Test tube fertilization

The task of the plant breeder can be made difficult by any of the following eventualities: the pollen fails to germinate on the stigma, the growth of the pollen tube in the style partially or completely stagnates, no fertilization takes place, the fertilized egg cell does not develop *in vivo* and aborts, or abscission of the ovaries occurs permanently. If no fertilization takes place after self pollination or cross pollination then it is referred to as self incompatibility or cross incompatibility. In some cases the plant breeder must resort to special procedures to bring about fertilization e.g. by ovule fertilization (here the pollen is artificially brought into contact with the ovules).

It was not until 1962 that did the idea arose of bringing about fertilization *in vitro* when this was not possible *in vivo*. Despite the fact that little research had been carried out in this area a few interesting examples of test tube fertilization were found.

In vitro fertilization is of particular importance if the incompatibility is present on the stigma or in the style.

In vitro fertilization can take place in three different ways:

1. **Stigma fertilization:** in this method an emasculated flower is extremely sterilized and then isolated *in vitro*. Pollen from a ripe anther (which has been externally sterilized) is then placed on the stigma. This method, which is similar to fertilization *in vivo*, can be used, if for example, the ovaries fall off the plant prematurely, resulting in lack of progeny. Using stigma fertilization success has been achieved with: *Nicotiana rustica*, *N. tabacum*, *Petunia violacea*, *Antirrhinum majus*, *Pisum sativum*, *Lathyrus odoratus*, *Zea mays* and *Glycine* species.

2. **Placental fertilization:** An intact flower is externally sterilized and placenta explants with unfertilized ovules are dissected under a stereomicroscope and inoculated onto a nutrient medium. At the same time anthers which are still closed and at a stage where they would be just about to open *in vivo* are externally sterilized. The anthers are opened under sterile conditions and the pollen grains placed near the ovules. After this, time is required to determine whether the pollen grains germinate, if they penetrate the embryo sac and whether fertilization follows. Placental fertilization is practiced with members of the *Caryophyllaceae*, *Gossypium* and *Zea mays*.

3. **Fertilization of an isolated ovule without a placenta:** This method is same as in 2 from the time that the ovule is isolated *in vitro*. There has been little success with this method since it is extremely difficult to induce embryo formation in *in vitro* fertilized ovules.

In vitro fertilization can be used in the following cases:

1. Placental pollination is sometimes possible when the plants are completely self incompatible *in vivo*. E.g. Petunia axilaris, Petunia hybrids.

2. Cross fertilization may be possible *in vitro* even if it is impossible *in vivo*. Hybrid plants after test tube fertilization of ovules of *Nicotiana alata* with pollen from *Nicotiana tabacum*. Intergeneric crosses can also be achieved *in vitro*, as seen with different members of the *Caryophyllaceae*; for this family it has been shown that the pollen grains germinate better with placental fertilization *in vitro* than on the stigma in vivo.

3. Production of haploids by parthenogenesis.

4. The abscission of a flower or ovary is sometimes unavoidable. In such a case stigma fertilization may be effective.

5. To study the physiology of the fertilization.

In general little is known about the conditions necessary for fertilization *in vitro*. However, it seems certain that:

1. The pollen grains and the ovules must be in the correct physiological and morphological state.

2. The choice of nutrient medium is extremely important. It is not surprising that this choice is very difficult processes have to take place one after the other: germination of the pollen grains, fertilization and growth of the embryo into a seed. A complex mixture of compounds is often used to induce growth of the embryo.

3. When sterilizing flowers for use with the stigma fertilization, care should be taken that the stigma is not in contact with the sterilizing agent for too long or the exudates on the stigma will be dissolved.

4. With stigma fertilization it is better not to remove the sepals from the flower, since they encourage the growth of the ovary.
5. Stigma fertilization may still be possible despite failure of placental fertilization.
6. Temperature may be a decisive factor.

Questions

1. In vitro fertilization can take place through ……
a) Stigma fertilization b) Placental fertilization
c) Fertilization of an isolated ovule without a placenta **d) All the above**

2. Stigma fertilization is successful in ……
a) *Nicotiana rustica* b) *Zea mays*
c) *Pisum sativum* **d) All the above**

3. Placental fertilization is successful in ……
a) *Caryophyllaceae* b) *Zea mays*
c) *Gossypium* **d) All the above**

4. The conditions necessary for in vitro fertilization include ……
a) Physiological state of pollen and ovule b) Morphological state of pollen and ovule
c) Nutrient medium **d) All the above**