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In Vitro Survival of Washed
Boar Spermatozoa

W. L. Singleton

Fertility of boar semen stored under present conditions is at best only adequate after 24 hr. of storage and decreases sharply thereafter. It is usually recommended that boar semen not be stored more than 48 hr. for artificial insemination use. The goal is to find a means whereby boar semen fertility could be retained indefinitely by freezing. Until a successful freezing technique is found, a method of storing boar semen for a period of four to five days at above freezing temperatures would be of value to artificial insemination of swine.

Several studies have shown that spermatozoa stored in the epididymis may remain immotile, but fertile, for several days in the sexually inactive male. It is thought that during ejaculation when the spermatozoa come into contact with the accessory gland secretions their metabolism and motility are stimulated. If this high rate of metabolism is not reduced, the length of in vitro survival time is rather short. The metabolic rate may be decreased and the duration of fertility and motility increased by several methods such as decreased temperature, addition of metabolic inhibitors and the alteration of certain ionic ratios.

The objective of this experiment was to study the effect of removing the seminal plasma immediately after ejaculation on the in vitro survival of spermatozoa.

Procedure

The sperm-rich fractions of 20 ejaculates from two mature Yorkshire boars were collected by the gloved hand technique. An equal volume of semen from each boar was pooled and the concentration and initial motility were determined. One portion of the pooled semen was centrifuged at 550 g for 7 minutes and the seminal plasma was removed by aspiration. The spermatozoa were resuspended and washed twice in an equal volume of an artificial medium containing 0.30 gm. glucose, 0.15 gm. sodium bicarbonate, 0.1 gm. dihydrostreptomycin, and 0.06 gm. penicillin "g" sodium per 100 ml. distilled water. One aliquot of washed spermatozoa was resuspended in the artificial medium and another aliquot in the artificial medium plus 20% egg yolk to a concentration of 10^8 sperm per ml. The unwashed portion was extended to 10^8 sperm per ml. in the artificial medium plus 20% egg yolk. Aliquots of each suspension were incubated for two hr. at 38° C. in a constant volume respirometer at 0 hr. to measure oxygen consumption. The semen was stored at 15° C. for 72 hr. and motility estimations (hanging drop technique) were made at 0, 24, 48 and 72 hr. by warming an aliquot to 38° C. and diluting 1:1 with a physiological dextrose solution.

Results and Discussion

The mean motility estimations for each method of storage are shown in table 1. The motility of washed boar spermatozoa resuspended in an artificial medium with egg yolk exhibited a higher percent motility at each 24 hr. period of storage than spermatozoa resuspended in only the artificial medium or unwashed spermatozoa. Oxygen consumption for a two hr. period indicates that the rate of metabolism for washed spermatozoa either resuspended in only the artificial medium or in the artificial medium plus egg yolk was lower (115 mcl. and 128 mcl./spermatozoa, respectively) than unwashed spermatozoa (187 mcl./10⁸ spermatozoa).

These data indicate that removing the seminal plasma immediately after ejaculation may be of some value in increasing the survival time of boar spermatozoa when stored at 15° C. in an artificial medium.

Table 1. Mean Percent Motility of Spermatozoa Stored at 15° C. for 72 Hr.

Treatment	Hours of Storage			
	0	24	48	72
Washed and stored in artificial medium	84	65.7	47.0	22.4
Washed and stored in artificial medium plus egg yolk	84	70.6	55.5	35.2
Unwashed and stored in artificial medium plus egg yolk	84	50.6	27.2	12.3