

The first method of Cryopreservation of Guinea Fowl semen

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France is the only country that practices the genealogic selection of guinea fowl genetic stocks and contains more than 80 % of the worlds guinea fowl breeders. This project was planned to deal with the increasing risk of line extinction for health and safety reasons (e.g.: bird influenza epidemic). To limit such risks, the aim of the present study was to establish the first method of freezing of guinea fowl semen that may introduce the *ex situ* management of guinea fowl livestock. For that purpose, we used the freezing methods currently employed to freeze chicken semen and progressively adapted them to guinea fowl spermatozoa. The method giving the highest rate of spermatozoa surviving and fertility rate after freezing-thawing was further chosen for the cryopreservation of guinea fowl spermatozoa.

Materials and Methods

Animals were of the Galor G55 line, and the males contained between 45 and 70% (mean 55%) of morphologically normal and living spermatozoa (%VNx) in their ejaculates. Different freezing methods previously established in the chicken (Seigneurin et Blesbois, 1995; Tselutin et al, 1999; Chalah et al, 1999) were tested in guinea fowl. Parameters of freezing including dilution rate and semen concentration, nature of the diluents and of the cryoprotectant agents, freezing rate and packaging of semen were then studied and optimized. From all these different aspects, packaging in straws gave the best results and was used in most of the experiments. The internal cryoprotectant DMF at the rate 6% was chosen because it is the most efficient for guinea fowl spermatozoa. Several saline diluents containing sugars (FEB, TSEL, BPSE, BPSE2, BHSV, IGGK; Tselutin et al, 1995; Sexton, 1977; Schramm, 1989; Surai, 1996) and for some of them an external cryoprotectant (polyvinyl pyrrolidone (PVP) for FEB, TSEL, BPSE2) or myoinositol (for BHSV and IGGK) were also tested. The semen quality tests used were the surviving rates (% VNx after freezing-thawing/VNx before freezing) measured after eosin-nigrosin stain and observation under the light microscope; and the fertility rates measured after artificial insemination and egg candling at day 9 of incubation. Analysis of variances and PLSD test. were used for statistical analyses.

Main results

The combination of an intermediate freezing rate (15°C/min) and a straw size of 0.5 ml gave rise to a surviving rate up to 38 % after semen freezing-thawing (Table 1). This survival rate was further enhanced (Table 2) by the use of diluents that contain myoinositol (BHSV and IGGK) as external cryoprotectant agent instead of PVP (FEB, BPSE2, TSEL) or no external cryoprotectant (BPSE). However, the two diluents that gave the highest survival rates differed in their ability to support the fertilizing capability of spermatozoa because the diluent IGGK that contains also a phosphate buffer gave significantly higher fertility rates than the diluent BHSV (Table3).

Conclusion

The initial semen quality of guinea fowl spermatozoa is lower than in most of the other domestic bird species (Massip et al, 2004; Blesbois et al, 2005). As a consequence, freezing induces severe injuries that lead to low survival rates and low levels of fertility after inseminations with frozen-thawed spermatozoa.

However, among the numerous factors of variation of the freezing method employed in this paper, the conditions of freezing rate and the nature of the diluent and of the cryoprotectant agents used were very important. Finally, the highest survival rates of spermatozoa after freezing-thawing (up to 50%) were obtained with semen diluted in IGGK diluent with 6% of the cryoprotectant agent Dimethylformamide (DMF), the freezing rate 15°C/min and the use of straws. This method led to a mean of 20 % fertility.

These results show the establishment of the first method of freezing of guinea fowl spermatozoa that may be used for the *ex situ* management of genetic variability in guinea fowl.

Table 1 Surviving rates of frozen-thawed spermatozoa (%) depends on the freezing rate and the type of straws.

Freezing rates (°C/min)	60	30	15	7
0.5 ml straws	25 ^c	28 ^b	37 ^a	25 ^c
0.25 ml straws	12 ^e	18 ^d	30 ^b	17 ^d

^{a, b, c, d} indicate significant differences (P<0.05)

Table 2 Surviving rates of frozen-thawed spermatozoa (%) depends on the diluents composition

Diluent	FEB	TSEL	BPSE	BPSE 2	BHSV	IGGK
Surviving rate (%)	33 ^a	34 ^a	38 ^a	38 ^a	47 ^b	53 ^b

^{a, b} indicate significant differences (P<0.05)

Table 3 Effects of diluents on the surviving rates (%) and fertilizing ability (fertility rate: % fertile/incubated eggs) of frozen-thawed spermatozoa.

	Surviving rates		Fertility rates	
	BHSV 3.0 × 10 ⁹ spermatozoa	28	12,2 ^b	
IGGK 3.0 × 10 ⁹ spermatozoa	35	20,7 ^a		

The fertility rates were obtained with two successive inseminations (each 3 days) per hen with 300×10⁶ spz/AI/hen. ^{a, b} indicate significant differences in the same column (P<0.05)

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