The constant ratio of the zinc content of boar spermatozoa at 4°C and room temperature

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Summary. The ratio of the zinc content of boar spermatozoa obtained from semen cooled to 4°C for 30 min to that of the original room temperature control (20–26°C) was constant at 2.28 ± 0.16 in 22 samples of fresh whole semen from 12 animals. The same ratio occurred when zinc (0 to 0.6 mM in citrate buffer) was added to semen or washed spermatozoa. The increase is dependent only on the initial sperm zinc content at room temperature.

Earlier investigations by Bournsnel & Mustill (1975) and Bournsnel & Noble (1975) showed that the zinc concentration in boar spermatozoa from fresh semen samples at room temperature was considerably increased when the semen samples were cooled to about 4°C and that this increase was not affected by the subsequent re-warming of the semen samples.

The ratio of the sperm zinc values from the semen samples at 4°C to those from the corresponding room temperature samples appeared to be relatively constant. In the present study the existence of such a ratio at 4°C and at other temperatures was explored, particularly in relation to current problems outlined by Polge (1956) of the preservation of boar semen at low temperatures.

Methods and results

Whole semen was collected from boars housed at the Animal Research Station, strained through a large nylon sieve to remove the gel and used immediately. At the stages indicated below semen aliquots were centrifuged at about 10,000 *g* for 5 min in a Beckman Microfuge B (Beckman Industries, Glenrothes, Fife) using pre-weighed capped polypropylene tubes of 1.5 ml capacity (39/10, W. Sarstedt (U.K.), Leicester). The methods for weighing the spermatozoa and measuring the zinc content are described by Bournsnel & Mustill (1975).

Water baths (Grant Instruments, Barrington, Cambs) were set up at three of the required temperatures. Covered beakers of water at intermediate temperatures (2°C above and below the bath temperature) were placed in the water baths. These beakers contained smaller water-filled plastic beakers within which were placed the polypropylene centrifuge tubes containing the semen samples. The temperatures within these plastic beakers remained constant throughout the required time. This system was suggested by the work of van Veersen (1943).

Ratio of sperm zinc content at 4°C and room temperature (20–26°C). Aliquots (1.5 ml) of a fresh collection of whole semen were dispensed into centrifuge tubes. Replicate sets of these aliquots were kept at 4°C for 30 min and then re-warmed to room temperature for 30 min. The sperm weight and zinc content were determined in the samples held at 4°C and after re-warming. Six other identical experiments were carried out on fresh semen from 5 other boars. In three of these, a further set of 4°C-treated aliquots were subsequently kept at room temperature for 90 min and the controls were retained at room temperature for 120 min. Results from a further five experiments, carried out with 1.5 ml fresh semen samples under similar conditions to those described above, are available. Some of these are taken from the experiments described in the following section on the temperature dependence of this ratio. Measurements of zinc content from the above experiments are plotted on Text-fig. 1. The mean ratio of the 22 points for zinc content at 4°C and room temperature is 2.28 ± 0.16 (S.D.).

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Text-fig. 1. The mean values of zinc in boar spermatozoa from 1.5 ml aliquots of fresh whole semen at room temperature (20–26°C) and after cooling to 4°C for 30 min with no re-warming (□) or with subsequent re-warming to room temperature for 30 min (○). +, These values are taken from Text-fig. 3 of Bournsell & Mustill (1975) and from 6 similar experiments: the values are those for the first absorption of zinc by boar spermatozoa at 4°C and in uncooled control samples (0.6 ml aliquots of fresh semen, no re-warming).

The wide scatter of the points along the line in Text-fig. 1 is partly due to the use of 0.6 ml semen aliquots in the sperm absorption experiments of Bournsell & Mustill (1975) and 1.5 ml aliquots in the present experiments. It is also due to differences in the sperm density between individual semen collections as revealed by different weights of the sperm pellets. The range of sperm dry weight (calculated for convenience to 1.5 ml semen) in the untreated room-temperature samples was 5.25–18.6 mg. The sperm weights obviously partly influenced the room-temperature sperm zinc values, which ranged from 0.398–0.887 μg zinc per mg dry spermatozoa. In spite of these variations, the mean 4°C–room temperature dry sperm weight ratios in these experiments is 1.21 ± 0.14 (S.D.).

Early observations which led to the graph given by Bournsell & Mustill (1975) in their Text-fig. 3 of sequential sperm zinc absorption at 4°C were made with larger volumes (5 ml) of fresh semen samples from two boars and the 4°C–room temperature ratios were 2.23 and 2.24.

Bournsell & Noble (1975) treated native semen or washed spermatozoa with increasing concentrations of zinc up to about 0.6 mM and the ratios of the slopes of the respective regression lines at 4°C and room temperature were, in both cases, 2.41.

The 26 experiments detailed above which are available for the calculation of this 4°C–room temperature ratio give a mean value of 2.28 ± 0.15 (S.D.).

Temperature dependence of sperm zinc uptake. Aliquots (1.5 ml) of fresh whole boar semen were dispensed into centrifuge tubes. Replicate sets of these aliquots were kept at controlled temperatures between 2 and 18°C as described and also at room temperature (26°C) for 30 min and all were subsequently kept at room temperature for 30 min. Dry weight and zinc determinations were carried out on the centrifuged sperm samples and zinc was also determined in the supernatant fluids. The results are shown in Text-fig. 2; similar curves were obtained in 5 other experiments employing collections from 4 other boars. The zinc in the supernatant plasma was reduced from the original room temperature value of 24 μg/1.5 ml to 18 μg/1.5 ml, and the mean sum of the sperm and supernatant zinc values obtained at all the temperatures was $27.8 \mu\text{g} \pm 0.8$ (S.D.). The ratio of the weight of dry spermatozoa at 2°C to that at room temperature was 1.23.

Text-fig. 2. Mean ($\pm$ S.D.) total zinc content in spermatozoa (O) after treatment of fresh semen aliquots for 30 min at various temperatures, followed by a 30 min period at room temperature. x, Mean dry weights of sperm samples. Vertical bars represent the S.D.

Discussion

The exactness of the ratio (2.3) of the zinc content of boar spermatozoa at 4°C compared with the value at room temperature was surprising. This ratio was obtained under a wide range of conditions and in spite of considerable differences in the room temperature zinc content of the spermatozoa separated from native seminal plasma. In previous work (Bournsnel & Noble, 1975) the regression lines of sperm zinc content with zinc added to whole semen revealed this ratio between the 4°C and room temperature slopes; and, more surprisingly, washed spermatozoa resuspended in buffered zinc citrate also demonstrated it.

The present results show that this ratio is temperature sensitive (Text-fig. 2), the portion of the curve showing maximal rate of change being between 20 and 10°C. Mathematical analysis shows that the inflexion point of this virtually symmetrical curve occurs at 15°C. This temperature is mentioned by Polge (1956) as the lowest to which boar spermatozoa may be cooled with subsequent recovery of a high degree of motility on re-warming; the relatively flatter portion of the curve below 10°C corresponds to the region of increasing sperm immotility on re-warming.

We are seeking an explanation for the apparent specificity of the zinc attachment to spermatozoa which could be connected with the cold-shock phenomenon. This reaction may be connected with the membrane as it occurs with washed spermatozoa in the absence of seminal plasma.

The 2.3-fold increase in sperm zinc at 4°C is dependent only on the initial zinc content of the spermatozoa at room temperature. It is postulated that a sperm membrane ligand is involved which, when combined with zinc, undergoes a specific transformational change on cooling, allowing attachment of a further amount of zinc.

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