

THE USE OF BIOTECHNOLOGIES OF REPRODUCTION TO ASSURE FOOD SECURITY

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Abstract

In recent years it has become increasingly clear that the role of research in agricultural production is of major importance and problems facing mankind in terms of securing decent and mostly healthy nutrients could be solved only by basic research, which should provide real solutions to revolutionize this field.

People who remain on the concept that agriculture and food industry are old and conservative activities will be contradicted in future by the natural development and will be eliminated from the competition.

It is true that science is considered a two-edged sword, but to abandon the real benefits because the fear of threats is equal to the unconsciousness to the future of mankind. The main force that stimulated the implementation of biotechnology in agri-food was the need to provide food for a growing population. Progress in these modern techniques has led, naturally, to commercial and financial opportunities that will ensure, in turn, the continuation and development of research findings.

Biotechnologies of reproduction, acting directly on primary processes of life, provide expertise means that can be influenced and directed by the will and needs.

The classification of the biotechniques in breeding, as Holtz (1989) is allowed three degrees:

I-st degree-artificial insemination;

II-nd degree -embryo transfer;

III-rd degree -"in vitro"fertilization, obtaining monozygotic twins and chimeras, transgenic animals and embryo sexing.

Keywords: artificial insemination, embryo transfer, „in vitro,, fertilization, monozygotic twins, chimeras, transgenic animals.

ARTIFICIAL INSEMINATION

This Biotechnology, has already become a current breeding technique from several decades for some species such as cattle, sheep and pigs (Bilton R.J., 1980; Adams C.E., 1982; Altenhof R.L. et al., 1982; Bogdan A.T. et al., 1999; Bogdan A.T. et al., 1999).

In addition to the benefits of veterinary order, must be mentioned those relating to the fight against sterility, more rational use of breeding males and increasing selection pressure (Feredean T., 1975; Feredean T. et al., 1982). All this has contributed to the widespread of the method, which tends even to replace, in some species (Mantea S., 2003) the system of natural mating and the deep freezing preservation of semen created the possibility of setting up warehouses of genetic material to use in the works of selection, improvement and conservation of animal biodiversity.

EMBRYO TRANSFER

This Biotechnique of II-nd degree, includes a complex of related activities to obtain the greatest possible number of fertilized ova (zygotes) and embryos in the early stages of development from donor females, of high genetically value, handling these "in vitro "(control of the quality, sorting, cultivation, conservation and storage), and finally deposit them in the uterus of receiving females (Bilton R.J., 1980; Adams C.E., 1982).

In the transfer of embryos, methods of management of sexual cycle (considered by some authors as an independent Biotechnique) means to induce ovulation in prepuberal females, synchronization of oestrus and ovulation, induction of poli-ovulation, induction and synchronization of parturition (Armstrong D.T., 1981; Chen H.B. et al., 1992).

Embryo transfer, in addition to that underlies and makes possible the III-rd degree biotechniques, and has already many practical applications in the import of genetic material without health risk, the work of selection and improvement of breeds, and biodiversity conservation for livestock breeds or lines being at risk of extinction (Kirkwood R.N. and Huges P.E., 1979) . We have to mention that this method has become a current technology in some species, such as in cattle, where sequences of recovery of oocytes and non-surgical implant, make this technique as accessible as artificial insemination (Hughes P.E., 1982; Mantea S., 1982; Himeno N. et al., 1984).

„IN VITRO,, FERTILIZATION

By this method all the physiological processes of maturation of oocytes, sperm treatment, contact and penetration by the sperm and oocyte pronuclear fusion, which normally takes place in the female body, are directed and produced partly or wholly "in vitro", in different culture systems (Chen H.B. et al., 1992; Morrel J.M. et al., 2002).

Although the last two decades, many scientists all over the world worked to deepen the mechanisms involved in this process and systems of

culture (incubation temperature, culture media and gas atmospheres), success in farm mammals is still limited for fertilization totally "in vitro".

The main sources to collect oocytes are:

- The mature follicles from slaughterhouse material;
- Puncture of mature follicles in vivo before ovulation;
- Flushing the oviducts soon after ovulation.
- Maturing oocytes can be achieved:
 - In vivo in the oviducts of the same species or different species;
 - "In vitro" in culture systems by incubation at 37-38 ° C in complex environments and controlled atmosphere with CO₂, N₂, O₂.

The process of treatment of the sperm can get: - In vivo in the oviducts; - "In vitro", by successive washes in hypertonic solution to remove surface antigens (Armstrong D.T., 1981, 1993; Christenson R.K., 1981).

Course completion in vivo of phases such as maturing oocytes or sperm increase the percentage of success, but contribute very little to define the components (as part of modelling the physiological requirements of medium pH, osmolarity, gaseous atmosphere etc.) required by total "in vitro" fertilization. It is therefore necessary to continue studies in these directions, and for the quality and further development of embryos from fertilization "in vitro" which so far is very low due to the emergence of anomalies (Murdock W.Y. et al., 1986).

Development of the work on "in vitro" fertilization in domestic animals must be to:

- Better management of production of embryos from donor selection;
- An effective method for assessing the ability of sperm and embryos for forecasting future fertility of females;
- Access to early stages of zygote development to make possible gene transfer and the cloning of domestic animals is of great interest for animal husbandry (Bilton R.J., 1980; Demnefors B., 1982).

MONOZYGOTIC TWINS, CHIMERAS AND TRANSGENIC ANIMALS

Using the micro-manipulators fine movements, equipped with micro-tools, made possible embryo microsurgery that can make real miracles.

Starting from the phenomenon of omnipotent blastomeres until the blastocyst stage, is possible to obtain identical monozygotic twins (by sectioning) and chimeras (by joining blastomeres from embryos genetically different).

The method involves cutting the zona pellucida, cutting the mass of blastomeres in two halves and put them, separately in two empty zona pellucida which are implanted in the uterus of recipient mothers using techniques of embryo transfer.

Results in obtaining monozygotic twins, both in cattle and pigs, are higher when working on the blastocyst, compared with first stages.

This Biotechnique is extremely valuable especially for the testing of pharmaceuticals or feed formulas, eliminating genetic factors from the experiment. Making chimeras from different blastomeres, while not as immediate practicability, was done by using microsurgery both in laboratory animals (mice, hamsters, guinea pigs, etc..) as well as cattle, sheep and pigs.

Making transgenic animals, using micro-injection of foreign molecules of DNA in the pronucleus of zygotes is of great interest both for basic research and for new biological creations in the future. Also in this area, the swine species proves to be more difficult to work, but of more practical interest to obtain transgenic animals with human DNA for heterotransplantation of organs.

SEXING THE EMBRYOS

Attempts for predetermination of sex by separating sperm bearing X or Y chromosomes by physical methods (sedimentation, centrifugation, electrophoresis, etc.) are now replaced by biochemical methods (Morrel J.M. et al., 2002).

Following the developments in the last period of time in terms of biochemistry of molecular genetics, genetic markers can identify the sex. Using a "probe" made by PCR (polymerase chain reaction), which react with DNA specific for Y chromosome from taken blastomeres and the sex of the embryo can be determined accurately. The possibility of sexing embryos before implantation, offers the prospect of establishing a desired sex ratio in different populations of breeds, after the necessary of animals for breeding, meat, milk etc.

AVOIDING HEALTH RISK

One of the main reasons for which was initiated research for the development of biotechnologies of reproduction has been the prevention of contagious infectious diseases spread through the exchange of genetic material in the form of adult breeding animals (Hughes P.E., 1982; Himeno N. et al., 1984).

Although until recently, it was accepted the idea that embryo transfer is of no health risk and may even be used as a substitute for SPF, recent research has nuanced this theory.

Possibility of transmitting infections through embryo transfer refers to:

Risks "in vivo":

-presence of a virus on or in the sperm who penetrate the oocytes;

- presence of a virus on zona pellucida;
- infection at the time of recovery of zygotes or during implantation.

Risks "in vitro":

-contamination by culture media, equipment or handling during the test phase, conservation and transportation.

To minimize the risks it is necessary to strictly observe the following measures:

a) prophylaxis in the donors.

Female donors are checked for health, biological and possibly quarantine tested. In case of export, running the testing at the request of the importer. The same test will run and complete for male semen.

b) prophylaxis in ova recovery and implantation.

These operations will be carried out as aseptically as possible, with sterile equipment. After collection, the embryos will be removed from the environment as quickly as possible.

c) prophylaxis in the embryo.

Since the zona pellucida is a natural barrier, its surface contamination is resolved with several washes with fresh medium, each time changing sterile pipette. All manipulations of embryos are running in perfect aseptic conditions, with glassware and sterile instruments.

The news on sanitation methods for embryo transfer was approved by the competent international bodies and authorities of the EEC, which made rules for "the official approval of embryo transfer teams."

For accreditation of these teams, there must be the following conditions:

a). competence, ie the presence of a veterinarian specialized in embryo transfer, which is technically in charge of the team;

b). material and adequate accommodation, with clear separation between "aseptic" rooms (which runs manipulation "in vitro") from the „septic” areas (where it comes into contact with animals);

c). personal commitments to obey the recommendations on handling embryos (eg repeated washing for decontamination);

d). team agreement to comply periodically checks for "quality" with the withdrawal of the statute if there is a discovery of pathogenic elements in the environment where the embryos are handled.

Recently the same regulations were extended to the "in vitro" fertilization.

Under the conditions and regulations provided, health risks are much lower than the exchange of live animals.

Compliance with these requirements could be made exchange of genetic material with minimal health risk, and even genetic conservation in contaminated flocks.

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REFERENCES

1. Adams C.E., 1982, Factors affecting the success of egg transfer in mammalian egg transfer, C.E. Adams, Boca Raton, Florida, USA.
2. Armstrong D.T., 1993, Recent advances in superovulation in cattle, *Theriogenology*, 39, 7-24.
3. Armstrong D.T., 1981, Prostaglandin and follicular function. *J. Reprod. Fert.* 62, 283 – 291.
4. Altenhof R.L., Tanksley T.D.Jr., Knabe D.A., Harms P.G., Bowen M.J., Kraemer D.C., 1982, Investigations of nonsurgical embryo collection in swine. *Theriogenology* 17, 75.
5. Bogdan A.T., Mantea S., Bogdan, D., 1999, *Tratat de reproducție și înșămânțări artificiale la suine*. Editura Tehnica Agricolă București, 409 – 436.
6. Bogdan D., Mantea S., Chelmu S., Vasile M., Costache C., 1999, *Monografia înșămânțării artificiale la porcine*, Editura Tehnica Agricolă.
7. Bilton R.J., 1980, Preservation of embryos of the large domestic species. *Proc. IX th Intern. Congr. Anim. Prod. Artif. Insem. Madrid* 245 – 253.
8. Burnett P.J., Walker N., Kilpatrick D.J., 1988, Effect of age and growth traits on puberty and reproductive performance in the gilt. *Anim. Prod.* 46, 427 – 436.
9. Chen H.B, Lu K.H., Polge C., 1992, In vitro fertilization (IVF) of bovine oocytes with frozen-thawed sperm after storage at different temperature and periods of time. *12th INT. Congr. Anim. Reprod.*, A.I. The Netherlands, vol 2, 628-631.
10. Christenson R.K., 1981, Influence of confinement and season of the year on puberty and estrous activity of gilts. *J. Anim. Sci.* 52, 821 – 830.
11. Demnefors B., Tygum J., Norstrom A., 1982, Collagen synthesis inhibition by PGF_{2α} within a human follicular wall. *Prostaglandins* 24, 295 – 302.
12. Feredeian T., Mantea Ș., Mocanu V., 1982, *Tehnologia înșămânțărilor artificiale la porcine*”, Editura Ceres.
13. Feredeian T., Mantea Ș., 1975, *Organizarea reproducției prin înșămânțări artificiale în complexele de creștere a porcinelor, Recomandari pentru producție în creșterea animalelor*; Editura Ceres.
14. Himeno N., Kawamura N., Okamura H., Mori T., Fucumoto M., 1984, The effect of PGF_{2α} on collagen synthesis in rabbit ovary during the ovulatory process. *Acta. Obstet. Gynecol. Jpn.* 36, 2494 – 2495.
15. Hughes P.E., 1982, Factors affecting the natural attainment of puberty in the gilt. In: *Control of Pig Reproduction* (ed. D.J.A. Cole and GR. Foxcroft) Butterwarths London, 117 – 138.
16. Kirkwood R.N., Hughes P.E., 1979, The influence of age at first boar contact on puberty attainment in the gilt. *Anim. Prod.* 29, 231 – 238.
17. Mantea Ș., 2003, *Manualul crescatorului de porci*, Editura M.A.S.T. Bucuresti.
18. Mavrogenis A.P., Robinson O.W., 1976, Factors affecting puberty in swine. *J. Anim. Sci.* 42, 1251 – 1255.
19. Morrel J.M., Keeler K.D., Noakes D.E., Mackenzie N.M., 2002, Sexing of sperm by flow cytometry. *Vet. Rec.* 122, 322-324.
20. Murdock W.Y., Peterson T.A., Kirk E., Vincent D.L., 1986, Interactive roles of progesterone, prostaglandins and collagenase in the ovulatory mechanism of the ewe. *Biol. Reprod.* 35, 1187 – 1194.