THE RELATIONSHIP BETWEEN FERTILITY AND POST-FREEZE MOTILITY OF BULL SPERMATOZOA (BY PELLET FREEZING) WITHOUT GLYCEROL*

C. D. GIBSON† AND E. F. GRAHAM

*Department of Animal Science, University of Minnesota,
St. Paul, Minnesota, U.S.A.*

(Received 1st April 1969)

Glycerol has been the most widely-used protective agent for the freezing of bull semen. However, Nagase, Graham & Niwa (1964) demonstrated that bovine spermatozoa could be frozen without the addition of glycerol. The purpose of this experiment was to determine whether a relationship exists between the motility of an individual bull's semen after subjecting it to the stress of freezing without the protection of added glycerol and the fertilizing capacity of that animal's semen after freezing by commercial means.

Semen used in this study was obtained from adult bulls in regular use at a commercial bull stud. Motility, volume and sperm cell concentration were recorded and a 0.5 ml sample was taken from the first ejaculate of each bull immediately after collection. The 0.5-ml semen sample was diluted in 1.5 ml of a solution containing 18.5 g of raffinose/100 ml of water and 20% egg yolk by volume. The sample was then cooled slowly from 37° C to 5° C and was frozen 4 hr after collection, on dry ice using the pellet freezing method developed by Nagase & Niwa (1964). This method consisted of placing three drops of the extended semen in a depression on the surface of a block of dry ice. The pellets were removed from the dry ice after a period of 15 min and placed in a 1-ml isotonic saline solution to thaw at 5° C. The thawed samples were then evaluated microscopically for motility immediately after being brought to 37° C on a warming plate.

Several samples from each bull were evaluated, using this technique, during a 3-month period to gain a representative assessment of the semen motility. The 60- to 90-day, non-return rate for the months most closely associated with the test-freeze period was used as the measurement of the fertilizing capacity of the semen from the bulls being tested.

Data from twenty-one different sires are shown in Table 1 and correlation coefficients were determined for the variables being tested, as shown in Table 2. The data in Table 2 shows that the relationship between the after-freeze motility and the 60- to 90-day, non-return rate was correlated with a coefficient of 0.67 which significantly differs from zero ($P < 0.01$). The correlation coefficient value between before-freeze motility and the after-freeze motility also was

* Scientific Journal Series Paper No. 6861, Minnesota Agricultural Experiment Station.

† Present address: Department of Veterinary Obstetrics and Gynecology, University of Minnesota, St Paul, Minnesota 55101.

TABLE 1
COMPARISON BETWEEN BEFORE-FREEZE (BF) MOTILITY, AFTER-FREEZE
(AF) MOTILITY AND 60- TO 90-DAY NON-RETURN RATES

Bull no.	Breed	No. of collections†	Ave. % BF motility	Ave. % AF motility	% 60- to 90-day non-return*
1	Hol.	10	53	25	68.5
2	Hol.	10	59	26	70.7
3	Hol.	10	53	28	70.0
4	Hol.	10	34	17	45.4
5	Hol.	6	60	30	68.0
6	Hol.	10	57	21	68.5
7	Hol.	10	50	23	69.8
8	Hol.	10	54	25	65.2
9	Hol.	10	50	25	62.7
10	Hol.	7	54	25	67.3
11	Hol.	7	58	22	64.7
12	Hol.	5	57	17	55.4
13	Hol.	10	67	27	70.8
14	Hol.	6	50	29	85.4
15	Ang.	9	54	25	67.3
16	Ang.	5	58	31	67.2
17	Ang.	10	61	29	69.5
18	Ang.	10	51	22	61.1
19	Ang.	8	54	26	61.7
20	Ang.	4	51	28	78.8
21	Ang.	6	50	27	85.7

* The 60- to 90-day non-return rate is an average of 3 months most closely associated with the test-freeze period.

† All of the semen samples taken are from the first ejaculate of the sire for that collection day.

TABLE 2
AFTER-FREEZE MOTILITY CORRELATION STUDIES BY CORRELATION COEFFICIENT MATRIX

	Volume	Cell no.	BF% motility	AF% motility
Volume				
Cell no.	-0.369			
BF% motility	-0.009	0.420		
AF% motility	-0.339	0.263	0.483*	
60- to 90-day non-return	-0.105	0.139	0.320	0.674

* $P < 0.05$ if $r > 0.423$.

** $P < 0.01$ if $r > 0.549$.

significantly different from zero ($P < 0.05$), but that was not valid for the correlation coefficient between before-freeze motility and fertility in this study. Therefore, it would appear that the after-freeze motility pellet method without glycerol would be a better indicator of fertilizing capacity than the before-freeze motility rate.

The procedure employed may have merit in the evaluation of sires and may be of clinical significance as a simple screening test to help predict a bull's potential for use as a frozen semen donor. Preliminary comparative studies have shown that there is very little variation in the after-freeze motility between individuals using conventional methods incorporating glycerol, while the same semen samples will show great variation in recovery rates using a freezing

TABLE 3
COMPARATIVE MOTILITY STUDIES OF
BOVINE SEMEN USING THE PELLET
FREEZING METHOD WITH GLYCEROL
AND WITHOUT GLYCEROL*

Bull no.	% Recovery of AF motility	
	Without glycerol	With glycerol
1	90	70
2	84	68
3	77	95
4	60	90
5	45	75
6	40	62
7	40	65
8	40	62
9	38	62
10	35	65
11	35	80
12	33	62
13	30	62
14	28	56
15	25	60
16	24	65
17	18	60
18	12	68

* Split ejaculates were used for the above study. The above values are averages of % recovery of the first and second ejaculate of each bull using each freezing technique.

method without glycerol (Table 3). The two bulls in this trial who had extremely low 60- to 90-day, non-return rates (45.4 and 55.4) also had low after-freeze motility rates with an average of 17%.

The procedure may also be useful in selecting semen to be used in experiments investigating freeze-drying of spermatozoa since addition of glycerol to semen has a deleterious effect in this process according to Meryman & Kafig (1959) and also Saacke & Almquist (1961).

The authors are indebted to the North Central Breeders Research Council for financial assistance and to the Midwest Breeders for furnishing materials and data.

REFERENCES

- MERYMAN, H. T. & KAFIG, E. (1959) Survival of spermatozoa following drying. *Nature, Lond.* **184**, 470.
 NAGASE, H., GRAHAM, E. F. & NIWA, T. (1964) Pelleted semen: the effect of glycerol level and composition of thawing material on fertility of bovine spermatozoa. *5th Int. Congr. Anim. Reprod. Artif. Insem., Trento, Italy*, **4**, 404.
 NAGASE, H. & NIWA, T. (1964) Deep freezing bull semen in concentrated pellet form. *5th Int. Congr. Anim. Reprod. Artif. Insem., Trento, Italy*, **4**, 410.
 SAACKE, R. G. & ALMQUIST, J. O. (1961) Freeze-drying of bovine spermatozoa. *Nature, Lond.* **192**, 995.