Micromanipulation with animals' embryos

Questions:

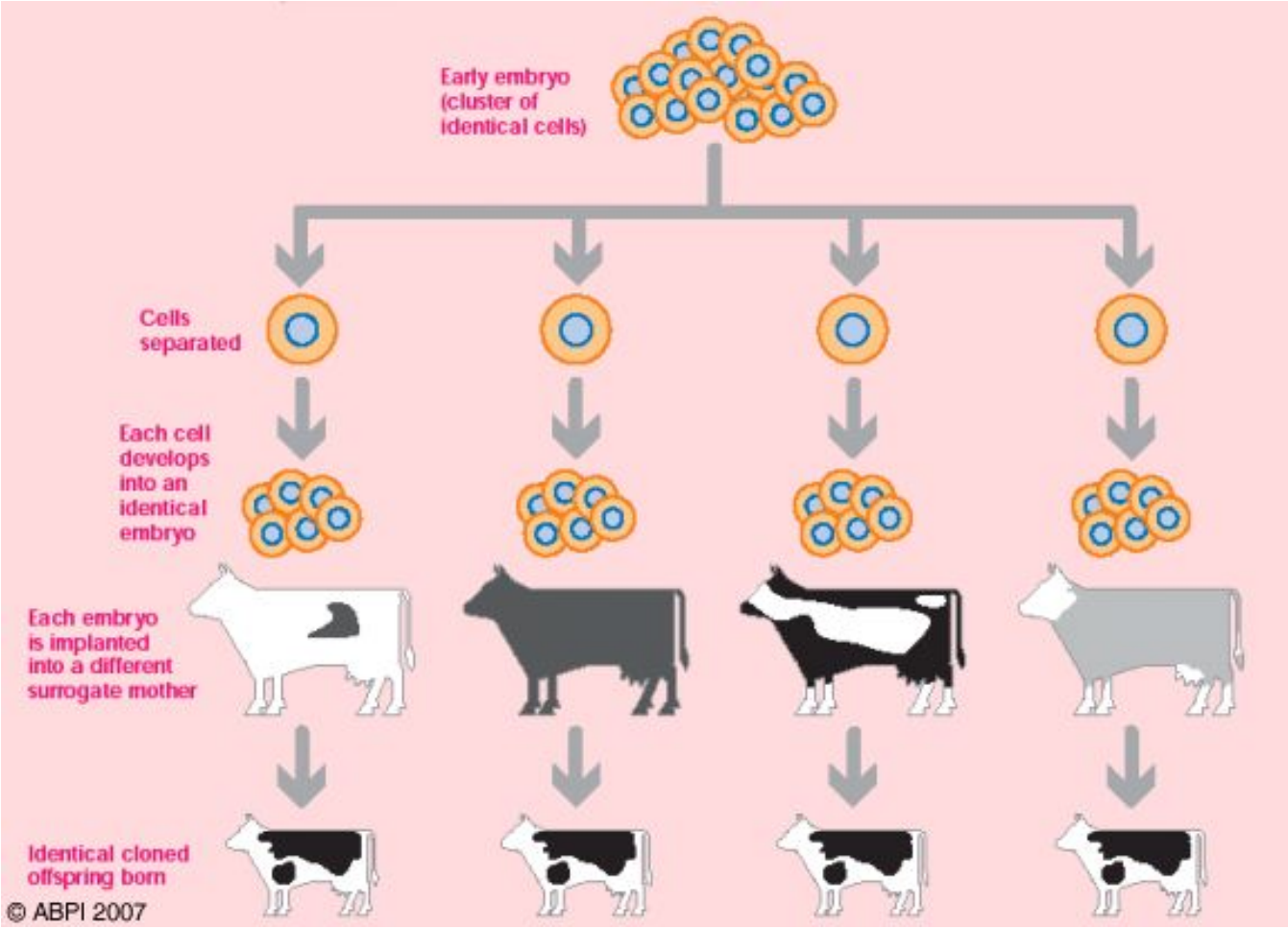
1.Obtaining identical twins

2. Chimeric animals

3. Cloning of animals

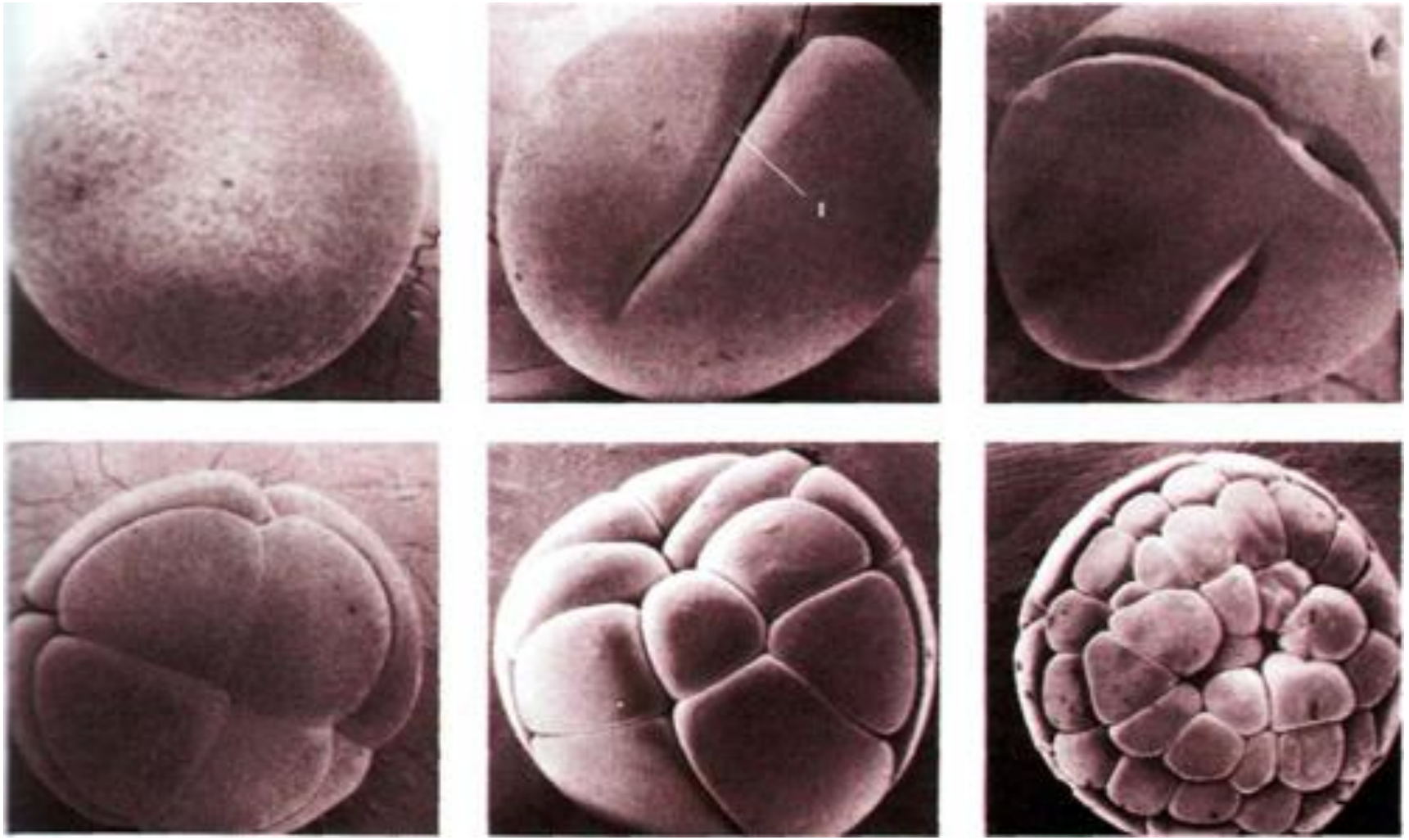
4. Obtaining homozygous diploid offspring

5. Definition and sex regulation.



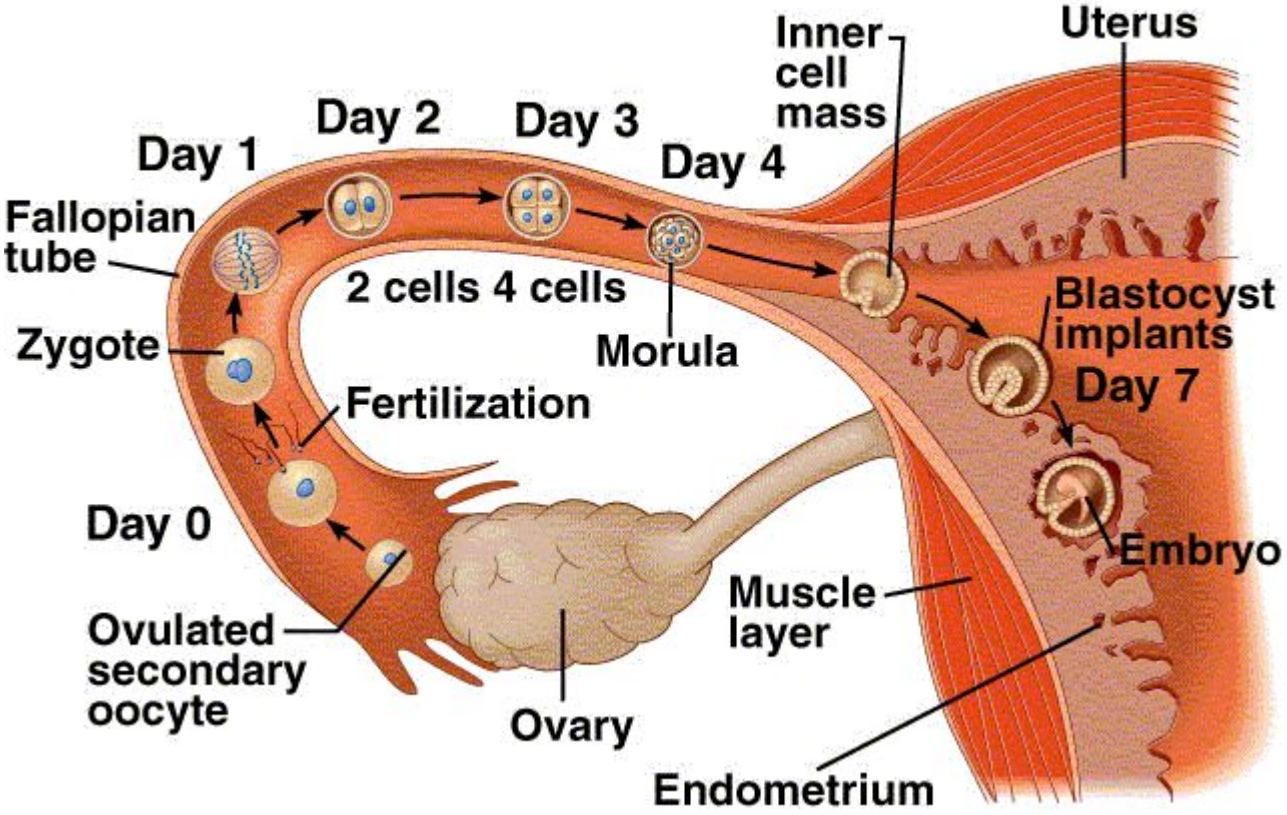
***Obtaining identical twins*. The basic premise of the natural manifestations of multiple pregnancy in mammals is the simultaneous fertilization of at least two mature eggs by different sperm. Cattle is characterized by low twins frequency (an average of 0,025). Identical twins are sometimes found among twin-calves.**

The likelihood of such genetically identical twins is only 0.01%. Low rates of twins incidence and heritability do not allow to expect the high efficiency of selection. Therefore genotypes copy methods of highly productive animals on the basis of early embryo separation by microsurgery and micromanipulation techniques into two or more blastomeres capable to develop during the entire ontogeny have a great practical importance.

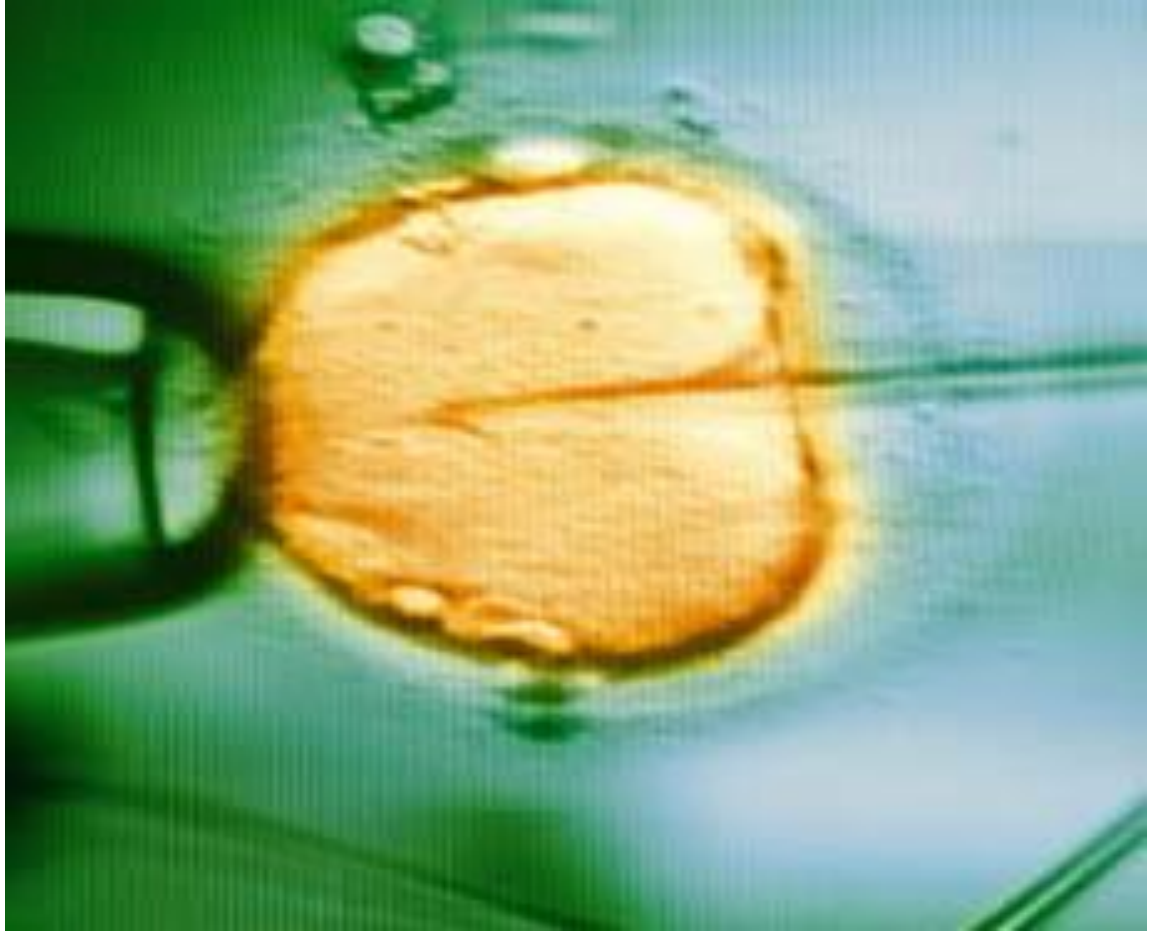


The zygote divides into two cells (in mammals approximately in 30 hours after fertilization). Further mitotic divisions form a group of cells called *Blastomeres.* Mammalian embryo, containing more than 12 blastomeres called *Morula*. 4 days after fertilization, Morula cells begin to differentiate into two layers of cells: *trophoblast* and *embryoblast*. As a result of the mammalian *Blastocyst* is formed.

From ovulation to implantation



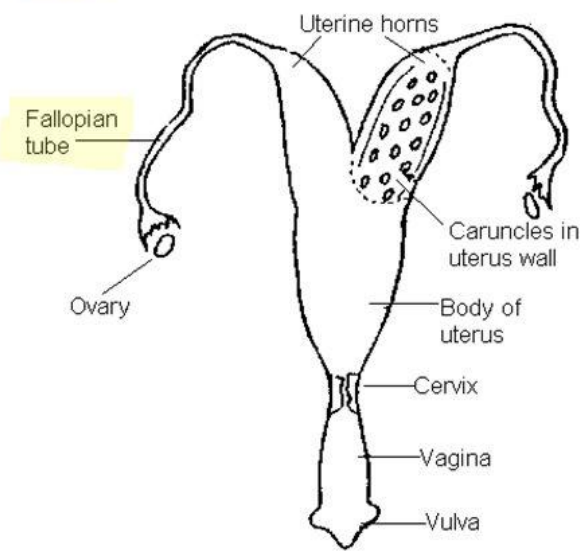
Blastocyst stage belongs to preimplantation period of development, that is the earliest period of embryogenesis of mammals (prior to attachment of the embryo to the uterine wall.



S.M.Willadsen first reported in 1979 about getting monozygotic twins in sheep by dividing 2-cells blastomere. He divided blastomeres into two separate cells, and impaired *pellucida zone* was clogged with agar. Pellucida is formation preventing spillage of blastomeres, as well as contact with other embryos, foreign cells, white blood cells, sperm cells and facilitates the passage of the embryo through oviduct.

Enclosing separated blastomeres in agar, which is practically insoluble in the female genital tract, allowed them to survive and develop *in vivo.*

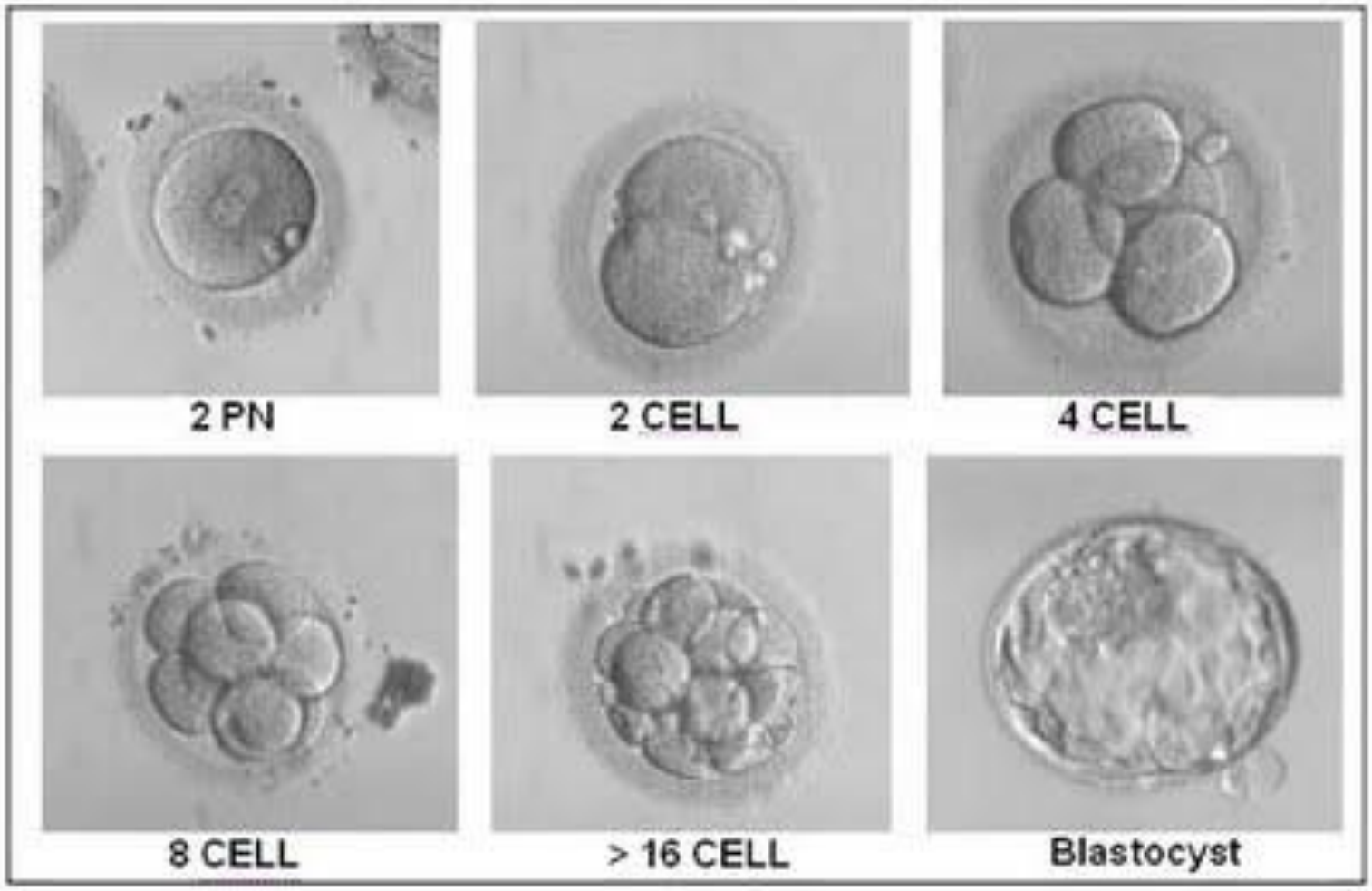
Oviducts (fallopian tubes)



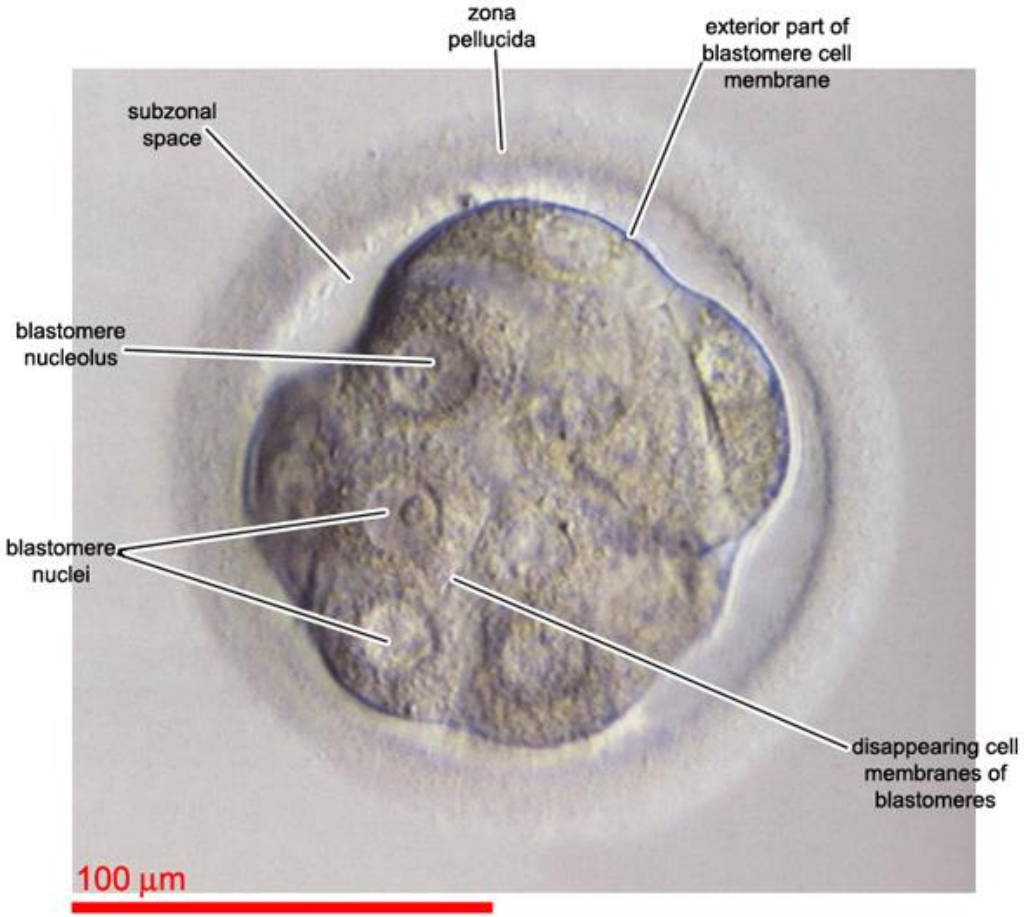
- Provide the site of fertilization and early embryo development before the embryo passes to the uterus.
- Transport the ova from the ovary to the site of fertilization which occurs midway down the oviduct.
- At the same time, moves the sperm cells in the other direction towards the ova from the uterus.

Sheep oviduct was used as a temporary recipient (sheep oviduct is the most suitable object for cows, horses and pigs embryo development up to blastocyst) for the cultivation of embryos enclosed in agar. Survival rate of "halves" of embryos at this stage after transplantation to recipients was about 50%.

In sheep, two-cells blastomeres can be obtained in a very short period of time. So, after 60 hours, most embryos are on the 4-cell stage of development. It is almost impossible during the operation of sheep to find embryos, which are on the 2-cell stage..



Later experiments showed that genetically identical twins can also be obtained from the 4 - and 8 - cell blastomeres by splitting them into two groups. These "half" were equally viable as a normal sheep embryos. It is established that embryos derived from 8-cell blastomeres have no vitality. It is believed that the sharp decrease in the number of cells of the embryo is a major factor reducing their ability to develop into viable blastocysts.



Technique of enclosing in agar blastomeres of cattle embryos divided into parts to get identical twins was successfully used by S.M.Willadsen et al. (1981) in obtaining calves - identical twins. Investigations were carried out on 5-6-day-old embryos at morula stage, because in cows non-surgically method more appropriate to get embryos.

Morula were divided in "half" or "quarters", enclosed in agar and transferred to the oviduct of a sheep for 1-2 days. Then they were removed and surgically transplanted to recipients on the 6th and 7th day of the sexual cycle. Engraftment of "halves" was high (75%), while this figure in "quarters" was significantly lower (41%).

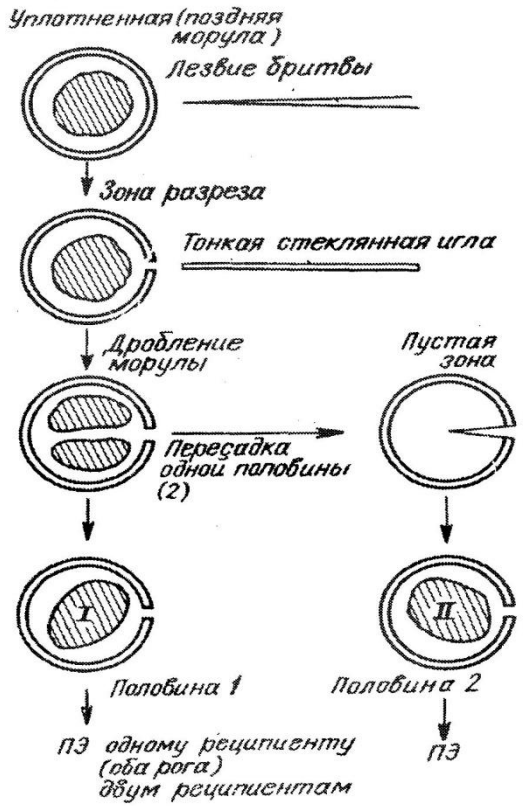
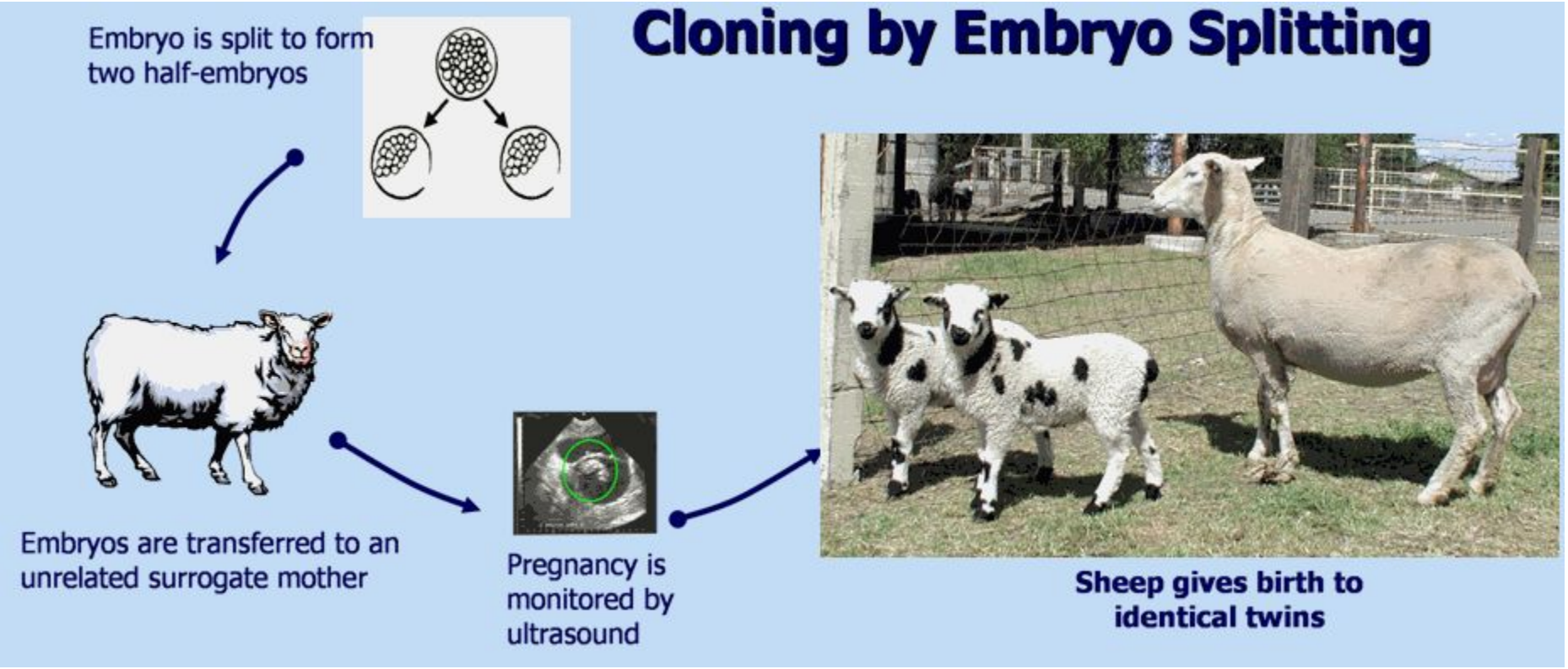
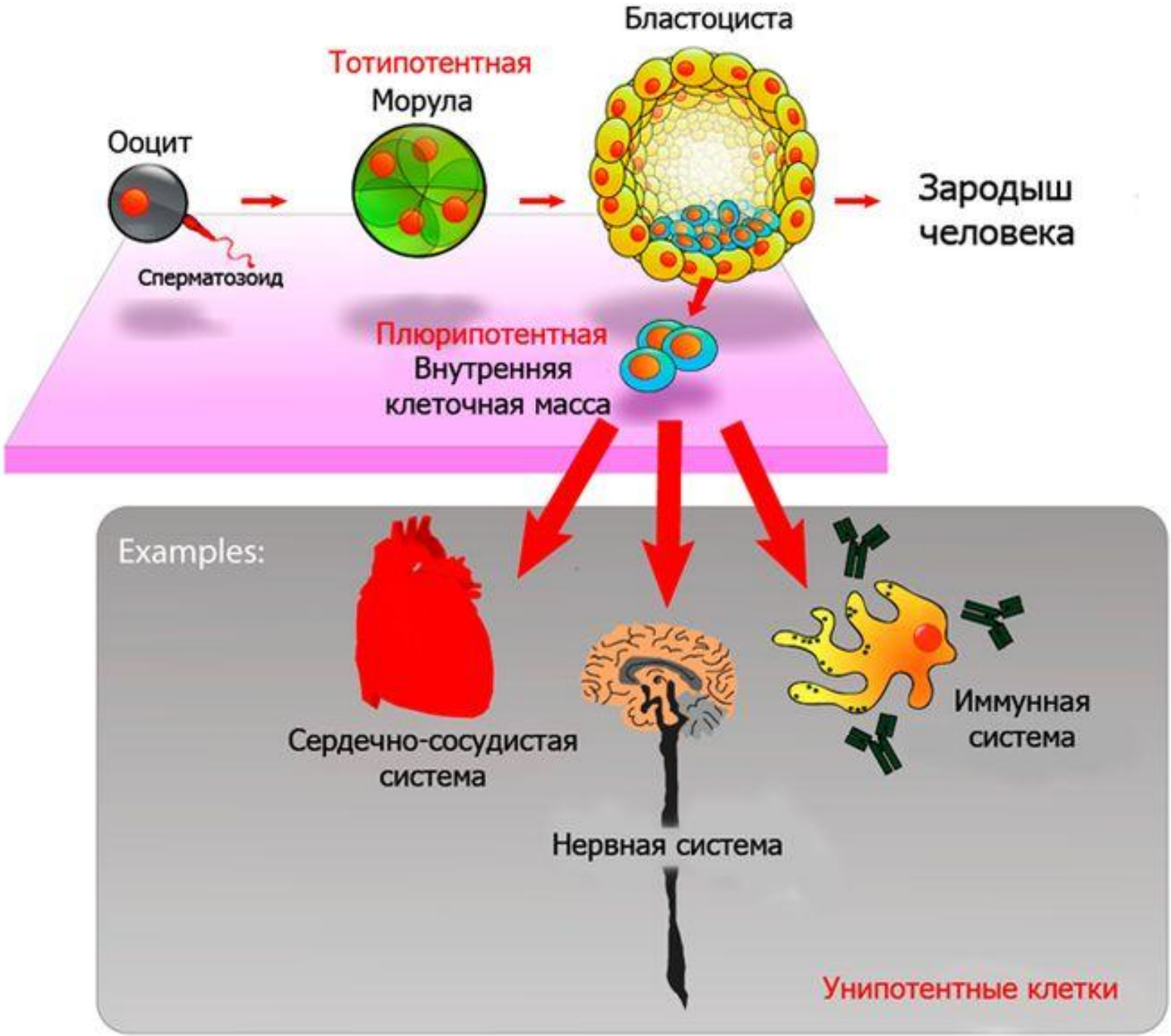


Схема деления эмбрионов крупного рогатого скота
(Б.П.Завертяев, 1989)

S.M.Willadsen and R.A.Godke (1984) carried out separation of sheep embryos at the *late morula stage*, at the stage of early, late and hatching blastocyst into two equal halves. In this case part of the halves of embryos remained inside torn areas of pellucida, while others were transplanted without it. Halves of embryos were transplanted to the same sheep from that they have been removed.



Researchers have not received any single egg twins after embryo transfer on morula stage, whereas they were obtained after transplantation of blastocysts on all three stages of development. And there are reports about possibility of using embryos of cows and pigs in the later stages of development for obtaining of single egg twins. Effective test for assessing viability of embryo halves is cultivating them within 2-4 hours between separation and the transplantation. Culturing embryo halves for a night or more than 12 hours, was accompanied by a marked decline in their viability.


Бластоциста
Тотипотентная
Морула
Ооцит
Сперматозоид
Зародыш человека
Плюрипотентная
Внутренняя клеточная масса
Examples:
Сердечно-сосудистая система
Нервная система
Иммунная система
Унипотентные клетки

One Stem Cell Line, One Growing Embryo

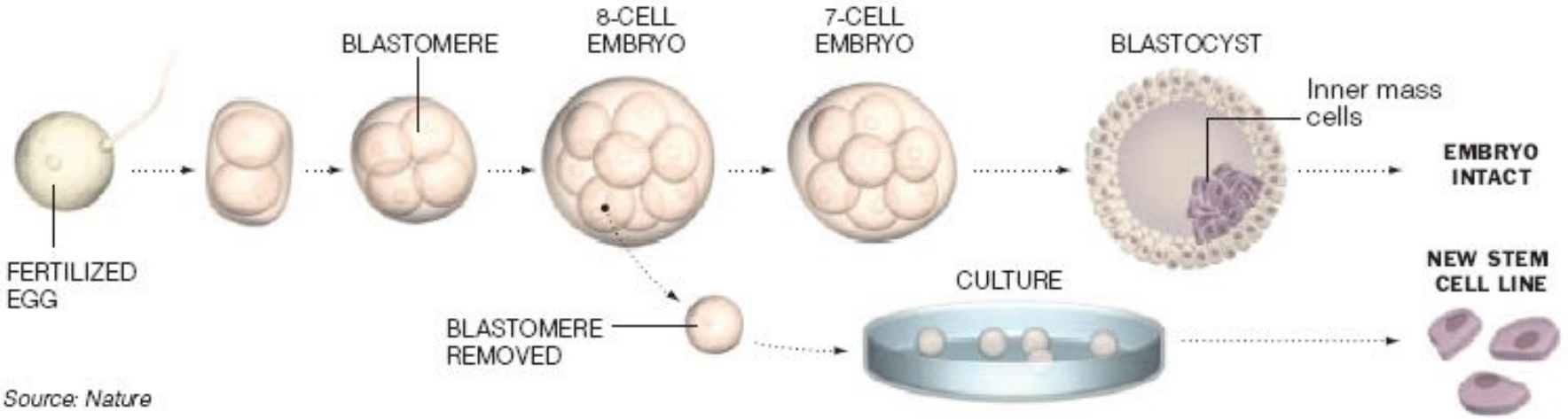
Scientists report a new method to make human embryonic stem cells that does not, as before, destroy the embryo.

1 An egg, fertilized by sperm, begins to divide.

2 After the third division, at the 8-cell stage, a single cell called a blastomere is removed.

3 The blastomere is cultured with an established embryonic stem cell line and then separated to form new lines.

4 Left intact, the embryo continues to develop into a ball of some 150 cells, the blastocyst. The older stem cell technique removes inner mass cells at this point, destroying the embryo.



Source: Nature

Al Granberg/The New York Times

Novel methods

- Extraction of single blastomere without damaging embryo and developed into independent hESC lines

Chung et al ,Nature 2006; 439:216-19

- Altered Nuclear Technique (ANT) genetically modifying the somatic nucleus so that induced pluripotent stem cells are produced

Meissener & Jaenisch Nature 2006;439:212-15

- Somatic Cell Nuclear Transfer / Therapeutic cloning

Chimeric animals*. The concept of a chimera means a compound animal.** ***In the modern concept the term chimera is mainly used for designation of composite organisms that have genetically different cell populations from more than one zygote or more than one embryo. **Obtaining genetic chimeras or mosaics is currently one of the promising areas of biotechnology The essence of this biotechnological method, based on the achievements of cell engineering and micromanipulation on early embryo consists in artificial combining of embryo cells from two or more animals, relating not only to one breed, but also to the different breeds and even species. Chimeric animals are signs of different genotypes.**

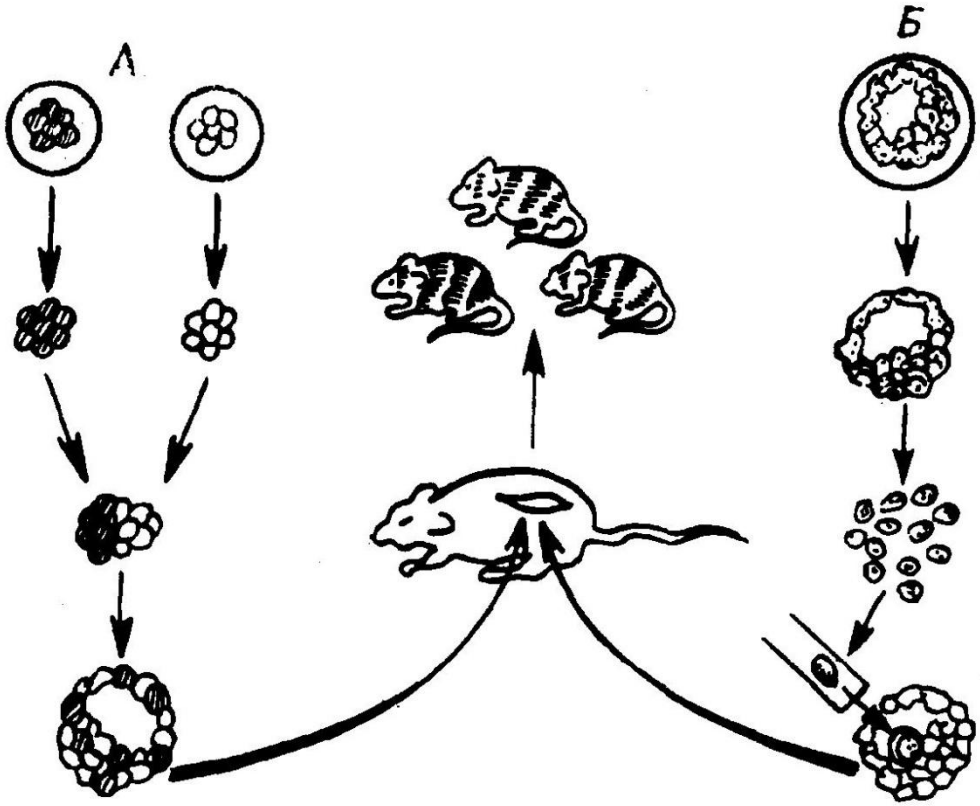
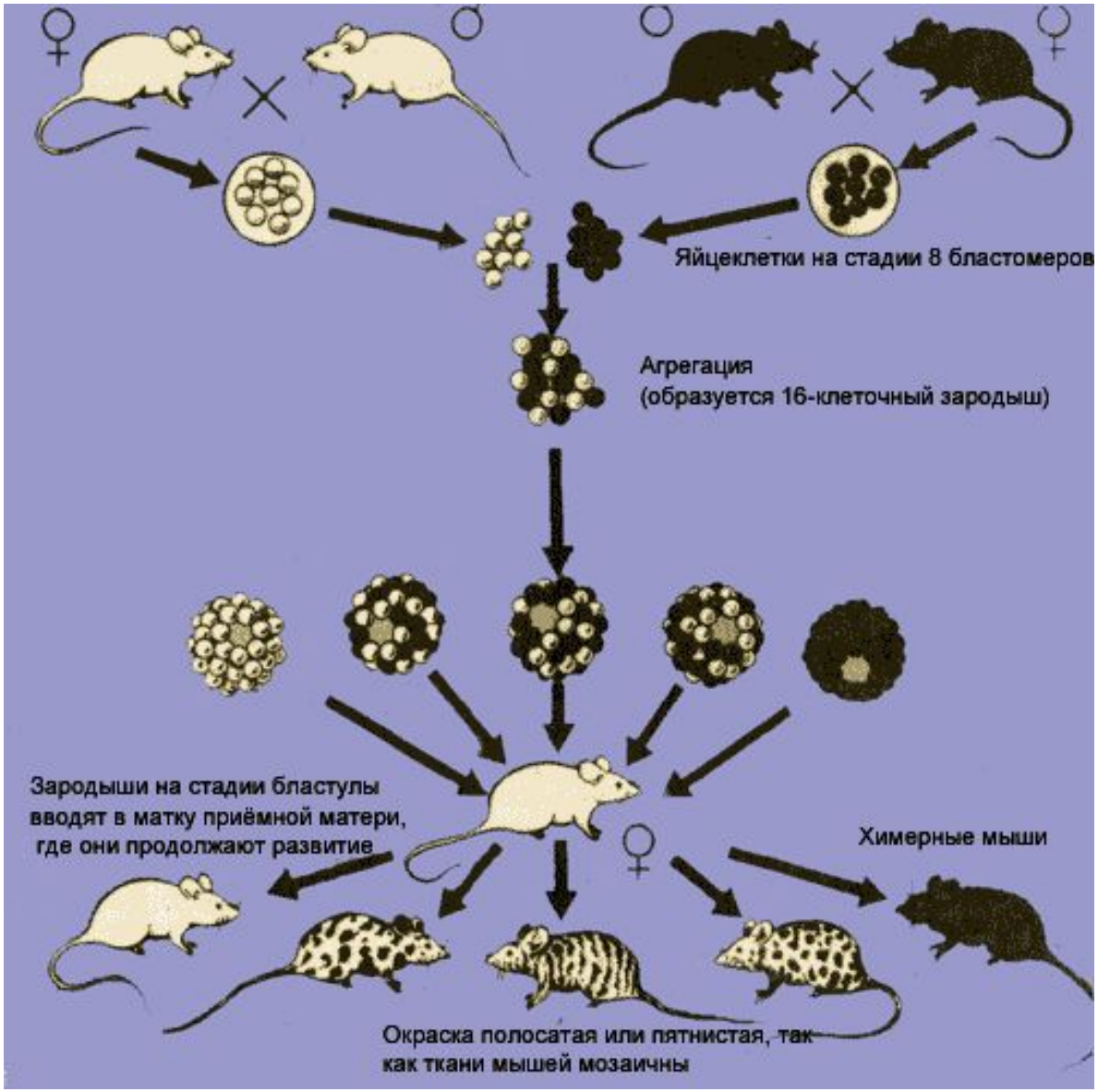


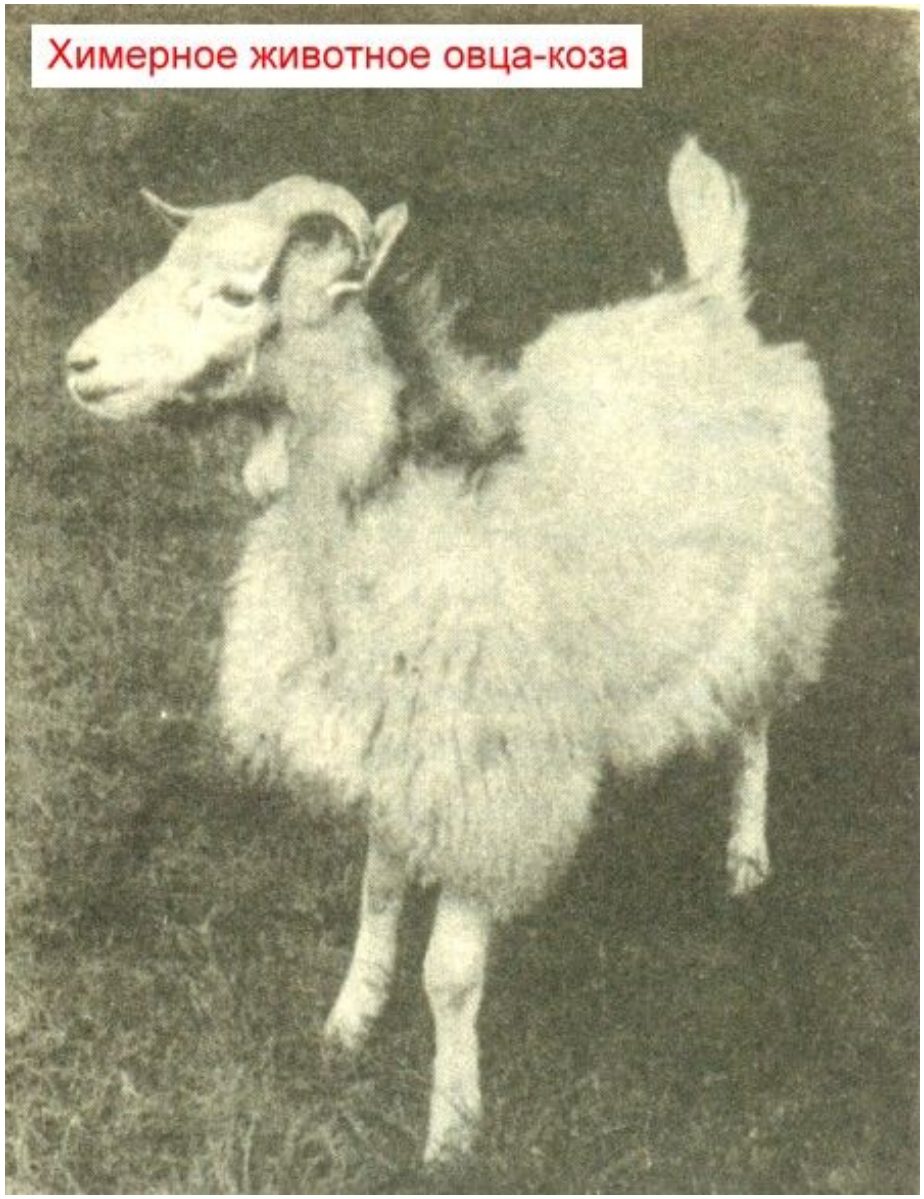
Схема получения химерных мышей агрегационным (А) и инъекционным (Б) методами (Б.П.Завертяев, 1989)

Chimeric animals are achieved by integrating blastomeres from two or more embryos or by injection of cells of one embryo into the cavity of another embryo blastocyst. The first method is called *aggregation*, and the second is known as *injection method.*


Яйцеклетки на стадии 8 бластомеров
Агрегация
(образуется 16-клеточный зародыш)
Зародыши на стадии бластулы
вводят в матку приёмной матери,
где они продолжают развитие
Химерные мыши
Окраска полосатая или пятнистая, так
как ткани мышей мозаичны

Complex chimeric sheep embryos by integrating 2 -, 4 - and 8-cell blastomeres were obtained by Fehilly et al. (1984). Each of these embryos consisted of an equal number of blastomeres of embryos from 2-8 parents. Results of the survey of 48 lambs at 2 months of age showed that 36 heads were chimeric by blood tests, by external signs or in other indicators.

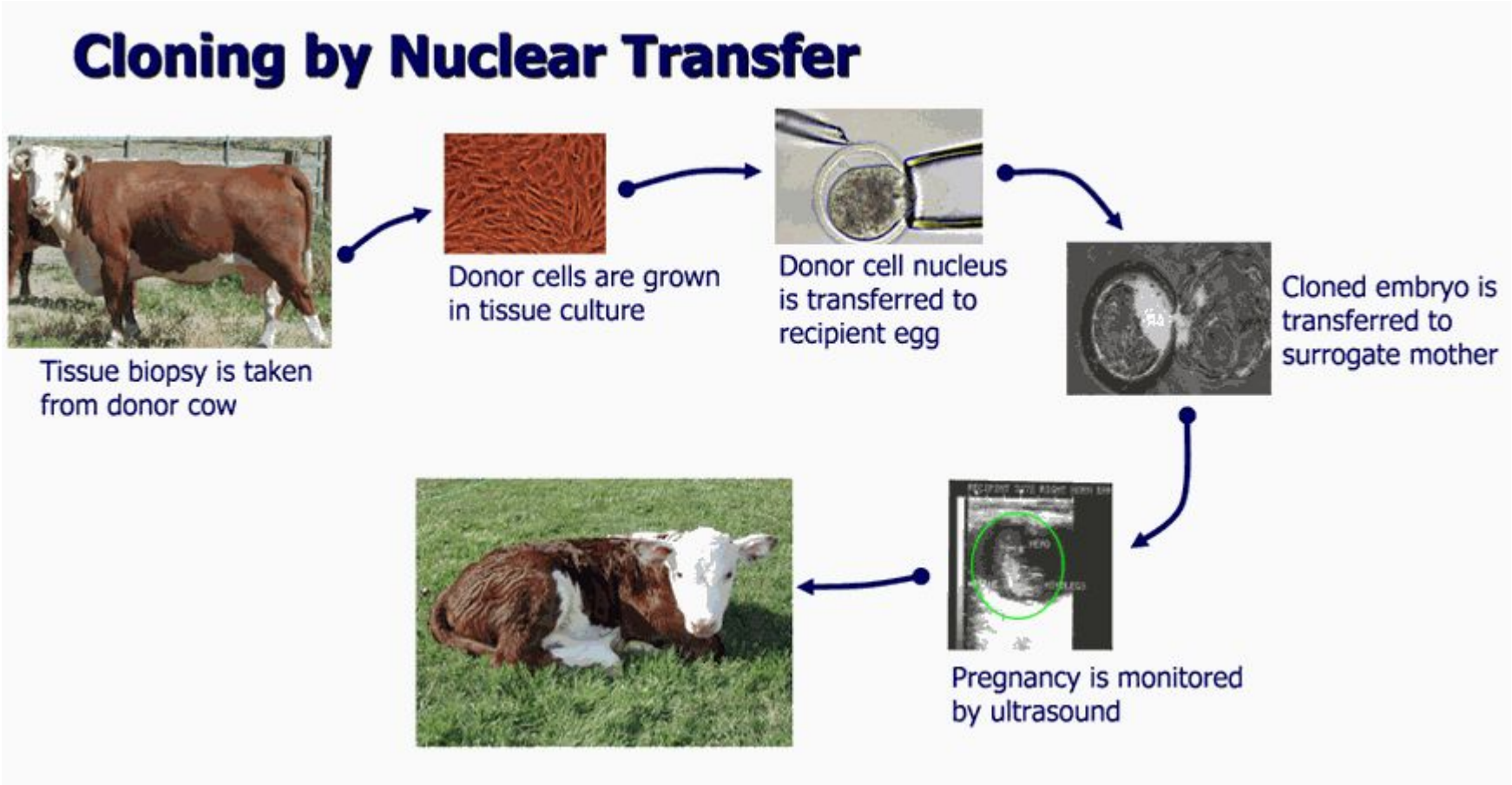
A year later, Butter et al. got chimeric lambs by injecting inner cell mass isolated from donor embryos into embryos' blastocyst of recipients. From these 15 lambs 5 heads were identified as chimeras on blood groups and 1 head appears by its external signs. Chimeras in cattle were obtained by Brem et al. (1985) combining halves of 5-6-day-old embryos. 2 of 7 calves had evidence of chimerism. 1 calf was a chimera on suit of brown schwyz breed and Holstein-Friesian, although blood group it inherited from their Holstein-Friesian breed parents. Another calf was uncertain chimera.



Typically, the embryos of an experimental hybrid pregnancy of sheep and goats at the end of the 2nd month are killed. The immediate cause of abortion in interspecific pregnancy is the strengthening of maternal immune responses to antigens of the fetus, leading to dysfunction of the placenta. Fehilly et al. (1984) showed that the blastomeres of sheep and goats that are enclosed in agar and placed for 4-5 days into oviduct of sheep can form a combined blastocysts that are viable and can develop to birth normal offspring.

17 blastocysts were received by mergering single blastomere of 4-cells sheep and goats' embryos, transplantation of which was ended with the birth of 7 lambs. They all looked like mostly in lambs, but 3 of them had wool that with transverse ridges and patches of hair sharply contrasting with the tight curly wool.

The above experimental results indicate the feasibility of transplantation of chimeric embryos between closely related species of animals.
Interspecies transplantation could be invaluable in preserving endangered species from extinction because embryo transfer may provide a small benefit, as the female recipient may not always be enough. Technique of obtaining chimeras can be used in breeding animals with desirable economic characteristics, as well as resistant to certain diseases.
Chimeric animals do not transmit to offspring their inherent genetic mosaicism. Like heterozygous or hybrid animals there is a splitting in the offspring, resulting in broken of valuable genetic combinations. Although chimeric animals support economic important signs only for a single generation they can be of great practical interest in the breeding of cattle. For example, you can create chimeric animals that combine features such as milk and meat productivity, which are antagonistic and incompatible in a single body. Creation of chimeras by injecting of certain embryo cell lines will improve the immune system and increase resistance to a range of diseases.



Cloning of animals **is getting identical offspring by transfer of nucleus of embryonic cell into oocyte with remote nucleus. Obtaining from high yielding donor cows five 32-cell blastomeres and transplantation each of nucleus into enucleated oocyts allows to receive from one donor 160 embryos simultaneously. Repeating this procedure with obtained "secondary" embryos give opportunities for an unlimited number of offspring.**

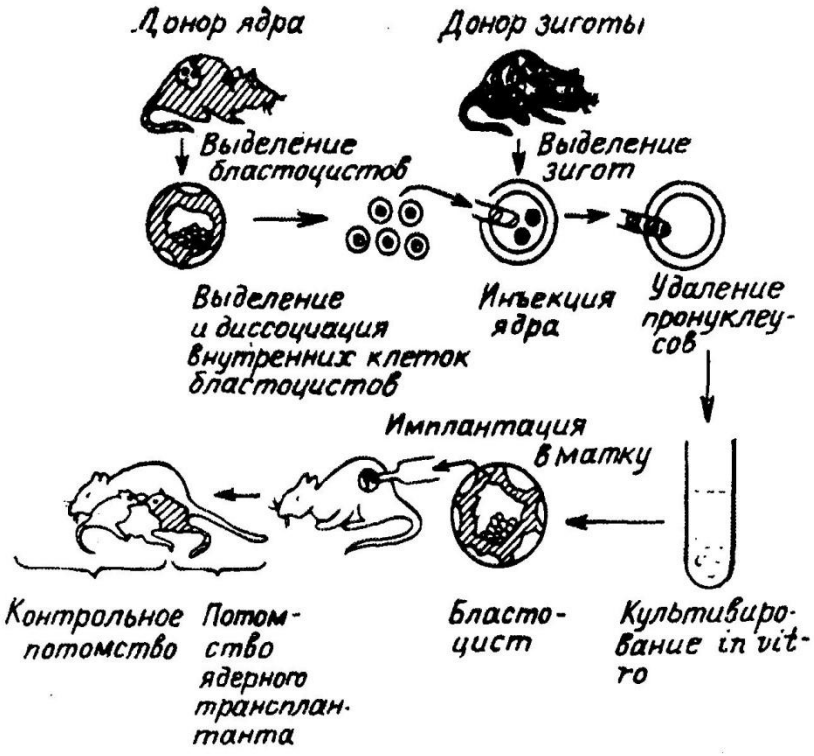
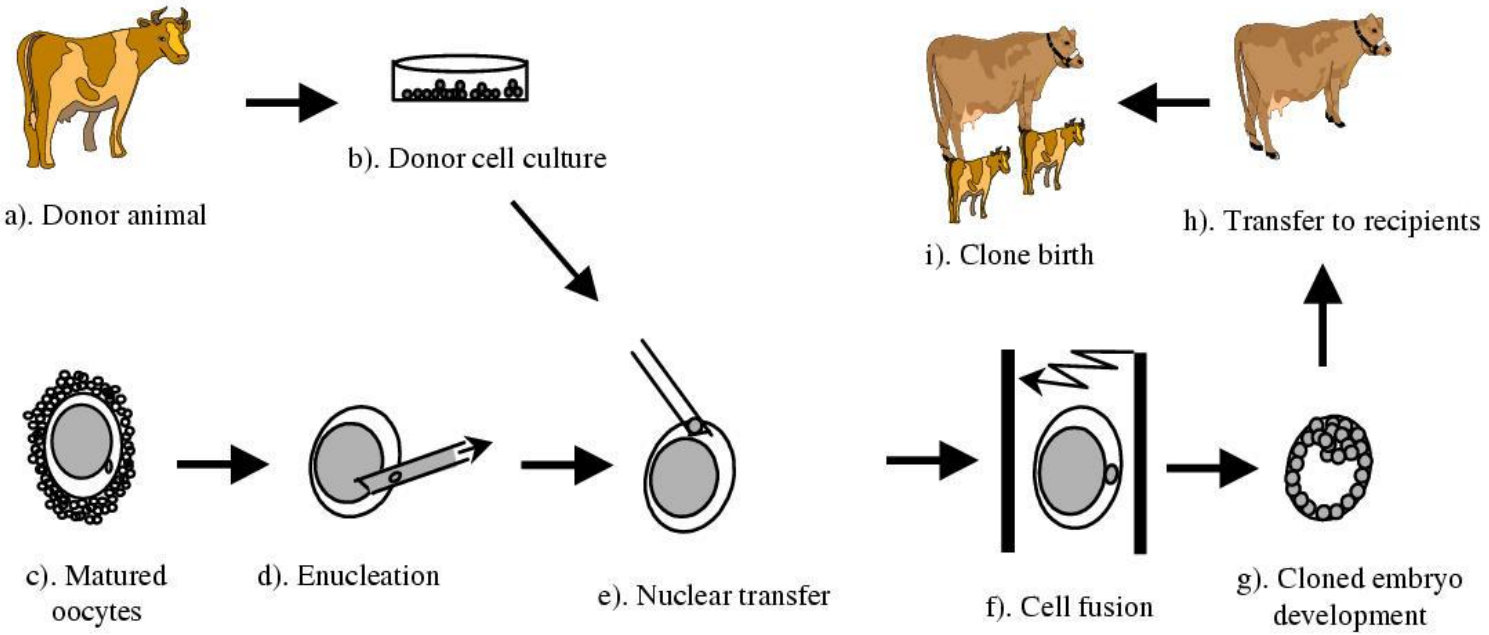


Схема клонирования мышей (Б.П.Завертяев, 1989)

The first report of successful transplantation of mammals nuclei in mice appeared in 1981 (Illmense and Hoppe). In this experiment, the nucleus were extracted from the cells of the inner mass of the blastocyst and with the help of a micropipette were transplanted into the zygote of different lines of mice. Own pronuclues of zygotes was removed by the same pipette. After *in vitro* culturing zygotes to blastocyst stage embryos were transplanted to recipient - to mice of third line. Three obtained offspring by genotype as well as by phenotype were identical with the line of the donor mice.

Cloning of animals



Prather et al. (1987) transplanted blastomeres of two 32-cell cow embryos into enucleated oocytes after maturation *in vivo* and *in vitro* by electrofusion. Oocytes extracted 36 hours after the start of hunting and used as recipients of the nuclei of embryonic cells more likely to achieve the stage of morula or blastocyst than ovum matured *in vitro* or extracted in 48 hours after the start of the hunt. 7 pregnancies achieved after transplantation of 19 embryos to 13 heifers. In 2 heifers calves were born alive. The above data suggest that the improvement of the efficiency of nuclear transfer technology of embryonic cells into enucleated oocytes allows to receive multiple copies of a single embryo.

ИНБРИДИНГ – близкородственное скрещивание, которое приводит к повышению гомозиготности. Применяется для получения **чистых линий.**

Часто приводит к снижению общей жизнестойкости из-за накопления вредных рецессивных аллелей.

Единственный метод, используемый для сохранения сорта или породы в чистом виде.

Сорт яблок «Бужбон»

Буденовская порода лошадей

***Obtaining homozygous diploid offspring*. At purebred line breeding is traditionally used, the aim of which is to maintain a high genetic similarity with outstanding ancestor. This is achieved by moderate inbreeding and targeted selection. Animals such lines, with a high degree of homozygosity distinguished by the genetic similarity.**

Thus high phenotypic uniformity in respect of physiological and morphological traits is created in the line. Unfortunately, the creation of inbred animals requires a lot of time, because this is due to the splitting and recombination of genes, low fertility and a long interval between the generations

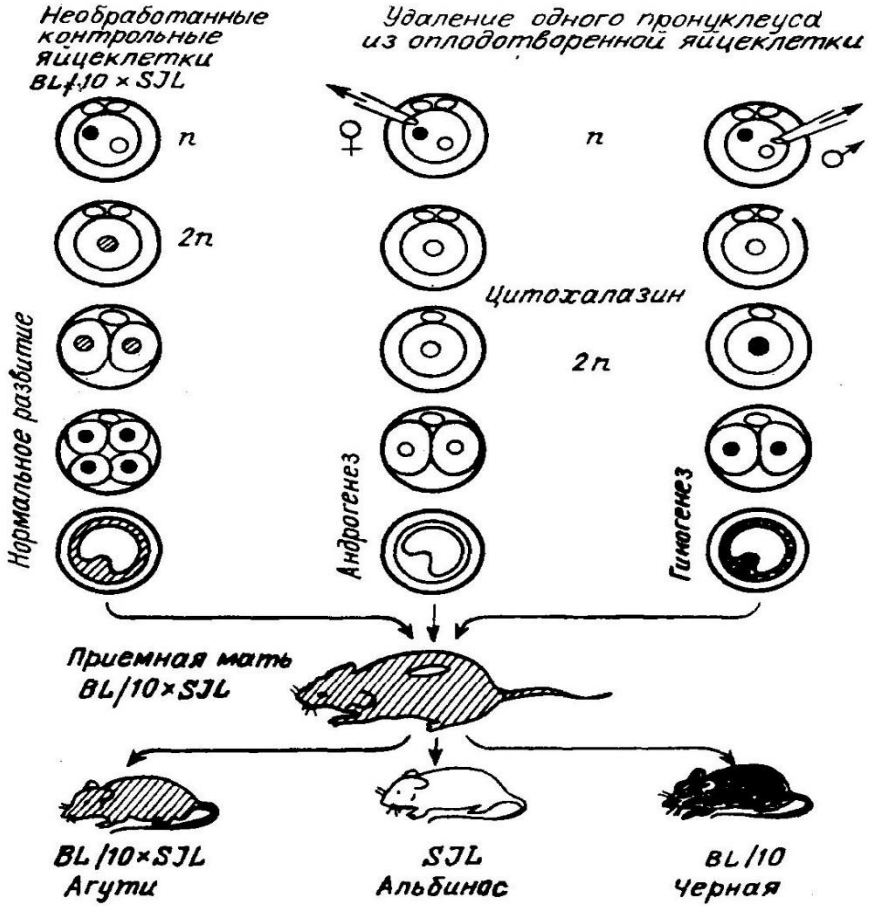
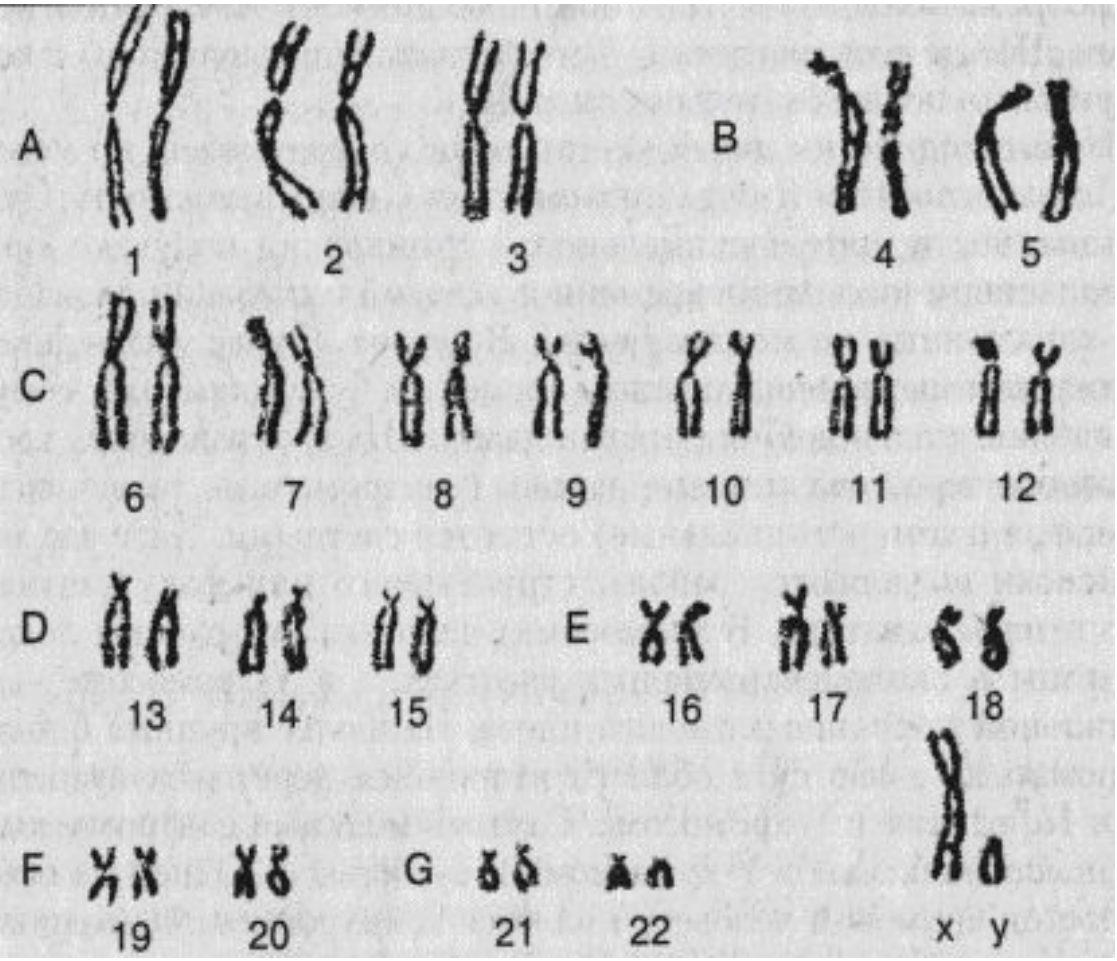


Схема получения гомозиготных животных (Б.П.Завертяев, 1989)

The method of obtaining homozygous diploid offspring in many ways similar to the technology of nuclear transfer of somatic cells into enucleated zygote. But in the latter case heterozygous animals are obtained, but not homozygous for all genes of one parent. The method is as follows: from zygote at the stage of two pronuclei male or female pronucleus is removed. As a result only one haploid set of chromosomes - male or female remains in the cell. For the development of the zygote, containing a haploid set of chromosomes, it is required to activate or restore the diploid set.

To this a brief incubation of haploid zygote in solution with cytochalasin B is carried out. The latter prevents the first cell division, but division of nucleus and diploidization of the remaining pronucleus are activated. As soon as happened nuclear fission, the embryo is washed away from cytochalasin B to prevent increase the number of chromosomes' sets.

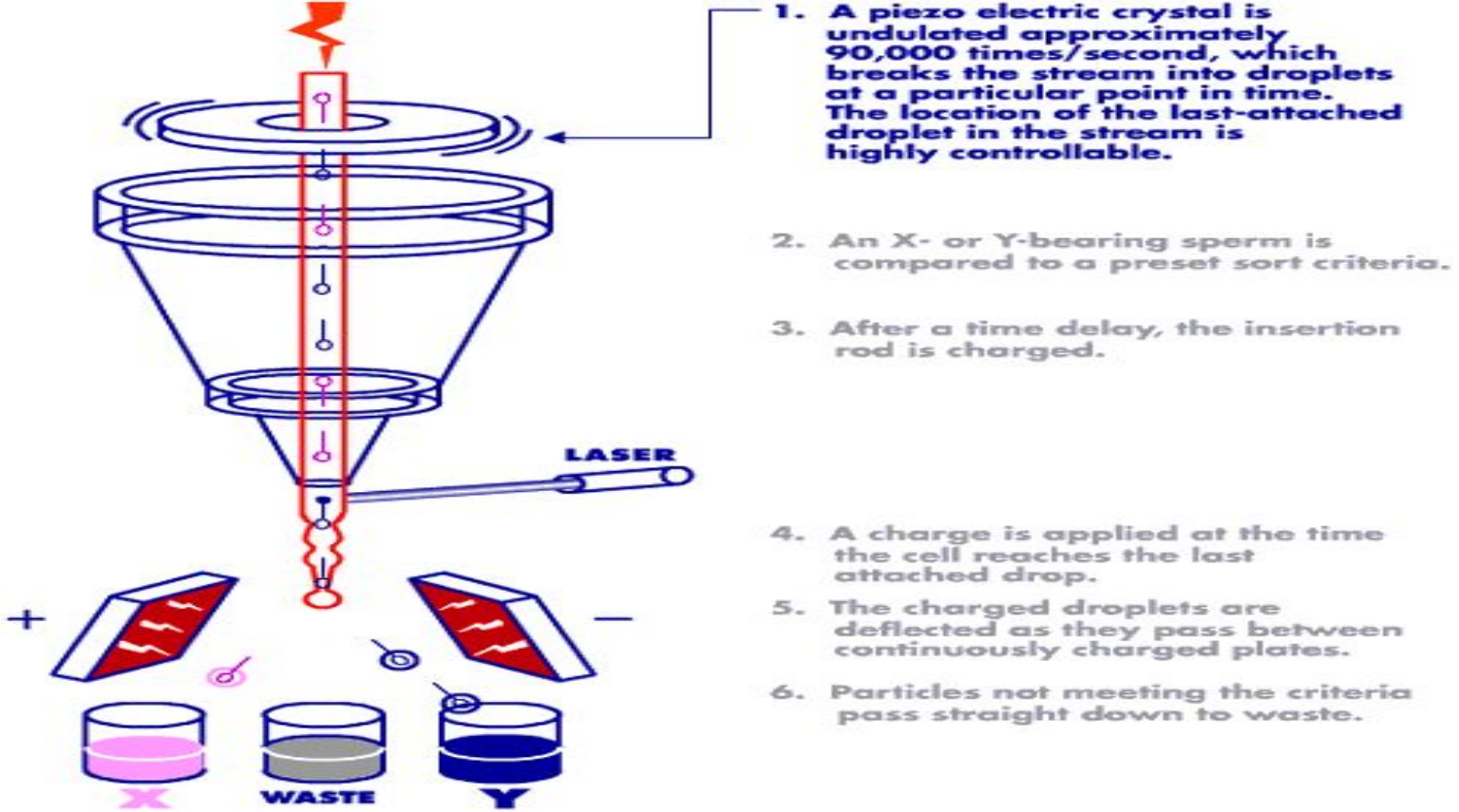
The experiments showed that in mammals for the normal development of the embryo up to the birth both male and female pronuclei are necessary. It is believed that diploid genome derived from only one of the parents of the same sex can not ensure the normal development of the embryo. It is believed that the paternal genome is required for the formation of extraembryonic tissues, and maternal genome is necessary to pass certain stages of embryogenesis. New data needed for theoretical understanding and experimental confirmation. Long-term research is necessary to overcome technical difficulties in obtaining homozygous diploid animals.



Definition and sex regulation.

Getting the animals of certain sex is not only biological, but also a practical problem. This is especially important for dairy cattle, whose main economic useful feature is milk production. And this feature refers to the attribute is bounded by sex. Therefore there is very important task of regulating the sex ratio needed for effective breeding. Genetic mechanism of sex determination provides splitting offspring by sex in a 1:1 ratio.

It is known that a set of homologous pair of sex chromosomes XX defines development of the female, and heterogeneous XY chromosomes determine development of the male. Over the years, investigations are underway to separate sperm carrying the X-and Y sex chromosomes. For this purpose, various methods have been tested: centrifuge, sedimentation, electrophoresis, filtration, cytometry, immunoassays, etc.



The most promising of them is the method based on the use of a laser. It is established that sperm with X chromosomes contain more DNA than sperm with Y-chromosomes. Thus the positive or negative charges of cells depends on the amount of DNA. First of all semen is processed by fluorescent dye, and then it is passed through a laser beam. Under the influence of negatively and positively charged plates sperm is deviated to the appropriate direction. Currently this method is being introduced into practice by «Sexing Technologies Navasota Texas». According to the manufacturer by means of this technologii it is possible to isolate fractions containing up to 92% of cells with X-or Y-chromosome. Since 2008 homosexual sperm is delivered to Kazakhstan