Busch, Klaus Henning

Development of an embryo transfer system for cattle breeding

The complex bioengineering method „Embryo transfer in cattle farming“ laid the foundation in the German Democratic Republic (GDR) in the 80s. This method was utilized beyond the use in research labs. The system made possible the broad use of such effective method for the reproduction of big animal herds. End of the eighties the proof of concept was provided for this method and this system in the agricultural environment.

Keywords
Embryo transfer, cattle farming, bioengineering, laboratory, development of device, in-vitro-cultivation, in-vitro-fertilization

Abstract
Landtechnik 64 (2009), no. 6, pp. 442-446, 4 figures, 17 references

The large-scale livestock production units emerging in the GDR in the 1960s demanded, among other factors, effective biotechnological organisation of the breeding process.

Systematic research in this direction within the responsible institutes of the Academy of Agricultural Sciences in the GDR began as early as the 1950s [1; 2; 3]. A first result was artificial insemination in cattle production which was in general use by the beginning of the1960s as part of the introduction of “industrial standard production” in agriculture. With this procedure it was recognised that – among other positive effects – very good exploitation of sire potential was possible [17].

A next step was to be embryo transfer with which planning security in fertilisation could be further improved and also genetic potential of the dam lines exploited more to offer substantial progress in breeding. On average a cow has three to four calves in her lifetime but actually can produce around 50,000 egg cells.

First basic work on this subject was conducted as early as the 1950s and 60s. Targeted work into creating a procedure for wide practical application began in the appropriate research facilities in the 1970s. An important result was the development of a procedure for non-surgical transfer instead of surgical embryonic recovery, used until then. This important simplification was achieved around 1980.

Further focuses of the research were embryo cell culture and deep freeze conservation which permitted the freezing of embryos at –196 °C. Components were also the early diagnosis of embryo sex, which can be possible as soon as 7 days of age, as well as microsurgical embryo splitting to give identical twins.

From this position it was possible at the beginning of the 1980s to succeed with the gradual introduction of the technique in practical farming. The first calves from deep-frozen embryos were born in 1981 in Dummerstorf and 1982 in Jürgenstorf. The research and transitional work was accompanied by investigations relating to its breeding and economic efficiency.

As early as 1973 there existed a “Temporary International Research Collective Egg Transplantation” under the administration of the GDR Agricultural Sciences Research Centre for Animal Production with 25 scientists from seven eastern European countries involved.

Embryo transfer procedure

Embryo transfer is a bioengineering procedure whereby embryos from donors are artificially placed in the uterus of host females. Hereby, the embryos can be from other, often artificially inseminated, females or from an in-vitro fertilisation (artificially inseminated in test tubes).

Spermatozoon are collected from a high-performance sire for the artificial insemination. Multiple ovulation – superovulation – in the dam, a female with desired characteristics, is activated through hormone treatment. The resultant egg cells are either artificially inseminated in the female animal or are taken out of the female and fertilised in a test tube. In this case they develop in-vitro into embryos. The egg cells fertilised within the female animal also develop into embryos and are flushed out of the uterus around seven days post-fertilisation. Frozen down to -198 °C the embryos can be conserved for transplantation into surrogate mothers wherever required.

Equipment for embryo transfer

The development of embryo transfer in farm animals took place in several innovation impulses which in each case were influenced by social and scientific-technological developments. A decisive step hereby was the transition of the procedure from

research laboratory into practical farming. For this, a complex equipment system was required, one able to function reliably and effectively under these conditions. Focal point of the subsequent presentation is the development of selected positions in this integrated solution system in which the author played a substantial role. The translation of such comprehensive natural scientific research results into technical solutions required the interdisciplinary cooperation of various specialist sectors which also included an important input from agricultural engineering [4; 5]. Hereby, the development of equipment for embryo transfer and its integration in the reproductive process of farm animals must follow the same rules as the development of system solutions or machinery systems for other processes in crop and livestock production [10; 11]. Under these aspects and according to the GDR standard TGL 22 290 "Agricultural Technological Terminology" the equipment for embryo transfer was understood as the total of all the various working materials adjusted to complement their various technical and technological parameters for carrying out the complete procedure. **Figure 1** presents in simplified form the result of the analysis of the embryo transfer system [9].

From this analysis it is possible to calculate:

- the target for the design of the whole process
- the basis for project management of research and transfer plans
- the precise problems involved in "an equipment system for fertilisation biology"
- the tasks for the individual instruments within the system

Necessary for the embryo transfer equipment system was – alongside the application of standard veterinary instruments - the development, testing and manufacture of the following instruments in particular:

- equipment for analysing uterus motor functions
- ultrasonically controlled follicle puncture equipment
- flushing equipment for oocyte harvesting
- computer-supported photo evaluation systems for identifying and evaluating egg cells and embryos
- equipment for cultivation of egg cells and embryos (incubation systems, manipulation box, flexible movement techniques for ovocytes in nutrition medium in incubators)
- transport containers for biological material
- micromanipulators for biological objects
- packets of plastic "straws" for embryo transfer
- programme-controlled deep freeze devices for embryos and somatic cell material
- programme-controlled thermostats
- generators for fusion of embryonic cells
- implantation catheters

The following equipment comprised the main elements of instruments used in embryo transfer.

Equipment for registering uterus motor function

The mechanical activities of fallopian tubes and uterus have an important function in the breeding-physiological processes of ovulation, the transport of sperm and egg cells, the fertilisation, the implantation the protection and the nutrition of the foetuses as well as during birth. The achieved development of sensory and electronic technology enabled application of modern equipment for recording bioelectric activities in various areas of the uterus [12].

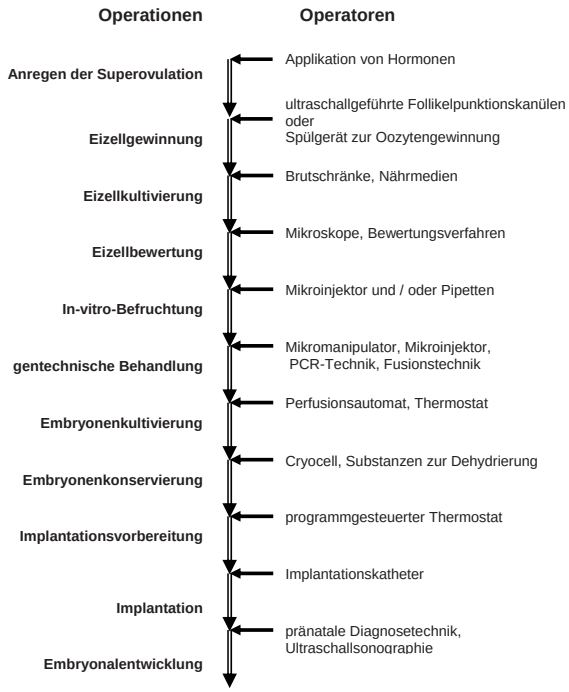
Hereby, the sensor comprised one or more bipolar precious metal electrodes for conducting the bioelectrical action potential from the muscle group of the myometrium cells as well as a stainless steel electrode for conducting the "zero potential" from the epidermis. For recording the bioelectric activity the investigated animals were equipped with especially developed recording apparatus.

Transport containers for biological material

The collection of biological material – e.g. ovary, oocytes, embryos and tissue samples – often occurred on farms a long way from laboratories. To avoid damaging the material transport had to be carried out in a portable container under defined conditions.

For practicality and control a temperature selector switch, a power switch and an visual and an acoustic warning system for signalling temperature deviations and for indicating the charge-

Fig. 1



System embryo transfer

level of the nickel-cadmium accumulators were all included in the electronic part. The temperature range of 35 to 39 °C could be regulated in 1 K steps.

Plastic straw box for embryo transfer

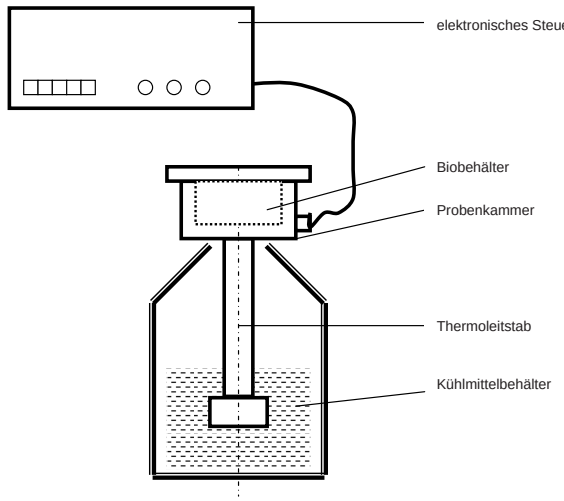
The storage of the embryos at the correct temperature up to time of transfer is a crucial requirement for success and permanent part of laboratory technology. The straws served as embryo carriers for non-surgical as well as for surgical transfer. This protective container thus represented integrated technology from the filling with straws in the laboratory through to the transfer of the embryos wherever that took place. The straws could also be used in the deep-freezing process. The straw box could contain up to 18 straws. Temperature range was selectable from 36 to 42 °C in steps of 1 K.

Incubator for in-vitro cultivation of cell cultures

For in-vitro maturation and fertilisation of oocytes as well as for the cultivation of embryos, biochemical and physical environmental conditions are required similar to in-vivo conditions. Hereto it is also required that the cultures are supplied continually with fresh, sterile nutrition medium with defined parameters regarding concentration and pH as well as with the required concentrations of gases (O_2, CO_2, N_2). Parallel to the supply, removal of metabolic products has to be carried out.

Basic within the incubator was a thermostat block featuring several chambers for cell cultures. A circuit system supplied nutritive medium and supply could be varied over an adjustable pump either continually or intermittently. In the nutrient medium container there was a pH sensor linked to an electrically-controlled valve via evaluation unit. Through the valve CO_2, N_2 und O_2 were channelled from the gas store into the nutrition medium. The temperature was adjustable in the range 35 to 39 °C (**figure 2**) [13; 14].

Fig. 3

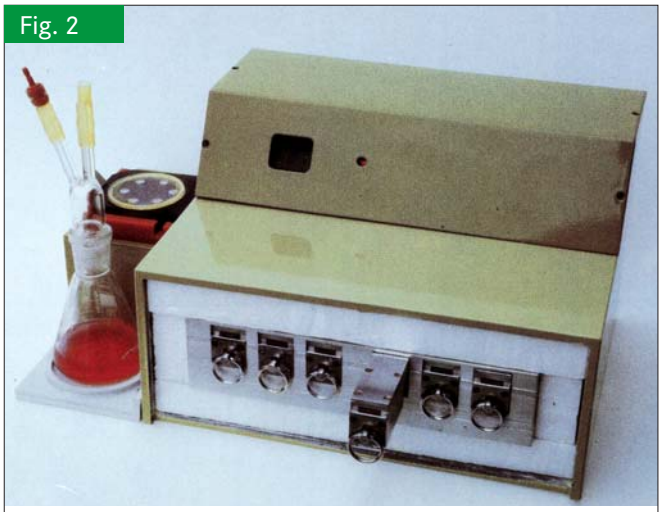


Deep freeze device Cryocell

Cryocell deep freezer

The programme-controlled Cryocell deep freeze device enabled through the cryobiological process the deep frozen conservation of oocytes, embryos, isolated blastomeres, spermatozoon, somatic cells, blood cells and vegetable material via freely selectable freezing and defreezing programmes (**figure 3**) [15]. Freezing occurred from 20 to –40 °C in continual or gradual mode with cooling rates of 0.1 to 1.5 K per minute. Halt phases in predetermined temperature areas were possible. The precision of temperature regulation was < ± 1 K. The device was capable of taking samples in straws („plastic straws"), ampules or tubes in a sampling chamber. The final temperature could be freely pre-selected and then kept constant after being reached.

Fig. 2



Incubation system for in-vitro-cultivation

Fig. 4

Incubation box

Manipulation box

Some of the manipulation and inspection of biological material – especially on oocytes and embryos – took place in open containers or in nutritive medium drops. During these experiments the biological materials were protected from environmental influences which did not represent natural conditions in-vivo such as temperature variations, optical influences and infections. The manipulation box realised on the one hand the required optimum conditions for the experiments while allowing, on the other hand, the experimenter sufficient freedom of movement without the person having to be subjected to the conditions in the direct surroundings of the object (**figure 4**). A sluice enabled introduction of material and sterile instruments. Sterilisation took place as UV or wet sterilisation.

Micromanipulator

This device was used for micromanipulation of embryos - e.g. splitting of embryos for rearing identical offspring or for injection of substances in cell nuclei. It enabled attachment of instruments or conducting of certain defined movement procedures under the microscope.

Conclusions

With the development of the complex bioengineering proce dure ”Embryo transfer in cattle farming“ a basic technology was created in the GDR in the 1980s with the help of which further bioscientific knowledge could be exploited effectively in production. From the 1990s the targeted reproduction of large dairy herds was no longer of such immediate importance for the development. Instead there was increasing diversification into the application area of embryo transfer with farm animals (cattle, pigs, sheep, goats) and horses through to smaller animals (dogs, rabbits, etc.) and also a concentration of the application on the targeted breeding of high-performance animals and, in special cases, for retention of rare breeds. Nowadays to help in this direction there are available stationary and mobile laboratories and embryo transfer stations.

Literature

[1] Schmidt, K. und G. Bachnik: Transplantation von Kaninchenеiern. Zuchthygiene, Fortpflanzungsstörungen und Besamung der Haustiere (1960), S. 349-355

[2] Schmidt, K., W. Altenkirch und G. Bachnik: Eitransplantation beim Schaf nach Progesteron-PMS-Synchronisation. Zuchthygiene, Fortpflanzungsstörungen und Besamung der Haustiere (1961), S. 339-350

[3] Forschungszentrum für Tierproduktion Dummerstorf-Rostock (Hrsg.): 50 Jahre Tierzucht- und Tierproduktionsforschung Dummerstorf 1939-1989. Ostsee-Druck, Rostock,1989, S. 51

[4] Dyhrenfurth, K. und K. H. Busch: Aufbau und Arbeitsweise der Abteilung Forschungstechnologie. Tagungsbericht 161 der Akad. Landwirtsch. Wiss, Berlin, 1978, S. 5–11

[5] Busch, K. H., K. Dyhrenfurth und R. Vilbrandt: Intensivierung der Tierproduktionsforschung durch forschungstechnologische Arbeiten. Tagungsbericht Nr. 287 der Akad. Landwirtsch. Wiss, Berlin, 1990, S. 213-216

[6] Forschungszentrum für Tierproduktion Dummerstorf-Rostock (Hrsg.): 50 Jahre Tierzucht- und Tierproduktionsforschung Dummerstorf 1939-1989. Ostsee-Druck, Rostock, 1989, S. 118-135

[7] Frick, R.: Erzeugnisqualität und Design. Verlag Technik GmbH, Berlin, 1996, S. 67–79

[8] Busch, K. H.: Handbuch Erfinden lernen – lernend erfinden. Trafo Verlag, Berlin, 2003

[9] Kurth, K.-J.: Forschungstechnologische Systemanalysen als Grundlage für die Entwicklung von Geräteketten. Tagungsbericht Nr. 265 der Akad. Landwirtsch. Wiss, Berlin, 1988, S. 21-27

[10] Soucek, R. und H. Regge (Hrsg.): Grundsätze für die Konstruktion von Landmaschinen. Verlag Technik, Berlin, 1979, S. 31-35

[11] Krombholz, K.: Landmaschinenbau der DDR. DLG-Verlags-GmbH, Frankfurt a. M., 2006, S. 96-97

[12] Hofmann, J. et al: Programmgesteuerte Tiefgefriereinrichtung für den Einsatz in der Kryobiologie. Medizintechnik 27 (1987), H. 2, S. 60–62

[13] Brüssow, K.-P., K.-J. Kurth und G. Blödow: Elektrophysiologische Untersuchungen zur Uterusmotorik im Östrus bei Jungschweinen nach Ovulationssynchronisation. Arch. Exper. Veterinärmedizin, Leipzig 42 (1988), H 6, S. 933–943

[14] Waselowski, B. und F. Geissler: Generator zur Fusion embryonaler Zellen. In: Tagungsbericht Nr. 265 der Akad. Landwirtsch. Wiss, Berlin, 1988, S. 193–199

[15] Patentschrift DD 279 030 C12M: Vorrichtung zur Kultivierung von Zellkulturen in vitro. Erfinder: Kurth, K.-J., U. Koch, H. Torner, M. Götze und P. Kauffold. 29.12.88/23.05.90

[16] Patentschrift DD 278 659 G01N: Regelkreis zur kontinuierlichen Veränderung des pH-Wertes in Nährmedien. Erfinder: Kurth, K.-J., U. Göllnitz, H. Alm, und H. Torner. 27.12.88/09.05.90

[17] Busch, W.: Lehrbuch der künstlichen Besamung bei Haus- und Nutztieren. Schattauer Verlag GmbH, Stuttgart, 2007

Author

Prof. Dr. sc. nat. Klaus Henning Busch was from 1975 to 1992 manager of the Research Technology Department (from 1991 Biotechnology Sector) in the former Research Centre for Animal Production in the GDR Academy of Agricultural Sciences. At that time he lectured at the University of Rostock on fine instrument construction. Now, he is chairman of itf Innovation Transfer and Research Institute Schwerin e. V., E-Mail: k.busch@gmx.de

Notes

The treasures of experience from previous generations offer not only much of interest but also can often give valuable stimulation for creating the future. Under this motto, the special VDI-MEG committee ”History of Agricultural Engineering“ has made it a target to ”dig out“ and publicise agri-historical facts. Such themes have also a place in the publication Landtechnik. The presentation from Professor Busch published here is to be the starting shot for this. The special committee has made it its aim to activate as many authors as possible in the great society of competent agricultural engineers from west and east for this interesting task. We hope that suitable material for publication will be offered by Landtechnik readers everywhere.

sgd. Dr. Klaus Krombholz,
Chairman of special VDI-MEG committee, ”History of Agricultural Engineering“, E-Mail: klaus.krombholz@t-online.de