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IN VITRO TOXICOLOGY STUDY: TO STUDY TOXIC EFFECT OF ANTI- BIOTIC ON MALE FERTILITY

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ABSTRACT

Reproduction is essential for survival, as it helps maintain population balance in nature. There are two types of reproduction: sexual and asexual. Humans reproduce sexually, which involves the fusion of male gametes (sperm) and female gametes (eggs). For fertilization to occur, high-quality sperm is needed. Various factors can affect male fertility, including pollution, an unhealthy diet, alcohol consumption, smoking, the use of narcotics, certain diseases, disorders, and in some cases, medications like antibiotics. In recent years, the use of antibiotics has increased due to the rise in viral infections. This poses a higher risk of infertility because antibiotics are designed to kill cells, and sperm are one type of cell. Antibiotics do not affect sperm immediately or completely, but over time and with prolonged use, they can have detrimental effects on sperm count and function. In my research, I tested six different types of antibiotics on sperm cells and found that they are capable of killing sperm cells. "Research has shown that prolonged use of antibiotics can reduce sperm quality and function." For this research work I chose 6 higher anti-biotics.

KEYPOINTS: Human reproduction, Human Sperm, Female egg, Anti-biotics, Toxicology.

INTRODUCTION

Reproduction is essential for survival, as it helps maintain population and balance in nature. There are two types of reproduction: sexual and asexual.[1,2] Humans reproduce sexually, which involves the fusion of male gametes (sperm) and female gametes (eggs). Sperm are

male reproductive gametes responsible for carrying male genetic material. Human sperm are divided into three parts:

i) head, ii) middle piece, and iii) tail as show in figure 1.

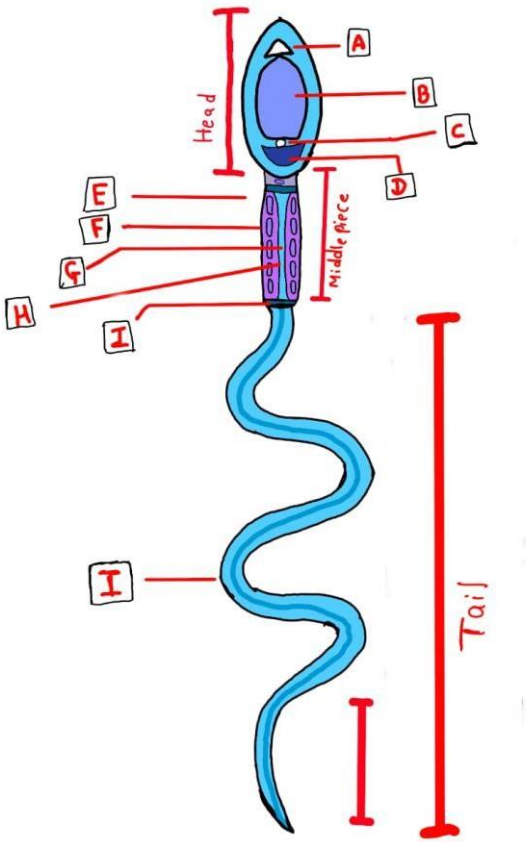

Fig. 1: Structure of sperm.

The head is further divided into four components: (a) acrosome, (b) nucleus, (c) nucleoplasm, and (d) centriole.

(a) Acrosome

The acrosome has two types of membranes: the sub-acrosomal membrane and the outer acrosomal membrane. It is a cap-like structure located at the top of the sperm's head. The acrosome contains two specific enzymes: hyaluronidase and acrosin, which help the sperm penetrate the outer layer of the egg during fertilization. Hyaluronidase is a key enzyme stored in the acrosome that aids the sperm in penetrating the cumulus oophorus, a layer of cells that surrounds and protects the inner layers of the egg. Acrosin, on the other hand, is a sperm-specific serine proteinase that is stored as an inactive precursor called proacrosin. It plays a critical role in fertilization by assisting the sperm in penetrating the zona pellucida, the outer protective layer of the egg.[3,4,5,6,7,8]

(b) Nucleus

The nucleus is the largest organelle in a cell and is present in every single cell, except for mature erythrocytes (red blood cells). It carries the genetic material of both men and women, which includes DNA (deoxyribonucleic acid) and RNA (ribonucleic acid). The nucleus is made up of three parts: a) Nuclear envelope b) Nucleolus The nuclear envelope is a double membrane that surrounds the nucleus, separating it from the cytoplasm. It acts as a barrier and regulates the movement of molecules into and out of the nucleus through nuclear pores. The nuclear envelope consists of an inner nuclear membrane (INM) and an outer nuclear membrane (ONM).[9,10]

(c) Nucleolus

A prominent structure within the nucleus responsible for producing ribosomes and ribosomal RNA (r – RNA).[11]

(d) Nucleoplasm

The gel-like substance within the nucleus, similar to cytoplasmic but specific to the nucleus it supports and cushions the nuclear components.[12,13]

(e) Centriole

Sperm cells contain two centrioles: the proximal centriole and the distal centriole. The proximal centriole is located at the bottom of the head, while the distal centriole is situated at the end of the tail. The distal centriole plays a crucial role in the formation of the flagellum, which is responsible for the sperm's movement.[14,15]

II) Middle piece

It is medal part of sperm and its divided into four parts.

a) Mitochondria

The average diameter of mitochondria is about 0.5 micrometers (µm), with a length ranging from 1 to 4 µm. Mitochondria are known as the powerhouses of the cell, as they generate ATP by performing the Krebs cycle. The ATP synthase is most efficient in the final stage of aerobic respiration. Mitochondria provide energy to sperm for movement.[16,17]

b) Dense fiber

Dense fibers in sperm, known as Outer Dense Fibers (ODFs), are protein structures found in

the sperm tail that provide stability and elasticity. Composed mainly of proteins such as ODF1 and ODF2, these fibers play a crucial role in sperm motility by serving as a passive structural component that aids in the bending and recoil of the tail. Damage or deficiencies in ODFs are associated with infertility and reduced sperm motility, underscoring their vital importance in male fertility.[18]

c) Axoneme

In sperm, the axoneme forms the structural and functional core of the flagellum, which is essential for motility. It is found in the principal piece and end piece of the sperm tail and has the classical 9 + 2 arrangement of microtubules: nine outer doublets of microtubules and two central singlets of microtubules. Dynein arms, both inner and outer, are attached to the A-tubule of each doublet and act as motor proteins. Radial spokes connect the outer doublets to the central pair, while nexin links connect adjacent doublets. Dynein hydrolyzes ATP, which is provided by mitochondria in the midpiece, to generate sliding forces between the microtubules. These forces are converted into a bending, whip-like motion of the sperm tail, which propels the sperm forward, allowing it to reach and fertilize the egg.[19,20,21]

d) Annulus

The annulus, also known as Jensen's ring, is a ring-shaped structure composed of septin proteins located at the junction between the midpiece and the principal piece of the sperm tail. It is primarily made up of septin proteins, especially septins 4, 7, and 12, and appears as a dense, ring-like band under an electron microscope. The annulus functions as a diffusion barrier, separating the proteins and lipids of the midpiece (a mitochondria-rich region) from those of the principal piece. It plays a crucial role in maintaining the integrity of the sperm tail by anchoring mitochondria in the midpiece. Proper function of the annulus ensures correct organization of the axoneme and mitochondrial sheath, which is essential for sperm motility.[22,23]

III) Tail

i) Axial filament

The axial filament is a central supporting structure of the sperm tail (flagellum). It consists of the axoneme and surrounding supporting fibers. This filament runs through the midpiece, principal piece, and end piece of the sperm tail. The core arrangement follows a 9+2 microtubule pattern, featuring nine doublet microtubules around the periphery and two single microtubules in the center. The axial filament acts as the motor core of the sperm tail, with

dynein arms on the microtubules utilizing ATP from the mitochondria located in the midpiece. This process generates sliding of the microtubules, which is converted into bending and a whip-like motion, propelling the sperm forward. This mechanism ensures the motility and fertilizing capacity of the sperm.[24,25,26]

ii) Terminal piece

The text describes the last and thinnest part of the sperm tail, known as the terminal piece. After the principal piece, it consists solely of the axoneme (axial filament) with a 9 + 2 arrangement of microtubules. This section does not contain dense fibers or a fibrous sheath; it is only covered by the plasma membrane. The terminal piece plays a crucial role in the final propagation of the wave- like motion generated by the axoneme, maintaining the continuity of motility from the principal piece to the tip.[27,28]

Ovum

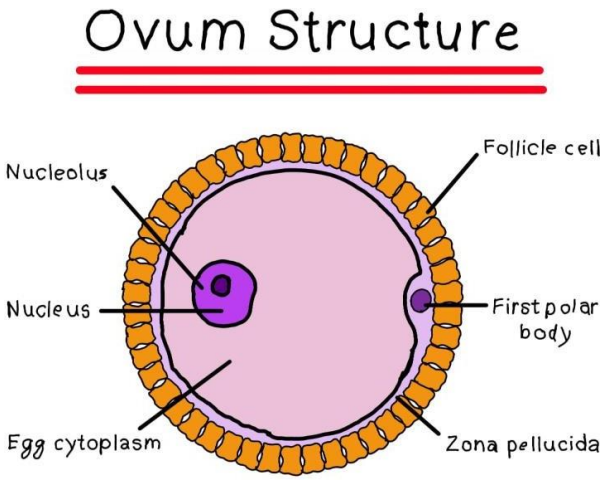

Fig. 2: Structure of ovum (egg cell).

An ovum is the mature female reproductive cell, commonly referred to as an egg cell. It contains half of the chromosomes required for reproduction and is the largest single cell in the human body. Its primary function is to be fertilized by a sperm cell, leading to the formation of a zygote, which then develops into a new organism. The structure of an ovum can be divided into six parts: 1.

Follicle cell 2. First polar body 3. Zona pellucida 4. Egg cytoplasm 5. Nucleus 6. Nucleolus

(i) Follicle cell

A "follicle cell" in a female typically refers to the granulosa and theca cells that surround the immature egg (oocyte) within an ovarian follicle. These cells are crucial for the development

of the egg, producing hormones that regulate the menstrual cycle, and provide structural support to the follicle.[29,30]

(ii) First polar body

The first polar body is a small, non-functional cell produced during the first meiotic division of a primary oocyte. It has the same number of chromosomes as the secondary oocyte but contains very little cytoplasm, which is retained by the larger secondary oocyte to provide resources for potential fertilization and development. The first polar body may divide into two more polar bodies during the second meiotic division or degenerate, and its genetic material can be used for genetic testing.[31,32]

(iii)Zona pellucida

The zona pellucida is a glycoprotein layer surrounding a mammalian egg that protects the oocyte and early embryo, and plays a crucial role in fertilization by binding species-specific sperm and preventing polyspermy (fertilization by multiple sperm). It also initiates the acrosome reaction, which is a critical step for sperm penetration, and ensures the correct development and size of the embryo before it implants.[33,34]

(iv)Egg cytoplasm

The egg cytoplasm's primary functions are to provide essential nutrients and cellular components for the developing embryo, act as a "factory" for early development by containing organelles like mitochondria and ribosomes, and contain molecules that can reprogram a somatic cell nucleus. It also plays a crucial role in protecting the genetic material and directing the initial development of a new organism.[35,11]

(v) Nucleus

The nucleus in a female egg (ovum) contains half the genetic material (23 chromosomes) needed for reproduction and acts as the cell's control center. Its main functions are to store the female's DNA, ensure the correct number of chromosomes for a potential new individual is present, and to control the development and maturation of the egg cell.[36,37]

(vi)Nucleolus

The female egg, or oocyte, contains a nucleolus that is crucial for early embryonic development. Initially, the nucleolus is involved in active ribosome production, but its function shifts to a more specialized role in chromatin organization and replication control as

development progresses. During oocyte maturation, the nucleolus undergoes a transformation referred to as the "surrounded nucleolus" (or karyosphere) stage, during which it loses its transcriptional activity and becomes encased in heterochromatin. Following fertilization, the maternal nucleolus plays an essential role in enabling the zygote to advance past the two-cell stage. When sperm cells enter egg cells, they release their genetic material. The 23 chromosomes from the male and the 23 chromosomes from the female fuse to create a total of 46 chromosomes, forming a new living organism.[38,39]

Sperm cells are primarily responsible for fertilization. Male fertility depends on sperm quality, and poor sperm quality is a leading cause of male infertility. Various factors can affect sperm quality and fertility, including pollution, an unhealthy diet, alcohol consumption, smoking, the use of narcotics, certain diseases, disorders, and, in some cases, medications like antibiotics. In my research, I am demonstrating that antibiotics can affect male infertility. Antibiotics are typically used to treat infections, but in some cases, they are also used to treat cancer. This is because antibiotics can function in two ways: they either destroy the DNA of cells or damage the cell wall, which can lead to the destruction of cancer cells. However, an overdose of antibiotics can negatively impact human sperm cells, thus affecting male fertility and sperm quality.

MATERIALS AND METHOD

MATERIAL

1. Drug profile

i) Linezolid[40,41,42]

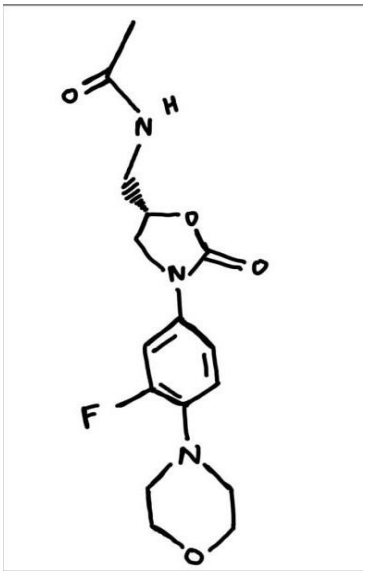

Fig. 3: Linezolid.

Chemical formula : $C16H20FN3O4$

Molecular weight : 337.35 g/mol.

Linezolid is a potent synthetic antibiotic used to treat serious bacterial infections, particularly those resistant to other antibiotics. It is the first drug in the oxazolidinone class of antibiotics.

Primary medical uses

- **Antibiotic-resistant infections**: Linezolid is often a drug of last resort for infections caused by multidrug-resistant bacteria. This includes:
  - **MRSA**: Methicillin-resistant *Staphylococcus aureus* infections.
  - **VRE**: Vancomycin-resistant *Enterococcus faecium* infections, including those with concurrent bacteremia.
- **Pneumonia**: It is used for hospital-acquired and community-acquired pneumonia. For severe cases caused by MRSA, it is an alternative to vancomycin.
- **Skin and soft tissue infections**: Linezolid treats complicated and uncomplicated skin infections, such as those that can occur with diabetes.
- Common side effects
- These typically do not require medical attention and may resolve over time.
- Diarrhea, nausea, vomiting, or stomach pain
- Headache
- Dizziness
- Metallic or unpleasant taste in the mouth
- Oral thrush (white patches in the mouth)
- Insomnia (sleep problems)
- Mild skin rash or itching
- Severe side effects
- Some adverse reactions to linezolid require immediate medical attention.
- Blood disorders (myelosuppression)
- Linezolid can decrease the production of blood cells, especially platelets, which increases the risk of unusual bleeding or bruising.
- **Symptoms:** Pale skin, unusual fatigue, shortness of breath, easy bruising, or unusual bleeding.
- **Monitoring:** Regular blood tests are recommended, especially with long-term use, to monitor blood cell counts.

Mechanism of Action

Unlike other protein synthesis inhibitors that target the elongation phase, linezolid blocks the very first step of bacterial protein production—the initiation phase.

- It binds to the 23S ribosomal RNA of the 50S ribosomal subunit.
- This binding prevents the formation of the 70S initiation complex, a critical component of the bacterial reproductive process.
- This unique mechanism makes cross-resistance with other protein-synthesis-inhibiting antibiotics very unlikely.

Use

- **Vancomycin-resistant *Enterococcus faecium* (VRE) infections:** These can occur with or without bacteria entering the bloodstream.
- **Pneumonia:** It is used for both hospital-acquired (nosocomial) and community-acquired pneumonia.
- **Skin and soft-tissue infections (SSTI):** This includes both complicated and uncomplicated infections, particularly those caused by resistant strains.
- **Methicillin-resistant *Staphylococcus aureus* (MRSA):** Linezolid is effective against this serious and often hard-to-treat bacterial strain.
- **Tuberculosis (drug-resistant):** It can be used as part of a treatment regimen for multidrug-resistant tuberculosis.

ii) Cefixime[43,44,45,46]

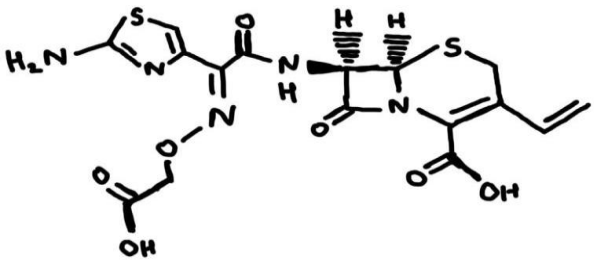

Fig. 4: Cefixime.

Chemical formula: $C_{16}H_{15}N_5O_7S_2$

Molar mass: 453.44g/mol.

Cefixime is a broad-spectrum cephalosporin antibiotic used to treat various bacterial infections.

USE

- **Respiratory tract infections:** Including pneumonia, tonsillitis, pharyngitis, and bronchitis.
- **Urinary tract infections (UTIs):** Such as cystitis and kidney infections.
- **Ear and sinus infections:** Including otitis media and sinusitis.
- **Sexually transmitted infections:** Such as uncomplicated gonorrhea.
- **Typhoid fever:** In some cases, depending on local resistance patterns.

Mechanism of Action

Cefixime is a third-generation cephalosporin antibiotic that works by inhibiting the synthesis of the bacterial cell wall. Its bactericidal effect is achieved through binding to and inactivating penicillin-binding proteins (PBPs).

Severe side effects

- Severe diarrhea (watery or bloody)
- Jaundice (yellowing of the skin or eyes)
- Seizures
- Unusual bruising or bleeding

Common side effects

The most frequently reported side effects are usually mild and include:

- Diarrhea or loose stools
- Nausea and vomiting
- Stomach pain or indigestion
- Headache

Common uses

- **Respiratory infections:** Such as tonsillitis, pharyngitis (sore throat), bronchitis, pneumonia, and sinusitis.
- **Ear infections:** Including otitis media.
- **Urinary tract infections (UTIs):** Such as cystitis and kidney infections.
- **Sexually transmitted diseases (STDs):** It is an alternative treatment for uncomplicated gonorrhea.
- **Other infections:** It is also used to treat typhoid fever and shigella.

iii) Chloramphenicol[47,48,49,50,51]

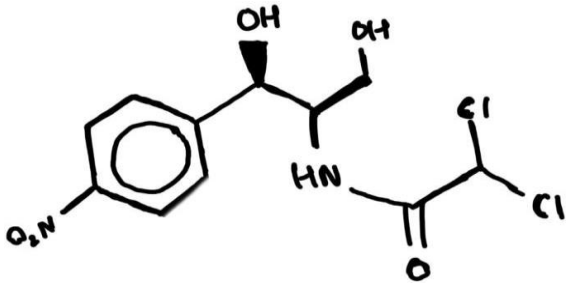

Fig. 5: Chloramphenicol Molecular formula: $C_{11}H_{12}CL_2N_2O_5$

Molecular weight: 323.132 g/mol

Chloramphenicol is a broad-spectrum antibiotic used to treat serious bacterial infections Medical uses.

Due to its risk of serious side effects, chloramphenicol is reserved for life-threatening infections when safer antibiotics are ineffective, contraindicated, or cannot be used.

- **Serious infections:** It is used to treat severe infections such as bacterial meningitis, typhoid fever, and plague.
- **Eye and ear infections:** It is often administered topically as eye drops, eye ointment, or ear drops for infections like conjunctivitis and otitis externa.
- **Other infections:** It can also be used for certain types of anaerobic and rickettsial infections, such as Rocky Mountain spotted fever.

Adverse effects

The toxicity profile of chloramphenicol is the main reason for its restricted use.

- Aplastic anemia: This rare, idiosyncratic, and often fatal side effect is the most serious risk associated with chloramphenicol treatment.
- Bone marrow suppression: A dose-related and reversible form of bone marrow toxicity can occur during treatment, which manifests as a drop in hemoglobin levels.
- Gray baby syndrome: This severe, potentially fatal condition can occur in newborn infants, especially premature babies, who have underdeveloped liver enzymes and cannot properly metabolize the drug. It leads to a toxic buildup of the drug, causing symptoms like abdominal distension, hypotension, cyanosis (blue/gray skin), and collapse.
- Gastrointestinal issues: Common side effects include nausea, vomiting, and diarrhea.
- Neurotoxic effects: Long-term therapy can cause headaches, confusion, and peripheral or optic neuritis.

Mechanism of action

Chloramphenicol is primarily a bacteriostatic agent, meaning it prevents bacteria from reproducing but does not kill them outright.

- It works by inhibiting protein synthesis in bacteria. Specifically, it binds reversibly to the 50S ribosomal subunit, blocking the activity of the enzyme peptidyl transferase.
- This action prevents the formation of peptide bonds between amino acids, stopping the elongation of the polypeptide chain and effectively halting the production of essential proteins necessary for the bacteria to function and multiply.

iv) Vancomycin Hydrochloride[52,53,54,55] Molecular formula **:** C66H75CL2N9O24.HCL Molecular weight : 1485.71 g/mol.

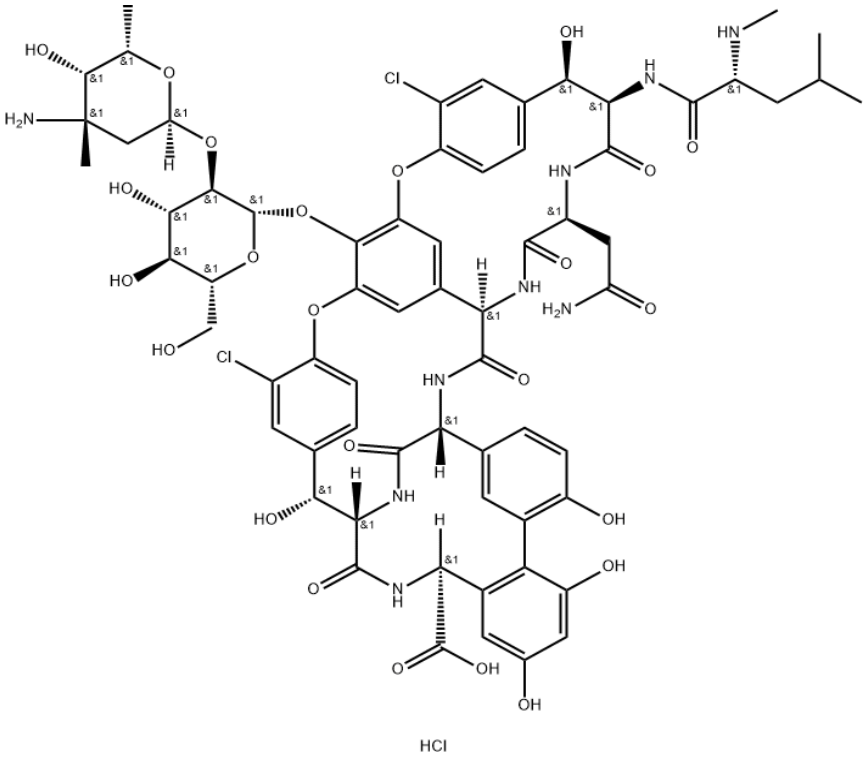

Fig. 6: Vancomycin Hydrochloride.

Vancomycin hydrochloride is a potent glycopeptide antibiotic used to treat severe, life-threatening infections caused by Gram-positive bacteria that are resistant to other antibiotics. It is most notably effective against methicillin-resistant *Staphylococcus aureus* (MRSA) and *Clostridioides difficile*.

IV administration use

- **Septicemia:** Blood infections.

- **Endocarditis:** Inflammation of the heart lining and valves.
- **Osteomyelitis:** Bone infections.
- **Meningitis:** Inflammation of the membranes surrounding the brain and spinal cord.
- **Pneumonia:** Severe lung infections.
- **Surgical prophylaxis:** Prevention of infection during certain surgeries, particularly in patients with heart valve disease who are allergic to penicillin.

Oral administration

When taken by mouth as a capsule or oral solution, vancomycin is not absorbed into the bloodstream. It stays in the gut to treat infections there. It is specifically used for:

- ***C. difficile*-associated diarrhea:** Inflammation of the intestines caused by an overgrowth of *C. difficile* bacteria.
- **Staphylococcal enterocolitis:** Inflammation of the intestinal lining caused by *Staphylococcus aureus* bacteria.

Serious side effects include

- Red man syndrome: A fast IV infusion can cause flushing, a rash on the face and upper body, and a drop in blood pressure.
- Nephrotoxicity: Kidney damage or renal failure.
- Ototoxicity: Hearing loss, vertigo, or ringing in the ears (tinnitus).

Common side effects include

- Nausea, vomiting, and abdominal pain
- Low blood pressure (hypotension)
- Fever and chills
- Injection site reactions (phlebitis)
- Peripheral edema (swelling of the extremities)

Primary mechanism: Inhibition of cell wall synthesis

- **Targeting precursors**

Vancomycin binds to the D-alanyl-D-alanine terminus of the peptide precursors that are used to build the bacterial cell wall.

- **Blocking synthesis**

By binding to these precursors, it inhibits the transglycosylase and transpeptidase enzymes that

are responsible for cross-linking the peptidoglycan polymers.

- **Resulting damage**

This prevents the formation of a strong, rigid cell wall. The bacterial cell wall becomes damaged, and the cell lyses (bursts) due to osmotic pressure.

v) Ceftriaxone[56, 57, 58]

Molecular formula $C_{18}H_{16}N_8NaO_7S_3.3.5H_2O$

Molecular weight 661.60 g/mol.

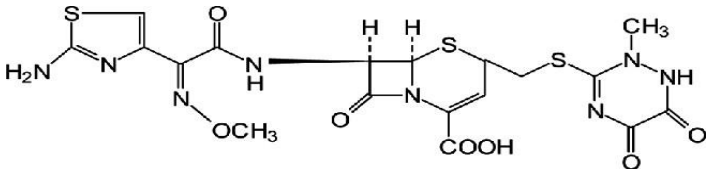

Fig. 7: Ceftriaxone.

Ceftriaxone is a broad-spectrum cephalosporin antibiotic administered by injection to treat a wide range of bacterial infections.

Uses

Ceftriaxone injection is used to treat or prevent various types of bacterial infections, including:

- Meningitis (an infection of the brain and spinal cord)
- Pneumonia and other lower respiratory tract infections
- Ear infections (otitis media)
- Urinary tract infections (UTIs)
- Skin and soft tissue infections
- Bone and joint infections
- Bloodstream infections (bacteremia)
- Lyme disease and gonorrhea
- It is also given before certain types of surgery to prevent postoperative infections.

Common side effects

Some common side effects may include

- Diarrhea
- Rash

- Nausea and vomiting
- Injection site reactions, such as swelling, pain, or a hard lump
- Changes in liver function tests or blood cell counts

Mechanism of action

1. Binding to penicillin-binding proteins (PBPs): Ceftriaxone mimics the D-alanyl-D-alanine portion of the bacterial cell wall's peptidoglycan precursor. It irreversibly binds to and inactivates penicillin-binding proteins (PBPs), which are enzymes on the bacterial cell membrane.
2. Inhibiting cross-linking: The PBPs are responsible for the final transpeptidation step, which cross-links the peptidoglycan chains to give the cell wall its structural rigidity. By blocking this process, ceftriaxone prevents the formation of a stable, rigid cell wall.
3. Bacterial lysis: Without a properly constructed cell wall, the bacterium is unable to withstand its internal osmotic pressure, leading to cell lysis and death.
4. Resistance to β-lactamases: As a third-generation cephalosporin, ceftriaxone is more stable against hydrolysis by β-lactamase enzymes produced by many bacteria, which gives it a broader and more potent spectrum of activity against gram-positive and gram-negative bacteria compared to earlier generations.

vi) Cefepime[59,60,61,62,63,64,65,66]

Molecular Formula C19H24N6O5S2

Molecular Weight 480.56 g/mol

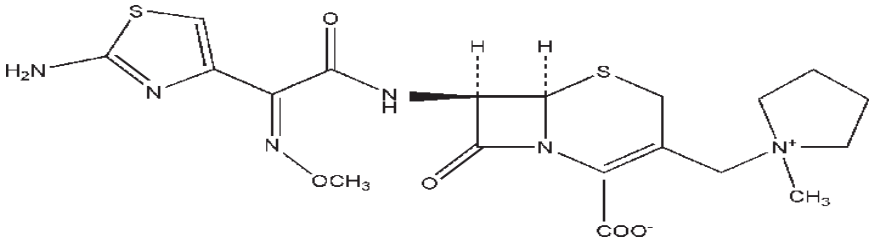

Fig. 8: Cefepime.

Is a fourth-generation cephalosporin antibiotic used to treat moderate-to-severe bacterial infections. It is a broad-spectrum antibiotic, meaning it is effective against a wide variety of both Gram-positive and Gram-negative bacteria.

Common uses

- Pneumonia

- Complicated and uncomplicated urinary tract infections (UTIs)
- Skin and soft tissue infections
- Complicated intra-abdominal infections (often with metronidazole)
- Febrile neutropenia, which is a fever in a patient with a low count of white blood cells.

Common side effects include

- Diarrhea
- Headache
- Rash
- Nausea and vomiting
- Injection site pain or inflammation
- Oral or vaginal thrush

Serious side effects include

- Severe diarrhea, which may be a sign of a *C. difficile* infection.
- Allergic reactions, which can include hives, trouble breathing, and swelling of the throat.
- Neurotoxicity, with symptoms such as confusion, hallucinations, and seizures, especially in patients with kidney problems.

Mechanism of action

Cefepime's mechanism of action is similar to other beta lactam anti-biotics, which is to kill bacteria by inhibiting cell wall synthesis. It does this by binding to penicillin-binding proteins (PBPs), enzymes that are essential for the final step in building the bacterial cell wall. This binding leads to structural defects in the cell wall, causing the bacteria to undergo autolysis (cell self- destruction) and die.

Interferes with cell wall synthesis

Cefepime disrupts the cross-linking of peptidoglycan, a crucial component of the bacterial cell wall.

- **Targets PBPs**

It specifically binds to and inhibits penicillin-binding proteins (PBPs), which are involved in the transpeptidation process that strengthens the cell wall.

- **Causes bacterial death**

The resulting defects in the cell wall lead to bacterial autolysis and death.

- **Broad spectrum**

It is a bactericidal agent with a broad spectrum of activity against many Gram-positive and Gram- negative bacteria.

- **Resistant to beta-lactamases**

Cefepime is highly resistant to many beta-lactamase enzymes, which are produced by bacteria to break down beta-lactam antibiotics.

- **Rapid penetration**

Due to its zwitterionic nature, it can penetrate Gram-negative bacterial cell walls more rapidly than some other cephalosporins.

2. Method

The fresh sample was isolated from a healthy, young person. For this research, I selected 22 participants who are runners, as the scientifically relevant age range is 18 to 30 for reproduction. After age 30, fertility is less of a concern since many individuals do not plan to have children after that age. Therefore, I chose participants who are 22 years old. To protect privacy, I have omitted the names and addresses of the donors. The donor has not had any diseases or infections during a specific period, does not smoke, and does not consume alcohol. They are a neat and healthy individual.

Details

Age: 22

Height: 5.8

Weight: 62

BMI: 21.1 normal (show in figure 9)

Fig. 9: BMI.

Precautions

All glass wares wash with hot water

Use different dropper for different test tubes

Sperm Details/condition

Colour : milky white

- Thickness : grater
- Dirty odor : no
- quantity in ml : 10 ml (show in figure 10)
- quantity in gm : 9.21 gm (show in figure 11)
- pH : 9 basic

Fig. 10: Sperm sample in ml.

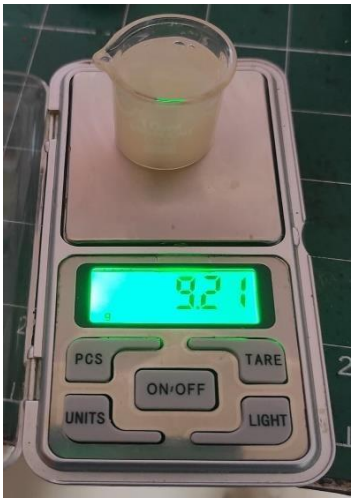

Fig. 11: Sperm sample in gm.

Procedure

To conduct the experiment, first collect a fresh sperm sample in a beaker. Next, dissolve the drug in 10 ml of distilled water in a test tube. Prepare two samples for comparison: the first sample will be a blank containing only the sperm, and the second will include the sperm combined with the drug (sperm + drug). Add 1.5 ml (1.53 g) of the sperm sample to the test tube. Then, add 1 ml of the dissolved drug to this sample and shake it gently. After mixing, add two drops of the prepared sample onto a slide and observe it under a microscope. Compare the results with the blank sample to determine whether the drug has caused any death to the sperm cells. Repeat this process every 30 minutes until you obtain conclusive results.

Fig. 12: Prepared sample sperm + drug.

OBSEARVATION TABLE

Table no. 1: Observation result after applying drug on sperm cells.

Sr. no	Drug	Sample (sperm+drug) Total Quantity (sperm 1.5ml drug - 1 ml)	Time		Observation Blank sample with out drug only sperm		Observation Sample with drug
			In min	In hrs			
1	Linezolid	2.5 ml in test tube	Freshly apply		100% alive and highly active		Alive
			30 min	1 hrs	100% and active	alive highly	Alive
			30 min		100% and active	alive highly	Dead but some alive
			30 min	1 hrs	100% and active	alive highly	All dead Only 1% alive
			30 min		100% and active	alive highly	All cells are dead
			Freshly apply		100% and active	alive highly	Alive
2	cefazolin	2.5 ml in test tube	30 min	1 hrs	100% and active	alive highly	Call dead but 15% alive
			30 min		100% and active	alive highly	Only 5% Alive with low activity
			30 min	1 hrs	100% and active	alive highly	Only 1% alive
			30 min		100% and active	alive highly	All cell was dead
3	chloramphenicol		Freshly apply		100% and active	alive highly	Alive
		2.5 ml in test tube	30 min	1 hrs	100% and active	alive highly	All most all cells was dead but few alive

			30	min		100% and active	alive highly	All cells are dead only few cells was alive with pour movement
			30	min	1 hrs	100% and active	alive highly	All cells are dead
			30	min		100% and active	alive highly	All cells are dead
4	Vancomycin Hydrochloride	2.5 ml in test tube	Freshly apply			100% and active	alive highly	Alive
			30	min	1 hrs	100% and active	alive highly	Dead only few alive
			30	min		100% and active	alive highly	All cells are dead only 1% alive
			30 min		1 hrs	100% and active	alive highly	All cells are dead
			30 min			100% alive and highly active		All cells are dead
5	ceftriaxone	2.5 ml in test tube	Freshly apply			100% alive and highly active		Alive
			30 min		1 hrs	100% alive and highly active		50% dead
			30 min			100% alive and highly active		All cells are dead
			30 min		1 hrs	100% alive and highly active		All cells are dead
			30 min			100% alive and highly active		All cells are dead
6	Cefepime		Freshly apply			100% alive and highly active		Alive
		2.5 ml in test tube	30 min		1 hrs	100% alive and highly active		Maximum cells dead But some alive
			30 min			100% alive and highly active		Cells are Dead only 1% alive
			30 min		1 hrs	100% alive and highly active		All cells are dead
			30 min			100% alive and highly active		All cells are dead

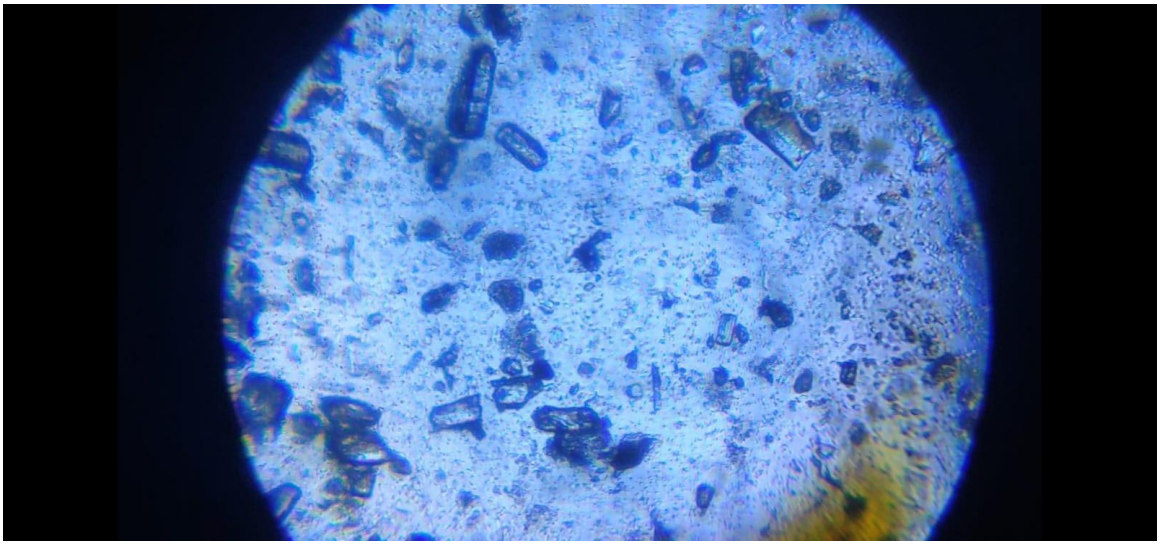

Fig. 13: Condition of sperm cells after 2 hours of applying Linezolid.

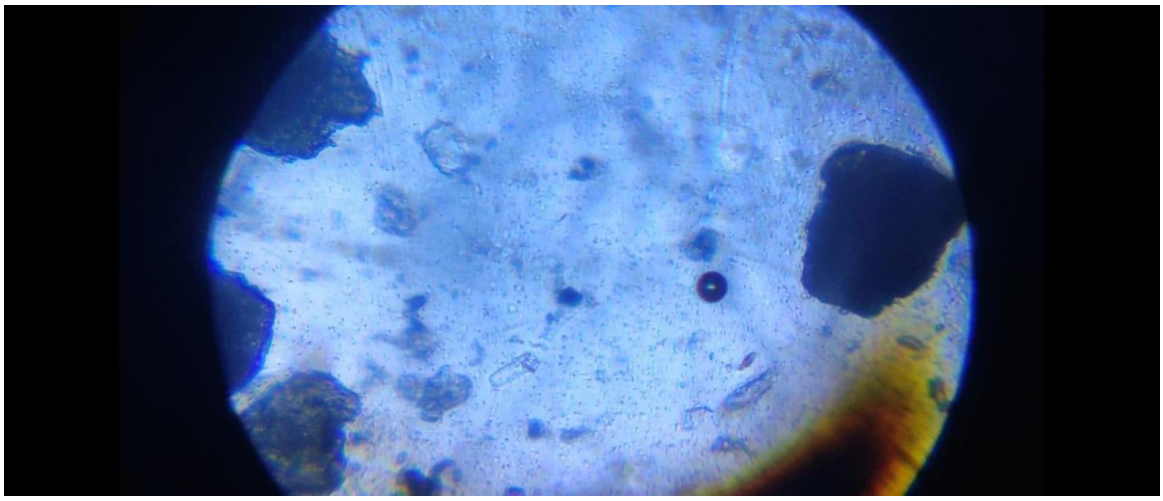

Fig. 14: Condition of sperm cells after 2 hours of applying Cefixime.

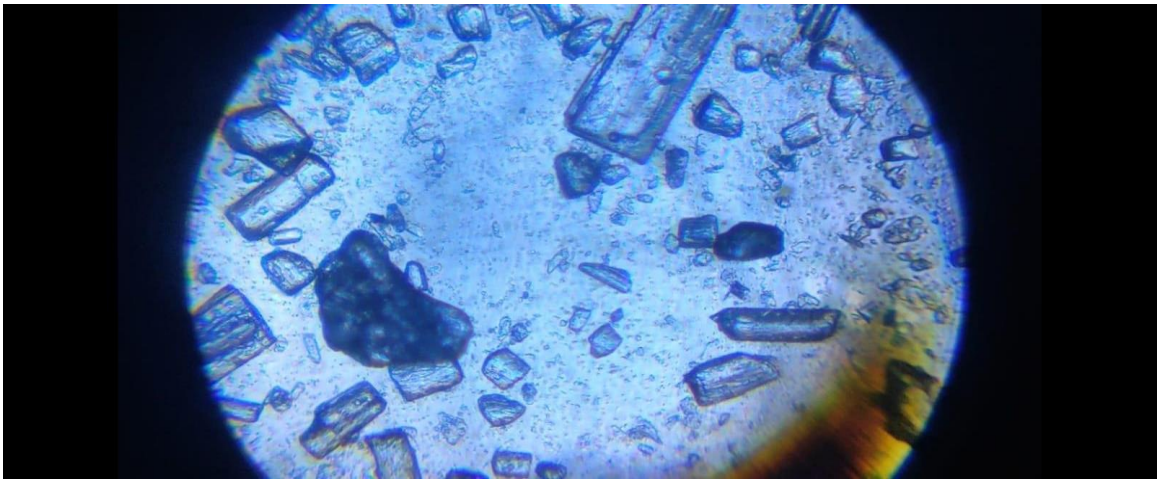

Fig. 15: Condition of sperm cells after 2 hours of applying chloramphenicol.

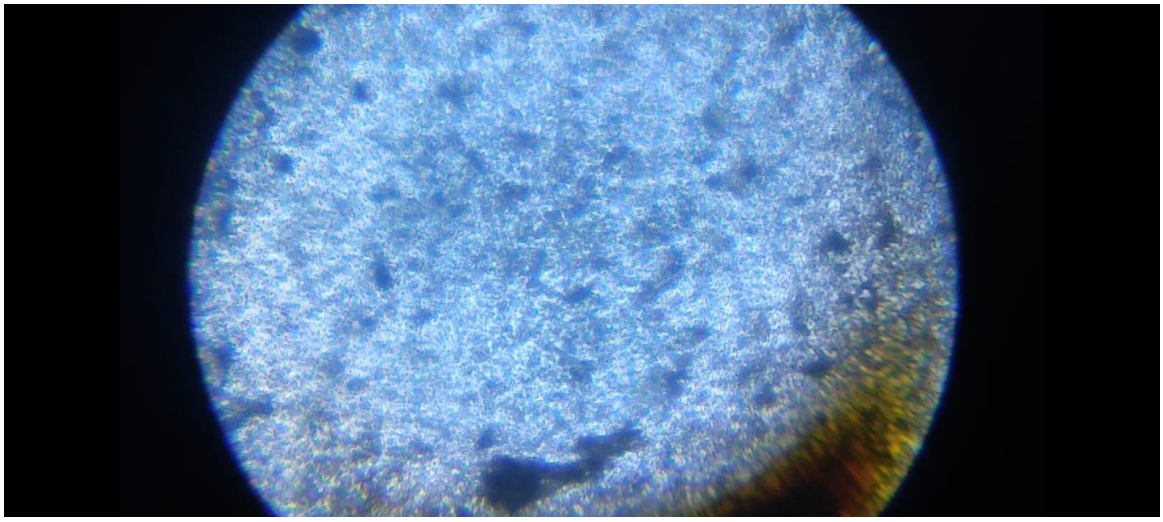

Fig. 16: Condition of sperm cells after 2 hours of applying Vancomycin Hydrochloride.

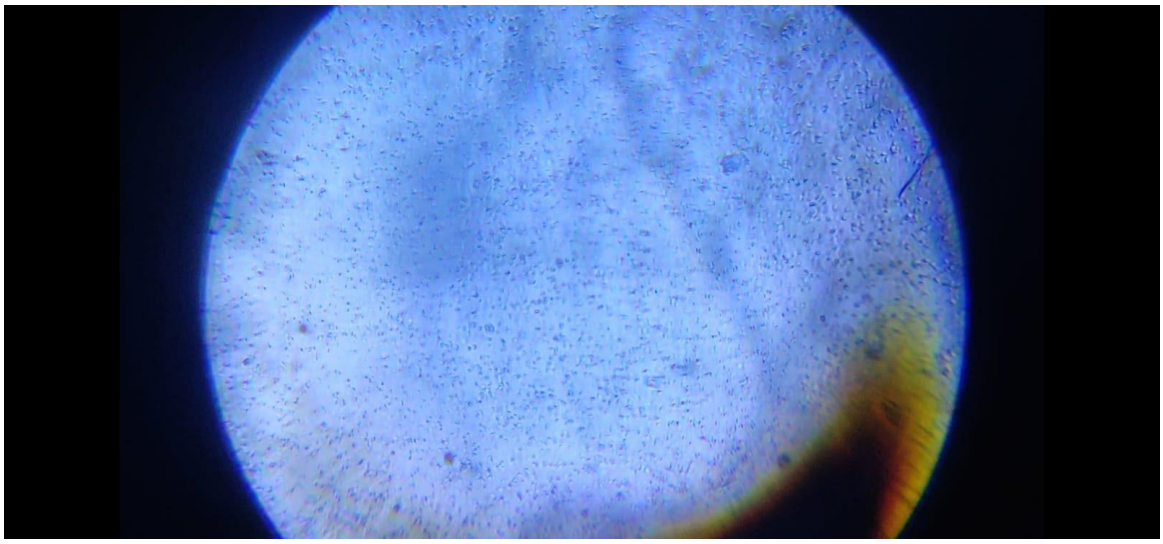

Fig. 17: Condition of sperm cells after 2 hours of applying ceftriaxone.

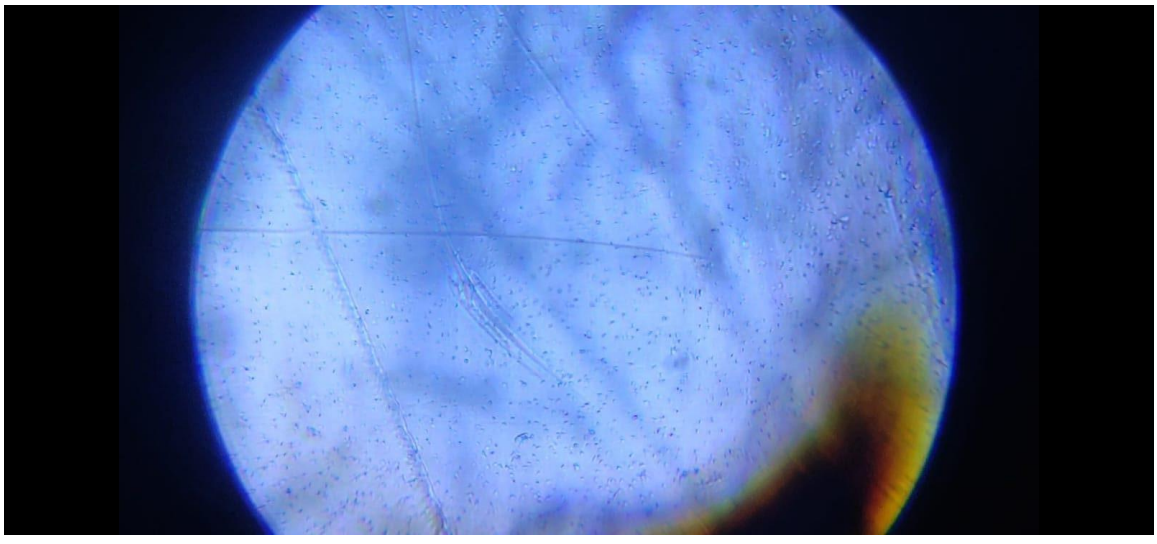

Fig. 18: Condition of sperm cells after 2 hours of applying Cefepime.

RESULT

Complet research work was proved that higher anti-biotic are capable to kill sperm cells by consuming for long time and its increase risk of male infertility.

DISCUSSION

Antibiotics are designed to kill bacteria and are not intended to harm sperm cells. These drugs do not completely affect sperm cells; only a small amount can have an effect. For example, if someone takes antibiotics for a few days to a week, they typically do not harm sperm quality because the body continually repairs itself. Within that time frame, old sperm are eliminated from the body, and new, healthy sperm cells are generated. However, when antibiotics are taken over a longer duration, such as three weeks or a month—especially for treatments related to cancer or long-term infections—there is a risk that antibiotics can affect sperm cells. This effect is not immediate; rather, it operates slowly and in small amounts, acting like a slow poison that can potentially increase the risk of infertility. This risk is particularly concerning for individuals aged 18 to 30, as studies have shown that this age range is optimal for reproduction. The impact of drugs on the body during this time may impair its ability to produce high-quality sperm, which could lead to decreased sperm count and potential problems in the future.

1. For instance, in the case of linezolid, an antibiotic, studies have observed the following: Initially, all cells are 100% alive. However, after 30 minutes, cells begin to die, and after one hour, nearly all cells are dead, with only a few remaining that exhibit slow motility. By the two- hour mark, all cells are completely dead except for about 0.1% of cells, which show very poor motility.
2. Cefazolin treatment results in the following observations: Initially, when applied, the cells remain alive. After 30 minutes, a minimum of 10% of the cells are still alive. After 1 hour, at least 5% of the cells remain viable. However, after 2 hours, only 1% of the cells are alive, and they exhibit very limited movement.

3. After 1 hour of exposure to chloramphenicol, almost all cells are dead, with only a few remaining alive. After 2 hours, all cells are dead.

4. Vancomycin hydrochloride

After 30 minutes, nearly all the cells are dead, with only 1% still alive. After 2 hours, all the cells are completely dead.

5. Ceftriaxone

After 90 hrs cells are completely dead.

6. Cefepime

After 1 hour, only 1% of the cells were alive but had very poor movement. Then, after 30 minutes, all cells were completely dead.

CONCLUSION

The overconsumption of antibiotics affects male fertility. This research helps reduce toxicity and increase therapeutic effects in the future.

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