OBSERVATIONS ON THE SURVIVAL OF WASHED BOAR SPERMATOZOA IN DIFFERENT MEDIA

I. G. WHITE* AND R. A. P. HARRISON

ARC Unit of Reproductive Physiology and Biochemistry, Cambridge†

(Received 13th January 1971)

Semen, collected from boars of proven fertility, was concentrated by centrifuging the strained ejaculate at 400 *g* for 15 min at 20° C and gently resuspending the sperm pellet in one-fifth the original volume of seminal plasma. The concentrated semen was then diluted 1:6 either in a chosen wash medium (see Table 1 for composition of media) and used immediately, or in a milk diluent (MFP), cooled slowly to 5° C over a period of 5 hr, and stored overnight at 5° C.

The spermatozoa were washed by centrifugation at 400 *g* for 12 min followed by gentle resuspension in a fresh aliquot of the chosen wash medium. Three centrifugations were carried out and samples of the final pellet were resuspended in chosen media. All manipulations of fresh semen were carried out at 20° C, whereas all manipulations of stored semen were carried out at 5° C, and various combinations of wash medium and resuspension medium were tested.

Spermatozoa in the final resuspension media were incubated at 37° C with frequent aeration with a Pasteur pipette, and scored for motility (Emmens, 1947) after 0, 1, 2 and 3 hr by examining a drop of the sperm suspension on a warmed microscope slide under low power magnification in a hot box maintained at 37° C. The identity of each preparation was known to the observer.

Cooling boar semen to 5° C at 0.5° C/min caused an irreversible depression of motility. In semen cooled slowly after dilution in MFP, motility was restored on warming. Washing the stored spermatozoa reduced their motility when they were finally resuspended in RFP (Table 1). While the use of the sperm-rich fraction (Polge, 1956), or the addition of 2.5 mg bovine serum albumin/ml (Choong & Wales, 1962) or 0.75 mg vitamin E/ml to the SP wash medium did not improve maintenance of motility after washing, final resuspension of the washed spermatozoa in MFP did restore their motility.

A further experiment was carried out using, as wash medium, MFP, SE or RFP and, as resuspension medium, MFP, MSP, MRFP, SP RFP or SE (Table 1). Samples of stored spermatozoa were also centrifuged three times and resuspended in their own supernatant as a control.

The results (Table 2) indicated that the composition of the final resuspension medium was much more important than that of the wash medium, highly

* Present address: Department of Veterinary Physiology, University of Sydney, Sydney 2006, Australia.

† Postal address: Animal Research Station, 307 Huntingdon Road, Cambridge CB3 0JQ, England.

TABLE 1
COMPOSITION OF MEDIA

MFP:	2% w/v dialysed heat-treated skim-milk solids, 200 mm-fructose, 19 mm-NaH ₂ PO ₄ , 31 mm-NaCl, 5 mm-KOH, adjusted to pH 7.0 with NaOH (Harrison & White, unpublished data).
FP:	As MFP, but omitting the milk solids.
MSP:	As MFP, but substituting sucrose for fructose.
SP:	As MSP, but omitting the milk solids.
MRFP:	2% w/v dialysed heat-treated skim-milk solids, dissolved in Ringer-fructose-phosphate (Mann, 1964).
RFP:	Ringer-fructose-phosphate (Mann, 1964).
SE:	300 mm-sucrose, 4 mm-Na ₂ EDTA, adjusted to pH 7.0 with KOH.

TABLE 2
THE MOTILITY OF BOAR SPERMATOZOA PREVIOUSLY COOLED
OVERNIGHT IN A MILK DILUENT AFTER WASHING AND RESUS-
PENDING IN VARIOUS MEDIA AT 5° C

Wash medium	Final resuspension medium						Mean
	MFP	MSP	SP	MRFP	RFP	SE	
Nil*	22	21	19	18	16	15	19
MFP	21	18	18	17	15	11	17
SE	23	17	18	16	17	12	17
RFP	25	19	18	16	16	14	18
Mean	23†	19	19	17	16	13‡	

The results represent the mean Motility Index for three ejaculates
(Motility Index = (sum of motility scores after 1, 2 and 3 hr at 37° C) × 4).

* Spermatozoa centrifuged and resuspended without washing.

† Different from all others ($P < 0.01$).

‡ Different from all others except RFP ($P < 0.01$).

significant differences being detected amongst the former. Motility indices were highest when spermatozoa were resuspended in MFP and lowest when they were resuspended in SE. With or without milk solids, RFP was not a good resuspension medium.

The effect of RFP was tested on spermatozoa washed at 20° C immediately after concentration. The results (Table 3) indicated that RFP at 20° C was as satisfactory as any of the other media for washing or resuspending boar spermatozoa. However, the FP medium was unsatisfactory both for washing and for resuspension.

When spermatozoa that had been washed at 5° C were resuspended and incubated at 37° C, prolonged incubation was often necessary to obtain maximum motility (Text-fig. 1), though this was a variable phenomenon. However, when the motility of a cooled sample showed an increase after 1 hr at 37° C, this increase was evident in all subsamples, irrespective of the washing or resuspension media.

While these results underline the sensitivity of boar semen to low temperatures (Lasley & Bogart, 1944), it is possible to obtain preparations of motile washed boar spermatozoa essentially free both of seminal plasma and of cytoplasmic droplets (Harrison & White, in preparation) by first diluting in a milk

TABLE 3
THE MOTILITY OF FRESHLY COLLECTED BOAR SPERMATOZOA
AFTER WASHING AND RESUSPENDING IN VARIOUS MEDIA AT
ROOM TEMPERATURE

Wash medium	Final resuspension medium					Mean
	MFP	FP	MRFP	RFP	SP	
MFP	31	16	35	31	41	31
FP	26	12	29	25	29	24†
MRFP	28	21	30	28	29	27
RFP	37	21	33	32	33	31
Mean	31	18*	32	29	33	

The results represent the mean Motility Index for three ejaculates (Motility Index = (sum of motility scores after 1, 2 and 3 hr at 37° C) × 4).

* Different from all others ($P < 0.01$).

† Different from MF and R ($P < 0.01$).

TEXT-FIG. 1. The motility of washed boar spermatozoa after resuspension in different media. Each point represents the mean for three ejaculates. Symbols used: O, resuspension in MFP after washing in RFP at 20° C; Δ, resuspension in RFP after washing in RFP at 20° C; ●, resuspension in MFP after washing in RFP at 5° C following storage overnight at 5° C; ▲, resuspension in RFP after washing in RFP at 5° C following storage overnight at 5° C.

diluent, and cooling very slowly to 5° C. RFP must be avoided as a resuspension medium after washing cooled boar spermatozoa at 5° C if good motility on rewarming is desired, even though RFP appears satisfactory as a resuspension medium for spermatozoa washed at 20° C (cf. Aalbers, Mann & Polge, 1961).

It is not possible from these experiments to explain the adverse effects of RFP at low temperatures. The absence of milk solids is not a factor since motility in MRFP was little better than in RFP, also the presence of bicarbonate and magnesium (as in RFP) might be expected to enhance motility. One feature of the Ringer diluent, however, is the high electrolyte to non-electrolyte ratio due to the high NaCl content. Many years ago, Milovanov (1934) advanced the theory that electrolytes were harmful to boar spermatozoa and destroyed the 'lipid capsule' surrounding the head; such an effect might be aggravated by cold shock to which boar spermatozoa are particularly susceptible. The most promising diluent used in A.I. practice for diluting boar semen, the modified Illinois Variable Temperature diluent, contains relatively little electrolyte (Bennett & O'Hagan, 1964).

The unsatisfactory nature of FP (Table 3) is surprising since the replacement of fructose by sucrose (SP) or the inclusion of milk solids (MFP) gave good results. High levels of fructose may be detrimental to the metabolism of boar spermatozoa in the absence of the high molecular weight material in milk, for Foley, Marsh, Heidenreich, Garwood & Erb (1967), studying washed boar sperm suspensions, found lower motility and metabolism associated with a diluent containing 72 mM-fructose.

The lag in the reactivation of boar spermatozoa after cooling to 15° C has been noticed by other workers (see Polge, 1956), and seems to be even more marked when the semen is cooled to 5° C. The present experiments underline the necessity to leave stored spermatozoa for some time at 37° C before rejecting such samples as dead. Anaerobically, boar spermatozoa convert fructose to lactate at a much lower rate than ram or bull spermatozoa and motility is also depressed (Aalbers *et al.*, 1961; Foley, Heidenreich, Harrington, Jones & Erb, 1964); however, in limited experiments, we did not detect any difference in the subsequent motility and revival of boar spermatozoa when suspensions in the milk diluent were aerated with O₂ or N₂ during storage at 5° C.

REFERENCES

- AALBERS, J. G., MANN, T. & POLGE, C. (1961) Metabolism of boar semen in relation to sperm motility and survival. *J. Reprod. Fert.* **2**, 42.
- BENNETT, G. H. & O'HAGAN, C. (1964) Factors influencing the semen of artificial insemination of pigs. *Proc. V Int. Congr. Reprod. Artif. Insem.* **4**, 481.
- CHOONG, C. H. & WALES, R. G. (1962) The effect of cold shock on spermatozoa. *Aust. J. biol. Sci.* **15**, 543.
- EMMENS, C. W. (1947) The motility and viability of rabbit spermatozoa at different hydrogen-ion concentrations. *J. Physiol., Lond.* **106**, 471.
- FOLEY, C. W., HEIDENREICH, C. J., HARRINGTON, R. B., JONES, H. W. & ERB, R. E. (1964) Changes in fructose, lactic acid, pH and motility of boar semen during incubation at 37° C. *J. Anim. Sci.* **23**, 558.
- FOLEY, C. W., MARSH, H. M., HEIDENREICH, C. J., GARWOOD, V. A. & ERB, R. E. (1967) Effects of zero and three-day storage of washed boar spermatozoa on subsequent incubation characteristics. *J. Anim. Sci.* **26**, 1072.
- LASLEY, J. F. & BOGART, R. (1944) Some factors affecting the resistance of ejaculated and epididymal spermatozoa of the boar to different environmental conditions. *Am. J. Physiol.* **141**, 619.
- MANN, T. (1964) *The biochemistry of semen and of the male reproductive tract*, 2nd edn. Methuen, London.
- MILOVANOV, V. K. (1934) *Principles of artificial insemination*. State Publishing House, Moscow-Leningrad.
- POLGE, C. (1956) Artificial insemination in pigs. *Vet. Rec.* **68**, 62.