Review Article

AN OVERVIEW OF THE JOURNEY OF DEVELOPMENT OF ARTIFICIAL INSEMINATION IN LIVESTOCK

ABSTRACT

Centuries of scientific inquiry and research have led to the widespread adoption of artificial insemination (AI) as a crucial tool in animal reproduction. This innovative biotechnology, first applied to farm animals, particularly dairy cattle, has transformed breeding practices worldwide. Through AI, male sperm undergoes collection, processing, and storage before being introduced into the female reproductive system to fertilize eggs. The evolution of AI has paved the way for the development of various reproductive technologies, including cloning, cryogenic preservation, sexing of semen, and management of the estrous cycle, among others. These advancements have revolutionized the field of animal genetics and breeding. In this article, we delve into the history of AI development, highlighting key milestones that have shaped its evolution and its profound impact on agriculture and animal husbandry.

Key Words: Artificial Insemination, Estrous, Livestock, Selection, Semen, Extender

Introduction

Before the advent of modern technologies, artificial insemination (AI) played a vital role in plant reproduction, as observed in bees and other flying insects. However, animal AI is a comparatively recent innovation of humans. Serving as one of the earliest significant biotechnologies, AI has been instrumental in enhancing farm animal reproduction and genetics. Remarkably, historical accounts from as early as 1322 A.D. recount instances of AI experimentation, such as an Arab chief stimulating a stallion with a cotton wand to facilitate mating with a prized mare. The semen was taken out and inserted into the mare with the use of a cotton wand, resulting in conception. However, in this article, we will discuss the concrete studies done by researchers to support remarkable accomplishments. Most AI research was done in the 1980s when electronic networks became readily accessible. The development of AI was based on research that led to an explosion of information in the area of animal reproduction. When artificial intelligence (AI) is applied to the management of dairy cattle, it can reduce harmful genetic traits, reduce the spread of infectious diseases, increase milk production, improve efficiency in work-related tasks, and effectively manage the aforementioned conditions. These applications have resulted in significant economic benefits. In 1678, Leeuwenhoek and Hamm made history by being the first to observe sperm,

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which they aptly termed "animalcules." Further, according to Leeuwenhoek, these spermatozoa are called "zaaddiertjes," which are microscopic organisms that are present in human semen and have slender, flowing tails. They are about a millionth the size of a little grain of sand [2]. Fast forward to 1784, Spallanzani achieved another milestone by conducting the inaugural successful insemination on a female buck. Using body-temperature semen, he impregnated one of the females exhibiting signs of heat, resulting in the birth of three pups 62 days later. This groundbreaking event marked the first documented instance of artificial insemination. Through meticulous experimentation, Spallanzani discovered that only the spermatozoa remaining after semen filtration could lead to fertilization. Spallanzani's pioneering work earned him significant recognition as a key figure in the development of artificial insemination. Spallanzani's investigations uncovered a crucial finding: the freezing or chilling of stallion sperm did not lead to their demise; rather, it temporarily immobilized them until they were warmed again [3]. In 1799, Hunter employed artificial insemination to address a gentleman's hypospadias condition in England. However, it wasn't until the mid-1800s that J Marion Sims conducted a series of 55 inseminations on female humans. Regrettably, only one pregnancy ensued from his postcoital assessments. This outcome may have been influenced by his belief that ovulation coincided with menstruation, potentially explaining the limited success. Additionally, in 1897, Heape documented isolated instances of artificial insemination employed in studies involving horses, dogs, and rabbits [1].

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The development of Artificial Insemination

In 1899, Ivanoff embarked on groundbreaking efforts in Russia to establish artificial insemination (AI) as a viable procedure. By 1907, his pioneering work extended to encompass research on AI across various species, including dogs, livestock, rabbits, foxes, and poultry. Notably, Ivanoff not only conducted extensive studies but also contributed significantly to the development of AI by devising semen extenders, training personnel in the selection of superior stallions, and effectively utilizing AI to enhance their offspring [4]. In 1938, Milovanov presented innovative plans for the breeding of sheep and cattle. His contributions included the invention of practical artificial vaginas, which remain in use today. This invention marked a substantial improvement over previous methods, such as extracting semen from sponges implanted into the ovaries of female animals.

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Dr. Ishikawa, a scientist from Japan, received mentorship from Ivanoff. After working with Ivanoff in 1912, Dr. Ishikawa brought artificial insemination (AI) methods for domestic animal husbandry to Japan. The Japanese government started AI programs aimed at cattle and poultry in the late 1930s [6]. However, the adoption of AI progressed slower than expected, initially focusing on horses. The Ivanoff study, which was published in 1922, helped other nations learn about AI methods from Russia. Walton's seminal book on AI, released in the West in 1933, further popularized the technique. Walton

achieved a milestone by successfully using ram semen to inseminate ewes, a technique later transported to Poland. Eduard Sörensen founded Denmark's first cooperative dairy AI association in 1933, motivated by the groundbreaking work in Russia. The association's efforts were fruitful, as 59% of the 1,070 cows produced calves. EJ Perry of New Jersey established the country's inaugural AI cooperation in 1938 [8]. A major turning point was reached on November 1, 1939, when the first artificially inseminated rabbit was displayed at the 12th Annual Graduate Fortnight of the New York Academy of Medicine. This feat was accomplished by Gregory Pincus, an American scientist, who fertilized an egg from a female rabbit's ovary with a salt solution and placed it in another female rabbit's uterus to serve as an incubator [1].

Through rectovaginal immobilisation of the cervix, Danish veterinarians invented a method for injecting semen deeply into the uterine body or cervix. This method offered the remarkable advantage of achieving fertilization with significantly fewer sperm, a notable breakthrough. Another significant advancement in Danish artificial insemination was the introduction of semen packaging using straws [7]. Subsequently, Cassou began commercial production of straws in 1964, and today they are widely utilized worldwide [9].

Table I. Important dates in the history of Artificial Insemination [1]

YEAR	DESCRIPTION OF EVENT
1677	Leeuwenhoek discovered spermatozoa.
1780	Spallanzani successfully inseminated a bitch
1799	Hunter used AI for a woman.
1803	Spallanzani observed that chilling sperm did not kill them.
1899	Ivanoff initiated organized AI research in Russia
1902	Sand recommended AI in Denmark, but no program was started.
1912	Ishikawa organized AI research in Japan
1914	Amantea devised the first artificial vagina for use in dogs.
1930s	Organized AI began in Denmark and the USA and quickly spread.
1937	Danish had established rectovaginal insemination, reducing sperm required
1940	Phillips developed phosphate-buffered egg yolk for preserving bull sperm
1941	Salisbury and others developed citrate-buffered egg yolk for preserving bull sperm.
1948	Almquist and Foote reported independently on the value of antibiotics in semen extenders to control microorganisms and increase fertility
1949	Polge *et al.* discovered that glycerol-protected sperm during freezing
1950s	Powerful tools for progeny testing were developed by Henderson and Robertson.
1954	Waterloo (Canada) was the first organization to use frozen semen 100%.

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1957	American Breeders Service developed liquid nitrogen tanks and services for frozen semen.
1963	Davis *et al.* (Cornell) developed Tris-buffered egg yolk-glycerol for fresh and frozen sperm, used later for many species
1970	AI was used commercially for superovulated cows and embryo transfer and provided the initial framework for many breeding strategies.
1990s	Sexing bull sperm was improved with limited potential application.

Introduction of Artificial Insemination in India

The inaugural artificial insemination (AI) procedure in India took place in 1939 at the Palace Dairy Farm in Mysore, conducted by Sampat Kumaran. Subsequently, in 1942, a pilot experiment was initiated at the Indian Veterinary Research Institute (IVRI) under the supervision of Dr. P. Bhattacharya to evaluate the feasibility of AI. Upon realizing the applicability of AI in Indian settings, it has since become a regular practice for cattle and buffalo breeding. In 1942, the Indian government established four regional hubs in Calcutta, Montgomery (now in Pakistan), Bangalore, and Patna to further promote AI adoption. The first AI-produced buffalo calf was successfully bred in 1943 at the Allahabad Agricultural Institute. To bolster the country's cattle and buffalo population, the Indian government established 150 significant village centers in 1951 and 1956 as part of the first five-year plan. Subsequently, the succeeding five-year plan (1956–1961) saw the incorporation of AI into 400 significant village centers, leading to a surge in AI activities.

Additional Advances in Artificial Insemination

The most popular test for sperm quality has been the determination of the proportion of normal, progressively moving sperm, as described by Anderson in 1945 [10], Maule in 1962 [11], and Salisbury in 1978 [12]. The adoption of brightfield microscopes, variable interference contrast microscopes, various stains, flow cytometry, and computer-aided sperm analysis (CASA) has increased our capacity to measure sperm's motility [12-16]. Our ability to discern the sperm's acrosomal status has enhanced as a result of Saacke and Marshall's investigation from 1968 [17]. In 1989, Barth and Oko examined the procedures for evaluating sperm morphology [18]. Ejaculate quantity and sperm concentration, which have an impact on the quantity of sperm generated, are the other two crucial elements in assessing semen. The capacity to procreate is the ultimate measure of sperm quality, although it's not always attainable. Due to this, several tests of semen quality, such as the hypoosmotic swelling test, mucous or gel penetration, DNA integrity, motility, and morphology, have been connected to fertility [13, 19, 18, 20]. Estimating sperm quantity as well as sperm motility and shape is a crucial component in determining fertility. Competitive fertilization

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with mixed sperm gives an accurate approach to evaluate a male's fertility using either in vitro fertilization tests or testing using animal insemination, according to studies by Beatty in 1960 [21], Saacke in 1981 [19], Dziuk in 1996 [22], and Foote in 1998 [16]. It is not viable to combine commercial AI and sperm, nevertheless. Cows that do not return for insemination were developed, by Thompson and Salisbury in 1947, as a key component of the AI program, and they are an appropriate technique for assessing fertility in commercial AI [23]. It provided an important brand-new way to measure breeding efficiency. Others have made strong arguments in favor of using pregnancy diagnosis. Still, it only involved a small number of cows, it happened seldom, and it didn't allow for centralized data collection and analysis. The efficacy of the non-return approach for evaluating fertility has diminished due to the availability of different semen suppliers to individual farmers and herd inseminators. The use of the light microscope and phase-contrast microscope with 20x and 40x objectives is one of the advancements in sperm motility measurement, and these are thought to produce substantially good results [24]. Recently, other techniques, including the CASA [25], flow cytometer [26], and NucleoCounter SP-100 [27], have been used to measure sperm concentration. With the aid of CASA, the concentration of sperm and its motility can be immediately assessed. Sperm concentration and membrane strength can both be determined with the NucleoCounter SP-100. The NucleoCounter SP-100 is quicker, easier, more efficient, and more accurate than the hemocytometer. Additionally, it is less expensive and easier to use than flow cytometry [27]. The first crucial method for examining the properties of sperm motility is known as computer-assisted sperm analysis. The concentrations and motility of sperm can be immediately measured. Phase-contrast microscopy cannot offer data on as many sperm kinetic properties as CASA, which is more precise and repeatable [28, 29]. In 1985, the first CASA system for application in domestic animals was made commercially accessible.

Finding a way to preserve semen fresh long enough to ship and use it in the field presented the biggest hurdle. The first big development in the AI process occurred in 1940 with the invention of a yolk-phosphate semen extender by Phillips and Lardy [30]. Sperm survival at 5°C allowed for the storage of sperm for up to three days, and citrate scattered the fat globules in egg yolk such that sperm were observable for microscopic examination. This semen extender was used on cattle all over the world. The bull sperm was then cryopreserved using glycerol. The next significant factor in favor of AI in dairy cattle was the 15% increase in fertility that was brought about by a better method, which at first protected sperm from cold shock as stated by Foote and Bratton in 1949 [31] and later controlled some venereal diseases by adding antibiotics [32]. Shannon enhanced the extender in the late 1950s to use liquid semen during the busy breeding season in New Zealand. By combining caproic acid, catalase, and 5% egg yolk by volume, "caprogen" was produced. Bull sperm can be preserved at room temperature by using caprogen, a

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helpful extender. The 1950 invention by Foote and Bratton known as the Cornell extender, which served as the industry standard for many years, contained the antibiotics penicillin, polymyxin B, and streptomycin [31]. Due to dairy farms adopting only artificial insemination (AI) to eradicate venereal infections, lower embryonic death, and achieve high fertility, the demand for AI surged. To achieve this need, the "extension" of each ejaculate was necessary by utilizing fewer sperm per insemination, given that this could be accomplished without harming fertility. Milk was also frequently used. The finest procedures for milk detox and glycerol inclusion for freezing semen, or the milk glycerol extender, were established in several publications published after Michajilov's study in 1950 [33-35]. The idea that only a few million sperm were required for each insemination was demonstrated in various publications published in 1978 by Salisbury and colleagues [12]. These investigations collectively demonstrated that semen extension may be prolonged by at least 25 times. The total amount of sperm per insemination fell from over 100 million to 4 million in insemination using liquid semen.

The other way to increase the number of successful inseminations per bull is to take the most sperm out of each bull. In the 1950s, Bratton and colleagues did research on semen collection intensity and sexual readiness [36]. The results of the collective investigations revealed a sperm output of 30 to 40 x 10^9 sperm a week per sire and 10 x 10^6 sperm each insemination dose, which helped people realize the need to assess the sexual behaviors of individual bulls. A yearly production of 200,000 doses of semen for artificial insemination is possible with these sperm counts. The amount and quality of spermatogenesis were assessed in bulls using established methods [37, 38], and the results showed a clear relationship between testicular size and sperm production. As a result, testis size is an important factor in choosing and evaluating sires.

Table II: Techniques for collecting semen in different animal species

Species	**Semen collection methods**
Boar	By using a gloved hand and an artificial vagina
Bull	By using an artificial vagina, directly from the vagina, electroejaculation, and massage technique
Dog	By using digital manipulation and artificial vagina
Ram and buck	Directly from the vagina, artificial vagina, and electroejaculation method
Stallion	Using an artificial vagina

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The males will transmit superior genes for milk production, which was one of the main motivations behind the introduction of AI. Population genetic changes are accelerated by genomic selection, which increases precision and reduces the generational gap. Bulls were formerly chosen based on phenotype, followed by progeny testing, and now the choice is being made using the genomic breeding value of the animals, which can be chosen at a very young age, even soon after birth.

In the 1950s, liquid nitrogen storage at -196°C replaced solid carbon dioxide storage at -79°C. By using glycerol, England [39] accomplished the astonishing feat of effectively freezing chicken sperm. The discovery was made in part by accident [40]. The study's primary objective, employing sugars as cryoprotectants, did not, however, provide positive outcomes. Polge tried again after spending six months at home, and this time he was successful. The container included the glycerol and protein that make up Meyers albumin, according to a chemical analysis. Chemicals had been wrongly labeled during storage. The play's central theme was serendipity. [40]. Bull sperm can be preserved in whole milk glycerol, which was developed in 1957 by Almquist and colleagues. Tris-buffered egg yolk-glycerol also afforded good preservation for sperm, both frozen and unfrozen [41, 42]. Cassou improved the technology developed by Sorensen in 1940 by adding a method for securing plastic straws and an insemination pistol in 1964 [8]. The foundation for the modern cryopreservation industry was created by the development of efficient liquid nitrogen storage devices and the successful cryopreservation of sperm.

For success in the field, accurate estrous identification and skilled insemination were necessary. In 1948, Trimberger created the A.M. to P.M. and P.M. to A.M. scheme, a traditional insemination norm [43]. It was based on observation, ovary palpation, and data from breeding. This rule states that for the best fertility, cows first discovered in estrous in the morning should be inseminated in the afternoon. Cows that first enter estrous in the evening ought to be sexing by noon the next day. Estrous synchronization, which was created by Hansel and Convey in 1983 [44], enables insemination at a predetermined time without estrous detection but frequently results in lower fertility. In 1971, Rowson predicted that combining artificial intelligence (AI) with superovulation, estrous synchronization, and embryo alteration would lead to huge increases in animal productivity that would go beyond the use of AI alone [45].

Semen sexing

One of the most dramatic advances in technology in recent years is the sexing of sperm by DNA quantification utilizing flow cytometry technology developed at Livermore Laboratories [46]. The main

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advancement was the development of an in situ probe that was able to segregate sperm while maintaining their structural integrity and producing a fluorescent signal [47]. The use of this method to deliver a live rabbit was then shown. It is astounding that the Hoechst 33342 dye preferentially binds to DNA, easily traverses cell membranes, and quantitatively distinguishes X and Y-sperm without appearing to be harmful to cells or compromising sperm function [48-50]. A new reproductive innovation called sexed semen aims to change the progeny's sex ratio to the chosen gender. It was discovered that this technology has a 90% effectiveness rate, meaning that 90% of the offspring will be female. The sexed semen technology is built on the distinctions between X and Y spermatozoa [51]. The fundamental and only characteristic that still distinguishes X- and Y-sperm is the differential in DNA content [50]. Other alternatives to this have been investigated and developed due to these drawbacks.

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AI in other species

- *Swine*

Ivanoff created the first swine AI system in Russia at the beginning of the 20th century. Inquiries were conducted in more depth in the 1930s. Early studies were conducted in Western Europe, Japan, and the United States. Artificial vaginas built for collecting boar semen were developed to provide pressure to the glans penis [52–54]. An alternative is to use a gloved hand. The concentration of electrolytes was maintained at low levels by Milovanov in 1938 [5], Anderson in 1945 [10], Polge in 1956 [54], and Maule in 1962 [11] using a glucose solution containing sodium sulphate or potassium tartrate and peptone. The yolk phosphate, yolk citrate, and milk extenders were modified for use with boar semen [11, 54]. After employing frozen bull sperm with success, attempts were made to freeze swine semen as well. Fresh or extended liquid semen is frequently used for artificial insemination (AI) in pigs, with a typical dosage of 3 109 sperm in 80 mL [6, 13, 55]. Later, post-cervical AI (PCAI), often referred to as intrauterine AI, began to be used on farms [56]. With this method, a catheter inner tube or cannula that reaches 15-20 cm deeper than Cervical AI is used to deposit the semen dosage into the uterine body.

- *Horses*

In 1899, Russia started researching artificial intelligence (AI) for horses, and in 1912, Ishikawa started testing the technology in Japan. The collecting, processing, and artificial insemination of stallion and jack semen were initially studied in the United States by McKenzie *et al.* in 1939 [57] and Berliner in 1942 [58]. Prior to the invention of the artificial vagina, an estrous mare's vagina was used to insert a rubber semen collection bag in order to gather semen. Numerous artificial vagina kinds were created in the 1930s and 1940s, and since then, they have

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undergone modifications. The methods for analysing semen are similar to those for analysing semen from bulls.

Interest in cryopreserving horse sperm was spurred by the development of techniques for preserving bovine sperm. Within 48 hours of collection, chilled, extended semen is usually used in equine artificial insemination (AI).

- *Sheep and Goats*

The season has an impact on the quality and effectiveness of the semen, and Gunn's invention of electroejaculation in 1936 is a practical way to gather semen from a lot of rams in the field [59]. Numerous advancements have been made to the methods and media for freezing semen, including those that use egg yolk-trisglycerol [60-63], as a result of the procedures developed for bull sperm [41].

- *Poultry*

The practice of artificial insemination in chickens is common. Burrows and Quinn created the method of abdominal massage and pressure to collect semen in 1937 [64]. The collection, processing, and AI of sperm were explored by Sexton in 1979, Lake in 1986, and most recently Donoghue and Wishart in 2000 [65-67].

Implications

Initially attempts to develop AI were faced with several obstacles such as the fear that AI would lead to abnormalities. However, the field-tested research that accompanied AI proved to the agricultural community that the technology could identify superior production bulls free from lethal genes, would control venereal diseases, and did result in healthy calves. The knowledge gained from AI was extremely helpful in the stepwise development of each successive reproductive technology, such as frozen semen, superovulation, embryo transfer, and cloning.

Conclusion

The journey of artificial insemination (AI) is a testament to human ingenuity and our relentless pursuit of improving agricultural practices. From its humble beginnings in ancient Arabia to the groundbreaking research of Spallanzani and Ivanoff, AI has evolved into a sophisticated biotechnological tool that has revolutionized animal breeding. Early pioneers like Leeuwenhoek and Spallanzani laid the foundation for AI by unraveling the mysteries of spermatozoa and demonstrating its potential for fertilization. Ivanoff's systematic research in Russia and the subsequent spread of AI to countries like Japan and Denmark marked significant milestones in its development. The introduction of AI in India in

the 1940s ushered in a new era of livestock breeding, leading to increased productivity and improved animal genetics. Over the years, advancements in semen preservation techniques, sperm quality evaluation, and estrous synchronization have further enhanced the effectiveness of AI. The advent of sexed semen technology has added a new dimension to AI, allowing for precise control over the gender ratio of offspring. This innovation has far-reaching implications for livestock production and genetic selection strategies. Moreover, AI has not only transformed cattle breeding but has also found applications in other species like swine, horses, sheep, goats, and poultry. Each adaptation has brought about its own set of challenges and innovations, contributing to the continuous evolution of AI technology. Despite initial skepticism and challenges, AI has emerged as a cornerstone of modern animal husbandry, offering solutions to issues like genetic disorders, venereal diseases, and low fertility rates. The cumulative knowledge gained from AI research has paved the way for further advancements in reproductive technologies such as embryo transfer and cloning. In conclusion, the journey of artificial insemination from ancient practices to modern biotechnology exemplifies the power of human innovation in reshaping agriculture and ensuring food security for generations to come. As we continue to push the boundaries of scientific discovery, AI will undoubtedly remain a vital tool in the arsenal of farmers and breeders worldwide.

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