

Standardizing initial cooling of sheep semen before freezing

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A practical and repeatable method for the cooling, during the processing phase, of sheep semen, with the aim of minimizing inter-experiment variation is described. The superior repeatability of the method can be ascribed to the fact that the cooling rate of the test tube with semen in a container of water, becomes independent of the volume of water in the container, above a certain critical volume.

'n Praktiese en herhaalbare metode vir die afkoeling van skaapsemen gedurende die prosesseringsfase, om intereksperiment-variasie te minimaliseer, word beskryf. Die verhoogde herhaalbaarheid van die metode word toegeskryf aan die feit dat die afkoelingstempo van 'n proefbuis met semen in 'n glashouer met water onafhanklik word van die volume water in die houer bokant 'n sekere kritiese volume.

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For experimental results to be comparable between different experiments, it is necessary to minimize experimental error. This can be accomplished by eliminating all possible sources of variation and standardizing the techniques as far as possible. This is true, especially in the case of semen freezing, where widely diverging results are usually reported. Colas (1975) reported 'a 50% rejection rate of ejaculates after freezing and thawing with the best treatment'.

During experimentation to try and achieve results comparable to those reported by Colas, using the method for freezing sheep semen in straws (Colas, 1975), a number of precautions were taken to minimize inter-experiment variation. Diluents were made up in large volumes and 100 ml aliquots were frozen at -20°C until needed. This would reduce variation caused by differences between batches. All steps during the dilution and freezing process were timed daily with a stopwatch to minimize variation and ejaculates were collected at the same time each day. Cell counts were carried out on each ejaculate by using a spectrophotometer which had been calibrated against a haemocytometer. Ejaculates were diluted to give the same final concentration (9×10^8 cells/ml). Freezing in liquid nitrogen vapour was repeated as accurately as possible.

The one aspect that is not satisfactorily described in the literature concerns the control over the cooling of the semen from 30°C to 4°C over a period of 2 h during the processing of the semen. The timing of this cooling rate is a compromise between cold shock of the sperm cells at a higher cooling rate and exhaustion of the cells when cooling is too slow (Colas & Courot, 1976). To find a practical and repeatable method for this cooling of the semen, the following short experiment was conducted.

Glass beakers or flasks of different shapes and volumes were used and the volume of water in them was varied to influence the rate of cooling. Temperatures were measured using copper-constantin electrodes coupled to a Honeywell Multipoint recorder, no. 121-1. The cooling process was carried out in a walk-in type refrigerator with thermostatic temperature regulation and an average temperature of 0°C ($\pm 1^{\circ}\text{C}$). The glass containers were filled with water to

different levels and placed in a waterbath at 30°C until transferred to the refrigerator. An electrode was then placed into each container and one electrode left in the open to measure ambient temperature inside the refrigerator.

Although the cooling rate decreased for larger volumes, this occurred only until a certain critical volume was reached, after which addition of water did not cause a decrease in the cooling rate. The desired cooling rate under the above-mentioned conditions was achieved using 250 ml beakers containing volumes of water equal to or larger than 150 ml (Figure 1) and placed inside an open polystyrene-lined stainless steel box to reduce the effect of direct turbulent air on the glass containers.

The curves for the three volumes of water equal to or larger than 150 ml were completely identical. Identical cooling curves were also found for 100 ml Ehrlenmeyer flasks with volumes of water equal to or larger than 70 ml. These flasks were more suited to cooling a test tube of semen in an upright position.

The fact that the desired cooling rate was achieved within the area where cooling rate is independent of the volume, is very convenient for standardization purposes, in that an error in measuring the volume of water or the inclusion of a 15 ml test tube with different volumes of processed semen did not change the rate of cooling.

References

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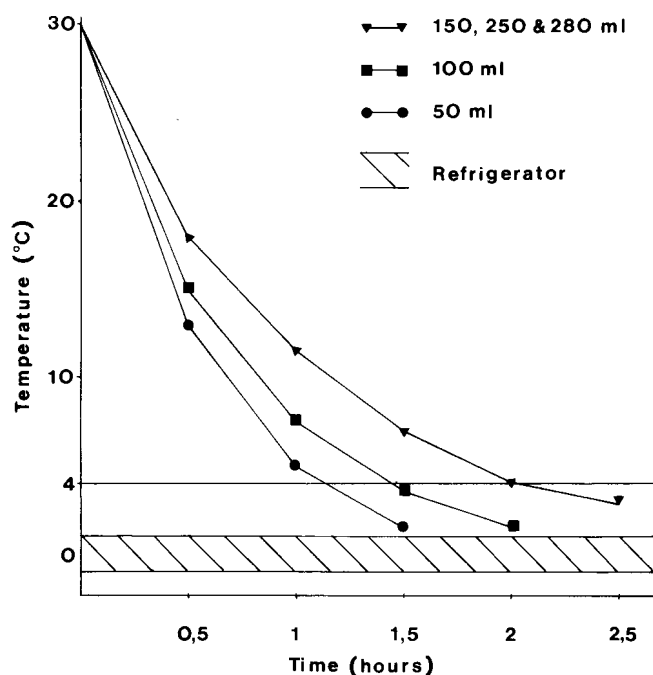


Figure 1 Cooling of 250 ml glass beakers containing different volumes of water