

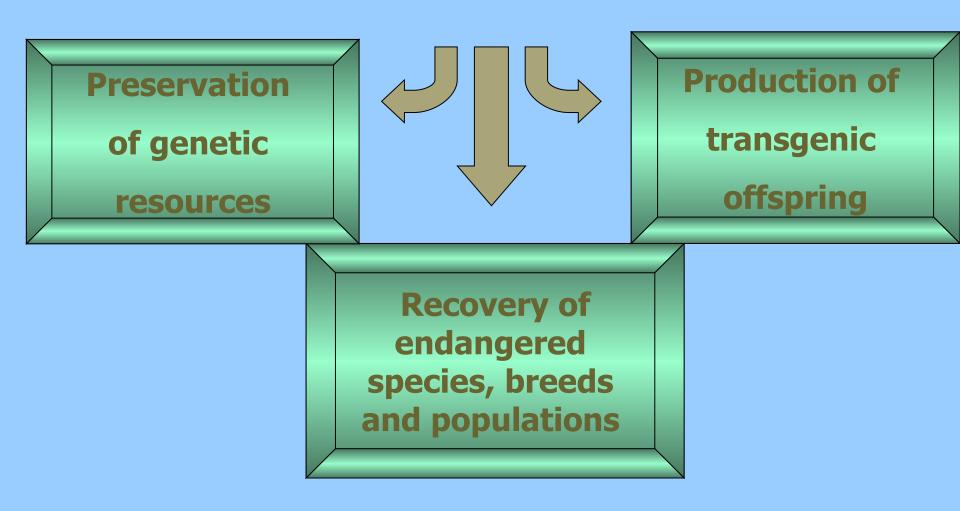
Use of modern biotechnological methods for preservation of rare and endangered bird species



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The main directions of the modern biotechnological research on bird



Biological objects serving for the preservation of bird genetic resources

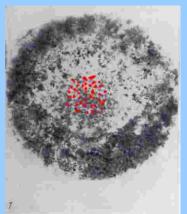
Spermatogonia

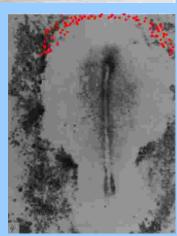


Blastodermal cells

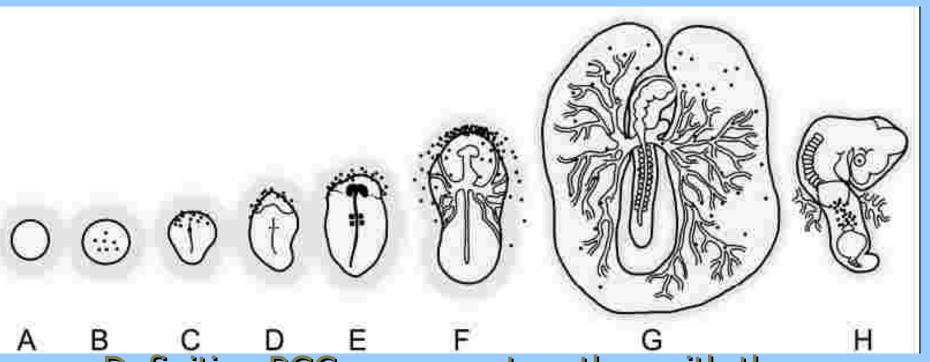
Embryonal stem cells (ESC)

Primordial germ cells (PGC)



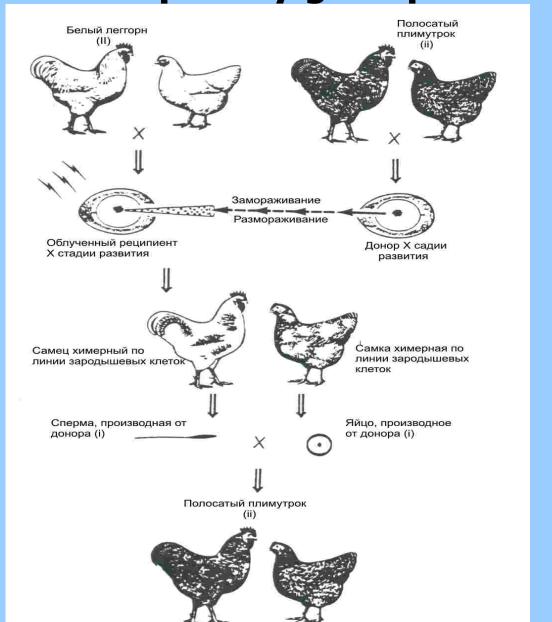


Development of the bird's primordial germ cells

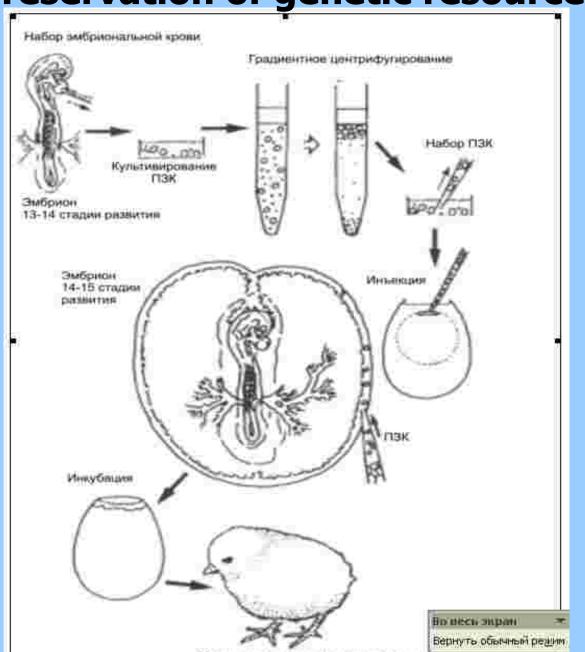


Definitive PGCs appears together with the germ crescent formation (C–E). F–H: PGCs penetrate the circulate during the embryo blood island formation (F) (G), actively migrate and settles the gonad anlages (H).

Use of blastodermal cells for the preservation of poultry gene pool



Use of primordial germ cells for the preservation of genetic resources

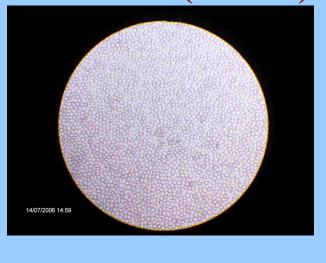


Methods used for the reduction of endogenous PGCs number

- Concentration of PGCs in the gradient of some substances
- Ultraviolet irradiation of recipient embryos
- Gamma irradiation of recipient embryos
- Laser irradiation of recipient embryos
- Chemical treatment of recipient embryos with busulfan
- Mechanical excision of germ cresent
- Culture of ESCs and PGCs

Culture of PGCs, separated from embryonal blood

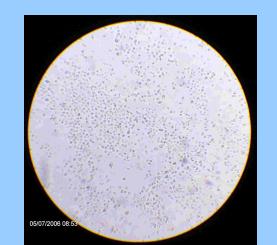
Embryo blood cells (50 hour)



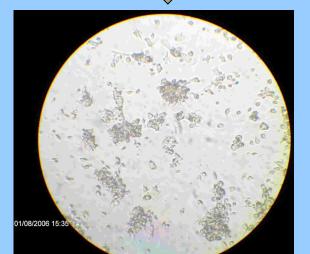
Special culture medium



Ordinary culture medium



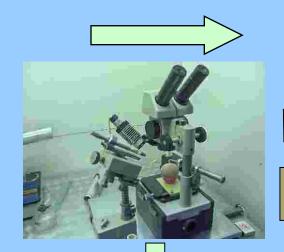
PGCs after purification



Production of intraspecific germ line chimera

Donor-Rod Island







Recipient-Leghorn

Microinjection of donor cells

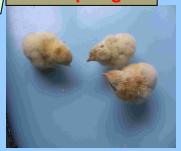
Chimera's offspring



Results: germ line chimera forming capability - 70%, the effectiveness of gonad settlement with donor germ cells- from 4 to 61%.

5-month old chimera birds

Chimera's offspring



Hybrids

Rod-island – in purity

Strategy of bird's chimera using for the recovery of endangered species, breeds and populations

Totipotent donor cells of endangered species

Microinjection

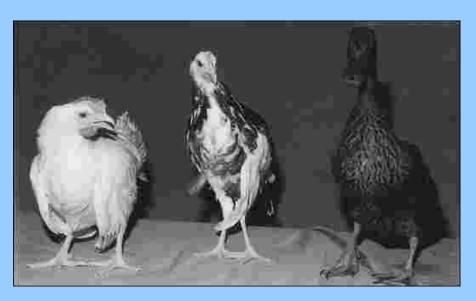
Recipient eggs of species with high reproduction qualities

Production of inter- or intraspecific germ line chimeras

Multiplication of donor germ cells in the body of individual with the high reproductive performance

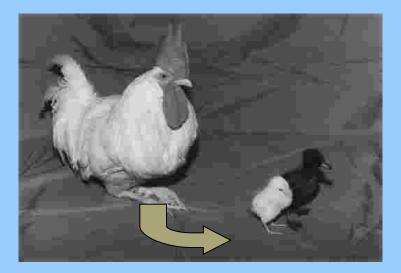
Reproduction of donor birds in the chimera progeny

Production of interspecific chimera hen-duck









The result of duck artificial insemination with sperm of chimera cock

Production of interspecific chimera henguinea fowl





Donor-guinea fowl



Chimera offspring is raising for germ line transmission analyse

Resume

- The modern bionechnological methods allow to solve the problem of gene pool preservation and recovery of endangered bird species completely.
- Nevertheless the affirmative results practically at all stages of chimera using technology, their effectiveness remains at the rather low level.
- One of the main problem is the choice of appropriate recipient capable of reproducing the allogenic gonocytes with high effectiveness.
- At present the most acceptable methods are the use of early blastodermal cells and PGCs. However if there would be developed the appropriate conditions for culture of PGCs it will allow to solve many problems related to the working out of as to the cell freezing regimes and the effectiveness of the genetic resources preservation technology.

Proceeding from the materials laid above the idea of interspecific chimera production between turkey and bustard for the genetic resources preservation of otis tarda seems to be very attractive. The strategy of these research would be presented as follows:

- 1. Preparation of turkey fertilized eggs as the recipients of allogenic cells;
- 2. Supression of the recipient germ cell line;
- 3. Microinjection of bustard blastodermal cells into the subgerminal cavity of the recipient eggs;
- 4. Incubation of handled eggs until hatching;
- 5. Production of chimera individuals;
- 6. Raising of chimeras till sexual maturation;
- 7. Artificial insemination of chimera with bustard sperm;
- 8. Incubation of eggs;
- 9. Analyse of chimera for germ line transmission.
- The affirmative results of the research will open the way for the solving of the following problems:
- 1. As the artificial insemination of turkeys is the routine procedure the problem of fertilization of bustard eggs in the turkey body will be achieved;
- 2. It is known that in nature conditions bustard clutch consists of 2-3 eggs, and turkey can lay more than 100 eggs for the breeding season. And so, depending upon the chimera forming capability and expressiveness of the feature tens of bustards may be obtained from one chimera individual. This will increase the number of bustards suitable for the raising in open-air cages with the following reintroduction to the nature.
- 3. The capability of bustard's gene pool preservation in the form of low temperature freezing of blastodermal cells opens up.