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# The history of artificial insemination: Selected notes and notables<sup>1</sup>

R. H. Foote<sup>2</sup>

Department of Animal Science, Cornell University, Ithaca, NY 14853-4801

**ABSTRACT:** Artificial insemination (AI) was the first great biotechnology applied to improve reproduction and genetics of farm animals. It has had an enormous impact worldwide in many species, particularly in dairy cattle. The acceptance of AI technology worldwide provided the impetus for developing other technologies, such as cryopreservation and sexing of sperm, estrous cycle regulation, and embryo harvesting, freezing, culture and transfer, and cloning. New, highly effective methods of sire evaluation were developed. The history of development of AI is reviewed, particularly in dairy cattle, in which the impact on genetic improvement and control of venereal diseases have been greatest. Other

species briefly included are swine, horses, sheep, goats, dogs, rabbits, poultry, and endangered species. Major landmarks in AI development are cited, along with the people most closely associated with these developments. Many of these pioneers helped to develop a new generation of reproductive physiologists and biotechnologists. A bit of the flavor of the times is included, along with the historical facts. Many of the references will take the reader back to an era before electronic networks were available, so these citations of classical studies will not be found with the press of a key on the electronic keyboard. Readers are invited to explore these historical treats that have provided a springboard for the future.

Key Words: Artificial Insemination, Estrus, Livestock, Selection, Semen, Sex Determination

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## Introduction

Artificial insemination (AI), as practiced by bees and many other flying insects, has played an important role in plant reproduction for a very long time. Use of AI in animals is a human invention and more recent. Undocumented tales exist of Arabs obtaining sperm from mated mares belonging to rival groups and using the sperm to inseminate their own mares.

However, our story starts with recorded history, where facts are available to document noteworthy achievements. Consequently, the story is related chronologically. Much of the development of AI occurred before the 1980s when electronic networks became available, so earlier references are included. The developments that made AI the most important animal biotechnology applied to date include improved methods of male management and semen collection, evaluation, preservation, and insemination. Detection of estrus and

control of the estrous cycle in the female also were important. The development of AI is a remarkable story of tireless workers dedicated to the pursuit of knowledge, to the replacement of fiction with facts, and the application thereof.

Dairy cattle will be emphasized because AI has had the greatest genetic impact in that species. Other species overviewed include swine, horses, sheep, goats, dogs, rabbits, poultry, and endangered species.

This review can only provide a taste of the important discoveries and developments associated with AI and the people most involved. A more comprehensive overview of the technical aspects of AI are available in many of the books on AI and reproduction (Walton, 1933; Anderson, 1945; Cole and Cupps, 1959; Maule, 1962; Mann, 1964; Milovanov, 1964; Perry, 1968; Salisbury et al., 1978; Watson, 1978; Brackett et al., 1981; Foote, 1981; Herman, 1981; Cupps, 1991). Also, several reviews are available (Nishikawa, 1962, 1964, 1972; Asdell, 1969; Bonadonna, 1975; Bonadonna and Succi, 1976; Foote, 1999).

## Early History of AI

Leeuwenhoek (1678) and his assistant, Hamm, were the first persons to see sperm, which they called "animalcules." Leeuwenhoek did not have an advanced formal education, so he did not study Latin, the scientific

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<sup>2</sup>Correspondence: 204 Morrison Hall (phone: 607-255-2866; fax: 607-255-9829; E-mail: [dgb1@Cornell.edu](mailto:dgb1@Cornell.edu)).

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language of the day. However, he was a clever, capable individual who ground lenses so precisely (one still exists today with 270 magnifications) that sperm were visible. His published paper (Leeuwenhoek, 1678) amazed, and perhaps amused, the reigning king of England, who regularly read papers submitted to the Royal Society, where Leeuwenhoek's paper was published.

Another century passed before the first successful insemination was performed by Spallanzani (1784) in a dog, which whelped three pups 62 d later. Spallanzani originally trained to be a priest, but he had a great interest in natural history and pursued the latter. He was a professor of natural history in Pavia by the age of 25. He collected, analyzed, and classified a large array of butterflies, shells, and other marine and land animals. His abode was overrun with many collections, somewhat to the consternation of relatives living there. But he used these for rigorous, comparative objective analysis to discern much about animal physiology and characteristics of fitness.

Another 100 yr passed before Heape (1897) and others in several countries reported that AI had been used in isolated studies with rabbits, dogs, and horses. Heape was an outstanding reproductive biologist, establishing much of the basis for the relationship between seasonality and reproduction. This led to Cambridge becoming a world center for reproductive studies (Marshall, Hammond, Walton, and students such as M. C. Chang).

### AI Becomes a Focal Point of Research

Pioneering efforts to establish AI as a practical procedure were begun in Russia in 1899 by Ivanow (see Ivanoff, 1922). By 1907 Ivanow (also transliterated as Ivanov or Ivanoff) had studied AI in domestic farm animals, dogs, foxes, rabbits, and poultry. Some of this research, especially in horses, is included in a paper in English (Ivanoff, 1922) submitted June 21, 1922, and published in record time in the July 1922 issue of the *Journal of Agricultural Science*. He developed semen extenders and trained technicians to select superior stallions and multiply their progeny through AI. Much of the AI work in Russia was taken over later by Milovanov (1938), described in a text translated into English (Milovanov, 1964). He established major projects for sheep and cattle breeding. He did not E-mail his orders for supplies. In his own workshop Milovanov designed and made practical artificial vaginas and other items, many similar to those used today. This was an enormous improvement over the earlier method of collecting semen from sponges placed in the vagina of mount animals.

The development of AI by Ivanow also stimulated research outside of Russia. The Japanese scientist Dr. Ishikawa studied with Ivanow. He returned to Japan and began a similar program in horses in 1912 (Nishikawa, 1962). This gradually developed into AI being applied in Japan in cattle, sheep, goats, swine, and

poultry. Other Japanese researchers became involved (Ito, Niwa, Sato, Yamane, etc.). Because most of the research was published in Japanese and few westerners knew Japanese, little was known about this research in the Western world until Niwa (1958) and Nishikawa (1962, 1964, 1972) summarized the research in English.

News of the extensive use of AI in Russia following the Ivanoff (1922) report became widespread in the Western world with the publication of the book on AI by Walton (1933). Walton conducted a number of experiments, including a pioneering shipment of ram semen to Poland, which 2 d later was used for successful insemination of ewes. However, commercial AI did not evolve rapidly in the United Kingdom.

Some AI work, particularly with horses, had been performed in the early 1900s in Denmark. Eduard Sørensen, at The Royal Veterinary College in Copenhagen, Denmark, was familiar with the Russian work. With Gylling-Holm, Sørensen organized the first cooperative dairy AI organization in Denmark in 1936. The program enrolled 1,070 cows the 1st yr and 59% conceived, slightly better than natural service in the same herds. This was an important stimulus for the development of AI in dairy cattle in the United States and other Western countries.

The Danish veterinarians established the method of rectovaginal fixation of the cervix, allowing semen to be deposited deeply into the cervix or into the body of the uterus. This technique provided a tremendous advantage because fewer sperm were required for insemination of each cow. Another Danish "invention" was the straw for packaging semen (Sørensen, 1940). In 1956 I saw some of the original oat straws that Dr. Sørensen kept in his desk. Subsequently he saw children at a birthday party for his daughter sipping punch with cellophane straws, and he recognized that he had found the straw that he needed. Later Cassou (1964) produced straws commercially that have been used worldwide. So, the French straw is a modified Danish straw. Dr. Sørensen also was a tough examiner in anatomy. Passing his exams was reported to be as tough as "getting a camel through the eye of a needle."

Meanwhile, the much earlier research by Spallanzani led eventually in Italy to the development of an artificial vagina for dogs by Amantea in 1914 (Perry, 1968). This work served as a model for the Russian development of artificial vaginas for bulls, stallions, and rams. Another Italian, Bonadonna (1937), continued research on AI in several species. His enthusiasm for the potential value of AI, along with Lagerlöf, resulted in the establishment of the highly successful International Congress on AI and Animal Reproduction held every 4 yr. The first one was held in Milan in 1948. One time I remarked about the extraordinary beauty of the Renaissance works of art in Italy, and Dr. Bonadonna said "Yes, but remember the future requires that you do not spend too much time dreaming about the past."

In Sweden, Lagerlöf became known for his research on infertility problems in bulls. This research was stim-

ulated by his visit with W. W. Williams, a Cornell D.V.M., who had published methods of staining spermatozoa. Meanwhile, Lagerlöf completed his classic Ph.D. dissertation titled "Changes in the spermatozoa and in the testes of bulls with impaired or abolished fertility" (Lagerlöf, 1934). He established a group with worldwide influence in training veterinarians in the various aspects of fertility and AI. Other Scandinavians, such as Blom (1950), followed, publishing a steady stream of excellent papers on abnormal sperm morphology (see Barth and Oko, 1989). These pioneers were all thinkers and doers, and they trained many who followed.

### Modern Development of AI in Dairy Cattle

Phenomenal growth of AI occurred in the 1940s in the United States. The procedures developed in the United States became established worldwide (Salisbury et al., 1978). In 1936, Brownell was inseminating cows in the Cornell herd (Sipher, 1991), and other AI work was started in the late 1930s in Minnesota and Wisconsin. In 1938, an AI cooperative was established in New Jersey, modeled after the Danish system (Perry, 1968). Another one in 1938 followed in the state of New York (Sipher, 1991). The development of the New York Artificial Breeders, Cooperative, Inc., currently Genex, Inc., in Ithaca, New York made possible the close collaboration between a farmer cooperative and researchers and extension personnel at Cornell University. This was a highly productive relationship resulting in the experimental insemination of hundreds of thousands of cows and publication of more than 100 research papers (Foote, 1998) on sire selection, testicular evaluation, semen collection, evaluation and processing; and fertility testing. The present status of AI in many countries has been summarized recently (Malafosse and Thibier, 1990; Foote, 1999).

#### *Semen Evaluation*

The most widely used test of sperm quality from the initial stages of AI development until the present time has been the assessment of the proportion of normal, progressively moving sperm (Anderson, 1945; Maule, 1962; Salisbury et al., 1978). Thus, a good microscope is the key. In addition to examining sperm with brightfield microscopes, differential interference contrast microscopes, multiple stains, flow cytometry, and computer-assisted sperm analysis (CASA) have contributed to improved quantification of sperm motion (Graham, 1978; Salisbury et al., 1978; Pace et al., 1981; Garner et al., 1997; Foote, 1998). With frozen semen, evaluation of post-thaw survival became important. The ability to evaluate the acrosomal status of sperm was enhanced by the work of Saacke and Marshall (1968). Techniques used for evaluating sperm morphology have been reviewed by Barth and Oko (1989).

Ejaculate volume and sperm concentration are the two other critical components of semen evaluation be-

cause they determine the number of sperm obtained. Volume originally was measured in graduated containers. Volume today often is determined more accurately by weight, assuming that the specific gravity is 1.0. Stallion semen was weighed years ago (Nishikawa, 1959). Rapid optical density methods for measuring sperm concentration have replaced tedious hemocytometric procedures.

Fertility of sperm is the ultimate test of sperm quality. Often it is not possible to measure fertility, so many tests of semen quality in addition to motility and morphology, such as the hypoosmotic swelling test, mucous or gel penetration, and integrity of the DNA have been correlated with fertility (Graham, 1978; Saacke, 1981; Foote, 1998, 1999). Competitive fertilization (Beatty, 1960; Saacke, 1981; Dziuk, 1996; Foote, 1998) with mixed sperm offers an efficient way to rank the fertility of males either using in vitro fertilization tests or tests with animal insemination. However, it is not generally feasible to mix semen in commercial AI. For commercial AI, an inexpensive method of estimating fertility, based on cows not returning for insemination, was developed as an essential component of the AI program (Thompson and Salisbury, 1947). This made possible the comparing of fertility of bulls, inseminators, semen processing procedures, and even herd performance under practical field conditions. It provided a remarkable new system of recording breeding efficiency. Others had argued strongly for using pregnancy diagnosis, but this clearly involved few cows, was performed sporadically, and did not provide for centralized collection and evaluation of data. The efficiency of the nonreturn method for monitoring fertility is reduced today because of multiple suppliers of semen to individual farms and within-herd inseminators.

#### *Semen Extenders, Semen Cooling, and Extension Rate*

Initially the most important problem to resolve was a method to store semen long enough for shipment and use in the field. The first major improvement in the AI procedure initiated in the United States was the development of a yolk-phosphate semen extender (Phillips and Lardy, 1940). Salisbury et al. (1941) improved the media by buffering the egg yolk with sodium citrate. Sperm survival at 5°C permitted use of the semen for up to 3 d, and the citrate dispersed the fat globules in egg yolk, making sperm visible for microscopic examination. This semen extender was used worldwide for cattle. Glycerol was added later for cryopreservation of bull sperm.

The next major stimulus to AI of dairy cattle was an improvement of about 15% in fertility resulting from a better method of initially protecting sperm from cold shock (Foote and Bratton, 1949) and the control of some venereal diseases by the addition of antibiotics (Almquist et al., 1949; Foote and Bratton, 1950). The Cornell extender (Foote and Bratton, 1950), containing the antibiotic mixture of penicillin, streptomycin, and poly-

myxim B, was used for many years as the standard. Many years were required to eradicate the diseases from bulls. During that time in vitro treatment of semen with antibiotics prevented transmission of several diseases. Antibiotics are still included as “insurance” protection against possible contamination. This treatment of semen was worth hundreds of millions of dollars to the dairy world. No patents were filed, and neither Pennsylvania nor Cornell received any remuneration. The reward was service to agriculture. Growth of AI was now ensured, because dairies using only AI eliminated venereal diseases, reduced embryonic death, and achieved high fertility.

With AI expanding rapidly, demands for semen from popular bulls increased. The simplest way to meet this demand was to “stretch” each ejaculate farther by using fewer sperm per insemination, providing that this could be accomplished without sacrificing fertility. Salisbury and coworkers published several classic papers (see Salisbury et al., 1978; Foote, 1998) clearly supporting the concept that only a few million sperm per insemination were required. In conducting these experiments Salisbury was criticized by some who declared that “dilution” of semen was like “watering the milk.” Consequently, Foote and Bratton (1950) introduced the word “extender” because the yolk-citrate-antibiotic medium enhanced and extended the usefulness of semen. This word has “stuck.” We considered using the word “suspender” as a snappy term, also. The net result of these experiments was that semen extension could be increased at least 25-fold. Sperm numbers per insemination with liquid semen were reduced from more than  $100 \times 10^6$  sperm per insemination to  $4 \times 10^6$  sperm per insemination (see Salisbury et al., 1978; Foote, 1998). Along with hard work in the laboratory, Salisbury’s graduate students remember relaxing over gourmet food in his home.

The yolk-based extender was improved with Cornell University Extender (CUE, Foote et al., 1960), which resulted in the highest fertility achieved in AI on hundreds of thousands of inseminations (Salisbury et al., 1978; Foote, 1998). Shannon visited Cornell in the late 1950s and modified the extender (Shannon et al., 1984) for use with liquid semen in the intensive breeding season in New Zealand. Caproic acid and catalase were included with 5% egg yolk by volume to form “Caprogen,” an effective extender for preserving bull sperm at the moderate ambient temperatures of New Zealand, with as few as  $2 \times 10^6$  sperm per insemination. Several researchers (see Salisbury et al., 1978; Foote, 1998) had reported that the volume of egg yolk used originally could be greatly reduced, particularly at ambient temperatures, and that catalase might be beneficial at room temperature.

Milk also was widely used. Following the report by Michajilov (1950), Almquist and coworkers (Thacker and Almquist, 1953; O’Dell and Almquist, 1957; Almquist and Wickersham, 1962) published a series of papers on skim milk and whole milk, establishing the

optimal procedures for detoxification of milk and addition of glycerol for freezing semen. The milk-glycerol extender continues to be used by many AI organizations.

#### *Bull Sexual Behavior, Intensity of Semen Collection, and Sire Power*

To meet the high demand for semen from selected bulls, harvesting the maximal number of sperm per bull was the other approach to increasing the number of inseminations possible per bull. Early research on sexual preparation and intensity of semen collection was initiated by Bratton and colleagues (Collins et al., 1951; Bratton and Foote, 1954; Hafs et al., 1959) and Almquist and colleagues (Hale and Almquist, 1960; Amann, 1970; Almquist, 1973, 1982). The extensive studies by Almquist and associates included beef bulls as well as dairy bulls (Almquist, 1973). These collective studies resulted in recognizing the importance of evaluating the sexual responses of individual bulls and applying stimuli along with an optimal frequency of about six semen collections per week. The result was a sperm output of 30 to  $40 \times 10^9$  sperm per week per sire. With a 50-wk-per-year collection schedule and  $10 \times 10^6$  sperm per insemination dose, these sperm numbers translate into 200,000 doses of semen for insemination each year.

Large differences occurred in sperm output of bulls when semen was collected under comparable conditions. Methods were developed to evaluate both the quality and quantity of spermatogenesis in bulls (Foote, 1969; Amann, 1970). These studies revealed that the differences in sperm output were largely due to testicular size. Testis size was easily measured and was highly inherited,  $h^2 = 0.67$  (Coulter et al., 1976). So, attention to testis size was important in selection and evaluation of sires.

Extensive studies were conducted on nutrition, performance, and aging of bulls (see Foote, 1998). Rapid growth was important in minimizing the time required for young bulls to reach puberty and be tested for AI. Unfortunately, these data are largely unknown because they were published in bulletin form (Bratton et al., 1959, 1961), although one classic paper appeared in a journal (Almquist, 1982). Almquist always kept an open mind about what might be possible. At one scientific meeting he handed out note pads less than 1 inch wide and several inches long to remind people not to be “narrow-minded.” Bratton was a perfectionist and did not publish results until the experiments were completed, even if they took 10 yr. Tenure then was translated as 10 yr.

#### *Genetic Selection of Bulls for Milk*

One of the major reasons for initiating AI was to make the males that transmit superior genetics for milk production available to more producers in the animal

industry. This was democracy in action. The elite bulls would not be limited to the wealthy. However, bull selection procedures practiced before the AI era gave disappointing results. Many bulls with high proofs in natural service did not repeat in AI. Robertson and Rendel (1950) in Scotland and Henderson (1954) at Cornell University pioneered new methods of sire selection (Van Vleck, 1981). Henderson continued his research to establish the principles required for optimal sire selection programs and to provide objective methods for adjusting records for unequal environmental influences.

Henderson received his training under Dr. Lush at Iowa. He combined this with his mathematical genius and attracted graduate students and scholars from all over the world. Along with these scholars, Henderson has had the greatest impact on dairy cattle genetics of any single person in history. He was a modest individual, always ready to take time with a yellow pad of paper to scratch out a solution to anyone's mathematical problem. He didn't need modern computers because he was born with one.

### *Frozen Semen*

While the geneticists were making breakthroughs on sire selection, an astounding achievement was reported from England (Polge et al., 1949), the successful freezing of chicken sperm by including glycerol. Glycerol soon was found to be useful for bull sperm. Many researchers had tried to freeze bull semen (I had even tried ethylene glycerol unsuccessfully in the late 1940s to store bull sperm at  $-10^{\circ}\text{C}$ ) but had failed. A bit of serendipity played a role in the discovery (Polge, 1968). The research had focused on using sugars as cryoprotectants, but they did not lead to successful results. However, Polge relates that he returned 6 mo later to try again, and results were promising, presumably with the same bottle of fructose stock solution. Why success now? What was in the bottle? Chemical analysis showed that the bottle contained no sugar, but rather glycerol and protein in proportions comprising Meyers albumin used for histology. Apparently, there had been a mistake in labeling when reagents were stored. Horace Wolpole coined the word *serendipity*, noting that it combined accidental discovery with sagacity. Both aspects were displayed here. Unfortunately, the dictionaries do not include sagacity in defining serendipity.

The basic medium used by Polge was the original yolk-citrate extender (Salisbury et al., 1941) plus glycerol. Almquist and coworkers (O'Dell and Almquist, 1957) developed whole milk-glycerol as a good medium to cryopreserve bull sperm. Tris-buffered egg yolk-glycerol also provided excellent protection for sperm either frozen or unfrozen (Davis et al., 1963; Foote, 1998). This soon became the most commonly used medium worldwide for cryopreservation of bull sperm and sperm from several other species (Iritani, 1980).

Packaging frozen semen for use with solid carbon dioxide (Dry Ice<sup>®</sup>) or liquid nitrogen was a problem. Glass ampules often broke during freezing or thawing. Cassou (1964) modified the system developed by Sørensen (1940), with a method for sealing plastic straws and a gun for insemination (Pickett and Berndtson, 1974). Originally 0.5-mL capacity straws were used, but 0.25-mL straws are popular because they require less storage space.

Another major change in storage occurred in the 1950s with the shift from solid carbon dioxide storage at  $-79^{\circ}\text{C}$  to liquid nitrogen at  $-196^{\circ}\text{C}$ . Researchers had demonstrated that sperm survival at  $-196^{\circ}\text{C}$  was virtually infinite, whereas biologic changes occurred with storage at  $-79^{\circ}\text{C}$ . Also, storage with solid carbon dioxide was not convenient, and frequent resupply was necessary.

Liquid nitrogen storage also was a problem, because insulation of the tanks was inefficient. Frequent refilling was required to maintain a safe temperature of about  $-196^{\circ}\text{C}$ . Manufacturers of tanks were not interested in improving tanks until J. Rockefeller Prentice, owner of American Breeders Service, privately provided a substantial sum of money, which convinced Linde Division of the American Cyanamid Company that there was a market for liquid nitrogen containers with improved insulation. The successful cryopreservation of sperm and development of efficient liquid nitrogen containers provided the foundation upon which today's entire cryopreservation industry is built.

Frozen semen is less fertile than fresh semen (Shannon and Vishwana (1995), as many semen additives to improve fertility of frozen semen have been tested with minimal success (Foote, 1999). However, a recent report (Amann et al., 1999) provided preliminary support for a peptide that increased fertility when added to frozen-thawed semen.

### *Detection of Estrus, Synchronization, and Timing Insemination*

Obviously, success in the field depended on the proper detection of estrus and skillful insemination. The classic rule referred to as the A.M. to P.M. and P.M. to A.M. system for insemination was established by Trimberger (1948). It was based on observation, palpation of the ovaries, and breeding data. This rule established that for best fertility cows first seen in estrus in the A.M. should be inseminated during the afternoon of the same day. Cows first seen in estrus in the P.M. should be inseminated before noon the next day. Trimberger was a keen observer and was one of the most successful coaches of intercollegiate dairy cattle judging teams.

Accurate detection of estrus is a problem on many farms (Foote, 1975). Intensive research to regulate the time of estrus and ovulation in the cow has been ongoing for about 50 yr (Ulberg et al., 1951; Hansel and Trimberger, 1952). Fertility was very low in early studies on estrus synchronization (Hansel and Convey, 1983).

Fertility still is lower in many cases, but cows can be inseminated at a fixed time without detection of estrus (Nebel et al., 2000). Rowson (1971) predicted that AI, combined with superovulation, synchronization of estrus, and manipulation of embryos would lead to major advances in animal production beyond the use of AI alone. Rowson was a prodigious worker with a great sense of humor. Many researchers came to Cambridge to gain from his experience.

Women played key roles. Few were inseminators, but taking the farmers' calls and washing and sterilizing the glassware (before the era of plastic) daily provided important teamwork.

### *Sexing Sperm*

One of the most dramatic technical advances in recent years is the sexing of sperm by DNA quantification using flow cytometry instrumentation developed at Livermore Laboratories (Gledhill, 1985) and improved since (Johnson and Seidel, 1999). The time currently required to sort the billions of sperm per ejaculate limits extensive commercial application, but the machines are getting faster.

### *Freeze-Dried Sperm*

Successful freeze-drying of bull sperm was erroneously reported by Meryman in 1960 (see Graham et al., 1974 for references). Attempts by many researchers to repeat the research produced negative results. However, Graham et al. (1974) produced one pregnancy. Had microinjection techniques been available then, Graham would have been at work at his usual 5 a.m. start of the day looking for another way to use AI. With the microinjection technique, preservation of the integrity of the DNA of freeze-dried sperm was demonstrated recently (Wakayama and Yanagimachi, 1998).

## Development and Use of AI in Species Other Than Dairy Cattle

### *Beef Cattle*

Beef cattle greatly outnumber dairy cattle in the United States. The technology of semen handling and insemination of beef cows is similar to that used for dairy cows, but beef cows are not managed as conveniently for AI. Many cows are on extensive ranges where detection of estrus and rounding up animals in estrus for insemination is not cost-effective. Therefore, the proportion of beef cattle bred by AI is low (Foote, 1981; Dziuk and Bellows, 1983). Where small groups of beef cows are kept in close confinement, estrous cycle-regulating agents can be used to synchronize the time of insemination for at least one insemination. In crossbreeding programs, AI has the advantage because semen can inexpensively replace maintaining bulls of separate breeds.

### *Swine*

The AI of swine was initiated by Ivanow in Russia in the early 1900s (Ivanow, 1907; Ivanoff, 1922). More extensive investigations were conducted in the 1930s (Milovanov, 1938; Anderson, 1945; Maule, 1962; Nishikawa, 1964). Early work was started in the United States in Missouri (McKenzie, 1931), in Japan in 1948 (Ito et al., 1948; Niwa, 1958), and in western Europe in the 1950s (Polge, 1956). Boars are easily trained to mount dummies (Milovanov, 1938; Polge, 1956).

All artificial vaginas developed for boar semen collection provided a means of applying pressure to the glans penis (McKenzie, 1931; Ito et al., 1948; Polge, 1956), or a gloved hand can be used directly. McKenzie trained many early outstanding reproductive physiologists (Andrews, Casida, Phillips, etc.). He attended animal science meetings long after officially retiring, asking questions about the latest developments. At the 75th meeting of the American Society of Animal Science, when a roll of long-time members was called, he was the only member standing at the end, a member for 61 yr.

Russian diluters for boar semen were based on glucose solutions with sodium potassium tartrate or sodium sulfate and peptone, keeping the concentration of electrolytes low (Milovanov, 1938; Anderson, 1945; Polge, 1956; Maule, 1962). The recommended storage temperature was 7 to 12°C. However, Ito et al. (1948) recommended storage at 15 to 20°C. When the yolk-phosphate, yolk-citrate, and milk extenders were developed for bull semen, they were used or modified for boar semen (Polge, 1956; Niwa, 1958; Maule, 1962), including use with cooled semen.

Major efforts were made to freeze semen, following the successful use of frozen bull sperm (Nishikawa, 1964; Graham, 1978; Iritani, 1980; Johnson and Larsen, 1985; Johnson and Rath, 1991; Rath et al., 1996; Johnson, 1998; Foote, 1999; Johnson and Guthrie, 2000). Many modifications of extenders and freezing procedures were developed (see Johnson, 1998), including the pellet method of freezing, which was developed originally in Japan (Nagase and Graham, 1964). Pregnancy rates and litter sizes are reduced with cryopreserved boar sperm, so frozen semen is limited to use in special breeding programs. Fresh or extended liquid semen is used for about 99% of AI in swine.

Rapid transport of extended semen makes it feasible for swine farmers to take advantage of commercially produced boar semen. Large corporations increase the services possible from their own boars by using AI. Insemination is done by the swine farm personnel. A typical dose is  $3 \times 10^9$  sperm inseminated in 80 mL. Over 50% of the sows in the United States are inseminated today, and about 80% farrow with 10 pigs per litter. Swine AI is growing at a phenomenal rate.

Techniques for evaluating quality of boar sperm are similar to those used for bull sperm (Johnson, 1998). Sexing of boar sperm is possible but is too slow to produce sexed sperm for commercial use. Detection of es-

trus is facilitated in sows because most come into estrus within a week after weaning their litters. Further technical details can be found in the references cited previously.

### *Horses*

Research on AI of horses started in Russia in 1899 (Ivanow, 1907; Ivanoff, 1922), stimulated particularly by the military's need for horses. Ishikawa initiated similar studies in Japan in 1912 (Sato, 1916; Nishikawa, 1962). The book by Nishikawa provides a fascinating account of the extensive, detailed studies by a great researcher and teacher and an extraordinary gentleman. After visiting in our home in the 1960s he wrote that he couldn't wait for me to visit him in Japan where he could "hospitalize" me. I was treated royally. McKenzie et al. (1939) and Berliner (1942) initiated studies on the collection, processing, and insemination of stallion and jack semen in the United States.

The earliest collections of semen were obtained by placing a rubber semen collection bag in the vagina of a mare in estrus. In the 1930s and 1940s, several types of artificial vaginas were developed (Anderson, 1945; Maule, 1962; Perry, 1968; Davies Morel, 1999) and they since have been modified. A smaller, lighter version was developed by Nishikawa (see Davies Morel, 1999). Semen evaluation is performed similarly to the evaluation of bull semen.

Military studs provided a convenient source of stallions (Anderson, 1945; Maule, 1962), but after World War II many of these were eliminated because the horse population had declined. In addition, the restrictive regulations on the use of AI by several equine breed organizations inhibited research and the application of AI. China was the major country using equine AI during this period.

Advances made in cryopreserving bovine sperm stimulated interest in cryopreservation of equine semen. Research results are summarized in several congresses and symposia (Nishikawa, 1964, 1972; Bonadonna and Succi, 1976; Sharp and Bazer, 1995) and by Davies Morel (1999). Although methods have been devised to freeze stallion sperm, most equine AI is done with cooled, extended semen used within 48 h of collection during the spring breeding season. The breeding season may be advanced by fall lighting.

### *Sheep and Goats*

The early development of AI in sheep on a major scale began in Russia (Milovanov, 1938, 1964; Maule, 1962), where the collective farms provided an ideal arrangement for establishing AI programs. China also has extensive sheep AI programs. Artificial insemination spread to central Europe and also was widely applied commercially in France and Brazil (Anderson, 1945; Maule, 1962; Foote, 1999). The techniques for semen collection and artificial insemination in sheep and goats

have been described in detail (Evans and Maxwell, 1987). Semen quality and breeding efficiency are affected by season.

Both rams and bucks can be trained to serve the artificial vagina. However, for obtaining semen from a large number of rams in the field, electroejaculation is a useful procedure, pioneered by Gunn (1936) and applied to many species (Dziuk et al., 1954). Much of the early research in the Western world on extenders for sperm, freezing of semen, and AI techniques was done by Emmens and Blackshaw, followed by Salamon and Maxwell in Australia, and Dauzier, Colas, and Cortell in France (Corteel, 1981; Salamon and Maxwell, 1995a,b; Maxwell et al., 1999).

Buck sperm cryopreservation is more successful than the cryopreservation of ram sperm. The techniques and media for freezing semen such as with egg yolk-tris-glycerol were modified (Corteel, 1981; Salamon and Maxwell, 1995a; Maxwell and Watson, 1996; Amoah and Gelaye, 1997) from procedures developed for bull sperm (Davis et al., 1963).

Frozen-thawed semen results in satisfactory fertility in goats provided that the sperm are deposited deep into or through the cervix. In the ewe this is difficult. Therefore, insemination into the uterus with the aid of a laparoscope has been necessary to achieve high fertility. Recently, Maxwell et al. (1999) used intracervical insemination successfully by adding seminal plasma to cryopreserved ram sperm before it was used for insemination. Because of the difficulty of insemination, general management and low value per animal, AI, particularly of sheep, is not widespread.

### *Poultry*

Artificial insemination has been widely applied to poultry. Semen collection, processing, and AI have been reviewed by Sexton (1979) and Lake (1986) and more recently by Donoghue and Wishart (2000). Pioneers in the poultry field were Burrows and Quinn (1937), who developed the method of abdominal massage and pressure to collect semen. With the ease of collecting poultry semen, and proximity of hens on large breeding farms, AI is used extensively with freshly collected semen. It is used 100% for turkey breeding because mating is difficult. Freshly collected chicken semen was among the first type of semen to be frozen (Shaffner et al., 1941; Polge et al., 1949). However, cryopreserved poultry sperm are less fertile and freezing poultry sperm still is experimental (Gill et al., 1999).

### *Other Domestic Mammals*

Although the dog was the first animal in which AI was documented, AI has only been used in special cases, such as for breeding guide dogs or for overcoming special problems. Many breed organizations do not register puppies produced by AI. Cryopreservation of dog sperm is successful, using modifications of the yolk-tris and



yolk-lactose extenders developed for cryopreserving bull sperm (Foote, 1990).

Rabbits have been extensively used as a model for large animals and humans (Foote, 1998; Foote and Carney, 2000). All the reproductive techniques employed with farm animals can be performed with the low-cost rabbit model, and certain placental membrane characteristics make them especially relevant for studies of human teratology (Foote and Carney, 2000).

### *Endangered Mammals*

The first animal reproductive biotechnology used to preserve endangered species was AI (Wildt et al., 1995). Again, many of the principles and procedures used were adapted from cattle (Watson, 1978).

### Implications

In the initial stages of attempting to develop AI there were several obstacles. The general public was against research that had anything to do with sex. Associated with this was the fear that AI would lead to abnormalities. Finally, it was difficult to secure funds to support research because influential cattle breeders opposed AI, believing that this would destroy their bull market. The careful field-tested research that accompanied AI soon proved to the agricultural community that the technology applied appropriately could identify superior production bulls free from lethal genes, would control venereal diseases, and did result in healthy calves. Thus, fear was overcome with positive facts. The extension service played an important role in distributing these facts.

The knowledge gained from the AI experience was extremely helpful in stepwise development of each successive reproductive technology, such as frozen semen, superovulation, embryo transfer, and, eventually, cloning. Simultaneously, the public became better informed and more willing to accept that technology developed with worthy goals, and built-in ethical application, could produce positive change, benefiting the whole community. Worthy goals, development of the necessary knowledge and skills, and ethical considerations all are essential components of any technology that will result in a positive impact on society and the environment. Thus, the impact of AI was much more profound than simply another way to impregnate females.

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