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Fertility Test of Frozen Boar Semen

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Abstract

The fertility results of two experiments are presented. In experiment 1, the semen was frozen in tris–fructose–EDTA or BF_3 diluents at $0{\cdot}25 \times 10^9$/ml sperm concentration and extended after thawing with either seminal plasma (SP) or the freezing medium (FM) containing no cryoprotective agent. In the second experiment the semen was glycerolated by two methods, frozen at $1{\cdot}0 \times 10^9$/ml sperm concentration, and extended with FM before insemination.

Fertility after double insemination within one oestrus with semen frozen in tris–fructose–EDTA or BF_3 diluents varied depending on the medium used for extension of thawed semen. The farrowing rates for semen frozen in the former diluent with FM and SP post-thawing media were 4/8 and 1/8 respectively, and for semen frozen in BF_3 diluent with FM and SP post-thawing extenders 1/8 and 5/8 respectively. The mean farrowing for the 32 animals inseminated was 34·4%.

Pregnancies for semen frozen in tris–fructose–EDTA and glycerolated at 30 or 5°C were 5/12 and 4/12 respectively, and for single and double inseminations 6/12 and 3/12 respectively. Of 24 animals inseminated 37·5% farrowed.

Introduction

Fertility of boar spermatozoa after freezing has been examined by a number of investigators (see Einarsson 1973; Paquignon and du Mesnil du Buisson 1973; Schmidt *et al.* 1974; Westendorf *et al.* 1975). For insemination of pigs via the cervix a relatively large volume of frozen–thawed semen is required which involves laborious processing, freezing and thawing procedures, and also limits the storage facilities. These disadvantages can be partly overcome by freezing a small volume of concentrated semen and extending the thawed semen before insemination (Pursel and Johnson 1975).

The experiments presented here were conducted to examine the effect of type of freezing diluent and post-thawing extender, and the effect of treatment of spermatozoa with glycerol on the fertility of boar spermatozoa.

Materials and Methods

Semen was collected by the manual method from Berkshire, Landrace and Large White boars in experiment 1, and from one Large White and two Saddleback boars in experiment 2. Only the sperm-rich fraction of the ejaculates showing good initial motility was used for processing. After removal of the gelatinous material by filtering through gauze, the semen of individual boars was pooled and the concentration of spermatozoa determined by a colorimeter calibrated against haemocytometer counts.

In experiment 1 the pooled semen was divided into two portions, then each part centrifuged at 600 *g* for 10 min and the seminal plasma removed. The spermatozoa in the two samples were re-suspended at 30°C with Beltsville-F_3 (BF_3, Pursel and Johnson 1971) and tris–fructose–EDTA diluents to a cell concentration of $0{\cdot}25 \times 10^9$/ml. Both diluents contained 5% (v/v) glycerol. The

samples were cooled to 5°C in 4 h, then centrifuged again at 1000 *g* for 10 min (5°C). After removal of the supernatant, the spermatozoa were resuspended to a concentration of $0{\cdot}25 \times 10^9$/ml with the initial diluent containing no glycerol.

In experiment 2 the pooled semen was extended at a ratio of 1 : 2 (semen : diluent, at 30°C) with tris–fructose–EDTA diluent and cooled to 5°C in 2 h. After cooling, the diluent seminal plasma mixture was removed by centrifugation (1000 *g* for 10 min at 5°C) and the spermatozoa resuspended with the initial diluent to a concentration of $1{\cdot}0 \times 10^9$/ml. The glycerol (2%, v/v) was included in the diluent used either for dilution at 30°C (and removed after cooling), or for resuspension at 5°C (and present during freezing).

The tris–fructose–EDTA diluent consisted of 250 mM tris, 111 mM fructose, 75 mM citric acid, 15 mM EDTA (disodium salt), and 15% (v/v) egg yolk. Potassium penicillin G (1 000 000 i.u./litre) and dihydrostreptomycin sulphate (1 g/litre) were included in all diluents. When the freezing diluent was also used for extension of thawed semen, it contained no glycerol or egg yolk (tris–fructose–EDTA), or no glycerol or casein (BF_3). Freezing of semen in both experiments was carried out by the pellet method, and the frozen pellets (0·2 ml) were stored in liquid nitrogen for 1–3 weeks until used for insemination.

For insemination, the frozen pellets were thawed in a series of large test tubes (3·5-cm diameter) held in a water-bath at 37°C (15–20 pellets per tube). The inseminate volumes were 120 ml (expt 1) and 80 ml (expt 2) which were obtained by adding 40 and 60 ml of the post-thawing extender to 80 and 20 ml of thawed semen respectively. The inseminate volumes in both experiments contained a total of 20×10^9 spermatozoa. The thawed semen was examined for motility of spermatozoa before and after addition of the post-thawing extender. In experiment 1 semen smears were also made from each inseminate and examined for acrosome morphology as described by Osinowo and Salamon (1976).

The animals used were Large White and Large White × Landrace sows and 8–9-month-old gilts. Oestrus was detected by teaser boars twice daily at 0900 and 1600 h. In experiment 1 each pig was inseminated twice within one oestrus, whilst in experiment 2 both single and double inseminations were carried out. Single insemination was performed 24 h after detection of oestrus. When double insemination was involved, the animals detected in oestrus in the morning were inseminated in the evening of the day of detection (8 h later), and in the morning of the following day (24 h after detection). The animals detected in oestrus in the evening received the inseminations in the morning and evening of the following day (17 and 24 h after detection). All inseminations were performed through the cervix using a rubber catheter.

Experimental Details and Results

Experiment 1

The factors included in this experiment (2 × 2 factorial) were:

(1)	Type of freezing diluent	Tris–fructose–EDTA *v.* BF_3
(2)	Type of post-thawing extender	Seminal plasma *v.* freezing diluent containing no egg yolk or glycerol (tris–fructose–EDTA), or containing no casein or glycerol (BF_3).

Two inseminations within one oestrus were performed on a total of 32 pigs, and the sows and gilts were distributed equally to each treatment.

The mean percentages of motile and normal spermatozoa in the inseminates are summarized in Table 1. While post-thawing extension of semen with the freezing diluent had no detectable effect, addition of seminal plasma to the thawed semen significantly depressed the motility of spermatozoa. Percentages of spermatozoa with acrosomes appearing normal after addition of post-thawing media were statistically indistinguishable (Table 1).

The farrowing results are presented in Table 2. Fertility after insemination with semen frozen in the two diluents varied depending on the post-thawing extender used (type of freezing diluent × type of post-thawing extender; $\chi^2_{(1)} = 6{\cdot}96$, $P < 0{\cdot}01$).

Farrowing for the semen frozen in tris–fructose–EDTA diluent was better when the same medium (containing no egg yolk or glycerol) was added to the thawed semen. On the other hand, the semen frozen in BF_3 diluent yielded better fertility when seminal plasma rather than the freezing medium (containing no casein or glycerol) was used for post-thawing extension.

Table 1. Percentage of motile and normal spermatozoa in the inseminates (experiment 1)

Values given are means ±s.e. PTE, Post-thawing extender

Time of examination	Tris–fructose–EDTA freezing diluent		BF_3 freezing diluent	
	Tris–fructose–EDTA PTE	Seminal plasma PTE	BF_3 PTE	Seminal plasma PTE
	Motile spermatozoa (n = 16)			
Before addition of PTE	28·5±0·76	29·2±0·56	30·0±0·87	27·7±0·79
After addition of PTE	26·0±1·25	25·4±0·42	27·9±0·96	22·7±0·79
	n.s.	$P < 0·001$	n.s.	$P < 0·001$
	Spermatozoa with normal acrosomes (n = 16, 8 duplicate samples)			
After addition of PTE	20±1·2	22±1·5	24±2·0	21±0·7

Table 2. Farrowing results following insemination with frozen semen (experiment 1)

Freezing diluent	Post-thawing extender	No. of pigs inseminated	No. farrowing	Litter size
Tris–fructose–EDTA	Tris–fructose–EDTA[A]	8	4	4, 8, 9, 10
	Seminal plasma	8	1	6
BF_3	BF_3[B]	8	1	4
	Seminal plasma	8	5	2[C], 3[C], 4, 8, 10
Totals and means		32	11 (34·4%)	6·8

[A] Contained no egg yolk or glycerol. [B] Contained no casein or glycerol. [C] One still-born in each litter.

Farrowing for the gilts and sows used was 5/16 and 6/16 respectively. The average litter size for the animals inseminated with semen frozen in tris–fructose–EDTA and BF_3 diluents was 7·4 and 5·1 respectively, and for the total of 11 animals that farrowed the value was 6·8. Five animals returned to service 27–60 days after insemination and of these, four had been inseminated with semen frozen in BF_3 diluent.

Experiment 2

The factors included in this experiment (2×2 factorial) were glycerol treatment (addition at 30°C *v.* 5°C) and number of inseminations within one oestrus (single *v.* double insemination).

The mean post-thawing motility of spermatozoa was similar for glycerol addition at 30 and 5°C (26·1±0·86% *v.* 26·7±0·90%; n = 18). A total of 24 pigs (6 per treatment) were inseminated with semen frozen in a concentrated state ($1·0 \times 10^9$ spermatozoa/ml). Nine animals farrowed a total of 78 live pigs and one still-born.

The average litter size was 8·7 pigs. The farrowing rates for semen glycerolated at 30 and 5°C were 5/12 and 4/12 (litter sizes 9·2 and 8·3) respectively, and for single and double inseminations the rates were 6/12 and 3/12 (litter sizes 8·0 and 10·3) respectively.

Discussion

Although the type of medium used in the first experiment for extension of the thawed semen had a detectable effect on motility, the number of motile spermatozoa in the inseminates varied only within a narrow range ($4·5–5·6 \times 10^9$). Farrowing after double insemination with semen frozen in tris–fructose–EDTA and BF_3 diluents varied depending on the post-thawing medium used (Table 2). Whilst seminal plasma depressed the fertility of semen frozen–thawed in the former diluent, it was beneficial when used for extension of semen frozen–thawed in BF_3 diluent. The frozen pellets were thawed in dry test tubes (without thawing solution) and the extending medium was added to the thawed semen shortly before insemination. Thus, the seminal plasma or the respective diluent buffer did not participate in the thawing procedure, but probably could have affected the transport of spermatozoa within the female genital tract. There are reports that thawing of frozen boar semen in seminal plasma improved the fertility (Crabo and Einarsson 1971; Crabo *et al.* 1972; Einarsson *et al.* 1973). This was attributed to the effect of seminal plasma on the contraction of the uterus and oviducts and better survival of spermatozoa in these parts of the tract (Einarsson and Viring 1973*a*, 1973*b*; Viring *et al.* 1974). On the other hand, it was reported that the decrease in the proportion of seminal plasma in the thawing solution improved the fertility (Hahn *et al.* 1974), and satisfactory fertility was also reported after thawing the pellet-frozen semen in other media (e.g. in INRA–ITP by Paquignon and du Mesnil du Buisson 1973; in BTS by Pursel and Johnson 1975).

In the second experiment the spermatozoa were in contact with glycerol either during the 2-h period of cooling from 30 to 5°C, or for a shorter time at 5°C (15–20 min) after centrifugation and resuspension. In the latter case the cryoprotective agent was present during freezing. Differences in fertility of semen processed by the two methods and frozen at a sperm concentration of $1·0 \times 10^9$/ml were indistinguishable. No explanation can be offered for the poorer results obtained after double compared with single insemination. The mean farrowing in experiment 2 (37·5%) was lower than the egg fertilization rates obtained with semen frozen at a sperm concentration of $0·6 \times 10^9$/ml by Pursel and Johnson (1975).

Finally, it should be mentioned that the animals which returned to service 27–60 days after insemination in experiment 1 probably conceived, but embryonic mortality occurred. Late returns to service following insemination with frozen–thawed semen were also observed by Baranov (1972) and Salamon and Visser (1973, 1974).

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