LIVESTOCK SEMEN BIOTECHNOLOGY AND MANAGEMENT

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Summary

Modern livestock breeding is basically dependent on the proper use of semen for artificial insemination of females and of other reproductive biotechnologies such as the production of embryos *in vitro* for embryo transfer. Both these techniques have made possible not only the wide dissemination of genetic material onto breeding populations but also enhanced the selection of best sires, owing to the development of better diagnostic techniques for sperm function and of preservation of seminal material over time. Although use of liquid semen cooled to room temperature, to intermediate temperatures (+16-20°C) or chilled (+5°C) dominates in some livestock species (swine respectively small ruminants), cryopreservation is rule in bovine and it is advancing in other species by the design of new containers, freezing methods and the use of better insemination strategies. Reliable semen diagnostics is absolutely essential to disclose which semen is to be processed/cryopreserved but also to aim determination of a potential fertilizing capacity in the laboratory, thus saving costs prior to artificial insemination. However, there is a yet no single laboratory method that accurately prognoses fertility in livestock, requiring use of a battery of diagnostic methods. Novel techniques for optimal use of ejaculates (low-dose) and intrauterine deposition of semen throughout species are those management techniques that shall increase our capabilities for better diagnostics/selection of semen/male potential fertility, of cryopreservation techniques and a more rational dissemination of genetics.

1. Introduction

Livestock semen is usually extended in an adequate medium to prolong its fertile life and either used immediately or preserved, following slight (pig) or intermediate (small ruminants) cooling for days or frozen and long term stored. In the latter case, the frozen

semen is maintained in liquid nitrogen until thawed for use, following evaluation of the process, for artificial insemination (AI) either for direct breeding, production of embryos for embryo transfer (ET, of fresh or frozen embryos) or *in vitro* insemination of cultured oocytes for ET of *in vitro*-produced embryos (either fresh or frozen). At present, frozen-thawed semen is routinely used in cattle AI, and becoming more applicable in other livestock.

Semen preservation has historical roots that document back to the 18th century, with a boom experienced during the first half of the 20th century in relation to the development of AI with liquid semen. From the 1950s, the application of cryoprotectants contributed to the wider use of semen freezing, particularly for the application of intrauterine AI in dairy cattle. Since the 1970s, the development and use of AI with preserved semen have grown exponentially and on a global scale, particularly in the breeding of dairy cattle (>200 million of the first AIs in the world use frozen semen) and pigs (>160 million used cooled liquid semen doses). Sows are -in Europe, the Americas and South-east Asia- basically only bred via AI, mimicking the situation already reached in dairy cattle.

Gestation rates obtained via AI vary largely, primarily depending on the species, the type of preservation method, the number of spermatozoa used, the moment when AI is performed and the site of deposition of the preserved semen in the female. In general, liquid cooled semen has a better fertility potential than frozen semen, intra-uterine AI favors fertility compared to intra-vaginal or intra-cervical deposition, and an AI near an imminent ovulation shows the highest gestation rates. Today, sows show fertility rates similar to those obtained after natural mating using liquid cooled semen while in dairy cattle fertility has deteriorated (decreases of up to 25%), mostly owing to the improper pressure on sire selection focused solely for milk yield, particularly affecting the dominating Holstein breed. In other breeds, fertility has been maintained despite using less and less spermatozoa per AI dose. Today, a single AI yields a 60% of gestation rate using only 1/300-500 of the total sperm number in the ejaculate. In pigs and small ruminants the situation is different, and they still require excessive sperm numbers when frozen-thawed semen is used, thus yielding fewer doses per processed ejaculate. Moreover, and particularly for pigs, sperm cryosurvival is still consistently lower than in bovine, owing to damage during a processing that is time-consuming, costly and yields few doses per ejaculate. Number of piglets born is lower than for cooled or neat semen implying that sperm lifespan, deposition site and closeness to ovulation are yet significant hurdles to be overcome. Similar constrains apply to *in vitro* fertilization (IVF), where excessive sperm numbers are still used leading –again particularly in pigs- to lethal polispermy. Processing of spermatozoa having been selected for chromosomal sex (the so-called "sexed semen") impose further limitations to cryosurvival. The technique used thus far; separation of spermatozoa via high-speed flow cytometry of fluorochrome-loaded spermatozoa, is rather rough on the cells where several factors (flow speed, nudity of sperm membrane etc) affects spermatozoa and weaken their survival prior to cryopreservation.

Semen is still the "cheapest" component of artificial breeding, something that explains the dominancy of AI over any other reproductive biotechnology, besides the classical advantages of AI: prevention of venereal diseases and the large dissemination of desirable genetic characters on a female population, propagating the genetic material of

selected stud sires, which are continuously replacing the best ones presently in use. Successful freezing of semen of all livestock is a long lasting priority, tied not only to acceptable cryosurvival and lifespan after thawing but also to the devise of rational techniques that -at the lowest possible cost- can provide largest possible numbers of doses for AI. Last but not least, the deposition of these doses is to be easy and yield acceptable fertility, i.e. close to use of cooled semen or even natural mating. The latter requires –unfortunately- a better knowledge of the moment of spontaneous ovulation, implying a holistic approach to the technology of semen processing and use.

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Bibliography

Arav A, Yavin S, Zeron Y, Natan D, Dekel I, Gacitua H (2002). New trends in gamete's cryopreservation. *Mol Cell Endocrinol* 187, 77-81 [Description of most methods to cryopreserve spermatozoa and oocytes, comparative].

Bolarín A, Roca J, Rodriguez-Martinez H, Hernandez M, Vazquez JM, Martínez EA (2006). Dissimilarities in sows' ovarian status at the insemination time could explain differences in fertility between farms when frozen-thawed semen is used. *Theriogenology* 65, 669-680 [Describes why frozen-thawed semen yields differences in fertility in the field].

Bwanga CO, Einarsson S, Rodriguez-Martinez H (1991). Freezing of boar semen in plastic bags and straws. *Reprod Domest Anim* 26, 117-125 [Describes methods for freezing boar semen].

Eriksson BM, Rodriguez-Martinez H (2000). Effect of freezing and thawing rates on the post-thaw viability of boar spermatozoa frozen in large 5 ml packages (FlatPack). *Anim Reprod Sci* 63, 205–220. [Describes the constraint of cooling and thawing methods for freezing boar semen].

Flowers WL (1999). Artificial insemination, in animals. In: Knobil E, Neill JD (Eds) *Encyclopedia of Reproduction*. Vol I, Academic Press, San Diego, 291-302 [Generic description of artificial insemination methods, historic review and methodological considerations].

Foote RH (1999). Development of reproductive biotechnologies in domestic animals from artificial insemination to cloning: a perspective. *Cloning* 1, 133-142 [Historic background to different reproductive biotechnologies and their relative value].

Hernandez M, Roca J, Ballester J, Vázquez JM, Martinez EA, Johannisson A, Saravia F, Rodriguez-Martinez H (2006). Differences in SCSA outcome among boars with different sperm freezability. *Int J Androl* 29, 583–591 [Describes the importance of sire variability when judging freezing of semen].

Hernandez M, Roca J, Gil MA, Vázquez JM, Martínez EA (2007). Adjustments on the cryopreservation conditions reduce the incidence of boar ejaculates with poor sperm freezability. *Theriogenology* 67, 1436–1445 [Describes the rationale for adjusting freezing methods to unselected sire populations of boars].

Holt WV (2000). Fundamental aspects of sperm cryobiology: the importance of species and individual differences. *Theriogenology* 53, 47–58 [Describes rationale for sperm cryobiology phenomena in relation to animal variation].

Holt WV (2000). Basic aspects of frozen storage of semen. *Anim Reprod Sci* 62, 3–22 [Describes the basics of sperm freezing].

Hossain Md S, Johannisson A, Wallgren M, Nagy S, Pimenta Siqueira A & H Rodriguez-Martinez (2011). Flow cytometry for the assessment of animal sperm integrity and functionality: state of the art. *Asian J Androl* 13,406-419 [Summarizes the value of use of flow cytometry as a tool for the assessment of sperm intactness and function].

Morrell JM & H Rodriguez-Martinez (2009). Biomimetic techniques for improving sperm quality in animal breeding: a review. *The Open Andrology Journal* (Open access) 1, 1-9 [Describes the value of using sperm selection for enrichment of robust spermatozoa in animal breeding].

Roca J, Parrilla I, Rodriguez-Martinez H, Gil MA, Cuello C, Vazquez JM, Martinez EA (2011). Approaches towards efficient use of boar semen in the pig industry. *Reprod Domest Anim* 46 S2, 79-83 [Summarizes strategies for best use of semen for breeding in commercial pig production].

Rodriguez-Martinez H (2000). Evaluation of frozen semen: Traditional and new approaches. In: Topics in Bull Fertility, Chenoweth PJ (ed), *Recent Advances in Veterinary Medicine*, International Veterinary Information Services (IVIS), w w w . I vis . org (Document No. A0502.0600) [Summarizes established and novel, experimentally designed methods and techniques for semen evaluation in bulls].

Rodriguez-Martinez H (2003). Laboratory semen assessment and prediction of fertility: still utopia? *Reprod Domest Anim* 38, 312-318 [A critical reappraisal of the relative value of different methods of semen evaluation in relation to livestock fertility].

Rodriguez-Martinez H (2006). Can we increase the estimative value of semen assessment? *Reprod Domest Anim* 41 S2, 2-10 [Describes novel methods to increase the impact of semen assessment techniques as prognostic tools].

Rodriguez-Martinez H (2007). State of the art in farm animal sperm evaluation. *Reprod Fert Dev* 19, 91-101 [A summary of current methods for semen evaluation in livestock].

Rodriguez-Martinez H (2007). Reproductive biotechnology in pigs: what will remain? In: "*Paradigms in pig science*". Wiseman J, Varley MA, McOrist S, Kemp B (eds), Nottingham University Press, Nottingham, UK, Chapter 15, 263-302 (ISBN 978-1-904761-56-3)[Comprehensive summary of reproductive techniques in livestock, their value and future prospects].

Rodriguez-Martinez H, Barth AD (2007). In vitro evaluation of sperm quality related to in vivo function and fertility. In: *Reproduction in Domestic Ruminants* VI. Juengel JI, Murray JF, Smith MF (eds), Nottingham University Press, Nottingham, UK, 39-54 (Soc *Reprod Fert* 64: 39-54, 2007) [Critical review of field and experimental techniques to evaluate fertility in livestock].

Rodríguez-Martinez H, Saravia F, Wallgren M, Roca J, Peña FJ (2008). Influence of seminal plasma on the kinematics of boar spermatozoa during freezing. *Theriogenology* 70, 1242-1250 [Describes the importance of seminal plasma on sperm function].

Rodriguez-Martinez H, Wallgren M (2011). Advances in boar semen cryopreservation. *Vet Med Int* 2011, Article ID 396181, 5pp (doi:104061 / 2011 / 396181), open access [Summarizes our advances in boar semen freezing].

Rodriguez-Martinez H, Kvist U, Ernerudh J, Sanz L, Calvete JJ (2011). Seminal plasma proteins: what role do they play? *Am J Reprod Immunol* (AJRI) 66 S1, 11-22 [Present a comprehensive, comparative review of the composition and physiological role of seminal plasma in relation to sperm function and female tolerance].

Rodriguez-Martinez H, J Hultgren, R Båge, A-S Bergqvist, C Svensson, C Bergsten, L Lidfors, S Gunnarsson, B Algers, U Emanuelson, B Berglund, G Andersson, M Håård, B Lindhé, H Stålhammar & H Gustafsson (2008). Reproductive performance in high-producing dairy cows: can we sustain it under current practice? In: IVIS *Reviews in Veterinary Medicine*, I.V.I.S. (Ed.). International Veterinary Information Service, Ithaca NY (w w w . ivis . org), Last updated: 12-Dec-2008; R0108.1208 (Open Journal)[Description of the factors ruling reproductive efficiency in cattle and the effects of selection for high production].

Saravia F, Wallgren M, Johannisson A, Calvete JJ, Sanz L, Peña FJ, Roca J, Rodríguez-Martínez H (2009). Exposure to the seminal plasma of different portions of the boar ejaculate modulates the survival

of spermatozoa cryopreserved in MiniFlatPacks. *Theriogenology* 71, 662–675 [Describes the modulatory effects of seminal plasma on survival of pig spermatozoa following freezing].

Saravia F, Wallgren M, Rodríguez-Martínez H (2010). Freezing of boar semen can be simplified by handling a specific portion of the ejaculate with a shorter procedure and MiniFlatPack packaging. *Anim Reprod Sci* 117, 279-287 [Describes the most novel method to ease freezing of boar semen for commercial purposes].

Biographical Sketch

Dr. Rodriguez-Martinez is currently Professor of Reproductive Biology at the University of Linköping, Faculty of Health Sciences, Linköping, Sweden. Born in Spain 1950, he grew up in Montevideo, Uruguay where he got his DVM-degree in 1975, alongside his initiation as University teacher/researcher in morphology (Fellow Faculty of Veterinary Medicine 1970-1976; Associate Professor of Histology & Embryology, Faculty of Medicine, Montevideo 1976-1979). He is a licensed Veterinarian in Spain (1994) and Sweden (1998) the latter where he graduated (MSc 1980, PhD 1983) in Obstetrics & Gynecology at the Swedish University of Agricultural Sciences (SLU), Uppsala, Sweden. Post-doc in the USA (Assistant Professor, Faculty of Veterinary Medicine, University of Illinois) in 1984, he returned to SLU where he got his habilitation (Docent) in Histology (1986) and in Embryology (1998) while holding a tenure associate professorship in these subjects (1985-1991). He became Full Professor of Reproductive Biotechnology at the Dept of Obstetrics & Gynecology (now Division of Reproduction), SLU, in 1991 (Dept Head 2004-2006), until moving to his current position in 2010. Doctor of Science (Spain 1994) and Founding Diplomate of the European College of Animal Reproduction (ECAR) since 1999, he has been intensively involved in undergraduate and graduate education, at national (Vice-Dean of the Faculty of Veterinary Medicine, SLU, undergraduate education 1999-2001, research and postgraduate education 2002-2003) and international levels (past-vice-President and member of the Joint Education Committee of the European Association of Establishments of Veterinary Education, EAEVE, Brussels, 2003-2006 and member of the Examination Committee and Executive Board of ECAR 2001-2007). Expert visitor and officer for European (TAIEX, EAEVE) and international agencies (IAEA), he has been Director of Mobility and Research Programs with Canada (EU/1997-2000), Japan (STINT/1997-2002), Indonesia and Thailand (EU/2002-2006). Active researcher in reproductive biotechnology, diagnostic andrology and cryobiology, he has a genuine interest in sperm-tubal-oocyte interactions, well documented with a profuse track-record (authored more than 400 original papers and reviews). Prof Rodriguez-Martinez has tutored 46 graduate students to degree and serves as international reviewer for many non-Swedish granting agencies. He also acts as Editor-in-Chief of "Reproduction in Domestic Animals" (Wiley-Blackwell) since 2000. He is a member of several Academies and learned societies (Royal Academy of Medicine, Murcia Spain 2005, Royal Swedish Academy of Forestry and Agriculture (KSLA), Stockholm 2006, Royal Academy of Veterinary Sciences, Madrid, Spain 2008, Polish Academy of Sciences, Warsaw, Poland 2009 and Honorary Member of the Japan Society for Animal Science, Tokyo, Japan 2009).