FREEZING THE BOARS SEMEN: SOME LIMITATIONS AND PERSPECTIVES*

STANKOVIĆ B. [1], PETRUJKIĆ T.[2], HRISTOV S.[1], RELIĆ RENATA[1]

ABSTRACT: Artificial insemination (AI) was the first great biotechnology applied to improve reproduction and genetics of farm animals. Next developed procedure, to the cryopreservate semen made great step towards different possibilities: almost unlimited preservation of pathogen free quality genotypes semen, its usage in different time and locations, making transport easier and manipulative fees more acceptabile. Limitations of the technique consider biological traits of semen and difficulties to reproduce them. The knowledge gained from the AI and the cryopreservation experience was extremely helpful in stepwise development of each successive reproductive technology, such as superovulation, embryo transfer, and, eventually, cloning.

Key words: boar semen, cryopreservation, extenders, perspectives.

BEGINNINGS OF AI, FROZEN/THAWED SEMEN USAGE BEGINNINGS AND PROCEDURE

The artificial insemination (AI) of swine was initiated by Ivanow in Russia (Ivanow, 1907; 1922) and continued using artificial vaginas or a gloved hand (McKenzie, 1931; Ito et al., 1948; Polge, 1956). Extenders were based on glucose solutions of Na-K-tartarate or Na_2SO_4 and peptone keeping the concentration of electrolytes low, including cooled semen (Ito et al. 1948). Although AI was succesfully performed by Ito *et al.* (1948) in Japan, it took time before it became common method in swine reproduction.

While breakthroughs were made on sire selection, a great achievement was reported from England (Polge et al., 1949): successfully freezing chicken sperm using glycerol. Glycerol soon was found to be useful for bull sperm, but not for the boars.

Accidental discovery of Polge, (1968), focused on sugars as cryoprotectants, showed that glycerol and albumin obtained hopeful results. The basic medium used by Polge was the original yolk-citrate extender (Salisbury et al., 1941) and glycerol. O'Dell and Almquist (1957) developed milk-glycerol as a cryopreserving medium to bull sperm, as well as Tris-buffered egg yolk-glycerol (Davis et al., 1963; Foote, 1998), which became the most commonly worldwide used cryopreservation medium for of bull semen and several

[1] Mr Stanković Branislav, teaching assistant, dr Hristov Slavča, associated professor, DVM Relić Renata, teaching assistant, Faculty of Agriculture, Belgrade - Zemun
[2] Dr Petrujkić Tihomir, teaching professor, Faculty of veterinary medicine, Belgrade
*This paper is financed by Ministry of Science of Serbia, BTN 7103B

other species (Iritani, 1980), but not boars. It was shown its toxicity to boar semen, increasing permeability of cell membrane (Larsson, 1978).

It took many years of work to freeze boar semen, following the successful use of frozen bull sperm (Nishikawa, 1964; Iritani, 1980; Johnson and Rath, 1991; Foote, 1999; Johnson and Guthrie, 2000). Polge *et al.* (1970) performed fhe first successful deep frozen boar semen AI. Crabo *et al*. (1971) developed succesfull thawing procedure with seminal plasma as extender, but Beltsville method, developed by Pursel and Johnson (BTS - Betsville Thawing Solution, 1975) was more applicable, as it is today, with few modifications, mostly considering type of extender, with these phases: 1. equilibration on 20 ^{o}C during two hours, 2. separation of seminal plasma by centrifuge, 3. addition of BF5 extender and cooling to 5 ^{o}C, d. addition BF5 and glycerol, 4. freezing semen (peletts/straws) to – 79 ^{o}C, and – 196 ^{o}C. Until 1991, new boar semen extenders composition was presented, but now it is not revealed because of the competition among manufacturing companies, competing for a huge market for long-term (7 - 10 days) boar semen extenders. Reasons for developing new long-term boar semen extenders relate to: more favorable work hours in the boar stud, reduced transportation cost of delivering semen to the farm, testing of semen for Porcine Reproductive and Respiratory Syndrome virus before use, and a longer period for using the semen with satisfactory reproductive performance (Levis, 2002). First of extenders of the denominated ones long play it was the **Zorlesco** (Gottardi ET al., 1980), with TRIS pH regulator, bovine serum albumin (BSA) and cistein, stabilizing membranes and inhibiting the qualification process (Johnson et al., 2000), without satisfactory results, as well as Moretti's (1981) **Modena** extender with more glucose and no BSA (Johnson *et al*., 1988; Laforest and Allard, 1996). In 1990 was presented **Androhep** extender, containing HEPES as pH regulator and BSA, which keep osmotic pressure. This extender was accepted as extender of long conservation, followed by **Acromax, X-Cell**, **Androhep Extra**, Vital, SpermAid, Mulberry III, etc.

It must be emphasized that average egg lactose-yolk extender is more frequently used and described by Pursel and Johnson (1975) denominated **BF-5**, in whose composition it includes glucose, yolk of egg and Tris as pH regulator.

LIMITATIONS

Few serious problems occurred in establishing of frozen/thawed semen AI technique. Cryopreserving boar semen results in an extreme reduction in fertilizing ability (Almlid and Johnson, 1987; Parks and Graham, 1992), possibly due to damage to sperm membranes (Watson and Plummer, 1985; Maxwell and Johnson, 1997). Spermatozoa must be concentrated before freezing. Plasma and acrosomal membranes, their structural and functional integrity, influence sperm shape and volume motility, so energy production, permeability, capacitation and acrosome reaction and interaction with oocytes are very important to sperm. Cryopreservation (dilution, cooling, freezing/thawing) alters selective permeability of sperm membranes, reduces motility and energy production and changes membrane dynamic behavior (He *et al*., 2001). Membrane lipid composition is intimately involved in the degree and nature of the damage caused by cryopreservation (Quinn, 1989). Sperm from different parts of the epididymis have different sensitivity to cold shock, correlated to changes in lipid composition during sperm maturation from

caput to cauda epididymis (Bwanga,1991). Buffer system of extender, simmilar to same system of the seminal plasma, plays important roll in frozen semen viability. It was demonstrated that bicarbonate and calcium play crucial roles in the capacitation process in pig sperm. Specific tyrosine-phosphoprotein appears during capacitation in a calcium-dependent manner, but its presence is not a prerequisite for capacitation of pig sperm (Tardif *et al.*, 2003)

Just 10% of tested boars population provides needed quality of semen in respect of spermatozoa viability, survival and fertilization ability after thawing. Pregnancy rates and litter sizes are reduced with cryopreserved semen, so fresh or extended liquid semen is used for about 99% of AI in swine. Frozen semen is limited to use in special breeding programs**.** Low conception rate and litter size obtained by thawed semen insemination is not satisfying (Johnson *et al*. 1980), as many semen additives to improve fertility of frozen semen have been tested with minimal success (Foote, 1999), but data were provided for a peptide that increased fertility added to frozen/thawed semen (Amann et al., 1999).

PERSPECTIVES

New semen extenders generated on long-term viability of semen, farrowing rate and litter size data are presented by the company with a business interest, with aggressive advertisement and marketing, not peer reviewed or published in scientific journals. Only few boar studs/pork production enterprises compare extenders scientifically.

The knowledge gained from the AI and cryopreservation was extremely helpful in stepwise development of various technologies, such as superovulation, embryotransfer, and, eventually, cloning. Sexing of boar sperm is possible but slow for commercial use. Equipment is permanently developed, such as spermatozoa sorters (Foote, 2001).

Design of new extenders and knowing more the spermatozoa metabolism are necessary, as well as using new systems of evaluating and optimizing the components of the extender (Pettit *et al.,* 1999). Diverse additives, like alkil-glycerol must be evaluated in respect of the viability of the spermatozoa (Cheminade et al., 2002), cold shock (Zeng and Terada, 2001), improving the spermatozoa transport synchronized with the ovulation and fertilization (Waberski, 1997; Kemp and Soede, 1997). Problem of the thermal shock must be resolved, so equipment the elasticity effect on the spermatozoa survival and fertility results is evaluating (Althouse et al., 1998; Paulenz et al., 2000). Levis (2000) suggests that aspects of the artificial insemination, such as the individual differences, are not studied well, since all the males do not interact of equal way with used means (Weitze, 1990). Development of new systems like deep the intrauterine insemination and reduced number of spermatozoa by dose and the volume of insemination (from three to billion spermatozoa per milliliter of extended semen) requires new conditions of conservation. Of course, it will be necessary to study the best extender and freezing technique (Rath, 2002).

CONCLUSIONS

In order to make usage of fozen/thawed boar semen common, further investigations must take place:

- Diverse extenders and additives must be devoped and evaluated in respect of the viability of the semen;
- Problem of the thermal shock must be resolved, as well as development of new technicques (deep intrauterine insemination, e.g.), with reduced number of spermatozoa by dose and the volume of insemination and adaptation of extenders to the individual differences.

Untill that happen, freezing of boar semen will be procedure limited in possibilities, time and space.

REFERENCES CITED

ALMLID T, JOHNSON LA 1987. Fertility evaluation of the straw freezing technique for boar semen under practical artificial insemination conditions. Zuchthygiene (Berl); 22:193–202.

ALTHOUSE G. C., WILSON M. E., KUSTER C., PARSLEY M., 1998. Characterization of to lower temperature storage limitations of fresh-you extend porcine semen. Theriogenology. 50, 535-543.

AMANN, R. P., G. E. SEIDEL, JR., Z. A. BRINK. 1999. Exposure of thawed frozen bull sperm to a synthetic peptide before artificial insemination increases fertility. J. Androl. 20:42–46.

BWANGA CO. 1991. Cryopreservation of boar semen I: a literature review. Acta Vet Scand; 32:431–453

CHEMINADE C., GAUTIER V., HICHAMI TO, ALLAUME P., HIM LANNOU D., LEGRAND A.B. 2002. 1-O-alkylglycerols improve to boar sperm motility and fertility. Biol Reprod. 66, 421-8.

CRABO B.G., BOWER R.E. GRAHAM E.F. PACE M.M. 1971. Current Problems in Fertility. Plenum Press, New York, p. 33

FOOTE R. H. 2001. The history of artificial insemination: Selected notes and notables. www.asas.org/Bios/Footehist.pdf - 95k

FOOTE, R. H. 1998. Artificial Insemination to Cloning: Tracing 50 Years of Research. Published by the author, Ithaca, New York.

FOOTE, R. H. 1999. Artificial insemination from its origins up to today. In: V. Russo, S. Dall 'Olio, and L. Fontanesi (ed.) Proc. of the Spallanzani Int. Symp., Reggio Emilia, Italy. pp 23–67.

FOOTE, R. H., C. W. CARNEY. 2000. The rabbit as a model for reproductive and developmental toxicity studies. Reprod. Toxicol. 14:477–493.

GOTTARDI L., BRUNEL L., ZANELLI L., 1980. New dilution average artificial for insemination in the pig. 9th ICAR. Madrid. 5, 49-53

IRITANI, A. 1980. Problems of freezing spermatozoa of different species. In: Proc. 9th Int. Congr. Anim. Reprod. Artif. Insemin., Madrid, Spain. 1:115–131.

ITO, T., T. NIWA, A. KUDO. 1948. Studies on artificial insemination in swine. Zootech. Exp. Sta. Res. Bull. 55:1–74.

IVANOFF, E. I. 1922. On the use of artificial insemination for zootechnical purposes in Russia. J. Agric. Sci. 12:244–256.

IVANOW [Ivanov], E. I. 1907. De la fe´condation artificielle chez les mammife`res. Arch. Sci. Biol. 12:377–511.

JOHNSON L. TO, AALBERS J. G., GROOTE H. J. G., 1988. Artificial insemination of swine: Fecundity of to boar semen stored in Beltsville Thawing Solution (BTS), modified modena (MM), or MR-A and inseminated on one, three and four days to after collection. Zuchthyg 23, 49-55.

JOHNSON L. TO, WEITZE K. F., FISER P., MAXWELL W. M. C., 2000. Storage of to boar semen. Anim. Reprod. Sci. 62, 143 –172.

JOHNSON, L. A., D. RATH. 1991. Boar Semen Preservation. II. Second Int. Conf. Boar Semen Preservation, Beltsville, MD. Suppl. I, Reprod. Domestic Anim.

JOHNSON, L. A., G. E. SEIDEL, Jr. 1999. Proc. Current Status of Sexing Mammalian Sperm. Theriogenology 52:1267–1484.

JOHNSON, L. A., H. D. GUTHRIE. 2000. Boar Semen Preservation IV. Proc. IVth Int. Conf. on Boar Semen Preservation, Beltsville, MD. Allen Press, Laurence, KS.

KEMP B., SOEDE N. M., 1997. Consequences of variation in interval from insemination to ovulation on fertilization in pigs. J. Reprod. Fertil. Suppl. 52, 79-89.

HE L., BAILEY J.L. BUHR M.M. 2001. Incorporating Lipids into Boar Sperm Decreases Chilling Sensitivity but Not Capacitation Potential. Biology of Reproduction 64, 69-79

LAFOREST J. P., ALLARD D., 1996. For Comparison of four extenders long-term storage of fresh to boar semen. Reprod. Domest. Anim. 31, 275-276.

LEVIS D. G. 2002. New Reproductive Technologies For The AI Industry www.nsif.com/Conferences/2002/NewReproductiveTechnologiesAIIndustry.htm

LEVIS D. G., 2000. Liquid Boar Semen Production: Current Extender Technology and Where Do We Go From Here. In: Semen Boar Preservation IV. L.A. Johnson and H. D. Guthrie, eds. Allen Press, Inc. Lawrence, KS. pp. 121-128.

MAXWELL WMC, JOHNSON LA. 1997; Membrane status of boar spermatozoa after cooling or cryopreservation. Theriogenology 48:209–219.

MCKENZIE, F. F. 1931. A method for collection of boar semen. J. Am. Vet. Med. Assoc. 78:244–246.

MORETTI 1981 mentioned by Johnson L.A., Aalberts J.G. 1984. Artificial insemination of swine: fertility using several liquid semen diluents. 8th IPVS Congress Ghent, Belgica, 293.

NISHIKAWA, Y. 1964. History and development of artificial insemination in the world. In: Proc. 5th Int. Congr. Anim. Reprod. Artif. Insem., Trento, Italy. 7:163–259.

O'DELL, W. T., J. O. ALMQUIST. 1957. Freezing bovine semen. I. Techniques for freezing bovine spermatozoa in milk diluents. J. Dairy Sci. 40:1534–1541.

PARKS JE, GRAHAM JK. 1992. Effects of cryopreservation procedures on sperm membranes. Theriogenology; 38:209–223.

PAULENZ H., KOMMISRUD, HOFMO P. OR, 2000. Effect of Long-Term Storage AT Different Temperatures on the Quality of Liquid Boar Semen. Reprod. Sunday Anim. 35, 83-85.

PETTIT M.J., PLANTS C., POLLARD J.W. 2000. Long term fresh to extend development by simplex optimisation. IV Int. Conf. Boar Semen Preservation. Beltsville. P16.

POLGE, C. 1956. The development of artificial insemination service for pigs. Anim. Breed Abstr. 24:209–217.

POLGE, C. 1968. Frozen semen and the A.I. Programme in Great Britain. In: Proc. 2nd Tech. Conf. Artif. Insem. Reprod. NAAB, Columbia, MO. pp 46–51.

POLGE, C., A.U. SMITH, AND A. S. PARKES. 1949. Revival of spermatozoa after vitrification and dehydration at low temperatures. Nature (Lond.) 164:666.

PURSEL, V.G., JOHNSON, L.A., 1975. Freezing of boar spermatozoa: fertilizing capacity with concentrated semen in a new thawing procedure. J. Anim. Sci. 40, 99–102.

QUINN, PJ. Principles of membrane stability and phase behavior under extreme conditions. J Bioenerg Biomembr 1989; 21:3–19

RATH D., 2002. Low dose insemination in the sow - to review. Reprod. Domest. Anim. 37, 201-205.

TARDIF S., DUBÉ C., AND BAILEY J. L. 2003. Porcine Sperm Capacitation and Tyrosine Kinase Activity Are Dependent on Bicarbonate and Calcium but Protein Tyrosine Phosphorylation Is Only Associated with Calcium. BIOLOGY OF REPRODUCTION 68, 207–213

WABERSKI D., MEDING S., DIRKSEN G., 1997. Fertility of long-term stored to boar semen: Influence of extenders (Androhep and Kiev), storage Time and shapes droplets in the semen. Anim. Reprod. Sci. 36, 145-151.

WATSON PF, PLUMMER JM. 1985. The responses of boar sperm membranes to cold shock and cooling. In: Johnson A, Larsson K (eds.), Deep Freezing of Boar Semen. Proceedings of the First International Congress on Deep Freezing of Boar Semen. Uppsala, Sweden: L. Swedish University of Agricultural Sciences;: 113–127.

WEITZE K. F., 1990. Long- term storage of you extend to boar semen. Reprod. Sunday Anim. Suppl 1, 231-253

ZENG W. X., TERADA T., 2001. Protection of to boar spermatozoa from cold shock damage by 2-hydroxypropyl-beta-cyclodextrin. Theriogenology 55, 615-27. Spanish Journal of Agricultural Research (2003) 1 (2):17-27