

Newsletter:

Antibiotics in culture media

Bacterial contamination in media may seriously impair gamete and embryo developmental capacity. Maintaining sterile conditions *in vitro* is a great concern in ART, and antibiotics are routinely added to most culture media today.

The most common antibiotics for this use are **penicillin**, **streptomycin**, and **gentamicin**. Typical concentrations are Pen: 100U/ml, Strep: 100µg/ml, Gent: 50µg/ml

Bacteria can be either *Gram-positive* or *Gram-negative*. Some antibiotics only target G-positive bacteria, some only G-negative, while others are "broad spectrum" and can target both:

Antibiotic	Type	Affects	Mode of action
Penicillin	β-lactam	Gram-positive bacteria	disturbs cell wall integrity
Streptomycin	Aminoglycoside	Gram-negative bacteria	disturbs protein synthesis
Gentamicin	Aminoglycoside	Broad spectrum = both G-pos and G-neg	disturbs protein synthesis

Toxicity of antibiotics

Generally, antibiotics in culture media are included at standard concentrations and are not believed to have toxic effects on the cells/embryo. It must however be noted that, as biologically active substances, antibiotics have the potential to affect cell function.

Studies on antibiotic toxicity in cell cultures are few and ambiguous. This is the general scenario:

- Of the mentioned antibiotics, **penicillin** generates the least concerns. Penicillin is not believed to have direct toxic effects on the embryo.
- The **aminoglycosides** (Gent+Strep) show more toxic effects. The cause/origin of the toxicity is still not fully understood and no studies are available describing effects of aminoglycosides on human embryo development.
- Of these, **gentamicin** seems to generate the most concerns. This is probably because adverse effects from gentamicin are well known from therapeutic use (patient treatments with much larger doses). Out of six antimicrobials, *Berry et al. 1995* found gentamicin to be the most toxic after 7 days exposure of human corneal cells.

Other media producers:

Several major commercial producers of IVF media use gentamicin exclusively in their product lines.

Others generally do not include aminoglycosides in their media, but e.g. use only penicillin (= targets only Gram-positive bacteria).

MediCult uses a **Pen/Strep combination** which ensures a broad-spectrum mode of activity, effective on most bacteria.

Broad modes of action are desirable, since inadequate cover may allow even a single bacteria individual to proliferate. If e.g. only penicillin is used, the Gram-positive bacteria will be killed, permitting overgrowth of Gram-negative organisms.

Why include antibiotics?

- MediCult wishes to provide the most stable conditions in our media. IVF laboratory practice involves genuine risk of bacterial infection, esp. from semen and handling. Reduction or exclusion of antibiotics from media is possible but requires very strict laboratory methods which minimise the risk of contamination.
- MediCult has noted that the demand for antibiotic-free media is very small. This is interpreted as a sign from the clinics that antibiotics are an asset in daily clinical practice, and that success rates are not affected negatively by the antibiotics.

Storage and use of media with antibiotics

Antibiotics are easily affected by factors such as temperature, pH, light, etc. Maintaining antibiotic activity in the medium therefore very much depends on **how the medium is handled by the customer**.

This means that stability is affected by how often the bottles are opened, taken in and out of the refrigerator, how long the medium is kept at warm temperatures in the lab, etc.

To maintain proper antibiotic activity throughout the product's shelf-life, the following is recommended:

1. Keep containers tightly closed and refrigerated at all times. Once the medium has been warmed, it should be discarded.
2. Use the media within a few days after opening.
3. Use smaller volume bottles (single-cycle vials where possible). Using a fresh, unopened container gives a much better security of retaining antibiotic activity.

MediCult a/s