What should I know about artificial insemination (AI) of animals?

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Why does AI have great impact?

Greater numbers of superior offspring is the major objective of AI. Secondary advantages include virtual elimination of disease transmission during breeding and reduction of human injuries from dangerous large animals. With natural mating 1 boar, bull, dog, ram, or stallion can impregnate >25 females in 1–3 months. With AI up to 25,000 cows or almost 100 pigs can be bred with 1 week's production of sperm from a male. Hence, with AI most males are not needed to produce future generations, so sire selection is intense. Potentially superior sires are produced by planned use of semen from a male with many progeny displaying outstanding production traits.

AI facilitates intensive paternal selection. This has markedly improved milk production per cow and provided lean and tender carcasses in swine remodeled in <10 generations. A few bulls have produced >150,000 insemination doses in a year or 1.5 million doses in their lifetime. Spreading costs among many insemination doses allows rigid health control of AI sires, special housing, quality control in the andrology and processing laboratories, monitoring of reproductive performance, and transmission of desired traits to offspring.

Most boars in an AI program are used extensively starting around 7 months of age and are replaced ≤12 months later. With dairy cattle, semen processed from 11-15 months of age is used to inseminate ~1,500 females in 75–150 herds. Up to 10,000 AI doses might be used from an occasional elite young bull. The bull then is held from use until the quality of his female progeny is established by phenotypic evaluation for actual ease of calving and production of milk protein after an additional year. Daughters of a given sire are compared with those of all other sires used in the same and other herds to determine which sires best transmit desired traits. Approximately 1 of 9 "evaluated" sires is returned to active use and the other 8 are eliminated. By 2012, analyses of single nucleotide polymorphisims should allow meaningful estimation of a new born female's genetic potential for facile calving and milk production. Such data for a bull's first 200 female calves will shift the paradigm for sire selection, especially as accuracy of predictions is refined. Currently, a typical AI bull might remain in service for 3-5 years. Dogs and stallions remain in use as long as there is demand for their semen.

AI usually is performed by transcervical intrauterine deposition of sperm. After ejaculation, sperm are extended in special medium and held at 5°C (most species) or 17°C (pigs) for use ≤6 days after ejaculation or cryopreserved and maintained at -196°C for use over months or years (Chapter 14). This allows national distribution of liquid semen to inseminate females and both national and world-wide distribution of frozen semen (consistent with international biosecurity and health regulations). Liquid semen dominates with pigs, because it efficiently utilizes sperm where thousands of sows are nearby; procedures for cryopreservation remain imperfect for this species. Both liquid and frozen semen are used with dogs and horses (AI banned for thoroughbred horses). Frozen semen dominates with dairy cattle. Frozen semen is used with beef cattle, although natural mating predominates. AI with liquid semen is obligatory with commercial turkeys as males become too large to mate before reaching puberty. In every case, proper semen handling is crucial.

Use of AI

Approximately 11 million cattle are inseminated annually in North America and 11 million in Europe. Worldwide, approximately 230 million doses of frozen semen and 12 million doses of liquid semen are prepared annually, with 2–3 doses required to impregnate most females. Most pigs are inseminated with liquid semen, totaling 21 and 18 million AIs annually in North America and Europe.

How is outcome from AI measured?

Fertility simply means "being fertile". Conception rate or fertilization rate is the proportion of oocytes exposed to sperm that form a zygote. Because in vivo fertilization rate cannot be measured except in special research projects, it has limited value. Pregnancy rate is useful provided the mean value is accompanied by the measure used to determine pregnancy, e.g., ultrasonic detection of embryos, and the interval after mating or insemination when the observations were made, number of females contributing to the mean value, and a statement if the percentage was calculated for all females eligible for breeding or only females actually bred. Pregnancy rate always will be lower than fertilization rate because some zygotes will not develop into detectable embryos or viable fetuses.

It is common to refer to subfertile or highly fertile males, based on pregnancy rate obtained with a population of females. However, this overlooks 3 important facts. First, the male might be penalized for lack of pregnancy in

females that never were bred. Second, first mating pregnancy rate will be lower than cumulative outcome at the end of a breeding season. Third, observed pregnancy rate = [male's fertility][(female's fertility)(management factor)]. The management factor includes insemination of animals not in estrus. Validity of this "equation" is best evidenced by extensive data showing that semen from a male gives very different pregnancy rates when used with nuli-parous vs. parous females or with females in different producer units.

Modifying sperm before AI

Animal andrologists learned >65 years ago that one can improve on nature. Exposure of animal sperm to certain lipoproteins or proteins, rather than dilution in a simple salts solution, improved retention of membrane integrity and increased pregnancy rate with AI. Thousands of publicationsdescribe improved "extenders" and procedures. Usually they targeted improved function or survival after storage at 17°C, 5°C, or -196°C. Additives include antioxidants, vegetable-derived molecules, and synthetic molecules based on molecules found in semen.

Use of "sexed sperm"

With food producing animals there can be substantial economic benefit from modifying sex ratio at birth. Females might be preferred in dairy cattle and pigs operations. The concept started to move from dream to reality in the 1980s with development of flow-sorting instruments capable of detecting the 3–5% greater amount of DNA in X-chromosome-bearing sperm than in Y-chromosome-bearing sperm. Research to bias sex ratio based on other attributes of sperm continued.

Moving flow-sorted sperm to a commercial commodity required that the patent holder solve myriad electronic, computer programming, fluidic, and biological problems. By 1999 progeny of the desired sex, for several species, were being produced with regularity. Technical improvements enabled establishment of commercial flow-sorting facilities in the UK in 2000 and USA in 2004. Starting in 2006, flow-sorters were positioned at major cattle genetics companies. Worldwide, by late 2008 there were sorters in 17 commercial facilities. Sorter throughput limits an AI dose to ~$2x10^6$ sperm and possible suppression of pregnancy rate is minimized by AI primarily of nuli-parous females. More than 60% of sperm are discarded during sorting because they are of the wrong type, ambiguous to the machine, or dead. Desired sperm are processed, packaged into 0.25-mL straws, and cryopreserved. Approx-

imately 90% of sperm in a commercial dose of flow-sorted semen have the desired sex chromosome.

Three million doses of sex-sorted cryopreserved semen were produced at 5 sites in USA in 2008, with ~1 million of these exported. To date, ~7 million doses have been produced in USA and another 3 million doses in other countries. Approximately 97% of production has been for X-chromosome-bearing sperm, and producers obtain ~90% female offspring. In a recent report, 47% of 33,879 nuli-parous females conceived after 1 insemination; this pregnancy rate was 80% of that after AI of conventional semen (15–20 $x10^6$ sperm) into other nuli-parous females in the same 157 herds.

Summary

AI was the first widely used technique for assisted reproduction and remains the most important for genetic improvement and production of newborn. In the USA, millions of dairy cows, pigs and beef cows are bred via AI, as are fewer horses. Flow-sorting sperm to predetermine sex at birth works with many species, but is a commercial reality only with bull sperm; 5 million cryopreserved doses produced in USA to date.

Suggested reading

DeJarnette JM, Nebel, RL, Marshall CE. Understanding Estimates of AI Sire Fertility. Plain City, OH; http://www.selectsires.com/UnderstandingAISireFertility.pdf. 2008.

DeJarnette JM, Nebel RL, Marshall CE. Evaluating the success of sex-sorted semen in US dairy herds from farm records. Theriogenology, 2009; 71: 49-58.

Garner DL, Seidel GE Jr. History of commercializing sexed semen for cattle. Theriogenology. 2008; 69: 886-95.

Salisbury GW, Van Demark NL. Physiology of Reproduction and Artificial Insemination of Cattle, 2nd Ed. San Francisco, CA: WH Freeman; 1978.

Seidel GE Jr. Brief introduction to whole-genome selection in cattle using single nucleotide polymorphisms. Reprod Fertil Dev. 2010; 22: 138-44.

Sharp JC, Evans KM. Advances in flow cytometry for sexing sperm. Theriogenology. 2009; 71: 4-10.