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Influence of Temporary Chemical Immobilization of Boar Spermatozoa Upon In Vitro Survival

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The use of artificial insemination (AI) in swine has been restricted because of the inability to store semen. It is usually recommended that for AI use boar semen not be stored more than 48 hours. Obviously, the goal is to develop a technique whereby boar semen could be stored indefinitely such as is the case with bull semen. However, until such a technique is developed, a method of storing boar semen for a few days would be of value for promoting AI in swine.

It is well documented that sperm cells stored in the epididymides may remain immotile, but fertile, for extended periods of time in the sexually inactive male. During storage in the epididymides, spermatozoa undergo both morphological and metabolic changes. Among these are a change in potassium (K):sodium (Na) ratio and the acquisition of fertility.

In 1963, researchers at the University of Illinois developed a method for the collection of ejaculated bull spermatozoa directly into an inhibitory medium which rendered the cells immotile and prevented the absorption of carbohydrates from the seminal plasma. Following dilution or removal from the media, the cells regained their motility and metabolism. One of the most important components of this media was the K:Na ratio created from a mixture of sodium bicarbonate, potassium bicarbonate and citric acid.

Therefore, the following experiments were conducted to answer these objectives: (1) to determine the optimum K:Na ratio in medium for prolonged in vitro storage of boar sperm, and (2) to determine the subsequent fertility of inhibited boar semen.

Experimental Procedures

The sperm-rich fraction of ejaculates from two mature Yorkshire boars were collected by the gloved-hand technique.

Experiment I

These studies were directed toward determining the optimum levels of potassium (500, 650 or 800 mg.) and the ratio of K:Na (1:1, 2:1, 3:1 or 4:1) as indicated by the level of reversible motility.

The sperm-rich fractions from the two boars were pooled at each collection and an aliquot (3 ml.) of the pooled semen was immediately transferred to 9 ml. of each inhibitory medium. These tubes were capped and incubated at $15 \pm 0.5^{\circ}$ C. (59° F.). Motility estimates were made at 0 hour (5 minutes after inhibition) and 72 hours by diluting the inhibitory media 1:1 with physiological dextose and exposing to air for 4 minutes. This allowed motility to be regained.

Experiment II

The K level and K:Na ratio in Experiment I which appeared nearest optimum was evaluated more thoroughly by determining its influence on pH (relative acidity) and secondary sperm abnormalities.

Experiment III

The dry ingredients of the inhibitory media were placed in a vacuum flask and 100 ml. of distilled water (body temperature) were added just prior to emission of the sperm-rich fraction. The sperm-rich fraction was collected directly into the vacuum flask which was quickly capped. The diluted semen was then stored at $15 \pm 0.5^{\circ}$ C. (59° F.) for 0, 48 or 96 hours.

A total of 30 crossbred gilts were used to determine subsequent fertility. The estrous cycles were synchronized by feeding ICI 33828 (Aimax) at 100 mg. per gilt per day for 20 days. Following Aimax withdrawal the gilts were checked daily for the onset of estrus and were inseminated 24 hours later.

Results and Discussion

Experiment I

The initial motility prior to placement in the inhibitory medium was 87%. This compares with a motility of 75 to 85% for sperm cells inhibited for 5 minutes (table 1, 0 hour). This suggests that some sperm cells (2 to 12%) failed to recover from the immotile state.

The medium with the lowest percent of sperm cells which failed to regain motility was 650 mg. K per 100 ml. at a ratio to Na of 3:1. Therefore, this media was evaluated more thoroughly in Experiment II.

Experiment II

Initial motility before reactivation was completely inhibited by the medium in all observations. Upon dilution, exposure to air and warming, motility was readily regained (table 2, 0 hour). Motility decreased to 64% at 72 hours and 41% at 144 hours. If semen were handled similarly, but without the inhibitory medium, expected motility at 72 and 144 hours would have been considerably less. Therefore, it does appear that the inhibitory media enhanced maintenance of motility.

The pH decreased (became more acid) during storage (table 2) and indicates that metabolism occurred during storage. There was essentially no increase in secondary abnormalities during storage which suggests very little cell damage resulted from the extension and storage techniques.

Experiment III

Fertility of semen from two boars stored in the inhibitory medium for 0, 48 and 96 hours is summarized in table 3. The numbers of matings made are small; therefore, these results should be considered preliminary.

The conception rates (pooled across hours of semen storage) were 43% and 82% for boars 173 and 156, respectively. This might suggest a difference between

boars, which would not be unusual since it is known that males within all species have differences in their ability to settle females.

However, probably the most important observation presented in this table is the 55% conception rate, on limited numbers, from semen stored for 96 hours. This storage time is twice as long as normally recommended for boar semen.

Summary

Even though the numbers are limited, preliminary results of boar spermatozoa collected directly into an inhibitory medium (consisting of potassium bicarbonate, sodium bicarbonate and citric acid) indicate a trend to prolong the fertile life of stored semen. This method of collection may be of value for swine AI and justifies further study.

Table 1. Effect of Potassium Level and Potassium:Sodium Ratio on the Reversible Inhibition of Spermatozoa Motility (Percent)

Potassium bicarbonate, mg.	K:Na ratio 1:1		2:1		3:1		4:1	
	Hours of *in vitro* storage							
	0[a]	72	0	72	0	72	0	72
500	84	47	80	52	56	80	80	33
650	85	36	80	57	85	67	77	39
800	76	31	75	49	81	38	75	32

[a] Zero hour refers to about 5 minutes in the inhibited state and then motility reversed. Average motility before inhibition was 87%.

Table 2. Effect of Storage Time on Motility, pH and Secondary Abnormalities of Spermatozoa Stored in an Inhibitory Medium Containing 650 mg. K/100 ml. and a 3:1 K:Na Ratio

Storage time, hr.	Motility %	pH	Secondary abnormalities, %
0	84	7.6	4.5
72	64	7.1	4.8
144	41	6.4	5.2

These are means from 2 boars and 4 ejaculates per boar.

Table 3. Fertility of Boar Semen Stored in the Inhibitory Medium for 0, 48 or 96 Hours Prior to Insemination

Boar	Hours of Storage			Total
	0	48	96	
173	3/4[a]	1/5	2/5	6/14
	75%	20%	40%	43%
156	3/3	3/4	3/4	9/11
	100%	75%	75%	82%
Total	6/7	4/9	5/9	15/25
	86%	44%	55%	60%

[a] Number of gilts pregnant/number of gilts inseminated.