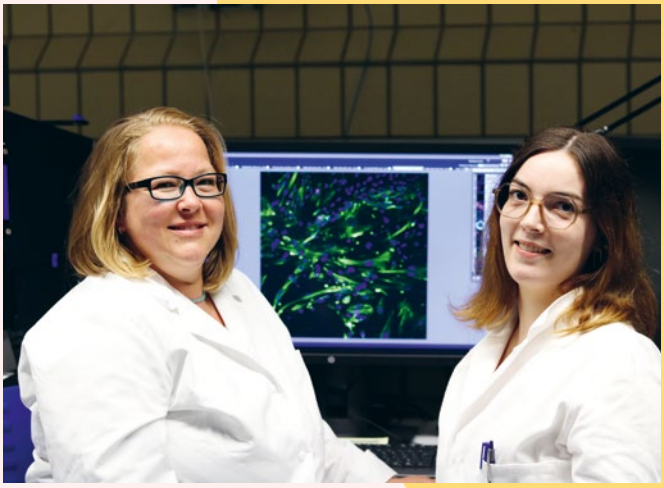
Understanding the beginning

Tissue cultures intended to “simulate” pregnancy and enable testing of potentially risky chemicals – without animal experiments.



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“We want to understand how an embryo implants in the uterus and how substances affect this process and embryonic development.”

DR FANNY KNÖSPEL (LEFT SIDE) AND MIRJAM NIETHAMMER, BFR

An unborn being is a sensitive creature. Approximately two in five pregnancies end prematurely, 80 percent of these during the first three months. In its early stages, the embryo is particularly vulnerable. Chemical substances are one of the reasons for unwanted miscarriages as well as abnormalities or developmental disorders. The clearest example is the thalidomide catastrophe: in the 1960s, the active ingredient prescribed against morning sickness for pregnant women in the first three months of pregnancy caused severe organ damage and missing limbs in babies.

Since the thalidomide catastrophe, chemicals and drugs must be tested for harm to the unborn baby before being put on the market. This is still predominantly done using animal experiments. At the German Centre for the Protection of Laboratory Animals (Bf3R), which is part of the German Federal Institute for Risk Assessment (BfR), scientists Dr Fanny Knöspel and Mirjam Niethammer are working on an alternative – embryoids from mouse cells. “Embryoids are embryo-like tissues created from stem cells,” explains Fanny Knöspel. “We want to understand how an embryo implants in the uterus and how substances affect this process and embryonic development.”

FEWER OFFSPRING – A WARNING SIGNAL

Established and legally required tests for testing chemical substances, such as pesticides, are usually carried out on rats and rabbits. The parental generation receives the test substance, for example with their food, to identify possible poisonous (toxic) effects on their offspring. It is then determined whether there are any health consequences, for example, whether the number of offspring decreases or if abnormalities and growth disorders develop.

One of the disadvantages of these tests is the fact that results from one animal species can only partially be transferred to others or to humans. This can mean that a dangerous substance is not detected – or, conversely, that a potentially useful drug is discarded as a seemingly harmful substance. And, of course, live animals are required for these experiments. The development of alternative methods by the Bf3R can help to reduce the number of laboratory animals in this area.

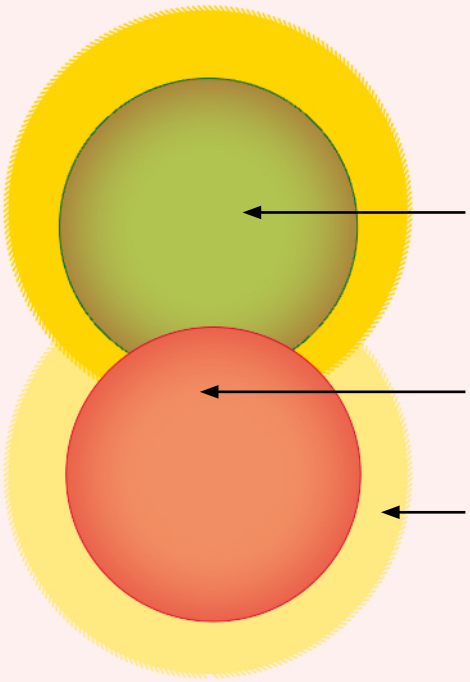
TESTS ON CELLS INSTEAD OF ANIMALS

Three alternative methods have already been independently tested (validated) in the past to ensure their suitability. They do not test living animals (in vivo) but work with cell and tissue cultures (in vitro).

The methods are based on rat embryos, connective tissue cells obtained from these and embryonic stem cells from mouse embryos. However, all three test systems are subject to significant limitations and have not yet become established methods. “A common cause of a pregnancy ending prematurely are interaction problems between the embryo and the mother’s uterus,” says Knöspel. “However, the existing test methods do not allow us to better understand these processes.”

Knöspel and Niethammer want to use an animal-free model to better understand how the uterine lining and the embryo communicate with each other. They are

Researching how an embryo implants



Simulated pregnancy with an embryoid (artificial embryo) consisting of three cell types

Trophoblast stem cells form the embryonic part of the placenta

Embryonic stem cells simulate the actual embryo

Extraembryonic endoderm stem cells form the yolk sac from which the early embryo is nourished

also investigating the extent to which medication and chemicals can influence the implantation of the early embryo in the uterus. These are ambitious goals that the two scientists want to achieve.

The first stage involves creating the embryo-like embryoids and a tissue culture from the uterine lining (endometrium) and connective tissue. The researchers are adopting a two-pronged strategy: Knöspel is focusing on the embryoids, Niethammer on the endometrium. They will only be combined when both biological models work on their own. However, it has not yet progressed that far.

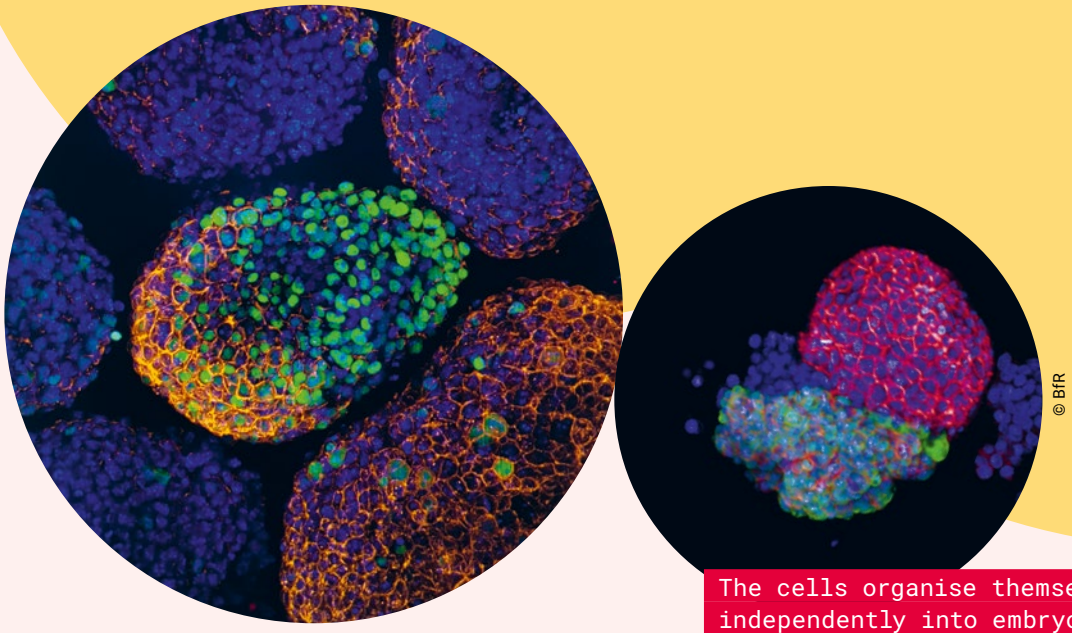
THREE CELL TYPES FORM THE EMBRYO

Fanny Knöspel works with three different cell types. Two of them were originally isolated from the early embryo (blastocyst) of a mouse. They are now stably cultivated as stem cells in a petri dish. Depending on their stage of development, stem cells have the ability to transform (differentiate) into different cell types. This ability has made it possible to obtain the third cell type. Stem cells can also multiply almost indefinitely. Therefore, they are a very good source for developing animal-free testing methods.

"We put these cells together in a nutrient solution and then something astonishing happens – the cells organise themselves into embryo-like structures," reports Fanny Knöspel. "This allows us to study the first stages of development, such as the formation of the amniotic sac, the amniotic cavity and the cotyledons, from which the organs then develop." So far, the embryoids have been kept alive for around a week.

CELL STRESS AND CELL DEATH

Even though it would be more realistic to study the embryoids on the uterine lining, they can also be tested directly for damage caused by potentially toxic substances. To do this, the cell structures are exposed to the chemical under investigation for one to two days.



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The cells organise themselves independently into embryo-like structures and demonstrate the first stages of development, such as the formation of the amniotic cavity.

“We then look to see if we can detect any changes,” says Knöspel. “These can be obvious processes such as the death of the cells, or we can observe metabolic processes that suggest increased stress.”

The creation of “artificial” embryos is already an established field of research. When it comes to attempts to recreate a uterus on a microscopic scale, the situation is different. They are still in their infancy, as it were. It is proving much more difficult to grow stable uterine cells. Another question is whether the uterine cells grow on a stabilising network of connective tissue as a basis (matrix) or whether they can form this independently, which would be closer to nature.

“Our aim is to depict the superficial mucous membrane with the associated connective tissue for the uterus-like structure,” says Mirjam Niethammer. Another important factor for the successful “implantation” of an embryo are hormones, which are essential for a prospective pregnancy in the “real” uterus. “Of course, an artificial system consisting of embryoid and uterine tissue would still be far from a perfect representation of nature,” summarises Knöspel. “But then we are already dealing with five different cell types that have to fit together and harmonise.” There is no doubt that the two scientists have set themselves some ambitious goals to investigate nature – and to detect substances that pose a risk to the unborn child. —

More information

Niethammer, M. et al. 2022. **In vitro models of human development and their potential application in developmental toxicity testing.** Development 149 (20). DOI: 10.1242/dev.200933

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