FERTILITY TEST OF FROZEN BOAR SPERMATOZOA*

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Abstract

Fertility of pellet-frozen boar spermatozoa was examined in three tests using single and multiple inseminations within one oestrus. One of 14 and two of 4 sows farrowed after one and three inseminations in test 1. Six of 10 gilts farrowed to three inseminations in test 2. In test 3 one of 4 and two of 9 sows farrowed after one and three inseminations respectively. In the three tests 41 animals were inseminated of which 12 produced a total of 100 pigs (96 live, 4 dead).

Introduction

Research on frozen storage of boar semen has been intensified during the last few years and several investigators (reviewed by Graham *et al.* 1971) have reported as obtaining moderate to satisfactory fertility following normal insemination. Deposition of spermatozoa directly into the oviducts of sows by a surgical method (Polge *et al.* 1970) seems to give more reliable fertility. Nevertheless, the use of this technique has its limitations and for practical application of frozen boar semen normal insemination via the cervix is necessary.

This communication gives an account of fertility tests in which single and multiple inseminations (via the cervix) were carried out using frozen–thawed semen.

Methods

Semen was collected by the manual method from one Berkshire and two Large White boars, and only the sperm-rich fractions of the ejaculates showing good initial motility were used. After removal of the gelatinous material by filtering through sterile gauze, the semen was diluted (1 : 2, semen : diluent) at 30°C by one addition of yolk–glucose (315 mM) diluent (Salamon *et al.* 1971) or of yolk–Tris (250 mM)–fructose (111 mM)–citric acid (79·5 mM) extender which was elaborated by us in a series of laboratory studies. The diluted semen containing 15% (v/v) egg yolk and 4% (v/v) glycerol was cooled to 5°C in 1·5 hr, then pelleted (0·30–0·40 ml) on dry ice (Nagase and Niwa 1964) and subsequently stored in liquid nitrogen for 2–5 weeks.

For insemination, 30–40 pellets were thawed in Erlenmeyer flasks (test 1) or 15–20 pellets were thawed in a series of large test tubes (tests 2 and 3) by shaking in a water-bath at 37°C until the semen melted.

Inseminating doses of 70–80 ml containing $8–15 \times 10^9$ motile spermatozoa were used. The sows allocated to single insemination were inseminated 24 hr after the detection of oestrus by a teaser boar. When multiple inseminations were carried out, the animals detected in oestrus in the morning received the inseminations in the evening of the day of detection (8 hr later) and in the morning and evening of the following day (24 and 32 hr after heat detection). The animals detected in oestrus in the evening were inseminated in the morning and evening of the following day (16 and 24 hr later) and in the morning of the third day (40 hr after heat detection). For the first and third inseminations pooled semen from the two Large White boars and for the second insemination semen from the Berkshire was used. Each female exhibited the standing reflex at the time of the third insemination. The insemination was performed with a rubber catheter (Melrose and O'Hagan 1961).

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Results and Discussion

The results are presented in Table 1. In the three tests 41 animals were inseminated, of which 12 farrowed a total of 100 pigs (96 live, 4 dead). The farrowing following single insemination in test 1 was low. Multiple insemination in tests 1 and 2 resulted in satisfactory fertility (2/4 and 6/10). In test 3 the same sows which already had litters from frozen–thawed semen in the previous tests were used for multiple insemination, yet the fertility was rather modest. When the farrowing rates obtained after single and multiple inseminations in all three tests were compared (2/18 *v.* 10/23), the difference did not reach statistical significance ($\chi^2_{(1)} = 3{\cdot}67$; $0{\cdot}05 < P < 0{\cdot}10$). The average litter size (8·3) compared favourably with those observed after natural mating in Australia (Penny *et al.* 1971). The animals which returned to service 49–56 days after insemination apparently conceived, but the pregnancies were terminated. Returns from 35 to 75 days following insemination with frozen–thawed boar semen were also reported by Baranov (1972).

TABLE 1

FARROWING DATA AFTER INSEMINATION WITH FROZEN–THAWED BOAR SEMEN

No. of inseminations	Diluent used for freezing	No. of females inseminated	No. of animals farrowing	No. of pigs born in litter
	Test 1 (insemination in January–February 1971)			
1	Yolk–glucose	14 sows*	1	7
3	Yolk–glucose	4 sows	2	4, 11‡
	Test 2 (insemination in October–November 1971)			
3	Yolk–glucose	2 gilts†	1	8
3	Yolk–Tris–fructose	8 gilts	5	9, 9, 7, 7, 6
	Test 3 (insemination in March 1972)			
1	Yolk–Tris–fructose	4 sows	1	11
3	Yolk–Tris–fructose	9 sows	2	10, 11

* Two returned to service 49 days and one 56 days after insemination.
† One returned to service 56 days after insemination.
‡ 7 live, 4 dead.

In the present study semen of the Berkshire boar was used for the second insemination; nevertheless, the efficiency of this insemination in the Landrace and Large White females used could not be detected, as the white colour is dominant to the black (Bogart 1959). It can be assumed that the second and third inseminations were more effective, as they were performed closer to the time of ovulation, which is generally reported to occur during the second half of the heat (30–46 hr after onset of oestrus).

The present study showed that normal pregnancies and litters can be obtained following normal insemination with semen frozen in either yolk–glucose or yolk–Tris–fructose diluting media. Multiple insemination may result in increased fertility. Successes in obtaining fertility have also been reported with spermatozoa frozen in other diluents, such as Beltsville F3 (Pursel and Johnson 1971, 1972), Beltsville L1

(Pursel *et al.* 1972), TES–glucose–yolk (Crabo and Einarsson 1971; Crabo *et al.* 1972), and TEST buffer (Graham *et al.* 1971; Graham and Crabo 1972). It remains to confirm under field conditions the results obtained in this study and in studies by other workers.

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