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# Fertility of inactivated bovine sperm

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## ABSTRACT

Bovine sperm was inactivated by centrifuging twice through a 7% Ficoll solution. The inactivated sperm could be reactivated for periods up to 10 days with B.S.A., egg yolk, seminal plasma and milk. To test the effect of inactivation on fertility semen was either (1) immediately diluted in Caprogen + 5% egg yolk; or (2) twice centrifuged and then diluted as above; or (3) twice centrifuged, stored in an inactivated condition for 60 h and then diluted as above. Cows were inseminated 72 h after collection of semen. Fertility following insemination was (1) 74.3%; (2) 72.5%; (3) 54.2%. Storage of sperm in the inactivated condition reduced fertility ( $P < 0.01$ ).

## INTRODUCTION

Bovine semen diluted in Caprogen and stored at ambient temperatures can be used at very high dilution rates ( $2 \times 10^6$ /insemination) without loss of fertility (Shannon, 1968). At this dilution rate fertility is maintained up to 60 h after dilution. Preservation of fertility for longer periods would obviously be an advantage but at present the only technique that allows long term storage of bovine semen (deep freezing) requires comparatively large numbers of sperm (20 to  $25 \times 10^6$ /insemination) to achieve optimum conception rates (Milk Marketing Board, 1971; Macmillan, *et al.*, 1978). The present study investigates the fertility of bovine semen stored for 3 days after inactivation by centrifuging through a Ficoll-containing medium.

## MATERIALS AND METHODS

The method is essentially an adaption of the method of Harrison (1976) for washing spermatozoa. Freshly collected bovine spermatozoa were diluted to contain  $400 \times 10^6$  sperm/ml in Caprogen (Shannon, 1964; 1965) with 5% egg yolk. One ml of diluted semen was layered on 5 ml 14 G (Shannon, 1964) + 7% Ficoll (mol wt 400 000) and then centrifuged at ambient temperatures in an M.S.E. table-top centrifuge at 2500 r.p.m. for 12 min. At this stage spermatozoa were present as a loose plug at the bottom of the centrifuge tube. The supernatant was aspirated, the sperm plug recovered, rediluted to 1 ml in 14 G and again layered on the Ficoll 14 G medium. The sample was then centrifuged for 12 min at 2500 r.p.m. and the sperm plug recovered. When diluted to contain  $20 \times 10^6$  sperm/ml less than 1% of these recovered sperm exhibited forward motility though between 30 to 70% showed slow tail movement. This is in close agreement with results with ram semen (Tervit *et al.*, 1982).

For fertility trials split ejaculates of 4 bulls were treated as follows: approximately one-third of the ejaculate was routinely diluted in 5% egg yolk Caprogen to contain  $4 \times 10^6$  sperm/ml (treatment 1). The remaining two-thirds was inactivated as above. Half of this material was then diluted in 5% egg yolk Caprogen to contain  $4 \times 10^6$  sperm/ml (treatment 2). This treatment was included to test the effect of centrifuging on fertility. The remainder was stored in dialysis sacs at  $400 \times 10^6$  sperm/ml and immersed in cylinders containing Caprogen buffer so that sperm concentration relative to total buffer volume was  $4 \times 10^6$  sperm/ml. After 60 h storage the inactivated sperm was diluted in Caprogen + 5% egg yolk to contain  $4 \times 10^6$  sperm/ml (treatment 3).

## RESULTS AND DISCUSSION

When inactivated sperm were stored in buffer it was observed that the slow tail movement shown in freshly inactivated sperm gradually decreased so that after 3 days storage this had disappeared. Up to 4 days of storage the sperm could be readily activated by a number of compounds, namely bovine serum albumin, seminal plasma, egg yolk and milk. After reactivation up to 80% of the sperm exhibited forward motility and were indistinguishable from freshly collected semen. Generally 10 to 30 min elapsed between the addition of the activating substance and full restoration of motility.

After 4 days storage the interval between addition of activating compound and restoration of motility became progressively longer until at 10 days the ability to reactivate sperm was generally lost. This inability to reactivate was not associated with disintegration of the mid-piece membrane usually associated with sperm senescence (Shannon and Curson, 1972; 1982). The fertility of cows inseminated with inactivated sperm is shown in Table 1.

**TABLE 1** Number of inseminations and 49-day non return rate % (NR) for semen stored for 72 h before use.

Treatment	1		2		3	
	No.	NR	No.	NR	No.	NR
Bull 1	223	74.9	230	62.6	309	53.7
2	279	74.6	288	75.0	303	53.8
3	495	71.9	255	69.4	55	61.8
4	398	76.9	263	81.0	74	52.7
Total/av.	1395	74.3	1036	72.5	741	54.2

The difference between treatments in non-return rate was highly significant ( $P < 0.01$ ). The major contributing factor was the depression caused by storing sperm in an inactivated condition (control minus inactivated storage, 20.1%). There was also a highly significant bull  $\times$  treatment interaction ( $P < 0.01$ ). This was largely attributable to the apparent detrimental effect of centrifuging on sire 1 (control minus centrifuged material, 12.3%).

The reasons for the poor fertility are unknown. This may be due to poor survival *in vivo* after reactivation, or possibly the loss of ability to fertilise after reactivation. The results do however show the beneficial effect of egg yolk on sperm survival during storage. Whether other proteins would be as effective is not known. Certainly adding seminal plasma back after centrifuging had a detrimental effect on sperm survival probably due to its toxic effect (Shannon, 1965). Testing of this treatment was considered unwarranted.

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