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Studies of assisted reproductive technology in the 19th and 20th centuries

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Summary

Artificial breeding technology has been an important subject of research from the very beginning of medicine and veterinary sciences. Several main directions should be distinguished within these methods: artificial insemination (including cryogenic semen preservation technology), transplantation (embryo transfer) and embryo culture *in vitro*. The first experiments in this field date back to the late 18th century, when Lazarro Spallanzani performed such experiments on animals. The late 19th and the early 20th centuries saw the development of artificial insemination, which became a routine procedure, significantly facilitating breeding. The first successful experiments in embryo transplantation were also carried out in 1890. The high potential of this method was recognized related to the intensification of breeding for specific individual traits without limitations imposed by breeding physiology. These procedures were limited for a long time to scientific experiments and were not introduced into medical practice until the early 1970s. Embryo culture *in vitro* was a separate problem and an even greater challenge for researchers. Significant experimental research in this field started only in 1949 and has been continued ever since. In the 1990s, the biotechnology of *in vitro* embryo culture was improved to the point of becoming one of the most promising fields of veterinary and zootechnical sciences.

Keywords: veterinary science history, artificial breeding techniques, artificial insemination, *in vitro* fertilisation

Researchers have been interested in assisted reproductive technologies ever since veterinary sciences separated as a discipline of medical science. In the case of animals, it was particularly important to obtain numerous progeny with selected genetic traits related to productivity. Development of scientific techniques and procedures was important for their future use in medicine. This study intends to present early studies in the field of assisted reproductive technology applied to animals worldwide, as well as their impact on Polish veterinary science and animal husbandry. Materials used in this work include scientific publications related to these topics, printed in Polish and international veterinary science journals, legal acts and archived documents from centres that introduced such procedures in Poland after World War II.

Animal reproduction biotechnology focused on three main areas: artificial insemination, embryo transplantation and *in vitro* fertilisation. Historical studies of insemination were the earliest and will therefore be presented first.

The first attempts at artificial insemination of animals were made by Lazarro Spallanzani, an Italian physiologist. After a positive result of this procedure in amphibians, he proceeded to test it in viviparous animals. Dogs were the species he selected for his experiment. The experiment was performed in 1780, and the inseminated female gave birth to three puppies on the 62nd day after the procedure. Spallanzani's experiment was repeated in 1782 by Rossi and Branchi. This time, insemination was performed five times. This experiment was also successful, and the female gave birth to four puppies on the 62nd day. An important observation made by Spallanzani was that artificial insemination is possible even if the semen is strongly diluted. After that, research on artificial insemination was neglected for more than a hundred years (19).

Subsequent experiments were also made on dogs. The American breeder Everet Millais inseminated 19 females between 1884 and 1896, with 15 of them becoming pregnant. Those experiments led to three conclusions, important for future studies:

1. Artificial insemination is relatively simple to perform.
2. The probability of pregnancy is similar as in natural mating.
3. With this method, a single ejaculate can be divided among multiple animals.

These results did not escape the attention of American farmers, who perceived artificial insemination as an opportunity to intensify production and a way to fight infertility. Studies of this technique were undertaken by Prof. Pearson of Pennsylvania University. However, it was only the work of the French veterinarian Repiquet, who in 1890 recommended artificial insemination in horses as a method against infertility that propelled these studies into the spotlight (6).

At that time, the rate of fertilisation in European stables was very low, and finding a method of improving it was extremely desirable. One of the first researchers to introduce artificial insemination into practice was Ferdynand Chełchowski, a Polish veterinarian and director of Russian stables, who described this technique in his monograph *Niepłodność konia, jej przyczyny i zwalczanie* (*Horse infertility: Causes and remedies*) (1894). Chełchowski used artificial insemination for zootechnical reasons, as well as to control stable disease in horses. He not only described the methods of artificial insemination, but also invented instruments to collect and inject semen (23).

Another insemination technique was proposed by Prof. Hoffmann from Stuttgart, who recommended artificial insemination with semen of the mating stallion after every actual copulation: "In every rationally managed horse stable, semen present in the vagina should be artificially introduced into the uterus via cervix immediately after every mating." He provided a detailed description of the procedure and tools required to perform it: "After the mating, the semen is collected using a speculum and a curette in a depression of the vagina, from where it is then collected into a special syringe and introduced directly into the uterus." Using the same semen with another mare is insignificant according to Hoffman, but in such a case he recommended dilution of semen in cow's milk (19).

Experimental studies began at the same time in Denmark. Sand and Stribolt presented results of their studies of insemination during the first Scandinavian Veterinary Congress. They used semen from the renowned Jutland stallion Aldrup Munkedal to inseminate 6 mares. Four of the animals became pregnant. Sand pointed out that artificial insemination is a valuable method of improving populations of bred animals because genetic material from a good male breeder can be distributed among many females (19).

This method was used for the first time to improve a population of bred animals in Russia. Ilya Ivanov is considered a pioneer of artificial insemination in animal husbandry. Initially (from 1899 to1905), he focused on insemination of mares, as did his predecessors. His research led to the conclusion that the efficiency and experience of the inseminator are decisive for the result of the procedure. In stables where he performed insemination himself or where it was performed under his supervision, the results were satisfactory, and the rate of pregnancies was higher than with natural mating. A total of 109 mares were artificially inseminated between 1901 and 1904, with 78% of them becoming pregnant (23).

The results of these experiments encouraged Ivanov to use this method in other animal species. He thus asked the Ministry of Agriculture for permission to experiment on cows and sheep, but objections of the higher scientific echelons prevented his experiments at schools under the ministerial aegis. He then purchased 10 cows and subjected them to the procedure. According to his report, "a few of them became pregnant." He also performed artificial insemination on sheep at the Askania Nova Zoo, which produced progeny.

Ivanov's work led to the foundation of a physiology department at a veterinary laboratory run by the ministry in 1909. The department focused on studying fertilisation physiology, improving the artificial insemination technique and training veterinarians in the field. Ilya Ivanow became its head and trained more than 300 veterinarians until World War I (12, 19).

Obviously, the results of this system were implemented fairly quickly. There was a significant increase in the number of artificial insemination centres and the number of procedures performed there. A total of 57 mares were inseminated in 1909 at 3 insemination stations, whereas 3 years later the number of mares inseminated was 3397 and the number of stations increased to 41. The rate of pregnancies was highly variable, from 4% to 90.7%, but irregularities were eliminated over time, and the average rate was 78%. Unfortunately, the start of the war and the change of political regime interrupted the studies. The programme was resumed only in 1923, and a number of insemination stations were created. About 1,000 mares were inseminated in 1923, and as many as 70,000 in 1928 (19).

One of the main objectives of Soviet Russia in the field of breeding was improvement of cattle and sheep herds, so attempts were also made at artificial insemination of these species. Initial experiments were unsuccessful, since the sponge method developed by Ivanov and used in horses proved ineffective in cattle and sheep. An improvement was achieved after the artificial vagina designed by Amontea in 1914 in Italy, which was used in dogs, was modified for use in bulls and rams (19).

Russian achievements were noticed in the United States, Denmark and Great Britain. In 1930, these countries began intensive studies of artificial insemination. The studies were pioneered by Danish veterinarian services, which developed methods used as an exemplary organisation of artificial insemination in other countries, including Poland (19, 23).

In Denmark, artificial insemination began on a large-scale in 1935, when Prof. Sørensen and his assistant Anker Hansen used Russian methods to successfully inseminate heifers. An experimental study in 1936-1938 was expected to provide an answer to the basic question whether artificial insemination results in higher pregnancy rates than natural mating. The study was performed on heifers of the same age receiving identical diets and inseminated artificially or mated naturally by the same bull. The results were as follows:

Artificial insemination – pregnancy rate of 97.4% in 1936-1937 and 97.5% in 1937-1938.

Natural insemination – pregnancy rate of 94.9% in 1936-1937 and 95% in 1937-1938.

The results showed that both methods were equivalent, and the advantage of the former method stemmed from a better use of valuable breeders in cattle breeding.

This experiment was of paramount importance for Danish cattle breeding because the publication of its results led to the implementation of planned and systemic artificial insemination programme. One of its key elements was the formation of associations (co-operatives) of cattle farmers who implemented the programme. But it was not only Danes who took interest in the results of these studies. Similar conclusions were arrived at in the United States, where Prof. J. E. Perry, assisted by the veterinary doctor A. F. Larsen, implemented similar systemic solutions.

In 1945, there were 100 cattle farmer co-operatives using artificial insemination in the United States (with 230,000 inseminated cows), about 25 such associations operated in England, and about 100 co-operatives existed in Denmark (with 500,000 inseminated animals) (23).

How was artificial insemination organized according to Sørensen's model? The first step was the formation of farmer co-operatives, whose main purpose was to use valuable bulls in breeding. A single co-operative needed to have 1,000-1,200 cows so that all of them had similar economic bases. Each co-operative employed a veterinarian or a veterinary technician, who worked according to a strictly defined schedule. After semen collection and evaluation in the morning, he telephoned farmers to collect information about cows in order to plan his visits at individual farms. After artificial insemination, pregnancy tests and procedures aimed at infertility elimination were performed. This schedule was repeated in each co-operative every day (19).

Despite encouraging results, some scientists were sceptical about this system. During the 1940s, the opponents pointed out that, paradoxically, the large number of females provided with semen was not only a benefit, but also a drawback, since inadequately studied, undesirable genetic traits of the breeders could be transferred to a relatively large number of progeny. With regard to the method of semen collection from the breeders, Goetller thought that it results in breeder degeneration, as the use of an artificial vagina in this procedure disturbs the breeder's sexual constitution (2).

These voices, however, were isolated, and the losses in agriculture caused by the war necessitated the use of techniques aimed at quick restoration of farm herds. It is worth noting that Poland suffered some of the highest material damages among European countries in World War II. Sixty-seven percent of its cattle population was destroyed, and almost all breeding animals were either killed or looted. As a part of war reparations, Poland received 112,000 cows, including 75,000 from Soviet military authorities, 20,000 from UNRRA, and 16,500 from Sweden. Most of those cows were provided to private farms. Cattle provided by the military was obtained from Pomerania, and UNNRA aid included mainly the Holstein-Frisian race of cattle (17). The aid was not limited to the delivery of the animals to Poland, but also included an artificial insemination programme conducted by the aforementioned Prof. E. Sørensen, together with a group of Polish veterinarians and zootechnicians (19).

Prof. dr Tadeusz Olbrycht began systematic experiments on insemination of mares, cattle and sheep in Lwów as early as 1928. He created the first mare insemination station in Poland, where over 500 mares underwent the procedure from 1935 to 1939. He also studied the technique of collecting and preserving semen from stallions, bulls, rams and hogs, developed a number of insemination tools, published numerous studies on insemination in Polish and foreign journals and participated in international congresses, actively participating in the sectional works on breeding physiology and insemination. Before the war, during the Husbandry Congress in Switzerland, he presented his artificial insemination methods, and during the Genetics Congress in Edinburgh he gave a lecture on the application of these methods in genetic studies (9, 12). After the war, in 1946, Tadeusz Olbrycht presented a plan for the organisation of artificial insemination, based largely on the Danish experience, in the journal *Medycyna Weterynaryjna* (14, 15).

The system for artificial insemination in Poland was organised at that time, on the order of the Ministry of Agriculture, by the Polish Zootechnical Society (PZS), which established the Chief Insemination Committee (Naczelna Komisja do Spraw Inseminacji, NKSI), initially headed by Prof. Prawocheński, and from 1947 by Prof. Olbrych (12, 13). PZS organised the first course on artificial cattle insemination in Pawłowice on August 17-28, 1946. Lectures and practical exercises were conducted by the aforementioned Prof. Ed

Sørensen from the Copenhagen University. The course was divided into three topic blocks:

1. Obtaining bull semen.
2. Testing semen for fertility.
3. Introduction of semen into the birth canal of a cow.

The course was attended by 10 veterinarians and 22 agricultural engineers (9).

The Veterinary Science Department and the Animal Production Department of the Ministry of Agriculture and Agricultural Reforms noted the benefits of mare insemination in the context of fighting stable disease. Thus, three insemination courses took place in Pawłowice between 18th November and 5th December 1947, intended for veterinarians, stallion herd managers, stallion herd inspectors and stable managers. The course for veterinarians dealt with theoretical and practical aspects of insemination, theoretical and organisational issues for herd managers as well as topics related to the activities of auxiliary staff for equerries. The course was organised by the Polish Zootechnical Society, and lecturers included Prof. Z. Moczarski, Prof. T. Olbrycht, Prof. J. Parnas, Prof. S. Runge, Dr. A. Tekliński, Dr. W. Bielański, Lech Jaśkowski DVM. A special guest was Prof. Kaplan from the FAO (11).

Five original insemination stations, in which only cattle was inseminated, were created in 1946-1947 (Zakład Doświadczalny Instytutu Zootechniki Pawłowice, Instytut Uprawy Nawożenia i Gleboznawstwa in Puławy, Instytut Zootechniki in Balice, in Wrocław and Trzęsacz). Initially, only fresh liquid-preserved semen was used. Frozen semen was introduced in 1968, and the stations were provided with equipment required to use it. Pig insemination was first attempted in Poland in 1965 at the Voivodeship Insemination Centre (Wojewódzki Zakład Unasienniania) in Gdańsk (13).

Taking into account further objectives of artificial insemination (after restoring population), it became the main element of the cattle improvement programme (regardless of the functional type of the given race), achieved through selection of sires of the next generation and the best bulls in the domestic population for breeding purposes. This objective was achieved thanks to the creation of a team of researchers including Prof. Z. Staliński, Dr. M. Stolzman, Prof. J. Romer and Dr. W. Głód, who in 1968 presented a plan for the programme "Evaluation and selection of bulls for artificial insemination in Poland." After minor changes, this plan was approved and implemented according to Ordinance of the Minister of Agriculture no. 56 of 21/04/1971. It was based on the rules of population genetics and took into account contemporary circumstances and conditions (17).

Artificial insemination proved easy to introduce into breeding and veterinary practice. This was not the only field of research, however, and experiments on embryo transplantation began as early as the 1890s. The first successful transplantation was performed by Walter Heape in Cambridge. On November 27, 1890, in a meeting of the Royal Scientific Society in England, he presented results of his experiment performed on April 27 of the same year. The experiment involved collecting two embryos from a female Angora rabbit, mated 32 hours earlier with a male of the same race. The embryos were in the phase of division into four blastomers, and they were immediately transplanted into the fallopian tube of a female Belgian Hare rabbit, mated 3 hours earlier with a male Belgian Hare rabbit. After a regular pregnancy, 6 rabbits were born on May 29, 1890, 4 of with were similar to their Belgian Hare mother and the other 2 were Angora rabbits. The latter had fur characteristic of Angora rabbits and were albinos, as the donor from which the embryos were obtained. The experiment of W. Heape was intended to determine the influence of the female donee on progeny born after embryo transplantation and whether the presence and development of a foreign embryo in the uterus of the mother influences her genetic progeny born at the same time. Heape did not observe any negative changes. Moreover, he noted that the progeny of the Angora rabbit showed behaviour typical of that race, manifesting in the habit slowly turning their heads when they observed an object (1, 3).

This experiment was repeated only in 1911 and 1913 by Biedl, but he did not report his results until 1921 (4). Further experiments, which confirmed previous observations, were performed 10 years later by Pinkus and Nicholas, who again transferred embryos between rabbits (1). These experiments paved the way to future experiments on large bred animals. In 1932, Warwick and Berry performed the first successful embryo transplantation in goats. A year later, the same researchers attempted an inter-species embryo transfer between goats and sheep, which proved unsuccessful.

An important direction of research was the induction of superovulation in females to increase the probability of high progeny numbers. In 1927, multiple independent studies showed a relationship between hormone excretion along the hypophysis-ovaries line. This observation was made by Smith and Engle and by Aschenhaim and Zondeck. The latter team subsequently separated estrogenic and gonadotropic hormones from the serum and urine of pregnant women (1928). In veterinary sciences, however, these hormones were used only to cure infertility in cattle or to provoke the ovarian cycle in sheep outside of the breeding period (3).

It was Soviet researchers who first used gonadotropic hormones to trigger superovulation in the 1930s and 1940s. Lopyrin et al. induced multiple pregnancy in sheep by administering PMSG. These studies were repeated in the United States, as well as in England, where in 1948 Chang obtained 46 rabbits from 53 embryos that had been transplanted to 4 donees from a female subjected to superovulation (5).

The first symposium dedicated to embryo transplantations took place in San Antonio, United States

in 1949. It concluded that "embryo transplantation makes the female an equivalent of a male participating in artificial insemination" (10). The first calf was born as a result of embryo transplantation already in 1951. The embryo was collected from a cow 20 minutes after slaughter and transplanted surgically into a donee cow. The pregnancy proceeded without complications, and the calf was born healthy (22).

After the initial success, further studies were performed only occasionally, and the idea of animal embryo transplantation did not resurface until the 1970s. The renewed interest in embryo transplantation was partly due to studies on embryo preservation in liquid nitrogen. The first calf obtained after transplantation of a frozen embryo was born in Cambridge in 1973. Embryo transplantation proved to be much cheaper than the purchase and transport of adult animals, so work in this field was continued despite the low rate of success of 30-40%. This was influenced especially by improvement of bloodless transplantation techniques, which are routinely used nowadays (5).

In vitro embryo cultures became another direction of research in breeding biotechnology. Schenk observed embryos outside the body of the mother as early as 1880. Interest in embryo development increased in 1907, when Ross and Harrison studied tissue cultures (7). Mark and Long made first attempts at *in vitro* fertilisation of mouse and rat eggs in 1912 at the University of California. They constructed a chamber that enabled liquid exchange and observation of eggs and sperm cells. At the same time, Brachet experimented on rabbit blastocyst cultures at Brussels University. The first film recording of embryo development to the stage of 8 blastomers was made in 1929. Its authors, Castle and Gregory, found that the number of blastomers after 48 hours was different in small and large rabbit races, with 16 blastomers in the former and 32 blastomers in the latter (7).

The next step were studies of the behaviour of fertilised an unfertilised eggs, both *in vivo* and *in vitro*. Their behaviour was described by Pincus, who also studied the influence of progesterone in blood plasma and serum on the development of embryos (16). Techniques used at the time were very unreliable. Progress was extremely slow despite the large number of experiments performed. Until 1949, rabbit eggs could only be stored *in vitro*. That year saw a breakthrough in the form of a study by John Hammond Jr., who used a broth based on physiological saline solution with addition of egg yolk and white to cultivate rodent embryos. He also observed that *in vitro* development of an embryo depends on the development stage at which it was collected from the mother (5). This phenomenon was defined as a "block" and confirmed by a study by Whitten in 1956. Whitten also modified the method of embryo cultivation by using a 5% CO_2 atmosphere (to improve pH control) and Krebs-Ringer solution enriched with glucose, antibiotics and egg white, which produced good results in 8-cell mouse embryo cultures leading to the blastocyst stage (20). The aforementioned development block at the 2-cell stage was explained in 1967 by Whittingham and Biggers, who showed that it may be overcome by placing the embryo in a fallopian tube ampulla, which clearly suggested that this part of the fallopian tube contains substances responsible for the development of a 2-cell embryo to the 4-cell stage (21).

Studies that made significant contribution to the development of breeding biotechnology included those on the metabolism and regulation of embryo development *in vitro*. The discovery of the role of energy substrates and amino acids in this process was particularly important. It turned out that removal of glucose and phosphates from the broths made it possible to overcome the development block because these two substances, even in minimum quantities, were toxic to embryos at the 2-cell stage. The 2-cell block could be overcome by replacing glucose with glutamine (18). This procedure proved to be more complicated, however, as glucose was required for embryo development at later stages, up to the blastocyst stage (8). As a result, during the second half of the 20th century, research on embryo culture *in vitro* was focused on broth composition. It was observed that the presence of other (auxiliary) cell types, such as ovary fibroblasts, trophoblast cells, and fallopian tube epithelial cells, is beneficial for embryo development. According to researchers, these morphotic elements exert their influence by producing mitogenic substances for the embryo or substances that may support cell differentiation. Foreign cells also influence embryo development through metabolism or deposition of embryotoxic substances present in the broth. These mechanisms were described by Kane et al. in their studies from 1992 (5).

Polish research centres that studied embryo transfer and *in vitro* procedures during the early period of their use in Poland included the Zootechnics Institute in Balice, where the Breeding Physiology Laboratory was created in 1952, operating from 1962 as the Department of Breeding Physiology and Artificial Insemination of Animals, and renamed the Department of Animal Physiology in 1991. These centres were headed by the best Polish veterinary scientists, and pioneers of artificial breeding of animals in Poland, such as Prof. dr hab. Władysław Bielański, Prof. dr hab. Stefan Wierzbowski or Prof. dr hab. Zdzisław Smorąg. Another such research centre was the National Veterinary Institute and its Bydgoszcz office, with the following units: Department of Infertility Treatment and Insemination created in 1950 and renamed Department of Breeding Physiopathology and Insemination in 1966, which was headed by Prof. dr Lech Jaśkowski, and the Animal Breeding Biotechnology Laboratory created in 1993, which was headed by Prof. dr hab. Jędrzej M. Jaśkowski and studied matters strictly related to artificial breeding technologies (24, 25).

In conclusion of this review, it should be noted that the technique used to obtain biological material has not posed significant problems and has been used practically unchanged (in the case of insemination) since the end of the 18th century. Problems occurred during transfer, preservation and culture. The studies described here improved various stages of artificial breeding, and research undertaken in the 20th century led to a much better understanding of semen and embryo preservation mechanisms in an artificial environment. This paved the way to interesting research in experimental biology. On the other hand, scientific research influenced the size of large herd animal breeding and obtaining semen, whereas embryo culture and cryopreservation made it much easier to transfer genetic material over large distances. Studies performed in the 20th century provided basis for a dynamic development of animal breeding biotechnology and significantly influenced reproductive technology in human medicine.

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