Chapter 39
What should I know about artificial insemination of animals? How is male fertility assessed in domestic animals and why is this important?

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

Artificial insemination (AI) is a method of impregnating a reproductively receptive (in heat), presumed fertile female with semen from a presumed fertile male of the same species collected by means of masturbation (rubber glove method), artificial vagina collection or electroejaculation. In cattle, a cryopreserved plastic straw with 20 million spermatozoa (standard AI dose) is thawed at a controlled temperature and emptied into the uterus of a cow/heifer with the help of a stainless steel pipette (AI gun) manipulated through the cervix by the inseminator's arm inserted in rectum (recto-vaginal insemination process). In pigs, the plastic, lubricated insemination catheter is attached to a plastic bag with extended (diluted) fresh boar semen and a dose of 1-3 billion spermatozoa, often pooled from 2-3 boars, are deposited deep in the cervix without guidance by palpation. Boar semen is notoriously difficult to freeze without damaging spermatozoa, for which reason most swine AI is performed with fresh, cooled semen. Variations of these procedures are used in other livestock species, and most AI in horses, dogs and turkeys is done with liquid semen.

Legends of the first AI involve horse thieves impregnating mares by inserting stallion semen-soaked sponges go back a millennium, while the first scientifically documented AI was done in 18th century in dogs, by Italian priest Lazzaro Spallanzani, considered the father of fertilization biology. Spallanzani was also credited with the first "test tube" fertilization experiments by using semen collected from male frogs and dispersed over female frog eggs. Livestock/domestic animal AI in a modern sense entered the scene in late 19th and early 20th century, and reached true

commercial application after WWII, coinciding with the development of semen dilutants (extenders) and cryoprotectants such as glycerol (a polyol compound extracted from plant and animal sources) for bull semen preservation. The most recent major advance in AI has been the development and commercialization of AI with sexed semen, i.e., semen separated to carry sex chromosome of the desired gender, either female (X) or male (Y), which ultimately predetermines offspring gender at birth.

To date, AI has been used on a worldwide commercial scale in food animals such as cattle, pigs, goats, sheep and camels, as well as in pets (dogs, cats, horses) and trophy game (elk, deer). AI is also being developed for food animals of regional importance (e.g., guinea pigs in Central/South America, ostriches in South Africa), and for conservation purpose in captive wildlife animals and rare livestock breeds (e.g., large cats, rare cattle and donkey breeds). AI has been used in human ART (assisted reproductive therapy/infertility treatment) for centuries (first recorded insemination in 1700s by Scottish-born surgeon John Hunter), long before the first successful human in vitro fertilization (IVF; 1978); it remains a front-line treatment in treatment-seeking couples that produce gametes (spermatozoa and eggs) of acceptable quality and quantity, before IVF is considered. At present time, about 33 million bull semen units are sold in the USA annually, with an additional 39 million units exported. Over 34 million pigs are inseminated annually in North America, with the use of swine AI over natural breeding approaching 100%. Bulls are first collected at around 10-12 months of age and remain in service for an average of 3 years. Boars are typically collected at around 7 months and replaced after one year of service.

In the modern era of precision agriculture, the major advantage of livestock AI is the ability to amplify offspring of males with superior genetic makeup favoring desirable production traits such as meat or milk production, or ease of calving in cattle. Another major advantage is the ability to limit the spread of infectious diseases that can occur by mating, and to eliminate the labor and costs associated with maintaining a large number of male genetic stock and managing the breeding herds. Also reduced are injuries to animal handlers and to animals themselves that are associated with natural breeding. Optimized AI, hand-in-hand with precise female estrus detection and synchronization (timed AI) is a powerful tool for maximizing the benefit of genomic selection while also maintaining a reasonable level of heterosis (hybrid vigor) in livestock herds.

How is male fertility and AI semen quality assessed in livestock animals? Why is this important?

Fertility of an AI dairy bull is expressed as sire conception rate (SCR, measured by female non-return to estrus) while boar fertility is expressed by both the pregnancy rate and litter size. Pregnancy success in beef cattle is generally determined by ultrasound evaluation or rectal palpation of the uterus. All of these measures are critically influenced by andrological health. The first step in the andrological evaluation of a male animal is the general physical examination (body condition, eye, foot and leg problems), as well as visual assessment and palpation of external genitals and accessory glands. In yearling bulls intended for AI service, this is done as part of breeding soundness examination (BSE), which also includes measurement of scrotal circumference (an informative parameter associated with normal sperm production) and light microscopic semen analysis. A major limitation of the bull BSE is that it is a pass/fail exam and is a better indicator of infertility than fertility. Although the first fertility test for (human) couples was described by female physician Trotta of Salerno in 11th century, the first observation of human spermatozoa by Van Leeuwenhoek and Hamm occurred in 17th century and the modern, microscopy based andrological semen analysis came to existence in late 19th century. Thus, semen quality for male fertility evaluation and AI quality control has traditionally been performed by subjective light microscopic analysis including sperm count (total sperm number in ejaculate), concentration (sperm number per milliliter of ejaculate), motility (movement including speed and direction), percent of visually normal spermatozoa (normal sperm morphology), and the presence of immature sperm forms and non-sperm contaminants (bacteria, white blood and epithelial cells); it remains the standard method of semen analysis. Such assessments are done on a small aliquot of fresh semen shortly after collection and, for cryopreserved semen also after thawing one dose from a single collection batch prior to AI. Visually, collected semen can be assessed for volume, density (opacity related to sperm concentration), color (may identify blood, urine or purulent exudate contamination) and swirl (liquid semen streaming caused by sperm motility). Due to inherent subjectivity and inter- and intra-laboratory evaluator differences, automated, objective measurements of semen/sperm quality have been developed, based on bright field microscopy image/video output capture by digital cameras and analysis by

dedicated computer software (CASA/computer-assisted semen analysis). To increase the speed, cell number and accuracy, methods based on flow cytometry techniques have been developed that utilize tagging and bulk measurement of sperm samples with fluorescent probes reflective of sperm structural integrity, viability and fertilizing ability. Such measurements appear to be superior to conventional and CASA analysis in their ability to accurately assess the percentage of normal spermatozoa and even predict fertility of a semen dose.

What is the difference between subjective and biomarker-based semen analysis?

Light microscopic semen analysis provides useful baseline assessment of a male's ability to produce spermatozoa in sufficient quantity and with acceptable concentration, viability and quality. In extreme cases, such evaluations identify transiently infertile males or males with chronic infertility due to disease, genetic/inherited factors, malnutrition or environmental effects (heat stress, pollution), separately or together causing reduction or cessation of semen production/quality. In males with acceptable semen and sperm parameters, such analysis provides clues as to what the minimal number of spermatozoa needs to be in an insemination dose, an important consideration with regard to cost effective production of doses from males with high genetic value that in some cases need to compensate for increased content of defective spermatozoa in semen. Automated, objective andrological methods bring semen analysis to the next level, with its speed (thousands of spermatozoa per sample measured in a matter of seconds) and application of specific biomarkers potentially reflective of current and/or predictive of a future sires' fertility in AI service. Thus, objective semen assessment could supersede the microscopic portion of BSE and eliminate need for retesting. The next step in the development of andrological analysis involves artificial intelligence (bringing AI to AI), which will facilitate fully automated, label-free (no fluorescent probes required) sperm quality assessment.

Can semen be improved? Supplementation, purification and ultimately, sexing.

Ultimately, the goal of the livestock industry is to improve the AI semen dose by clinical, nutritional and environmental management

of the male, as well as by the optimization of protocols and media for semen processing, storage, distribution and deposition. This can be done indirectly by nutritional supplementation or by direct alteration of semen extenders with antioxidants, to scavenge semen's intrinsic reactive oxygen species harmful to spermatozoa, and naturally occurring antimicrobials reducing the need for antibiotics to prevent bacterial growth and disease transmission. An additional level of improvement can be attained by semen purification, removing defective and dead/moribund spermatozoa using centrifugation, sedimentation, filtration or magnetic/nanoparticle-based purification. Flow cytometric semen sexing/sorting could be viewed as an ultimate purification technique; in addition to removal of spermatozoa carrying the undesired gender of sex chromosome, it also discards dead spermatozoa, spermatozoa with abnormal chromosome complement (aneuploidy) and those with defects of DNA packaging. However, sexing of spermatozoa is a very slow procedure and thus AI doses are prepared with 10 to 20% of the spermatozoa in traditional semen dose. Procedures that remove subfertile or dead spermatozoa before sex sorting may be valuable.

Summary

Artificial insemination revolutionized animal production in the 20th century. At present time, it remains the leading method of reproductive management in pigs and dairy cattle in the US and worldwide, with its adoption increasing in other livestock species. Precision agriculture approaches of genomic selection and gender determination/semen sexing would not be possible without AI. Application of biomarker-based, automated semen analysis coupled with artificial intelligence/machine learning will further expand the use and precision of livestock semen analysis for fertility determination and AI semen quality control. Semen dose improvements are being made through feeding of male-optimized, balanced diets, nanotechnology, and judicious semen extender supplementation with sperm friendly additives such as antioxidants and naturally occurring antimicrobials. Altogether, such improvements will increase cost efficiency and reduce the number of males needed for AI service, thus maintaining profitability and reducing the environmental impact of livestock operations on air, water and soil.

Acknowledgements

We thank Dr. Thomas Geary (USDA-ARS Fort Keogh, MT), Dr. Eriklis Nogueira (EMBRAPA, Campo Grande, MDS, BRAZIL), Dr. Jordan Thomas (University of Missouri Columbia MO) and Dr. Timothy Safranski (University of Missouri, Columbia MO) for helpful advice and reading of the manuscript.

Suggested reading

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