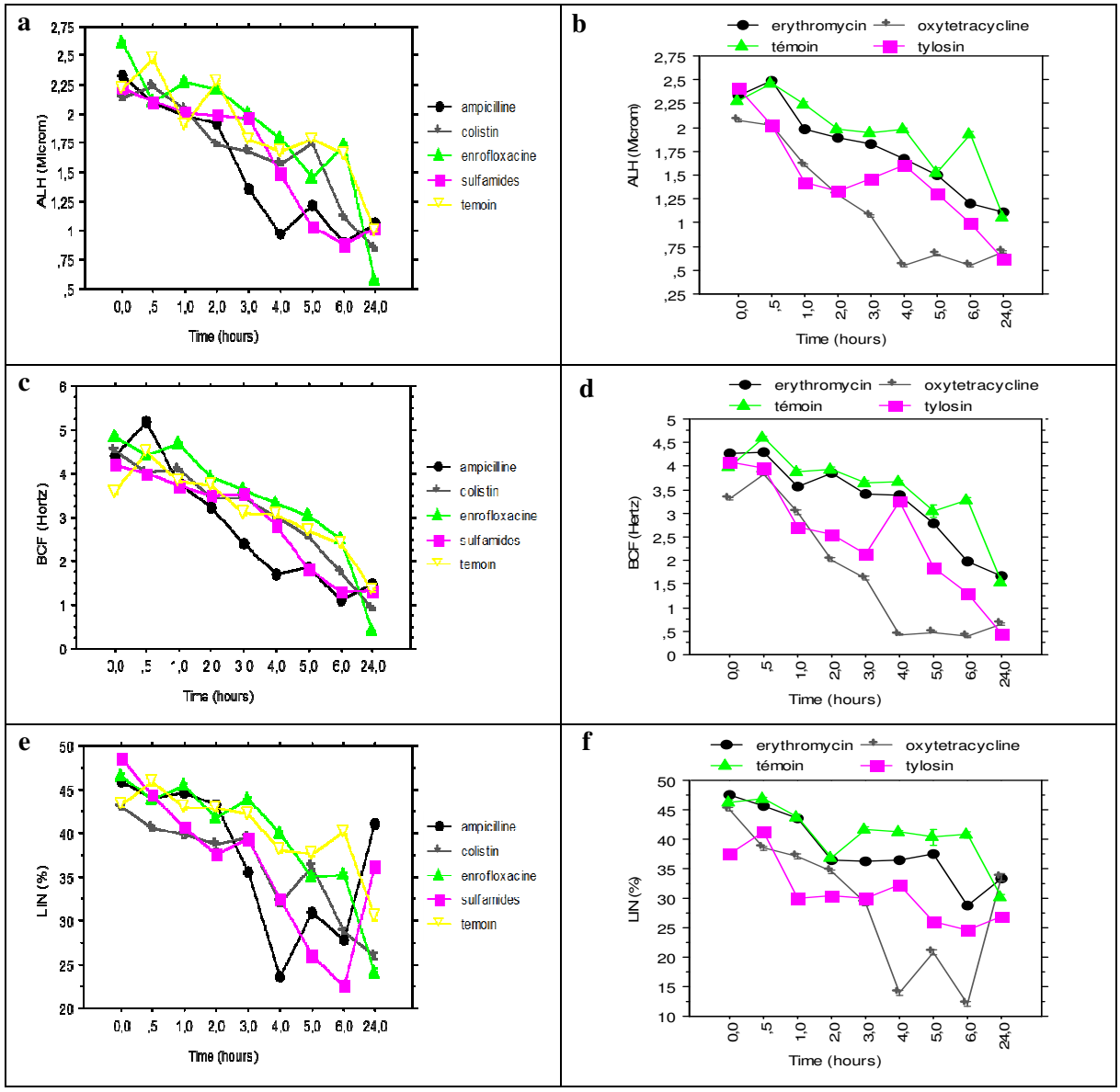


***Figure 7**. In-vitro effects of oxytetracycline, ampicillin, colistin, tylosin, erythromycin, enrofloxacin and sulfonamides on rooster' sperm amplitude of lateral head displacement (ALH) **(a and b)**, beat-cross frequency (BCF) **(c and d)** and linearity (LIN) **(e and f)** after 0, 1, 2, 3, 4, 5, 6 and 24 h of incubation at 37°C. Values are presented as mean (± S.E.M).*

A drastic reduction in the amplitude of lateral head displacement (ALH) and beat-cross frequency (BCF) attracted a great deal of attention after 2 to 3 hours of incubation in oxytetracycline, sulfonamides, tylosin, and ampicillin groups (Figure 3 a, b, c and d) to be slightly improved after 3 and 4 hours in ampicillin and tylosin groups respectively. However, for the sulfonamides and colistin-treated samples, the highest impact was observed after 4 and 5 hours respectively. The sperm movement linearity (LIN) expressed the same tendency and

developed as well as velocities in most antibiotic groups (figures 3 e and f). However, there was no significant difference in enrofloxacin group compared to the control. The parameter was slightly reduced in tylosin and erythromycin groups. Moreover, it was enhanced significantly after a longer incubation period (4 and 6 hours) in ampicillin and sulfonamides samples respectively.

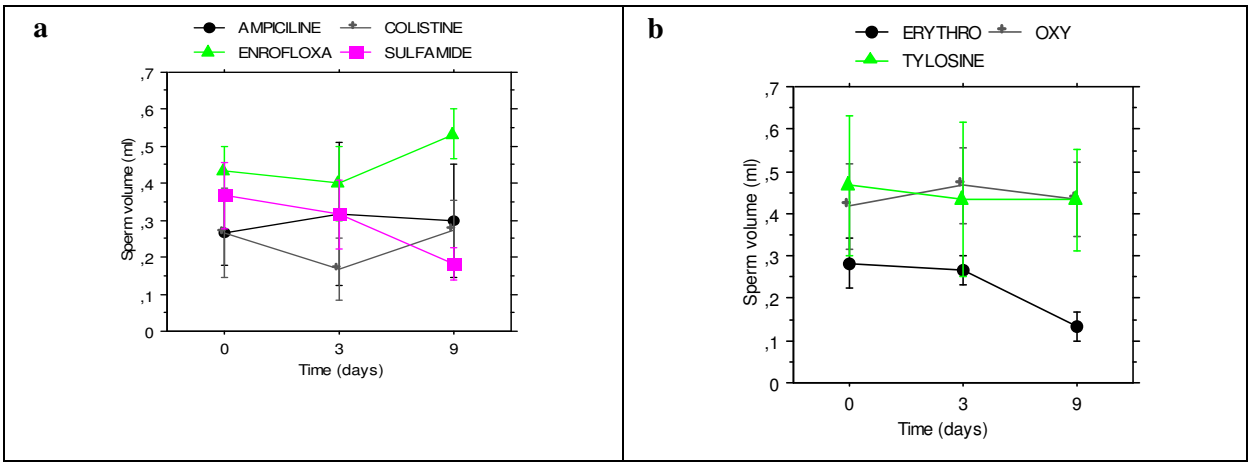
There are still many unanswered questions concerning the *in-vitro* effects of antibiotics on rooster spermatozoa motile parameters. The scarcity of information regarding the protocols of cryopreservation and the storage of that semen for AI prompts us to carry out further investigations such as the present study.

V.2. *IN-VIVO* experimental results

V.2.1. Fresh semen traits

Immediately after collection, Neat semen samples were checked for differences in sperm color, acidity, seminal fluid viscosity, and agglutination. All antibiotics had no significant effect ($P < 0.05$) on either of these parameters over the concentration range tested.

V.2.2. Tested antibiotics' effect on sperm volume, concentration, and viability



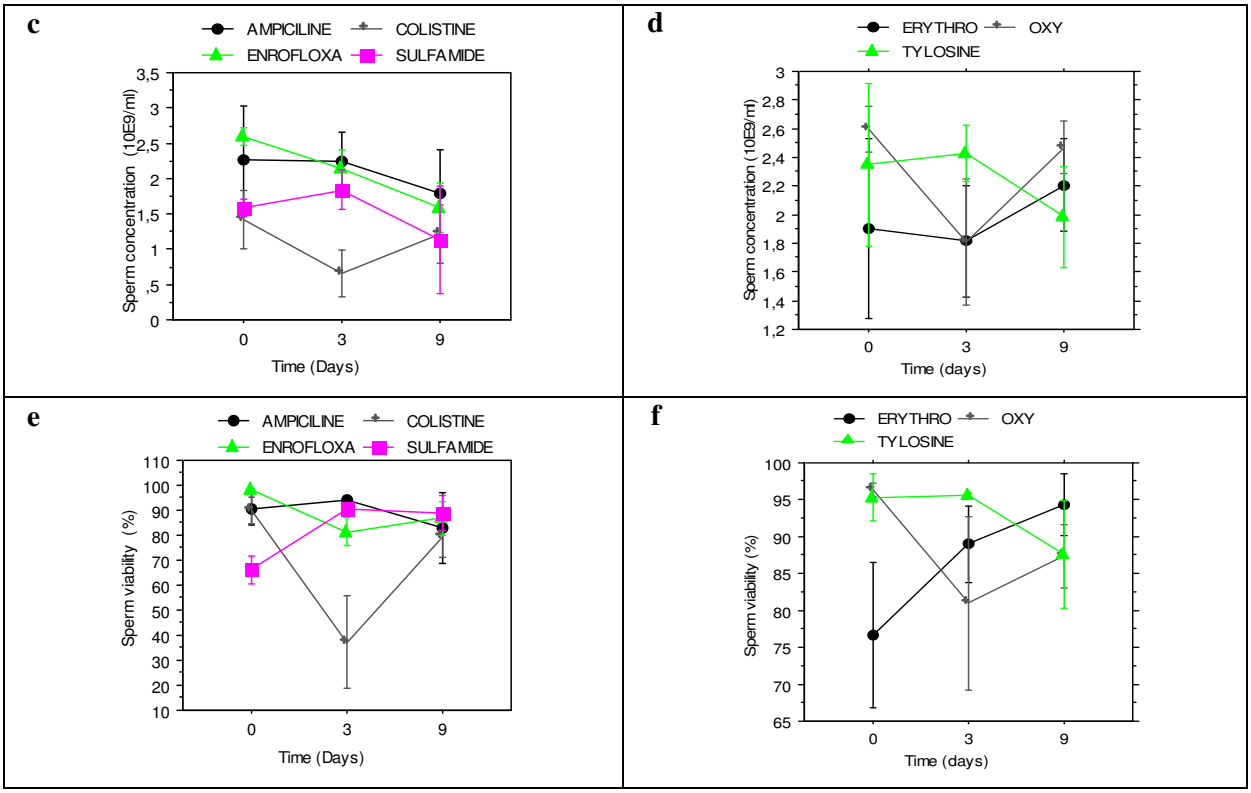


Figure 8. *In-vivo effects of oxytetracycline, ampicillin, colistin, tylosin, erythromycin, enrofloxacin, and sulfonamides on rooster' sperm volume* ***(a and b)****, concentration* ***(c and d),*** *and viability* ***(e and f)*** *at days 0 (T0), 3 (T3) and 9 (T9) of treatment. Values are presented as mean (± S.E.M).*

Our results revealed that ampicillin, tylosin, and oxytetracycline had no significant ($P < 0.05$) effect on sperm volume. This parameter did not deteriorate; it was slightly enhanced by oxytetracycline and ampicillin treatments at T3 and then remained relatively stable during the study period. However, erythromycin, colistin, enrofloxacin, and sulfonamides significantly decreased ($P < 0.05$) the ejaculate volume compared to the pre-treatment samples that represent the control (T0). Except for sulfonamides and erythromycin, the values lightly increased at T9, especially for enrofloxacin (figures 1. a and b). According to Vicari *et al* (2016), this heterogeneity could be related to variable antibiotics effects on reproductive secretory glands as the ejaculate volume for a large part is determined by the production of seminal fluid. Figure 1 c and d shows that sperm concentration was adversely affected by oxytetracycline, enrofloxacin, and colistin after 3 days of treatment. Similarly, previous research revealed adverse effects of fluoroquinolones such as enrofloxacin on testis tissue, sperm count, viability, and motility (Aral *et al.* 2008; Ebadimanas *et al.* 2018). In our study, tylosin did not have any significant effect during the first days of treatment. However, it decreased obviously and significantly ($P < 0.05$) the concentration of spermatozoa from T3 to

the end of the experience. In the opposite and contrary to erythromycin, sulfonamides enhanced sperm concentration at T3 to be reduced at T9. In erythromycin and ampicillin groups, the highest negative impact was respectively on the 3rd and the 9th days of treatment. Gametes viability was significantly ($P < 0.05$) enhanced by erythromycin and sulfonamides (figures 1 e and f). However, this parameter was dramatically affected by enrofloxacin, colistin, and oxytetracycline after 3 to 6 days of treatment, and then it was raised at T9 to be similar to T0.

Similar results were reported by Raji *et al*. in 2006, Elsawy *et al*., and Ebadimanas *et al*. in 2018 who showed that enrofloxacin and ampicillin decreased sperm concentration and viability with some histopathological alterations in rats' reproductive organs. Furthermore, a variety of negative effects, particularly in rats, was caused by colistin (Bouchicha *et al*., 2022). It increased sperm abnormality, apoptosis, oxidative stress and decreased sperm motility (Aksu *et al*., 2018, 2020). These negative effects could be attributed to decreased fructose and protein levels, which affect glycoproteins secreted by the epididymis (Gupta *et al.* (2013). Or it could be related to a disorder in proliferation cells in the tubules, a decline in anti-oxidant enzymes through the production of reactive-oxygen-species, sperm cell membrane toxicity, or reproductive hormones alteration (Drobnis and Nangia, 2017; Manas and Najafi, 2017). However, Qadeer *et al*. in their study (2013), suggest that the combination of colistin-penicillin does not deteriorate semen quality.

V.2.3. Tested antibiotics' effect on sperm total and progressive motility

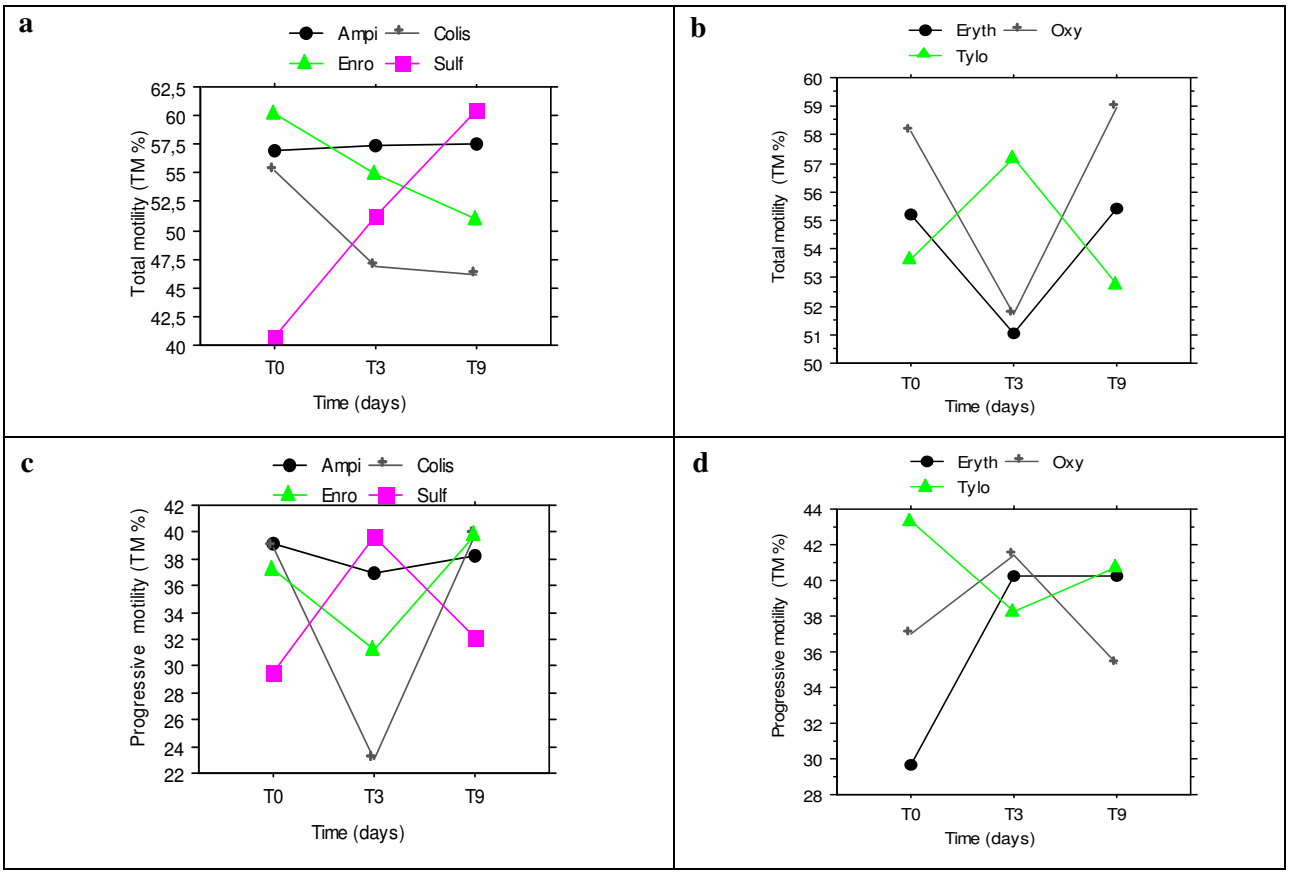


Figure 9. *In-vivo effects of oxytetracycline, ampicillin, colistin, tylosin, erythromycin, enrofloxacin, and sulfonamides on rooster' sperm total motility TM%* ***(a and b)*** *and progressive motility PM%* ***(c and d)*** *at days 0 (T0), 3 (T3) and 9 (T9) of treatment. Values are presented as mean (± S.E.M).*

As shown in Figure 2, varying effects on sperm motility were recorded, contrary to tylosin, most antibiotics (oxytetracycline, erythromycin, enrofloxacin, and colistin) induced a significant ($P < 0.05$) negative effect on total motility (TM %) at T3 (figures 2 a and b). Except for enrofloxacin and colistin, values were raised again at T9. Whereas, no significant impact was observed in the ampicillin group. Progressive sperm motility (PM %) was decreased in the ampicillin, enrofloxacin, colistin, and tylosin groups, especially in T3 (figures 2 c and d). At T9, a remarkable increase was noticed. Forward motile spermatozoa (PM %) was enhanced by erythromycin and sulfonamides. The same tendency was observed in total motility (TM %) that was increased regularly by sulfonamides during the hall experiment.

These results highlighted the effectiveness of erythromycin and sulfonamides to positively affect sperm motility. Probably, it could be through the elevation of testosterone levels (Tanyildizi and Bozkurt, 2003b). Conversely to Alavi-Shoushtari *et al*. (2007) who found that erythromycin had no negative effect on the buffalo' sperm, significant alteration of semen traits was observed by Berndtson and Foot (1976), Hargreaves *et al.* (1998) and Bodetti *et al.* (2003), in Holstein bulls, normozoospermic men and koalas respectively.

V.2.4. Tested antibiotics' effect on the kinematic parameters

The kinematic parameters before and after therapy are shown in Figures 3 and 4. Compared to the control (T0), the results indicated that oxytetracycline, ampicillin, colistin, enrofloxacin, and tylosin impaired significantly ($P < 0.05$) most CASA motile variables including velocities (VSL, VCL, and VAP), ALH, LIN, and BCF. The highest impact was recorded on the 3rd day of treatment for the majority of antibiotics and on the 6th day for the oxytetracycline group. On T9, values increased but remained lower than T0. Our results are consistent with previous research that showed the deleterious effects and lower fertility in each case where the previous antibiotics were used (Lorton *et al*. 1988; Hargreaves *et al*. 1998; Elzeinová *et al*. 2013; Elsawy *et al*. 2018).

No significant difference was observed in the ampicillin group concerning LIN. The parameter remained stable during the test (figure 4. e). In this study, the drug presents fewer negative effects on spermatozoa motile parameters compared to enrofloxacin and colistin. However, in 2006, Raji *et al*. reported that ampicillin caused a significant reduction ($P < 0.05$) in the testes weight, seminal vesicles, epididymis, and testosterone levels in addition to the obvious decrease in sperm motility, viability, and concentration. In contrast, the most spectacular amelioration was recorded in erythromycin and sulfonamides groups with a significant ($P < 0.05$) increase in all kinematics. The inclusion of 20 mg/kg/bwt of erythromycin improved VCL, VSL, VAP, ALH, BCF, and LIN throughout the experience, especially after a long period of treatment. Except for LIN that was slightly decreased on T9 in the group of roosters treated with 140 mg/kg/bwt of sulfonamides, the same observation was recorded with sperm velocities, ALH, and BCF.

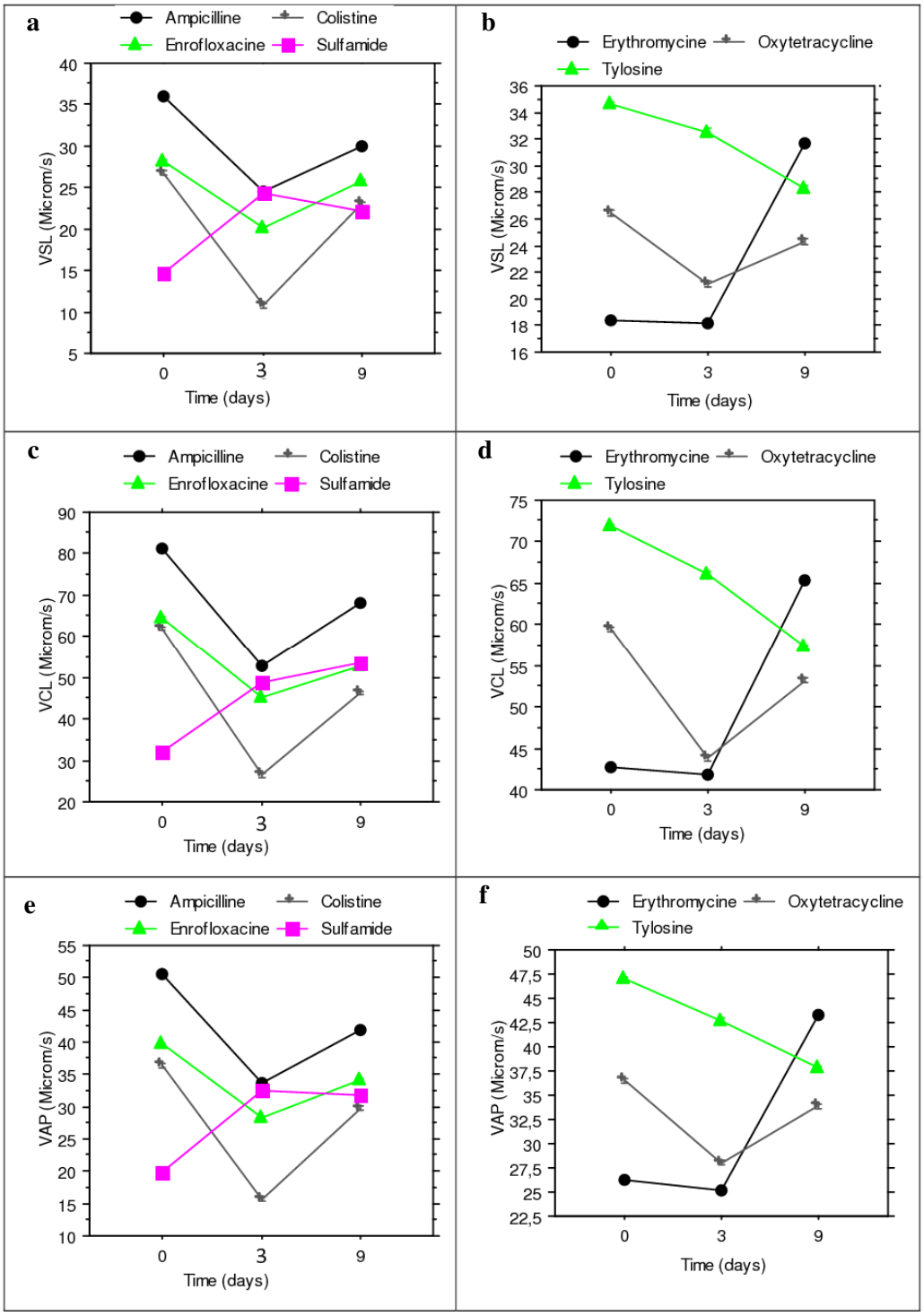


***Figure 10**. In-vivo effects of oxytetracycline, ampicillin, colistin, tylosin, erythromycin, enrofloxacin, and sulfonamides on rooster' sperm straight linear velocity (VSL) **(a and b)**, curvilinear velocity (VCL) **(c and d)** and average path velocity (VAP) **(e and f)** at days 0 (T0), 3 (T3) and 9 (T9) of treatment. Values are presented as mean (± S.E.M).*

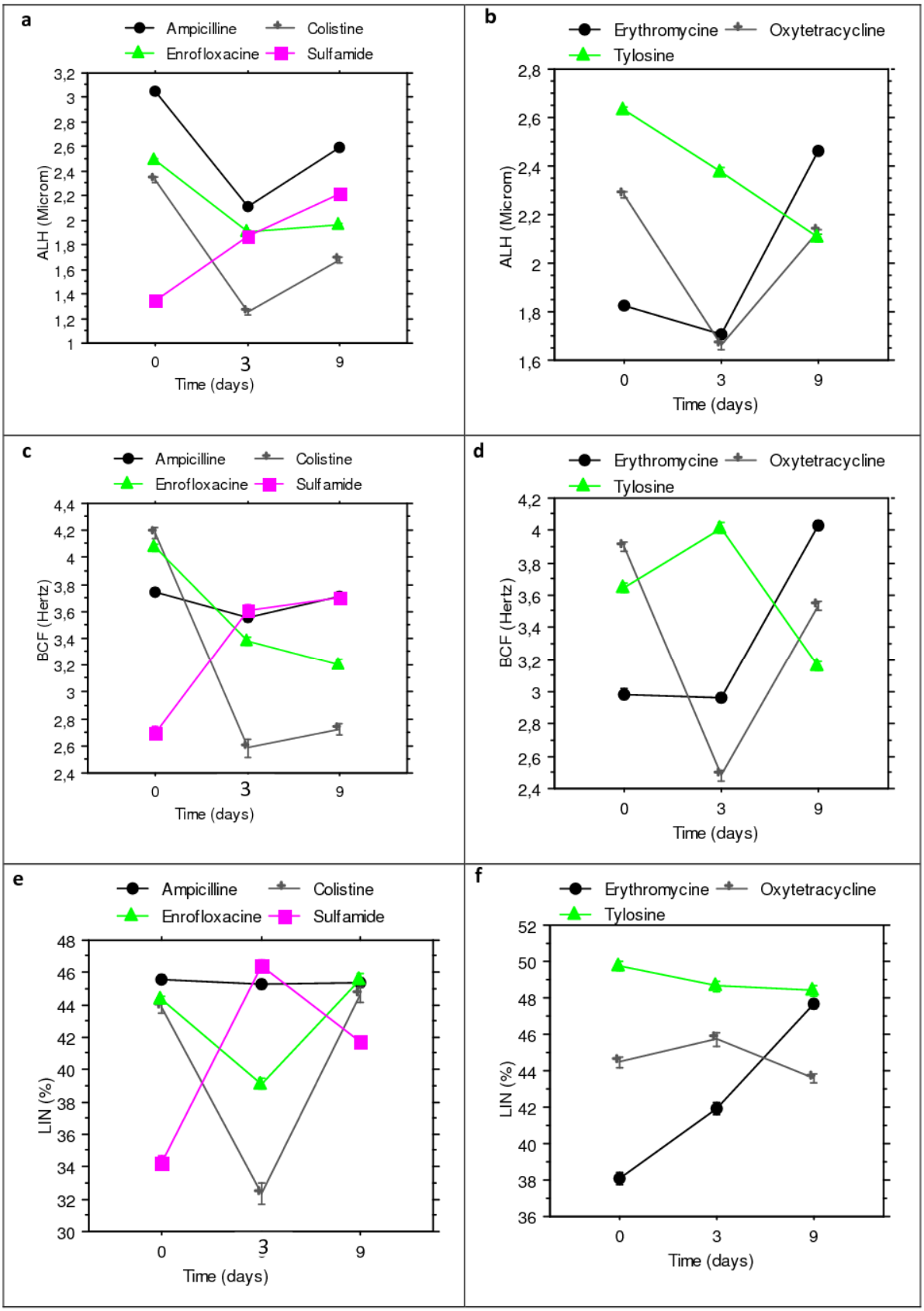

a
Ampicilline
Colistine
Enrofloxacine
Sulfamide
ALH (Microm)
Time (days)
b
Erythromycine
Oxytetracycline
Tylosine
ALH (Microm)
Time (days)
c
Ampicilline
Colistine
Enrofloxacine
Sulfamide
BCF (Hertz)
Time (days)
d
Erythromycine
Oxytetracycline
Tylosine
BCF (Hertz)
Time (days)
e
Ampicilline
Colistine
Enrofloxacine
Sulfamide
LIN (%)
Time (days)
f
Erythromycine
Oxytetracycline
Tylosine
LIN (%)
Time (days)

Figure 11. *In-vivo effects of oxytetracycline, ampicillin, colistin, tylosin, erythromycin, enrofloxacin and sulfonamides on rooster' sperm amplitude of lateral head displacement (ALH)* ***(a and b)****, beat-cross frequency (BCF)* ***(c and d)*** *and linearity (LIN)* ***(e and f)*** *at days 0 (T0), 3 (T3) and 9 (T9) of treatment. Values are presented as mean (± S.E.M).*

Third Section: Conclusion and Perspectives

Conclusion and Perspectives

Antibiotics are commonly used in animal husbandry to treat or prevent bacterial infections. The findings from numerous studies have shown that their use in livestock has been associated with negative effects on reproductive parameters, including sperm quality and quantity. Antimicrobials could reduce reversibly or irreversibly the motility and viability of spermatozoa. These effects are influenced by factors such as the specific antibiotic class, duration of treatment, and individual variations in response; they can be long-lasting; and they may persist even after antibiotic treatment is discontinued. Therefore, it became evident that while antibiotics are crucial for combating bacterial infections, their impact on sperm quality should not be overlooked.

To our knowledge, this thesis represents the first investigation on the potential effects of commonly prescribed antibiotics, Ampicillin, enrofloxacin, colistin, erythromycin, tylosin, oxytetracycline, and Sulfonamides on breeding rooster sperm quality, exactly on the sperm parameters of motility that strongly refer to male' fertility, using the CASA system, known to generate objective parameters. The current study delved into the intricate relationship between these antibiotics and sperm volume, concentration, viability, motility, and all kinematics *in vitro* and *in vivo*, shedding light on the most significant effects.

In the *in-vitro* culture, spermatozoa were particularly sensitive to almost all antibiotics. The percentage of total and progressive moving spermatozoa in addition to all CASA motile parameters decreased significantly after 2 to 4 hours in the presence of oxytetracycline, colistin, ampicillin, tylosin, erythromycin, and sulfonamides respectively to reach the lowest values after 6 hours comparing to the control. Consequently, they appear not suitable in cryopreservation extenders except enrofloxacin which showed a positive relationship with sperm kinematics. It means that the drug was highly tolerated by the rooster's sperm cells by enhancing their motility and prolonging their survival by up to 6 hours compared to the other antibiotics. The negative effect of tylosin on sperm motile parameters was greater than erythromycin although both drugs are macrolides. After 6 hours of incubation, all spermatozoa were static in most sperm treated samples compared to the control group. These implications should be considered when prescribing antibiotics, particularly for prolonged periods. Further *in-vitro* research is warranted to better understand the mechanisms underlying these effects, as well as to identify potential strategies to mitigate or counteract any negative impacts on sperm parameters.

In the *in-vivo* study, Initial evaluations of ejaculate appearances were uniform. There was no significant difference between the pre-treatment samples (T0) and those of T3 and T9 representing the 3rd and the 9th days of treatment concerning sperm color, pH, seminal fluid viscosity, and agglutination. The ejaculate volume did not deteriorate in the ampicillin, tylosin, and oxytetracycline groups; it remained relatively stable during the study period compared to T0. However, it was significantly decreased in erythromycin, colistin, enrofloxacin, and sulfonamides groups. Except for sulfonamides and erythromycin, the values lightly increased after 9 days of treatment, especially for enrofloxacin. Oxytetracycline, colistin, tylosin, and enrofloxacin caused a significant negative effect on motile parameters including velocities (VSL, VCL, and VAP) compared to ampicillin-treated samples; so lesser dosage and duration is recommended. The results highlighted the effectiveness of sulfonamides and erythromycin on sperm movement characteristics. Both antibiotics considerably improve total and progressive motility, viability as well and all kinematics.

The findings presented in this thesis underscore the complexity of the effects of antibiotics on sperm motile parameters. While some antibiotics have demonstrated adverse effects on sperm parameters, others have shown limited, neutral, or even improved influence. The mechanisms underlying these opposite effects are multifaceted, encompassing direct impacts on sperm production, potential disruptions to the testicular microenvironment, and alterations in hormonal balances. This discrepancy highlights the need for standardized research protocols and larger-scale studies to provide more insights into the interaction between breeding roosters' sperm and different classes of antibiotics. Moreover, successful artificial insemination programs in breeding rooster farms are dependent on the semen quality. Therefore, it is imperative that the effects of antibiotics on sperm characteristics and function are known when treating cocks. As we delve deeper into this realm of research, we uncover new avenues for enhancing rooster’s fertility and advancing the broader fields of reproductive biology and animal health.

In summation, this thesis illuminates and extensively explores the different effects of antibiotics on sperm parameters. The research journey highlighted both *in-vitro* and *in-vivo* influences of antibiotic administration on roosters’ sperm characteristics, shedding light on the complexities of this relationship.

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