

THE PROCESS FOR THE PRESERVATION OF BULL SPERM

This invention relates to a process which permits the freezing of ^{samples of} bull sperm and upon rethawing yields samples which can be used with good efficiency for fertilizing cows. The bull sperm samples frozen according to this invention will show upon rethawing that at least 10% of the spermatazoa which showed progressive motility in the original sample will have progressive motility at an essentially undiminished speed. According to this invention, a sample of bull semen which may be diluted by one of the conventionally used extenders - such as for instance, egg yolk citrate buffer - but which sample contains no glycerol or only a small amount of glycerol is diluted slowly over an extended period of time to which we shall refer as the dilution time t and for which we shall give an explicit value below by adding a number n (to be described below) of small aliquots of an extender containing glycerol in ^a concentrations described below and upon addition of the last aliquot of the glycerol containing extender the ~~mixture~~ final mixture which at this time shall have a concentration not very much lower and not very much higher than 10% glyderol by weight and which mixture at the time when the dilution process is completed might be at a ~~temperature~~ temperature of a few degrees centigrade is upon the addition of the last aliquot at once cooled down at a moderately ~~n~~ fast rate to about -30 centigrade and beyond that cooled down at a conveniently fast rate about to/the temperature of a conventional dry ice bath (acetone and carbon dixide or alcohol and carbon dioxide, etc.) or to any temperature which is lower and maintained at ~~some~~ such low temperature and shortly before the time when it is desired to use it at that time the sample is rethawed as fast as possible without heating the sample appreciably about 37 degrees.

One example for the process of slowly diluting the semen containing sample with a glycerol containing egg yolk citrate buffer semen extender is as follows:
 A bull semen sample in egg yolk citrate buffer extender is slowly cooled down to 5 degrees centigrade at this temperature equal aliquots of the glycerol containing extender which contains 11% of glyderol by weight are added every 10 minutes over a period of 8 hours. The total volume of the glycerol extender added V_1 is ten times as large as the volume ^{V_0} of semen contained ⁱⁿ the sample which contains no glycerol. ~~THE VOLUME~~ Therefore, the final mixture will contain 10% of glycerol by weight upon completion of the dilution process, i.e., after the last aliquot of the glycerol containing extender has been added, the mixture is immediately cooled down at a moderately fast rate to -30 centigrade and is then further cooled down at a conveniently fast rate to dry ice temperatures.

According to this investigation

We shall designate the dilution ratio $\frac{V_0 + V_1}{V_0}$ with r . In the above
 example we had $r = 11$. If the aliquots of the glycerol extender which are added
 are equal in quantity ~~or~~ are decreasing in quantity and if the dilution ratio ~~XXXXXX~~ r
 is 4 or larger good results are obtained ~~when~~ the dilution time in hours obeys
 a formula ~~reads~~ $t = \frac{4r}{r-1}$

addition is in hours or longer

In the above example this condition
 is satisfied for me because $r = 11$
 $n = 32$, $t = 8$ whereas our formula

merely requires that $t > \frac{4 \times 11}{10} = 4.4$ hours

Equal aliquot

Distillation from
concentration of solvent c_0
final distillation c_f

Volume ratio = ~~r~~
 $c_f =$

$T > 3 \text{ hours} +$

$$T > 3 + \frac{T}{r}$$

$$rT > 3r + T$$

$$(r-1)T > 3r$$

$$T > \frac{3r}{r-1}$$

$$T > \frac{4r}{r-1}$$

$\frac{4}{10}$

6 2

for $r > 4$
~~XXXXXXXXXX~~
~~XXXXXXXXXX~~