

# Perspectives for antibiotic replacement in semen diluents and the NeoGiANT approach

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 NeoGiANT, WP6 "Assessment of antimicrobial formulation for sperm preservation"

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## Why are antibiotics a problem in animal reproduction?

**Assisted reproductive technologies (ART)** are widespread in modern animal breeding

**Artificial insemination (AI)** with refrigerated or cryopreserved semen is one of the leading technologies.

**>90%** sows, dairy cows and turkeys are inseminated in EU and USA, with growing numbers in China and other countries.

**BUT**

Semen diluents contain **antibiotics** for biosecurity and preserving sperm quality.

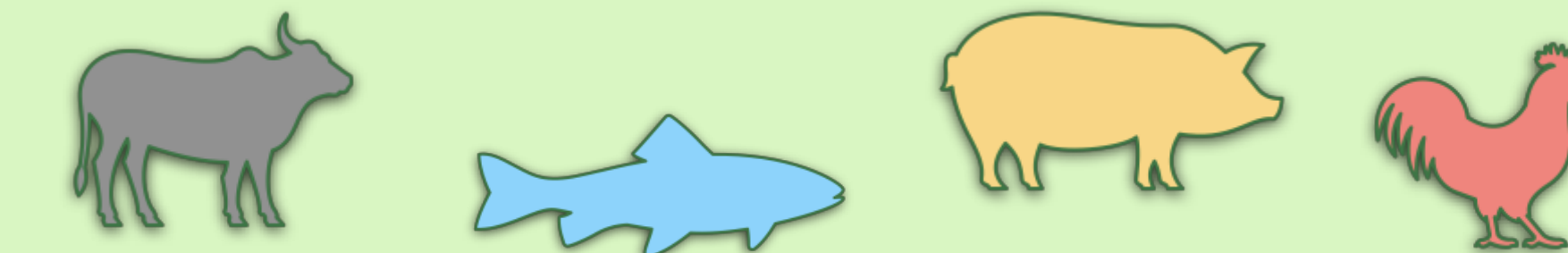
µg/ml  
 penicillin  
 spectinomycin  
 lincomycin  
 gentamycin  
 tylosin  
 polimixin

Only the pig industry uses **8 000 000 L** of semen extenders per year in the EU

This situation implies a continuous leak of broad-spectrum antibiotics to the environment.

**NeoGiANT** is an H2020 consortium focused on using antimicrobial natural extracts from grape marc for replacing antibiotics in animal production.

**WP6 - Assessment of the antimicrobial formulation for sperm for preservation**  
**Objective:** Using the natural and sustainable antimicrobials developed in NeoGiANT for replacing antibiotics in semen extenders



## What can be done for substituting antibiotics in semen diluents?

### Physical methods



**How**

Separate seminal plasma with its microbial load from spermatozoa, usually by single-layer centrifugation (SLC) through a low-density colloid.

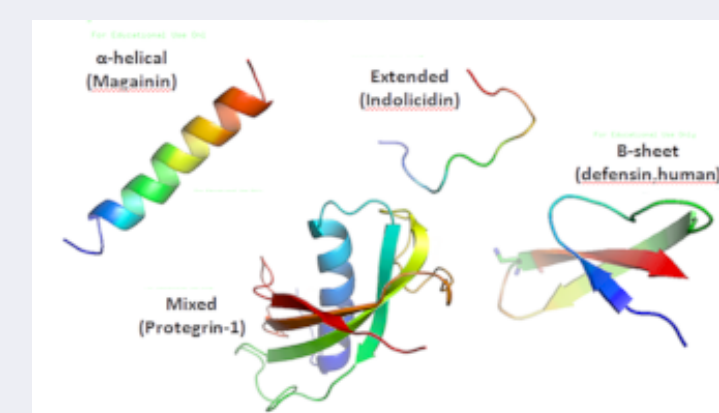
**Strengths**

Since most bacteria are removed, adding antibiotics is not necessary.

**Weaknesses**

Limited applicability, due to processing time, cost, and sperm loss.

### Antimicrobial peptides

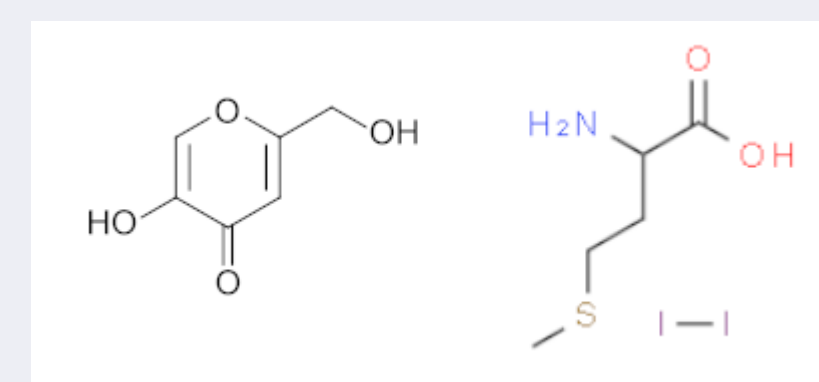


Gene-encoded polypeptides with roles in the immune defense of different organisms. Disrupt prokaryotic lipid membranes. Synthetic cyclic hexapeptides and bacteriocins have similar effects.

Effective against many bacteria of interest and few AMR.

Detrimental effects on spermatozoa due to special membrane composition (different than somatic cells). Risk of future AMR.

### Non-peptidic antimicrobial molecules



Non-antibiotic molecules or complex substances affecting different bacterial functions at concentrations not toxic for non-bacterial cells.

Kojic acid and iodine methionine inhibit bacterial proliferation at concentrations not detrimental to spermatozoa (tested in pig).

Only preliminary trials available. Optimization required. No information on fertility.

### Natural extracts



Aqueous or organic extracts, mainly from plant origin and containing a set of molecules with antimicrobial properties.

Natural origin, sustainable production, low cytotoxicity, small AMR risk, and potentially positive side effects (antioxidants).

Only preliminary trials available. Optimization required. No information on fertility.

**NeoGiANT** explores this approach by using bioactive extracts sustainably obtained from grape marc:

- Low cytotoxicity.
- High antimicrobial potential

Currently testing formulation compatibility with current diluents and safety for spermatozoa (taxon-specific parameters).

## Conclusions and implications

The threat of AMR and foreseen regulations are pushing research in the animal reproduction field for removing antibiotics from semen diluents. Whereas many methods are promising, cost, increased workload, or sperm toxicity make them impractical for now. The **NeoGiANT** approach combines a sustainable, circular economy extraction method with a low-toxicity product potentially suitable for semen diluents. Removing antibiotics from animal reproductive techniques would prevent the continuous release of these substances and resistant microorganisms to the environment, effectively contributing to a solution to increasing AMR.