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SURVIVAL OF BOAR SPERMATOZOA INTENDED FOR *IN VITRO* FERTILIZATION (IVF) FOLLOWING DIFFERENT SPEED, DURATION AND FREQUENCY OF SPERM WASHING

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ABSTRACT

A 3 x 2 x 2 factorial experiment in completely randomized design (CRD) was conducted to determine the effects of various washing treatments on the survival of boar sperms intended for IVF. Fresh boar spermatozoa survived various speeds (2000, 1500, or 1000 rpm), duration (10 or 5 min) and frequency (once or twice) of washing with modified Bracket and Oliphant (BO) solution. There are no interaction effects (P>.05) among factors and the main effects are likewise not significant (P > .05). Average initial sperm motility was 78.33% whereas post treatment motility varied from 66.67 to 75.00% at 0 hr, 58.33 to 71.67% at 1 hr; and 53.33 to 70.00% at 2 hr. Mean viability index ranged from 62.67 to 76.00. Indication of incomplete removal of the seminal plasma was evident in treatments with single washing.

INTRODUCTION

The production of mammalian embryos *in vitro* has been a major focus of animal biotechnology work for nearly two decades now. This is because of the expected enormous benefits that could be derived from this technique viz: production of more animals, induction of twinning or the possibility of embryo micromanipulation so as to produce animals with the desired sex, high milk yield, faster growth rate, or high immunity/tolerance to infection. The present work on IVF is really very interesting because of the possiblity of growing and cultivating immature oocytes in suitable laboratory culture conditions. We have very cheap sources of immature oocytes that could be utilized for IVF studies, i.e. from ovaries of slaughtered animals in the abbatoirs. Embryos produced can later be transferred to surrogate mothers or preserved cryogenically for future use.

For successful fertilization not only oocytes, but also spermatozoa require special treatment necessary for the induction of terminal events. These events, capacitation and acrosome reaction, occur in natural condition inside the female reproductive tract and form sperms competent for fertilization. Capacitation involves the removal of macromolecular material, a decapacitation factor (DF) located on the surface of the sperm. Without capacitation bovine, porcine and equine spermatozoa would not be able to accomplish fertilization. It is reported that the seminal plasma of bull, boar and stallion is very rich in DF although the exact nature of this substance is still unknown (Kanawaga, *et al.,* 1989). Hence, the importance of sperm washing in the preparation of spermatozoa for IVF.

Different speeds, durations and frequencies of sperm washings have been used by various scientists working on IVF on different species. Techakumpu et al. (1994) found satisfactory results from a single washing of boar semen for 5 minutes at 1000 rpm. The Institute of Animal Science, Chinese Academy of Arts and Sciences (IAS, CAAS, 1991) uses a 3x washing procedure for bovine spermatozoa with Bracket and Oliphant (BO) solution as the washing medium at 1000-2000 rpm for 5 minutes at room temperature. Kaganawa et al. (1989) on the other hand, washed bull sperms at 37°C with a high ionic strength medium for 5 minutes at a slow speed of 250 rpm.

This study was conducted to evaluate the motility of boar spermatozoa after subjecting semen to various washing treatments and determine sperm viability following sperm washing at different speed, duration and frequency.

MATERIALS AND METHODS

Semen was collected by hand massage technique from a trained boar at the Institute or Animal Science Farm. It was filtered through a course net to remove the gelatinous portion, and placed in a thermo jug to prevent change in temperature. It was immediately brought to the laboratory. The semen was then further filtered through a cheese cloth and extended using egg yolk, dextrose and distilled water.

Twelve (12) 4 ml samples were put into test tubes and were mixed with 1 ml each of modified Brackett and Oliphant (BO)

solution (Table 1). They were subjected to following washing treatments:

TREATMENT NO.	CENTRIFUGATION		
	SPEED (rpm)	DURATION (min)	FREQUENCY
1	2,000	10	1
2	2,000	10	2
3	2,000	5	1
4	2,000	5	2
5	1,500	10	1
6	1,500	10	2
7	1,500	5	1
8	1,500	5	2
9	1,000	10	1
10	1,000	10	2
11	1,000	5	1
12	1,000	5	2

The spermatozoa were evaluated for motility immediately after the washing procedure and after 1 and 2 hrs. Viability for various treatments were estimated. Final mixtures of sperms and BO solution were incubated at 37-39°C for 48 hours. Thereafter the solution was examined for color change.

Six trials (replicates) using different semen samples from the same boar were done and averages of parameters were estimated.

Results were analyzed using the analysis of variance (ANOVA) technique for a (3 x 2 x 2) factorial experiment in completely randomized design (CRD).

RESULTS AND DISCUSSION

The average motility and viability of boar spermatozoa as affected by speed, duration and frequency of sperm washing are shown in Tables 2-4. There are no significant interaction effects among factors and the main effects are likewise not significant.

Success in IVF depends not only on the proper maturation of oocytes but also greatly counts on the quality of the semen used.

Results of this experiment showed that boar spermatozoa could be washed and cultured with modified BO solution. In all of the treatments the activity or motility of the sperms did not change considerably after the washing procedures. Recent experiments yielded satisfactory to excellent sperm motility and viability using different protocols for sperm preparation: 10,000 rpm for 3 minutes (Jean, *et al.,* 1995), 500 rpm 2x for 10 minutes (Grippo, *et al.,* 1995), or 300 rpm for 5 minutes (Senyoku, *et al.,* 1995). Still there is no reported standard procedure for washing of sperms prior to culture.

After removing the seminal plasma by washing (centrifugation) with appropriate solution, the sperms should be provided with a culture medium (in this case, the same BO solution) that contains substances that would maintain high spermatozoa activity. The BO solution used in this experiment does not contain caffeine as originally recommended by Brackett and Oliphant (1975). This substance is known to stimulate sperm capacitation *in vitro*. Results of the present study showed that even without caffeine the viability of spermatozoa in some treatments remained high.

The BO solution used in this experiment also does not contain sodium pyruvate which is very essential for respiration of spermatozoa. After washing and culture of sperms in BO solution for 48 hours there was a change in color of the solution form pink to yellow cream and light pink color. The washing medium contained phenol red as pH indicator. Inside the test tube the sperms continue their metabolism and respiratory activity. The yellow color of the solution indicates that most of the spermatozoa in a particular test tube died during culture and produced ammonia making the pH of the solution alkaline. Further, the BO solution contains glucose (0.0025 g/ml) which is utilized by spermatozoa for fructolysis. Lactic acid is produced during fructolysis and fuels the respiratory activity of spermatozoa. However, under anaerobic condition, like what happens inside the test tube, surplus lactic acid accumulates and makes the BO solution acidic indicated by the light pink color of the solution. This light pink coloration was noted more in those treatments with 2x washing while yellow and yellow cream tinge were evident in those treatments with single washing. Probably, washing the sperms only once did not remove most of the seminal plasma that possibly brought about the immediate decay of the BO solution.

Under existing laboratory conditions extended boar spermatozoa intended for IVF can be washed at 2,000, 1,500 or 1,000, rpm for 10 or 5 minutes once or twice without significant adverse effects on motility and viability.

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Table 1. Modified BO solution from Brackett and Oliphant (1975).

A solution	380	ml
Glucose	1.25	g
Penicillin	0.375	g
Streptomycin	0.025	g
B solution	120	ml
	500	ml

Table 2. Average motility and viability of boar spermatozoa as affected by speed of washing.

	Speed of Washing (rpm)		
	2000	1500	1000
Motility (%)			
Initial	78.33	78.33	78.33
Post treatment			
0 hr	71.66	70.00	70.42
1 hr	62.92	67.09	65.42
2 hr	59.17	64.17	62.50
Viability Index	67.34	71.33	70.00

Table 3. Average motility and viability of boar spermatozoa as affected by duration of washing.

	Duration of Washing (min)	
	10	5
Motility (%)		
Initial	78.33	78.33
Post treatment		
0 hr	69.72	71.67
1 hr	61.95	68.33
2 hr	58.89	65.00
Viability Index	67.11	72.00

Table 4. Average motility and viability of boar spermatozoa as affected by frequency of washing.

	Frequency of Washing	
	once	twice
Motility (%)		
Initial	78.33	78.33
Post treatment		
0 hr	70.55	70.83
1 hr	66.39	63.88
2 hr	63.61	60.28
Viability Index	70.89	68.22