PRACTICAL ASPECTS OF PORCINE SEMEN COLLECTION FOR CONSERVATION AND UTILIZATION OF FARM ANIMAL GENETIC RESOURCES

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Abstract

The aim of this study was to present the results of boar semen freezing for animal genetic resources. Our activity in this field was done under the supervision of The United Nations Food and Agriculture Organization (FAO) especially under the global network on Animal Genetic Resources (AnGR) for Food and Agriculture. The main objective of this activity is focus on the long-term conservation needs, but there is a room and material for research enough. The Westendorf method (FAO recommended this one, Westendorf et al., 1975) or its minor modification (we use the Minitüb modification with some our changes) is still widely used for boar semen cryopreservation for this purpose. We produced 4446 straws from 67 boars of Přeštice black-pied pig during 7 years period. Each ejaculate was tested before and during processing and after thawing. Average volume of ejaculate was 190.5 ml with 489.12 x 10^3/mm^3 spermatozoa. Sperm motility was 78.87 % before processing and 32.83 % after thawing. Forty-eight sows were inseminated with 52.08 % conception rate. The main success of this activity was the revitalization of four extinct genealogical lines of boars.

Key Words: Genetic resources, pig, cryopreservation

The United Nations Food and Agriculture Organization (FAO) plays a major role in assisting individual countries with conservation programs and provides a forum for international consultation and planning (Patterson and Silversides, 2003). The FAO definition of animal genetic resources (AnGR) eligible for conservation includes animal populations with economic potential, scientific use and cultural interest (Henson, 1992). AnGR are here defined as those animal species that are used, or may be used, for food production and agriculture and the populations within each (FAO, 2007). Animal genetic resources exist in the form of a vast array of breeds and livestock populations which have evolved and adapted over many centuries, to the range of environmental conditions encountered throughout the world (Henson, 1992).

The progress and future development of animal production for humans are dependent on the genetic variability between and within breeds. The researchers involved in animal conservation are fully aware that the stored material will be their legacy to future generations (Mariante at al., 2002). A conservative global estimate suggests that at least 28 % of farm animal breeds became extinct, rare or endangered in the past century (World Conservation Monitoring Centre, 1992). Conservation of genetic diversity leaves the option to use alternative traits and to develop new ones in the future (Roosen at al., 2005). In the current situation, where so many breeds are in danger of extinction, it is essential that the limited resources (personnel and financial) available be best used to ensure that as much valuable genetic diversity as possible survives into the future (Ruane, 1999a). Modern animal industry now uses only a few breeds of any species. Of the many breeds once commonly seen on farms, many have declined greatly in numbers and others have disappeared completely (Patterson and Silversides, 2003). A key question then is: which breeds should be chosen for conservation? In attempting to choose specific breeds for conservation programmes, the following seven criteria might be considered (Ruane, 1999b): (a) degree of endangerment; (b) species of the breed; (c) adaptation to a specific environment; (d) possession of traits of current or future economic importance; (e) possession of unique traits that may be of scientific interest; (f) cultural or historical value; and (g) genetic uniqueness.

There are three methods for the conservation of animal genetic resources. The first involves the conservation of animal genetic material in the form of living ova, embryos or semen stored cryogenically in liquid nitrogen (-196 ° C). The second is the preservation of genetic information as DNA, stored in frozen samples of blood or other animal tissue or as DNA segments. The third is the conservation of live populations (Henson, 1992). The main reproductive technologies that have been used or considered are artificial insemination (AI), embryo transfer (and its combination with *in vitro* fertilization) and sexing, gamete and embryo micromanipulation, sperm sexing, genome resource banking, and cloning. In most of the above examples, AI techniques and strategies have been significantly improved, however, there are large differences between species in insemination techniques and pregnancy rates using fresh or frozen semen (Andrabi and Maxwell, 2007).

At the 1989 FAO Expert Consultation it was agreed that frozen embryo and semen technology was cost effective for long term genetic preservation. Systematic cryopreservation and storage of semen from endangered species can facilitate maintenance of genetic heterozygosity, while minimizing movement of living animals between captive areas/zoos/research centres or countries (Johnston and Lacy, 1995). Using frozen-thawed spermatozoa would facilitate the infusion of new genetic material across populations by AI. The use of frozen sperm from semen banks increases the generation interval indefinitely and allows fewer males to be held in captivity because some of the genetic diversity is maintained strictly as frozen spermatozoa. Semen banks are currently developed for rare domestic breeds (bovine, ovine, caprine and porcine) (Andrabi and Maxwell, 2007). Cryopreservation semen, ova, and preferably embryos are successful for cattle, but are unfortunately difficult for some species (Patterson and Silversides, 2003). For other mammalian species the percentage post-thaw motile sperm or membrane-intact sperm is generally somewhat lower, but a fair post-thaw viability can be expected for most species. For many species the fertility of frozen semen is found to be lower than that of fresh semen. This may depend on the site of semen deposition, the morphology of the female genital tract, and the ability to detect heat or ovulation. There may be considerable differences between breeds and between males, in the 'freezability' of the semen (Hiemstra, 2005). Cryopreservation technologies for livestock, although advancing rapidly, are still well developed only for a handful of species. Freezing procedures for semen cryoconservation are species-specific, but the general procedures are as follows: a) following collection, semen is diluted in a suitable ionic (salt) or non-ionic (sugar) solution adjusted to near physiological osmolarity; b) suitable cryoprotectant is added – glycerol is most commonly used, but Dimethyl Sulfoxide (DMSO), Dimethylacetamide (DMA) or Dimethylformamide (DMF) are, depending on the species, of high practical interest; c) diluted semen is cooled, sampled and then frozen in liquid nitrogen (-196 °C); d) individual semen doses are generally frozen in straws rather than pellets to guarantee optimal sanitary conditions and permanent identification of each dose. Following AI with frozen and thawed semen (FAO, 2007). Deep intrauterine insemination techniques have been developed in pigs (Vazguez at al., 2005) and may, in general, contribute to the more efficient use of semen (less sperm per insemination). In pig production disadvantages of using frozen semen (reduced fertility, high freezing, storage and transport costs) are still larger than the advantages (Hiemstra, 2005).

In the Czech Republic, the need to save the remaining heritage breeds which have been largely and systematically replaced or "improved" in accordance with former state breeding policy raised at the turn of 80's and 90's of the passed century. The multiyear conservation project was approved by the Ministry of agriculture and the Institute of Animal Science in Prague – Uhrineves was designated for its implementation. Institute of Animal Science was qualified as the National Reference Centre for Conservation of Farm Animal Genetic Resources. The National Centre is member of the European Regional Focal Point for Animal Genetic Resources (ERFP). The Přeštice black-pied pig is an original local breed and since 1996 is included in the programme of preservation of animal genetic resources in the Czech Republic. Importance of this breed reflects in genes which provide excellent reproduction performance, adaptability, rather easy care, good vitality and resistance to diseases.

Material and methods

Sixty-seven boars of Přeštice black-pied pig destined for conservation of AnGR was cryopreserved during seven years period. Ejaculates were obtained from healthy and fertile mature boars on chosen farms. Sperm rich fractions were collected by the gloved-hand method and the gel portion was removed using double gauze. Semen volume and sperm motility were evaluated in fresh native boar semen. The sperm motility was assessed subjectively using phase contrast microscopy with a heating stage (38 ° C) at 100x magnification. Semen was extended at a semen dilution rate of 1 + 1.5 in Androhep, packaged in insulated container at 17 °C, and delivered to the laboratory. There was next semen evaluation. The sperm concentration was determined by a cytometric method using Bürker's chamber. Morphologically abnormal spermatozoa were assessed according to the staining method of Čeřovský (1976) and evaluated microscopically under oil immersion and 1500x magnification.

Ejaculates were cryopreserved using the straw freezing procedure described by Westendorf et al. (1975) and modified by Minitüb (Tiefenbach, Germany). Extended semen, next day after collection, was centrifuged at 1800g for 10 min at 17 °C, seminal plasma was removed and spermatozoa resuspended in refrigerated extender (11% lactose solution, egg yolk), cooled and kept 2 h at 5 °C. Then freezing extender with glycerol and Eques-Paste was added to sperm suspension. Final mixture was immediately loaded into 0.5 ml plastic medium-straws containing 0.5 x 10^9 active sperm before freezing/straw, put in fumes of liquid nitrogen at 20 min. Straws were finally transfered to a liquid nitrogen container for storage at –196 °C until utilization. Straws were thawed in a water bath at 38 °C for 40 s and extended in commercial extenders (38 °C). Sperm motility was evaluated immediately.

Examination of AI with frozen semen in 48 sows (Přeštice black-pied, Czech Large White and Czech Large White x Czech Landrace) was performed in semiindustrials conditions on the farm. Sows with a mean parity of 3.68 and normal reproductive performance were inseminated (and re-inseminated) with frozen-thawed semen in post-weaning heat. The insemination dose contained a total number of 7-8 x 10^9 spermatozoa. An intra-uterine insemination technique was used by comercial AI intra-uterine catheter VERONA (Minitüb).

Results and discussion

In terms of conversation of farm AnGR was cryopreserved 75 ejaculates from 67 boars of Přeštice black-pied pig. 4446 straws were produced for this purpose during 7 years. Results of the evaluation of the initial semen quality are presented in Table 1. The semen volume was higher in the reality than data shown in this study because most of boars were used to the next natural service, therefore, collections were limited.

The cryopreservation of boar spermatozoa is problematic up to now. High variability exists in freezability of boar semen. Our work found out that about 30 % of boars was unsuitable for cryopreservation. Average sperm motility after thawing was 32.83 % in boar with good freezability. Considerable variation is in post-thaw semen viability among boars.

The number collected ejaculates for cryopreservation according to the period are displayed in Figure 1. Only 2 ejaculates were recorded in the last year (by May 2008) because this period is not yet finished. There is an evident decline in breeding base, Přeštice black-pied pig is become a very endangered pig breed.

Table 2 describes the results obtained in the intra-uterine insemination with frozen semen in sows. The fertility of a frozen-thawed AI dose was markedly reduced when compared with liquid-preserved semen and natural service, post-thaw motility of boar spermatozoa was moved 35-40 %. Average length of pregnancy was 115.23 days. Tventy-one sows farrowed and four sows (16 %) aborted for obscure reasons at the first trimester of pregnanacy.

The main success of this activity was the revitalization of four extinct genealogical lines of boars.

Table 1. The mean values of boar semen quality parameters in Přeštice black-pied pig for conversation of AnGR

Semen volume	ml	**190.5**
Sperm concentration	$10^3/mm^3$	**489.12**
Sperm motility	%	**78.87**
Morphologically abnormal spermatozoa	%	**19.67**
Sperm motility after thawing	%	**32.83**

Figure 1. The total number of collected ejaculates for cryopreservation through the certain years

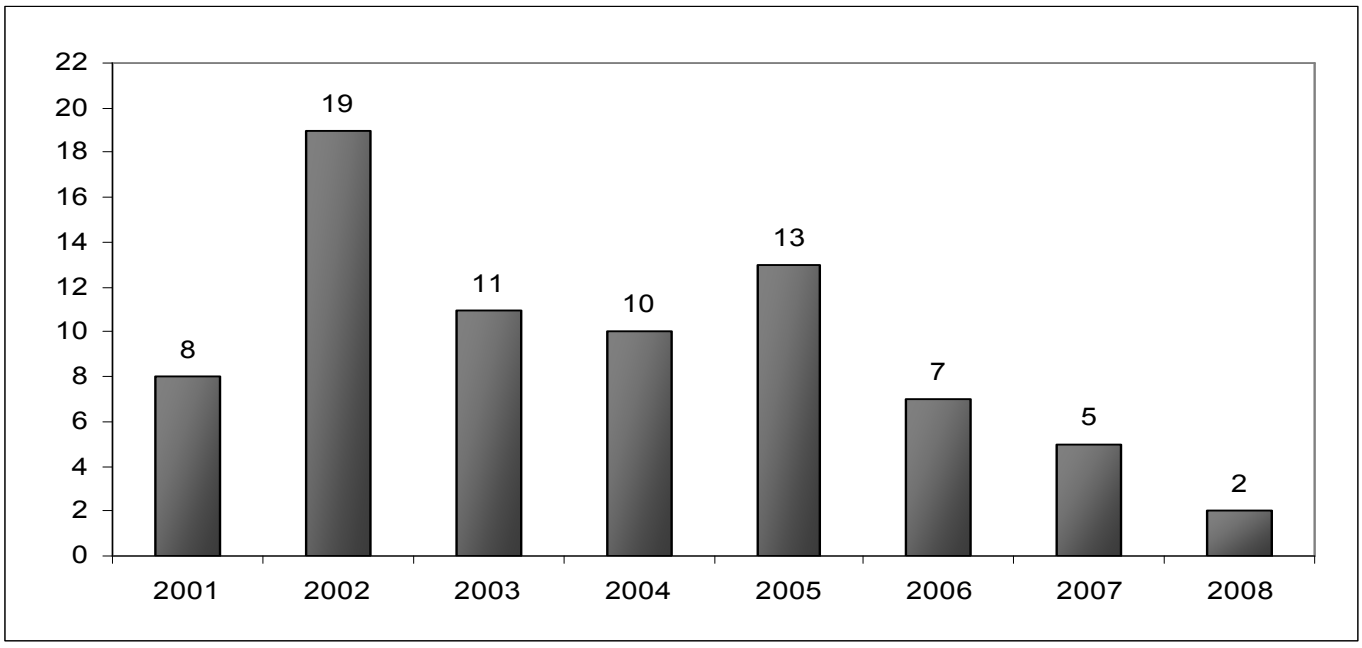

Table 2. The results of intra-uterine insemination with frozen semen of Přeštice black-pied boars

Number of inseminated sows	Weaning - oestrus interval	Conception rate	Farrowing rate	Litter size		Weaned piglets	Piglet losses
				All piglets born	Born alive		
	days	%	%	per litter	per litter	per litter	%
48	**5.46**	**52.08**	**43.75**	**8.32**	**7.66**	**7.11**	**9.23**

Conclusion

The present study evaluated the results of boar semen freezing for animal genetic resources in the Czech Republic. Using frozen-thawed spermatozoa with subsequent AI would facilitate the effort to maintain endangered pig breed, Přeštice black-pied pig, and adequate genetic diversity for future generations.

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