THE EFFECT OF CONTAINER TYPE ON MOTILITY OF DEEP FROZEN BULL SPERMATOZOA

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INTRODUCTION

The glass container for frozen bull semen has been used primarily in routine artificial insemination work. However, many units have expressed interest in the use of a plastic container in the freezing of semen. A few bull studs have frozen semen in limited quantities in plastic containers with rather poor results. Since there has been little work of an experimental nature to substantiate or refute this observation, it was felt that additional information was necessary.

The purpose of this experiment was to determine whether or not any practical differences existed in frozen semen that were due to the effect of the plastic or glass container in which it was stored; and to what factors such differences might be attributed.

REVIEW OF LITERATURE

Container Effects on Deep Frozen Bull Spermatozoa

Dunn et al. (1) have reported a study in which the split sample technique was used to compare the fertility of semen frozen and stored at -79° C. in hermetically sealed glass ampules and in polyethylene bulbs. There were 298 inseminations with semen stored in glass ampules and 313 inseminations with semen stored in polyethylene bulbs. The 60-90 day non-returns were 60.7% and 62.3%, respectively, favoring the polyethylene bulbs. This difference was not significant at the 5% level of probability.

History of Frozen Semen

The rapid expansion in the field of artificial insemination during the past few years has stimulated much research designed to overcome some of its immediate shortcomings. Although bull semen has been routinely stored quite satisfactorily for two days, the inability to preserve bull semen in a functional condition for a longer period of time has caused wastage of much valuable semen. It was evident that if a technique could be developed by which bull semen could be stored for long periods of time, it would be of much value to the industry.

Luyet and Gehino (6) implied that this might be possible when they reported in a review that many small cells and organisms withstood exposure to temperatures approaching absolute zero, were subsequently thawed, and resumed normal functioning. Since biochemical changes were arrested at the exceedingly low temperatures of liquid gases, there was little doubt that,

in theory, spermatozoa could survive in a state of suspended animation for an almost unlimited length of time.

Luyet and Hodapp (7) then reported that frog spermatozoa could be frozen in liquid air if the spermatozoa were first partially dehydrated by the addition of a strong sucrose solution, and then plunged into the liquid air. Similiar successes were reported by Shaffner, Henderson, and Card (11) with fowl spermatozoa. The semen was frozen in thin films or capillary tubes. This work was done under the premise that the essential condition for the survival of cells at low temperatures was the prevention of the formation of ice crystals; and, that water in aqueous colloids became vitreous rather than crystalline when frozen ultra rapidly in thin films to low temperatures.

Hoagland and Pincus (5) using the semen of several different mammals, were unable to duplicate this work. Parkes (8) demonstrated that human spermatozoa did not survive freezing in thin films, but did survive in substantial numbers when frozen in larger tubes.

A new avenue of experimentation was opened by the chance discovery of Polge, Smith, and Parkes (10) while working with fowl spermatozoa, that glycerol had remarkable protective properties against the harmful effects of low temperature. Of the several alcohols tested, only propylene and ethylene glycol in concentrations of 15-20% gave protection against freezing and thawing of fowl spermatozoa.

Further work of Smith and Polge (12) showed that this technique was ineffective with mammalian semen. However, it was found that if the semen was frozen relatively slowly, instead of by the ultra rapid technique of plunging it into liquid gases, a large portion of bull and goat spermatozoa could be revived after freezing to 79° C. Emmens and Blackshaw (4),

confirming the work of Smith and Polge, reported that bull spermatozoa had been satisfactorily revived after freezing to -79° C. in a solution of 7.5% ethylene glycol. They also found that additions of 1.25% of a pentose sugar increased revival rates.

With this information Polge and Rowson (9) developed a technique of freezing bull semen to -79° C. This technique has been generally accepted and with some modifications is in common use at the present time.

EXPERIMENTAL PROCEDURE

This thesis deals with the effect of certain factors on semen quality following freezing and storage in a dry ice chest. Trial 1 was a study of the effect that storage in glass and plastic containers had on semen quality. Semen in these containers was stored for 1-2 weeks in an alcohol bath at -79° C. Trial 2 was a study of the effect of storage in the same medium for more than 30 days. The effect of storage in air on semen quality under the same conditions as before constituted trials 3 and 4, respectively. The effect of simulated shipment (trial 5) and method of sealing (trial 6) were studied for each type of container.

Data for this experiment were assembled from 13 ejaculates from eight different bulls. No attempt was made to select bulls or ejaculates for any particular trait except for the restriction that the semen was of sufficiently good quality to insure that after freezing, the semen was of acceptable quality. Semen was collected by the use of an artificial vagina using standard collection procedure.

The procedure for processing and freezing of the semen was as follows:

1. The egg yolk-citrate-sulfanilimide diluter consisted of equal parts of 2.9% sodium citrate containing 0.6% sulfanilimide and egg yolk. Crystalline penicillin and dihydrostreptomycin were added at the rate of 500 units per ml. and 500 micrograms per ml., respectively.

2. After collection, a portion of the egg yolk-citrate-sulfanilimide diluter was added immediately and at a temperature corresponding to that of the semen. After the final dilution rate had been established, enough egg yolk-citrate-sulfanilimide buffer was added to bring the total volume up to

one-half of the final anticipated volume.

3. At this point the partially diluted semen was placed in a refrigerator and allowed to cool slowly to 5° C. It was left in this state for 4-5 hours.

4. A gylcerolated diluter consisting of 14% glycerol and 86% sodium-citrate-sulfanilimide by volume was then added in a sufficient amount to constitute 50% of the total final volume. The glycerolated diluter was added in four steps 5 minutes apart. The final dilution rates were varied, due to the quality and concentration of spermatozoa in the semen so that an estimated 10-15 million spermatozoa per ml. would revive after freezing. The dilution rate ranged from 1-10 to 1-40.

5. The semen was allowed to equilibrate for a period of 12-18 hours. During the equilibration period the semen was transferred to single service ampules in amounts of approximately one ml. and sealed. The plastic containers were sealed by fusing the open end of the vials with automatically controlled electric heating elements. The glass ampules were sealed manually with an oxygen-acetylene torch. After sealing, 5 plastic ampules and 5 glass ampules were randomly selected from each of 7 ejaculates and observed immediately for per cent and rate of motility. An analysis of variance was computed to measure the effect of sealing the glass and plastic containers on these semen characteristics.

6. After equilibration the sealed ampules were placed in an alcohol bath at 5° C. and the temperature was lowered at a rate of 1-2° C. per minute from 5° C. to -15° C.. From -15° C. to -79° C. the temperature was lowered at a rate of 3-4° C. per minute.

After freezing, a portion of the semen was evaluated immediately, and the remainder was stored either in an alcohol bath or in air in an open container in a dry ice chest for future observations.

Quite often the semen from a particular ejaculate was used for several different observations. In this case, the procedure followed was to sample randomly from the total ejaculate frozen, the amount required for any particular trial. Therefore, it is apparent that for any particular variable of interest, variation included not only experimental treatment, but also sampling error. There was no attempt to correct for sampling error in this report and all observations assumed this to be zero.

Criteria for the measurement of these data were the per cent and rate of motility as estimated by microscopic observation. Per cent motility observations were based on the percentage of live spermatozoa, and, in theory, ranged from 0-100. Rate of motility, based on the amount of progressive motion exhibited by the spermatozoa, was assigned values from 0-4.0.

The procedure for simulated shipment was to obtain a sample from the dry ice chest, thaw one-half of the semen immediately and observe for per cent and rate of motility. These observations served as a control. The remainder of the semen was then transferred to a field thermos containing dry ice and held for 24 hours. At the end of this period the semen was transferred back into the dry ice chest and held for an additional 24 hours. Finally the semen was again transferred to the thermos, held for 24 hours, thawed and observed for motility. This treatment simulates the conditions to which the semen would be subjected from the time of shipment from the collection center to the insemination of the cow.

The statistical analyses of these data, with the exception of the data on the effect of container sealing, were on an individual ejaculate basis. This procedure was followed since it was felt that the large amount of variation between ejaculates would make an analysis of variance by groups invalid. The new multiple range test (3) was used to compare treatment differences of individual ejaculates.

RESULTS AND DISCUSSION

The results of trials 1-6 are presented in tables 1-11, inclusive. The data from which these values were obtained are found in appendix tables I to XVI. A summation of each trial is presented at the bottom of the multiple comparison table for each trial. When a comparison was rejected, the treatment in which the rejection was made in favor of was entered in the appropriate column.

Trial 1

The data, as observed in table 1, seem to indicate that while the plastic container was superior for 3 of 5 ejaculates after freezing (per cent motility criterion), this advantage was quickly lost after storage in an alcohol bath for periods of from 1-2 weeks. It appeared that the semen in the glass containers remained fairly stable from freezing to final observation. Conversely, it appeared that the semen in the plastic containers deteriorated rather rapidly until reaching the quality level of the semen in the glass container.

Referring to comparisons in table 1, all 5 ejaculates for the comparisons $G_1 = G_2$ and $G_1 = P_2$ were equal. This indicated that for this trial there was no apparent difference between semen in glass containers after freezing (G_1) or after storage (G_2) and the plastic container after storage (P_2). Therefore, any comparison involving P_1 with either P_2, G_1 or G_2 should have been rejected. This was true for ejaculates 1, 3 and 4. For ejaculates 2 and 12 this was not the case and it was inferred that for these two ejaculates there were no apparent differences between P_1, P_2, G_1 and G_2.

Table 1

Multiple comparisons of treatment means - Trial 1

Per cent motility					Rate of motility				
Ejac.	Treatment means				Ejac.	Treatment means			
1	G_1^a	P_2^d	G_2^c	P_1^b	1	G_1	G_2	P_2	P_1
	15	20	22	35		1.0	1.6	1.6	2.0
2	G_1	P_2	G_2	P_1	2	G_1	P_1	P_2	G_2
	24	26	30	32		2.0	2.0	2.5	2.7
3	G_2	G_1	P_2	P_1	3	G_2	G_1	P_1	P_2
	12	19	22	29		1.4	1.6	2.0	2.3
4	G_2	P_2	G_1	P_1	4	G_2	G_1	P_2	P_1
	.2	.8	.8	3.8		.2	.4	.8	1.0
12	G_1	G_2	P_1	P_2	12	G_1	G_2	P_1	P_2
	8.0	9.4	11.0	13.0		.9	.9	1.1	1.2

Summary

Comparison	Ejaculate									
	Percent motility					Rate of motility				
	1	2	3	4	12	1	2	3	4	12
$P_1 = G_1$	P_1	≠	P_1	P_1	≠	P_1	≠	≠	P_1	≠
$P_1 = G_2$	P_1	≠	$P_.$	$P_.$	≠	≠	G_2	≠	P_1	≠
$P_1 = P_2$	P_1	≠	≠	P_1	≠	≠	P_2	≠	≠	≠
$G_1 = P_2$	≠	≠	≠	≠	≠	P_2	P_2	≠	≠	≠
$G_1 = G_2$	≠	≠	≠	≠	≠	G_2	G_2	≠	≠	≠
$P_2 = G_2$	≠	≠	P_2	≠	≠	≠	≠	P_2	P_2	≠

a - Initial glass observation (G_1)
b - Initial plastic observation (P_1)
c - Final glass observation (G_2)
d - Final plastic observation (P_2)

Table 2

Ejaculates on which data were based

Ejac.	Bull	Date collected	Date Read	
			Initial	Final
1	4	12/27/55	12/28/56	1/3/56
2	21	12/29/55	12/30/56	1/23/56
3	15	1/3/56	1/4/56	1/10/56
4	36	1/10/56	1/11/56	1/22/56
12	23	5/5/56	5/6/56	5/20/56

For ejaculates 1, 3 and 4 there was an advantage after freezing in favor of semen in plastic containers (comparison $P_1 = G_1$). After storage only ejaculate 3 maintained this advantage. This indicates that ejaculate 3 did not deteriorate as rapidly as did ejaculates 1 and 4. The results of the comparisons $P_1 = P_2$ and $P_2 = G_2$ substantiate this conclusion.

The explanation of these results can be attributed to one or more factors. Semen in plastic containers survived freezing better although it did not store as well as did semen in glass containers. Or, differences observed after freezing and attributed to the effect of the plastic container might have been due to chance sampling error and so there might have been no original differences due to container effect. This seems to satisfy the data in trial 1 with the exception of ejaculate 3. Random sampling would seem to give a more logical explanation than an initial superiority of the semen in plastic containers after freezing with a subsequently greater deterioration than was observed for semen in the glass containers. It is difficult to imagine deterioration proceeding to a given level (that of the semen in the glass containers) and then stopping. Also, it is not too plausible that there could have been so much deterioration in such a short time (two weeks at the most).

While there is some correlation in the results obtained from relating per cent motility data to rate of motility data, there are some obvious discrepancies. These may be due to several factors. For any given ejaculate, rate and per cent of motility may not be perfectly related. Rate of motility represents more discrete values (1.0, 1.5, 2.0 etc.) than does per cent of motility and it is dubious whether an analysis of this type of information is valid.

In the opinion of the author, the majority of the discrepancy between per cent and rate of motility should be attributed to observation error and analysis. If it were possible to remove these variables, it would appear that the two measurements would be closely correlated.

Trial 2

Trial 2, as presented in table 3, measured the effect of storage in alcohol for periods of 30-90 days. The data for this trial indicated no differences due to container effects after freezing (per cent motility criterion). With the exception of ejaculate 9, the same observation was true after storage for 30-90 days. Ejaculate 9 should be considered as having some experimental error since $P_2 > P_1$ (P_2 greater than P_1). Contrary to the results of trial 1, the data from trial 2 seem to indicate that the semen in glass containers deteriorated as rapidly as did semen in plastic containers.

These results would tend to strengthen the reasoning that the "differences" observed in deterioration rate in trial 1 were due to sampling error. It would seem logical that, if there were differences in deterioration rate between semen in plastic and glass containers, they would be more apt to appear after storage for 90 days than for only 2 weeks. However, it should be kept in mind that if there were an interaction between ejaculates and containers, this could account for the discrepancy in results between trials 1 and 2.

The conclusions obtained for per cent of motility for trial 2 seem to fit the data for rate of motility. Ejaculate 10 appeared to be the only serious exception.

Table 3

Multiple comparisons of treatment means - Trial 2

Per cent motility					Rate of motility				
Ejac.	Treatment means				Ejac.	Treatment means			
9	G_2*	G_1*	P_2*	P_1*	9	G_2	P_2	G_1	P_1
	12	22	23	25		1.1	1.7	2.1	2.2
6	G_2	P_2	G_1	P_1	6	P_2	P_1	G_2	G_1
	9	11	13	13		1.2	1.3	1.5	1.6
7	P_2	G_2	G_1	P_1	7	G_2	P_2	G_1	P_1
	3.2	3.4	18	24		.9	1.1	1.8	2.1
8	P_2	G_2	P_1	G_1	8	P_2	G_2	P_1	G_1
	8.8	12	24	31		1.2	2.0	2.7	2.8
10	G_1	G_2	P_2	P_1	10	G_2	G_1	P_2	P_1
	16	17	18	26		1.5	1.7	1.9	2.4

Summary

Comparison	Ejaculate									
	Per cent motility					Rate of motility				
	9	6	7	8	10	9	6	7	8	10
$P_1 = G_1$	≠	≠	≠	≠	≠	≠	≠	≠	≠	P_1
$P_1 = G_2$	P_1	≠	P_1	≠	≠	P_1	≠	P_1	≠	P_1
$P_1 = P_2$	≠	≠	P_1	P_1	≠	P_1	≠	P_1	P_1	≠
$G_1 = P_2$	≠	≠	G_1	G_1	≠	≠	≠	G_1	G_1	≠
$G_1 = G_2$	G_1	≠	G_1	G_2	≠	G_1	≠	G_1	≠	≠
$P_2 = G_2$	P_2	≠	≠	≠	≠	P_2	≠	≠	≠	≠

*Notation same as for table 1.

Table 4

Ejaculates on which data in Table 3 were based

Ejac.	Bull	Date collected	Date read	
			Initial	Final
9	23	3/22/56	3/23/56	5/22/56
6	15	2/17/56	2/18/56	5/24/56
7	37	2/24/56	2/25/56	5/21/56
8	MW	3/1/56	3/2/56	5/5/56
10	15	3/22/56	3/23/56	5/23/56

Trial 3

Trial 3 (table 5) measured the effect of storage in air for periods of 1-2 weeks. These data indicated that the plastic container was superior for 2 of 5 ejaculates after freezing (per cent motility criterion), but that this advantage was lost after storage. These were essentially the same results as observed in trial 1. Rate of deterioration appeared to be constant with the exception of ejaculates 3 and 4 which have been discussed in detail under trial 1. Explanation of these results seems to follow the logic applied to trials 1 and 2.

Rate of motility data was much less variable for trial 3 than per cent motility. Note that ejaculate 2 was the only case in which any comparisons were rejected. These results were almost the reverse of those obtained for per cent motility.

Trial 4

Trial 4 (table 7) measured the effect of storage in air for periods of 30 to 60 days. Data for this trial (per cent motility criterion) seemed to be less variable than for the preceding trials. Ejaculates 7 and 11 were the only ones for which any comparisons were rejected. Ejaculates 6, 7 and 11, 12 appeared in tables 3 and 5 respectively and presented much the same picture there as obtained in trial 4. From this, indications are that the variability observed in ejaculates 7 and 11 was inherent in the ejaculates themselves and was independent of treatment.

Rate of motility data followed closely that for per cent of motility data. However, ejaculate 5 appeared to have deteriorated more rapidly for rate than for percent of motility.

Table 5

Multiple comparisons of treatment means - Trial 3

Per cent motility					Rate of motility				
Ejac.	Treatment means				Ejac.	Treatment means			
2	G_1*	G_2*	P_1*	P_2*	2	G_1	P_1	G_2	P_2
	24	29	32	34		2.0	2.0	2.8	2.8
3	G_2	G_1	P_2	P_1	3	G_2	G_1	P_1	P_2
	10	19	24	29		1.3	1.6	2.0	2.1
4	G_2	P_2	G_1	P_1	4	G_1	G_2	P_2	P_1
	.4	.8	.8	3.8		.4	.4	.6	1.0
11	P_2	G_2	P_1	G_1	11	G_2	G_1	P_1	P_2
	9	10	21	25		.8	1.0	1.0	1.2
12	P_1	G_1	G_2	P_2	12	G_1	G_2	P_2	P_1
	5	6	8	11		.9	.9	.9	1.1

Summary

Comparison	Ejaculate									
	Per cent motility					Rate of motility				
	2	3	4	11	12	2	3	4	11	12
$P_1 = G_1$	≠	P_1	P_1	≠	≠	≠	≠	≠	≠	≠
$P_1 = G_2$	≠	P_1	P_1	P_1	≠	G_2	≠	≠	≠	≠
$P_1 = P_2$	≠	≠	P_1	P_1	≠	P_2	≠	≠	≠	≠
$G_1 = P_2$	≠	≠	≠	G_1	≠	P_2	≠	≠	≠	≠
$G_1 = G_2$	≠	G_1	≠	G_1	≠	G_2	≠	≠	≠	≠
$P_2 = G_2$	≠	≠	≠	≠	≠	≠	≠	≠	≠	≠

*Notation same as for table 1.

Table 6

Ejaculates on which data in Table 5 were based

Ejac.	Bull	Date collected	Date read	
			Initial	Final
2	21	12/29/55	12/30/56	1/23/56
3	15	1/3/56	1/4/56	1/10/56
4	36	1/10/56	1/11/56	1/22/56
11	99	5/5/56	5/6/56	6/5/56
12	23	5/5/56	5/6/56	6/5/56

Table 7

Multiple comparisons of treatment means - Trial 4

Per cent motility					Rate of motility				
Ejac.	Treatment means				Ejac.	Treatment means			
5	G_1*	G_2*	P_1*	P_2*	5	G_2	G_1	P_1	P_2
	24	25	26	29		2.4	2.5	2.6	2.7
6	G_2	G_1	P_1	P_2	6	G_2	P_1	G_1	P_2
	10	13	13	19		1.1	1.3	1.6	2.0
7	P_2	G_2	G_1	P_1	7	P_2	G_2	G_1	P_1
	6	7	18	24		.9	1.3	1.8	2.1
11	G_2	P_2	P_1	G_1	11	G_1	P_1	G_2	P_2
	12	14	21	25		1.0	1.0	1.5	1.5
12	G_2	P_2	G_1	P_1	12	G_1	P_1	G_2	P_2
	4.4	7	8	11		.9	1.1	1.5	1.6

Summary

Comparison	Ejaculate									
	Per cent motility					Rate of motility				
	5	6	7	11	12	5	6	7	11	12
$P_1 = G_1$	≠	≠	≠	≠	≠	≠	≠	≠	≠	≠
$P_1 = G_2$	≠	≠	P_1	P_1	≠	≠	≠	P_1	≠	≠
$P_1 = P_2$	≠	≠	P_1	≠	≠	≠	≠	P_1	≠	≠
$G_1 = P_2$	≠	≠	≠	G_1	≠	≠	≠	G_1	≠	≠
$G_1 = G_2$	≠	≠	≠	G_1	≠	≠	≠	≠	≠	≠
$P_2 = G_2$	≠	≠	≠	≠	≠	≠	P_2	≠	≠	≠

*Notation same as for table 1.

Table 8

Ejaculates on which data in Table 7 were based

Ejac.	Bull	Date collected	Date read	
			Initial	Final
5	35	1/22/56	1/23/56	3/3/56
6	15	2/17/56	2/18/56	4/14/56
7	37	2/24/56	2/25/56	4/14/56
11	99	5/5/56	5/6/56	5/20/56
12	23	5/5/56	5/6/56	5/20/56

Trial 5

Trial 5 (table 9) measured the effect of shipment on semen quality when semen was stored in plastic or glass containers. In the 5 ejaculates constituting this trial, there were no container differences due to simulated shipment. Ejaculate 9 indicated that plastic was superior to glass at the beginning of the trial. However, the same relative difference was maintained throughout the trial indicating no difference due to simulated shipment (per cent motility criterion).

It was noted that ejaculates 6, 8 and 10 deteriorated considerably. Possible explanations for this could be due to:

(1) The technique for handling semen could have been faulty. This does not seem to give an adequate explanation since all ejaculates did not follow the same abnormal deterioration rate. It is possible that some unknown factor was introduced in the handling of ejaculates 6, 8 and 10 and not for 7 and 9. This does not seem likely since all ejaculates were handled in a similar way.

(2) Semen constituting this trial had been stored previously for 60-90 days and had possibly deteriorated to some extent. This may have been of sufficient degree to cause the semen to lack the vigor required to withstand the hazards of simulated shipment and handling.

In this trial there appeared to be some discrepancy in the relationship between the data for rate of motility and per cent motility. Factors responsible for this phenomenon, as discussed in trial 1, would seem to apply here.

Trial 6

An analysis of variance utilizing the data for 7 ejaculates (table 11) showed that there was no significant difference between plastic and glass

Table 9

Multiple comparisons of treatment means - Trial 5

Per cent motility					Rate of motility				
Ejac.	Treatment means				Ejac.	Treatment means			
7	P_2*	P_1*	G_1*	G_2*	7	P_2	G_1	P_1	G_2
	1.4	3.2	3.4	4.2		.6	.9	1.1	1.3
9	G_2	G_1	P_2	P_1	9	G_1	G_2	P_1	P_2
	10	12	22	23		1.1	1.4	1.7	2.2
10	G_2	P_2	G_1	P_1	10	G_2	P_2	G_1	P_1
	0	0	17	18		0	0	1.5	1.9
6	G_2	P_2	G_1	P_1	6	G_2	P_2	P_1	G_1
	.6	1.0	9.0	11.0		.6	.6	1.2	1.5
8	P_2	G_2	P_1	G_1	8	P_2	P_1	G_2	G_1
	0	1.8	4.4	5.4		0	.7	.8	1.4

Summary

Comparison	Ejaculate									
	Per cent motility					Rate of motility				
	7	9	10	6	8	7	9	10	6	8
$P_1 = G_1$	≠	P_1	≠	≠	≠	≠	≠	≠	≠	G_1
$P_1 = G_2$	≠	P_1	P_1	P_1	≠	≠	≠	P_1	≠	≠
$P_1 = P_2$	≠	≠	P_1	P_1	P_1	≠	P_2	P_1	≠	P_1
$G_1 = P_2$	≠	P_2	G_1	G_1	G_1	≠	P_2	G_1	G_1	G_1
$G_1 = G_2$	≠	≠	G_1	G_1	≠	≠	≠	G_1	G_1	≠
$P_2 = G_2$	≠	P_2	≠	≠	≠	≠	P_2	≠	≠	G_2

*Notation same as for table 1.

Table 10

Ejaculates on which data in Table 9 were based

Ejac.	Bull	Date collected	Date read	
			Initial	Final
7	37	2/25/56	5/21/56	5/24/56
9	23	3/23/56	5/22/56	5/25/56
10	15	3/23/56	5/27/56	5/30/56
6	15	2/17/56	5/24/56	5/27/56
8	MW	3/2/56	5/27/56	5/30/56

containers due to sealing effects ($P < .05$). This was true for both per cent and rate of motility. Indications from this data are that the semen immediately before freezing was of essentially the same quality for both the plastic and glass containers. Any subsequent change, therefore, should be attributed to factors other than unequal treatment means at time of freezing.

Table 11

Analysis of variance of trial 6

Source	Per cent motility				Rate of motility			
	d.f.	S.S.	M.S.	F	d.f.	S.S.	M.S.	F
Total	68	19355.1	xxxx	xxxx	68	17.9	xxxx	xxxx
Error	55	4620.0	84.0	xxxx	55	6.3	.11	xxxx
Container	1	252.2	252.0	2.98	1	.2	.20	1.82
Ejaculate	6	13950.4	2325.1	xxxx	6	11.1	1.85	xxxx
Interaction	6	532.5	88.8	1.06	6	.3	.05	.45

Deterioration

The large amount of deterioration observed in these data can be explained by the inadvertant addition of sulfanilamide to the diluter. This is in agreement with previous experimental work (2).

Generalizations

It would be difficult to draw any set of conclusions that would explain all sets of the data perfectly. There seem to be some assertions, however, that would fit the majority of the data. Whether or not these are correct remains for further experimentation to determine.

(1) Freezing ability - indications were that there was little difference due to container effect. For certain ejaculates there was some advantage in favor of the plastic container. This would appear to be slight and variable for individual ejaculates.

(2) Storing ability - here, also, there appeared to be little difference due to container effect. In this case there was some indication that the glass container may store to better advantage than the plastic container. This would appear to be slight and a function of the individual ejaculate.

(3) Storing medium - From the data there were no indications that the plastic or glass container stored to any better advantage in either air or alcohol.

(4) Shipping ability - container type seemed to have no effect on the ability of semen to withstand simulated shipment.

SUMMARY

A short term study was conducted on 13 ejaculates of 8 different bulls to determine if any practical difference existed between plastic and glass containers for the storage of frozen semen. Also of interest were factors which might have been responsible for any observed container differences.

Results obtained were variable and inconclusive. Some advantage was found in freezing ability of semen in the plastic container, however, in general this was slight. Much the same relationship was true for storing ability with the exception that the glass container indicated an advantage in this case.

No indication was found that semen in plastic or glass containers stored to any better advantage in air or alcohol. Glass or plastic containers seemed to have no effect on ability of semen to withstand simulated shipment.

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APPENDIX

TABLES APPENDIX PAGE

TABLE I

Original data - trial 1 (per cent motility)

Initial Observations

	Glass					Plastic				
	Ejaculate					Ejaculate				
	1	2	3	4	12	1	2	3	4	12
	20	30	20	1	5	30	40	30	5	0
	20	20	25	0	10	40	30	15	3	20
	5	20	20	0	0	35	30	40	1	5
	15*	20	15	0	10	35	20	30	5	15
	15*	30	15	3	15	35*	40	30	5	15
Mean	15	24	19	.8	8	35	32	29	3.8	11

Final Observations

	Glass					Plastic				
	Ejaculate					Ejaculate				
	1	2	3	4	12	1	2	3	4	12
	15	30	15	0	5	10	30	20	1	5
	20	15	10	0	0	30	30	25	1	20
	25	35	15	0	15	15	30	15	0	20
	20	30	10	0	25	20	15	30	1	10
	30	40	10*	1	2	25	25*	20	1	10
Mean	22	30	12	.2	9.4	20	26	22	.8	13.0

*Indicates value calculated for missing data.

Table II

Original data - trial 1 (rate of motility)

Initial Observations

	Glass					Plastic				
	Ejaculate					Ejaculate				
	1	2	3	4	12	1	2	3	4	12
	1.0	2.0	1.0	1.0	1.0	2.0	2.0	2.0	1.0	0
	1.0	2.0	2.0	0	1.0	2.0	2.0	1.0	1.0	1.5
	1.0	2.0	2.0	0	0	2.0	2.0	3.0	1.0	1.0
	1.0*	2.0	2.0	0	1.0	2.0	2.0	2.0	1.0	1.5
	1.0*	2.0	1.0	1.0	1.5	2.0*	2.0	2.0	1.0	1.5
Mean	1.0	2.0	1.6	.4	.9	2.0	2.0	2.0	1.0	1.1

Final Observations

	Glass					Plastic				
	Ejaculate					Ejaculate				
	1	2	3	4	12	1	2	3	4	12
	1.0	3.0	1.5	0	1.0	1.0	2.0	2.0	1.0	1.0
	2.0	2.0	1.0	0	0	2.0	3.0	2.5	1.0	1.5
	2.0	3.0	2.0	0	1.5	2.0	3.0	2.0	0	1.5
	1.0	2.5	1.0	0	1.0	1.0	2.0	2.5	1.0	1.0
	2.0	3.0	1.5*	1.0	1.0	2.0	2.5*	2.5	1.0	1.0
Mean	1.6	2.7	1.4	.2	.9	1.6	2.5	2.3	.8	1.2

*Indicates value calculated for missing data.

Table III

Analysis of variance - trial 1

Ejac.	Source	Per cent Motility				Rate of Motility			
		d.f.	S.S.	M.S.	Sm	d.f.	S.S.	M.S.	Sm
1	Total	16	1670.0	xxxxx		16	4.9	xxxxx	
	Container	1	405.0	405.0		1	1.2	1.2	
	Time	1	80.0	80.0		1	0	0	
	Interaction	1	605.0	605.0		1	1.3	1.3	
	Error	13	580.0	44.6	2.99	13	2.4	.185	.192
2	Total	18	1126.0	xxxxx		18	3.7	xxxxx	
	Container	1	20.0	20.0		1	.05	.05	
	Time	1	0.0	0.0		1	1.8	1.8	
	Interaction	1	180.0	180.0		1	.05	.05	
	Error	15	920.0	61.3	3.50	15	1.8	.120	.154
3	Total	18	1295.0	xxxxx		18	6.64	xxxxx	
	Container	1	500.0	500.0		1	2.33	2.33	
	Time	1	245.0	245.0		1	.02	.02	
	Interaction	1	0.0	0.0		1	.09	.09	
	Error	15	550.0	36.7	2.71	15	4.2	.28	.237
4	Total	19	60.8	xxxxx		19	4.8	xxxxx	
	Container	1	16.2	16.2		1	1.8	1.8	
	Time	1	16.2	16.2		1	.20	.20	
	Interaction	1	7.2	7.2		1	0.0	0.0	
	Error	16	21.2	1.33	.516	16	2.8	.175	.187
12	Total	19	1086.5	xxxxx		19	4.74	xxxxx	
	Container	1	54.4	54.4		1	.32	.32	
	Time	1	14.4	14.4		1	.02	.02	
	Interaction	1	.5	.5		1	.0	.0	
	Error	16	1017.2	63.6	3.57	16	4.4	.275	.234

Table IV

Original data - trial 2 (per cent motility)

Initial Observations

	Glass					Plastic				
	Ejaculate					Ejaculate				
	9	6	7	8	10	9	6	7	8	10
	25	5	5	30	15	20	10	15	5	20
	30	10	30	15	20	20	10	40	40	25
	20	10	30	40	10	25	20	10	15	40
	20	20	10	35	15	35	5	30	25	20
	15	20	15	35	20	25	20	25	35	25
Mean	22	13	18	31	16	25	13	24	24	26

Final Observations

	Glass					Plastic				
	Ejaculate					Ejaculate				
	9	6	7	8	10	9	6	7	8	10
	20	5	2	5	10	20	10	2	2	15
	20	10	5	20	30	20	15	2	10	10
	5	20	5	5	15	25	15	5	25	15
	10	5	0	10	20	25	5	5	0	20
	5	5	5	20	10	25	10	2	7*	30
Mean	12	9	3.4	12	17	23	11	3.2	8.8	18

*Indicates value calculated for missing data.

Table V

Original data - trial 2 (rate of motility)

Initial Observations

	Glass					Plastic				
	Ejaculate					Ejaculate				
	9	6	7	8	10	9	6	7	8	10
	2.0	1.0	1.5	3.0	1.5	1.5	1.0	2.0	2.0	2.0
	2.5	1.5	1.5	2.0	2.0	2.0	1.0	2.5	3.0	2.5
	2.0	2.0	2.5	3.0	1.0	2.5	1.5	1.5	2.5	2.5
	2.0	1.5	2.0	3.0	2.0	2.5	1.0	2.5	3.0	2.0
	2.0	2.0	1.5	3.0	2.0	2.5	2.0	2.0	3.0	3.0
Mean	2.1	1.6	1.8	2.8	1.7	2.2	1.3	2.1	2.7	2.4

Final Observations

	Glass					Plastic				
	Ejaculate					Ejaculate				
	9	6	7	8	10	9	6	7	8	10
	1.0	1.5	1.0	2.0	1.0	1.5	1.0	1.0	1.5	1.5
	1.5	1.5	1.0	3.0	2.5	1.5	1.0	1.0	1.0	2.0
	1.0	2.0	1.5	1.0	1.5	2.0	1.5	1.0	2.5	2.0
	1.0	1.0	0	1.0	1.5	1.5	1.5	1.5	0	2.0
	1.0	1.5	1.0	3.0	1.0	2.0	1.0	1.0	1.0*	2.0
Mean	1.1	1.5	.9	2.0	1.5	1.7	1.2	1.1	1.2	1.9

*Indicates value calculated for missing data.

Table VI

Analysis of variance - trial 2

Ejac.	Source	Per cent motility				Rate of motility			
		d.f.	S.S.	M.S.	Sm	d.f.	S.S.	M.S.	Sm
9	Total	19	1045.0	xxxx		19	5.24	xxxx	
	Container	1	244.9	244.9		1	.62	.62	
	Time	1	180.0	180.0		1	2.82	2.82	
	Interaction	1	80.1	80.1		1	.30	.30	
	Error	16	540.0	33.8	2.60	16	1.5	.0938	.137
6	Total	19	655.0	xxxx		19	2.80	xxxx	
	Container	1	5.0	5.0		1	.45	.45	
	Time	1	45.0	45.0		1	.01	.01	
	Interaction	1	5.0	5.0		1	.04	.04	
	Error	16	600.0	37.5	2.74	16	2.3	.1438	.169
7	Total	19	2788.5	xxxx		19	7.74	xxxx	
	Container	1	42.0	42.0		1	.32	.32	
	Time	1	1566.4	1566.4		1	4.51	4.51	
	Interaction	1	48.1	48.1		1	.01	.01	
	Error	16	1132.0	70.8	3.76	16	2.9	.1813	.191
8	Total	18	3420.9	xxxx		18	17.14	xxxx	
	Container	1	130.0	130.0		1	1.02	1.02	
	Time	1	1462.0	1462.0		1	6.62	6.62	
	Interaction	1	18.1	18.1		1	.60	.60	
	Error	15	1810.8	120.7	4.91	15	8.9	.5933	.345
10	Total	19	1163.7	xxxx		19	5.44	xxxx	
	Container	1	151.2	151.2		1	1.52	1.52	
	Time	1	61.2	61.2		1	.62	.62	
	Interaction	1	101.3	101.3		1	.10	.10	
	Error	16	850.0	53.1	3.26	16	3.2	.20	.20

Table VII

Original data - trial 3 (per cent motility)

Initial Observations

	Glass					Plastic				
	Ejaculate					Ejaculate				
	2	3	4	11	12	2	3	4	11	12
	30	20	1	25	5	40	30	5	15	0
	20	25	0	25	10	30	15	3	25	20
	20	20	0	25	0	30	40	1	20	5
	20	15	0	20	10	20	30	5	30	15
	30	15	3	30	15	40	30	5	15	15
Mean	24	19	.8	25	8	32	29	3.8	21	11.0

Final Observations

	Glass					Plastic				
	Ejaculate					Ejaculate				
	2	3	4	11	12	2	3	4	11	12
	40	10	0	5	0	40	20	0	10	0
	25	10	1.0	0	15	20	15	2	5	10
	20	10	0	15	5	40	30	1	15	10
	20	10*	0	10	5	30	25	0	0	5
	40	10*	1.0	20	5	40	30	1	15	0
Mean	29	10	.4	10	6	34	24	.8	9	5

*Indicates value calculated for missing data.

Table VIII

Original data - trial 3 (rate of motility)

Initial Observations

	Glass					Plastic				
	Ejaculate					Ejaculate				
	2	3	4	11	12	2	3	4	11	12
	2.0	1.0	1.0	1.0	1.0	2.0	2.0	1.0	1.0	0
	2.0	2.0	0	1.0	0	2.0	1.0	1.0	1.0	1.5
	2.0	2.0	0	1.0	1.5	2.0	3.0	1.0	1.0	1.0
	2.0	2.0	0	1.0	1.0	2.0	2.0	1.0	1.0	1.5
	2.0	1.0	1.0	1.0	1.0	2.0	2.0	1.0	1.0	1.5
Mean	2.0	1.6	.4	1.0	.9	2.0	2.0	1.0	1.0	1.1

Final Observations

	Glass					Plastic				
	Ejaculate					Ejaculate				
	2	3	4	11	12	2	3	4	11	12
	3.0	1.0	0	1.0	0	3.0	2.0	0	1.5	0
	2.5	1.0	1.0	0	1.5	2.0	2.0	1.0	1.5	2.0
	2.5	2.0	0	1.0	1.0	3.0	2.0	1.0	1.0	1.5
	3.0	1.5*	0	1.0	1.0	3.0	2.5	0	0	1.0
	3.0	1.0*	1.0	1.0	1.0	3.0	2.0	1.0	2.0	0
Mean	2.8	1.3	.4	.8	.9	2.8	2.1	.6	1.2	.9

*Indicates value calculated for missing data.

Table IX

Analysis of variance - trial 3

Ejac.	Source	Per cent Motility				Rate of Motility			
		d.f.	S.S.	M.S.	Sm	d.f.	S.S.	M.S.	Sm
2	Total	19	1423.7	xxxxx		19	6.25	xxxxx	
	Container	1	211.2	211.2		1	1.76	1.76	
	Time	1	61.2	61.2		1	.05	.05	
	Interaction	1	11.3	11.3		1	.24	.24	
	Error	16	1140.0	71.3	3.78	16	4.20	.263	.229
3	Total	17	1545.0	xxxxx		17	6.25	xxxxx	
	Container	1	720.0	720.0		1	1.76	1.76	
	Time	1	245.0	245.0		1	.05	.05	
	Interaction	1	20.0	20.0		1	.24	.24	
	Error	14	560.0	40.0	2.83	14	4.2	.300	.244
4	Total	19	60.9	xxxxx		19	4.8	xxxxx	
	Container	1	14.4	14.4		1	.8	.8	
	Time	1	14.4	14.4		1	.2	.2	
	Interaction	1	8.5	8.5		1	.2	.2	
	Error	16	23.6	1.48	.544	16	3.6	.225	.212
11	Total	19	1593.7	xxxxx		19	3.5	xxxxx	
	Container	1	31.2	31.2		1	.2	.2	
	Time	1	911.2	911.2		1	0.0	0.0	
	Interaction	1	11.3	11.3		1	.2	.2	
	Error	16	640.0	40.0	2.83	16	3.1	.194	.197
12	Total	19	725.0	xxxxx		19	7.45	xxxxx	
	Container	1	5.0	5.0		1	.05	.05	
	Time	1	80.0	80.0		1	.05	.05	
	Interaction	1	20.0	20.0		1	.05	.05	
	Error	16	620.0	38.8	2.79	16	7.3	.456	.302

Table X

Original data - trial 4 (per cent motility)

Initial Observations

	Glass					Plastic				
	Ejaculate					Ejaculate				
	5	6	7	11	12	5	6	7	11	12
	10	5	5	25	5	20	10	15	15	0
	30	10	30	25	10	30	10	40	25	20
	40	10	30	25	0	30	20	10	20	5
	20	20	10	20	10	20	5	30	30	15
	20	20	15	30	15	30	20	25	15	15
Mean	25	13	18	25	8	26	13	24	21	11

Final Observations

	Glass					Plastic				
	Ejaculate					Ejaculate				
	5	6	7	11	12	5	6	7	11	12
	30	15	5	5	5	20	20	5	20	5
	25	15	5	5	5	30	10	0	10	10
	25	15	5	15	5	25	20	10	15	5
	25	5	5	10	5	40	25	10	15	10
	20	0	15	25	2	30	20	5	10	5
Mean	25	10	7	12	4.4	29	19	6	14	7

Table XI

Original data - trial 4 (rate of motility)

Initial Observations

	Glass					Plastic				
	Ejaculate					Ejaculate				
	5	6	7	11	12	5	6	7	11	12
	2.0	1.0	1.5	1.0	1.0	2.0	1.0	2.0	1.0	0
	2.5	1.5	1.5	1.0	1.0	3.0	1.0	2.5	1.0	1.5
	3.0	2.0	2.5	1.0	0	3.0	1.5	1.5	1.0	1.0
	2.5	1.5	2.0	1.0	1.0	2.0	1.0	2.5	1.0	1.5
	2.5	2.0	1.5	1.0	1.5	3.0	2.0	2.0	1.0	1.5
Mean	2.5	1.6	1.8	1.0	.9	2.6	1.3	2.1	1.0	1.1

Final Observations

	Glass					Plastic				
	Ejaculate					Ejaculate				
	5	6	7	11	12	5	6	7	11	12
	2.0	1.5	1.0	1.0	1.5	2.5	2.5	1.0	2.0	1.5
	2.5	1.5	1.5	1.0	1.5	2.5	1.5	0	1.5	2.0
	2.5	1.5	1.5	2.0	1.5	2.5	1.5	1.0	1.0	1.5
	2.5	1.0	1.5	1.5	2.0	3.0	2.0	1.5	1.5	2.0
	2.5	0	1.0	2.0	1.0	3.0	2.5	1.0	1.5	1.0
Mean	2.4	1.1	1.3	1.5	1.5	2.7	2.0	.9	1.5	1.6

Table XII

Analysis of variance - trial 4

Ejac.	Source	Per cent Motility				Rate of Motility			
		d.f.	S.S.	M.S.	Sm	d.f.	S.S.	M.S.	Sm
5	Total	19	980.0	xxxxx		19	2.45	xxxxx	
	Container	1	45.0	45.0		1	.20	.20	
	Time	1	20.0	20.0		1	0.0	0.0	
	Interaction	1	5.0	5.0		1	.05	.05	
	Error	16	910.0	56.9	3.37	16	2.20	.138	.166
6	Total	19	893.7	xxxxx		19	6.5	xxxxx	
	Container	1	101.2	101.2		1	.45	.45	
	Time	1	11.2	11.2		1	.05	.05	
	Interaction	1	101.3	101.3		1	1.8	1.8	
	Error	16	680.0	42.5	2.92	16	4.2	.263	.229
7	Total	19	2393.7	xxxxx		19	7.24	xxxxx	
	Container	1	31.2	31.2		1	.02	.02	
	Time	1	1051.2	1051.2		1	3.62	3.62	
	Interaction	1	61.3	61.3		1	.56	.56	
	Error	16	1250.0	78.1	3.95	16	3.00	.188	.194
11	Total	19	1120.0	xxxxx		19	2.75	xxxxx	
	Container	1	5.0	5.0		1	0.0	0.0	
	Time	1	500.0	500.0		1	1.25	1.25	
	Interaction	1	45.0	45.0		1	0.0	0.0	
	Error	16	570.0	35.6	2.67	16	1.5	.938	.434
12	Total	19	548.8	xxxxx		19	5.74	xxxxx	
	Container	1	39.2	39.2		1	.12	.12	
	Time	1	72.2	72.2		1	1.52	1.52	
	Interaction	1	.2	.2		1	0.0	0.0	
	Error	16	437.2	28.3	2.38	16	4.10	.256	.226

Table XIII

Original data - trial 5 (per cent motility)

Initial Observations

	Glass					Plastic				
	Ejaculate					Ejaculate				
	7	9	10	6	8	7	9	10	6	8
	2	20	10	5	10	2	20	15	10	0
	5	20	30	10	2	2	20	10	15	0
	5	5	15	20	5	5	25	15	15	10
	0	10	20	5	5	5	25	20	5	2
	5	5	10	5	5	2	25	30	10	10
Mean	3.4	12	17	9	5.4	3.2	23	18	11	4.4

Final Observations

	Glass					Plastic				
	Ejaculate					Ejaculate				
	7	9	10	6	8	7	9	10	6	8
	1	20	0	0	0	3	20	0	2	0
	0	10	0	0	5	1	25	0	0	0
	15	5	0	1	2	0	25	0	2	0
	3	5	0	1	1	3	15	0	0	0
	2	10	0	1	1	0	25	0	1	0
Mean	4.2	10	0	.6	1.8	1.4	22	0	1.0	0

Table XIV

Original data - trial 5 (rate of motility)

Initial Observations

	Glass					Plastic				
	Ejaculate					Ejaculate				
	7	9	10	6	8	7	9	10	6	8
	1.0	1.0	1.0	1.5	2.0	1.0	1.5	1.5	1.0	0
	1.0	1.5	2.5	1.5	1.5	1.0	1.5	2.0	1.0	0
	1.5	1.0	1.5	2.0	1.5	1.0	2.0	2.0	1.5	1.5
	0	1.0	1.5	1.0	1.0	1.5	1.5	2.0	1.5	1.0
	1.0	1.0	1.0	1.5	1.0	1.0	2.0	2.0	1.0	1.0
Mean	.9	1.1	1.5	1.5	1.4	1.1	1.7	1.9	1.2	.7

Final Observations

	Glass					Plastic				
	Ejaculate					Ejaculate				
	7	9	10	6	8	7	9	10	6	8
	1.5	2.0	0	0	0	1.0	2.5	0	1.0	0
	0	1.5	0	0	1.0	1.0	2.5	0	0	0
	2.0	1.0	0	1.0	1.0	0	2.5	0	1.0	0
	1.5	1.0	0	1.0	1.0	1.0	1.5	0	0	0
	1.5	1.5	0	1.0	1.0	0	2.0	0	1.0	0
Mean	1.3	1.4	0	.6	.8	.6	2.2	0	.6	0

Table XV

Analysis of variance - trial 5

Ejac.	Source	Per cent Motility				Rate of Motility			
		d.f.	S.S.	M.S.	Sm	d.f.	S.S.	M.S.	Sm
7	Total	19	212.9	xxxxx		19	6.24	xxxxx	
	Container	1	11.2	11.2		1	.32	.32	
	Time	1	1.2	1.2		1	.02	.02	
	Interaction	1	8.5	8.5		1	1.00	1.00	
	Error	16	192.0	12.0	1.55	16	4.9	.306	.247
9	Total	19	1163.7	xxxxx		19	5.3	xxxxx	
	Container	1	661.2	661.2		1	2.45	2.45	
	Time	1	11.2	11.2		1	.8	.8	
	Interaction	1	1.3	1.3		1	.05	.05	
	Error	16	490.0	30.6	2.47	16	2.0	.125	.158
10	Total	19	2043.7	xxxxx		19	16.55	xxxxx	
	Container	1	1.2	1.2		1	.20	.20	
	Time	1	1531.2	1531.2		1	14.45	14.45	
	Interaction	1	1.3	1.3		1	.20	.20	
	Error	16	510.0	31.9	2.53	16	1.70	.106	.146
6	Total	19	678.8	xxxxx		19	6.24	xxxxx	
	Container	1	7.2	7.2		1	.12	.12	
	Time	1	423.2	423.2		1	2.82	2.82	
	Interaction	1	3.2	3.2		1	.10	.10	
	Error	16	245.2	15.3	1.75	16	3.2	.200	.200
8	Total	19	245.8	xxxxx		19	8.24	xxxxx	
	Container	1	9.8	9.8		1	2.82	2.82	
	Time	1	80.0	80.0		1	2.12	2.12	
	Interaction	1	.8	.8		1	.00	.00	
	Error	16	155.2	9.7	1.39	16	3.30	.206	.203

Table XVI

Original data - trial 6 (per cent motility and rate of motility)

Per cent Motility

	Glass							Plastic						
	Ejaculate							Ejaculate						
	1	2	3	4	5	6	7	1	2	3	4	5	6	7
	30	30	50	30	50	80	50	20	20	50	50	60	60	50
	30	50	40	60	60	70	60	20	20	50	50	60	70	70
	20	50	50	50	40	60	60	20	20	35	40	40	80	60
	20	40	50	40	60	70	60	30	30	40	30	40	80	60
		30	50	30	50	60	80	40	40	30	40	40	80	50
Total	100	200	240	210	260	340	310	130	130	205	210	240	370	290

Rate of Motility

	Glass							Plastic						
	Ejaculate							Ejaculate						
	1	2	3	4	5	6	7	1	2	3	4	5	6	7
	3.0	2.5	3.0	2.0	3.0	4.0	3.5	2.0	2.5	3.0	3.0	3.5	3.5	2.5
	3.0	2.5	3.0	3.0	3.5	4.0	2.5	2.0	2.5	3.0	3.0	3.0	3.5	3.5
	2.0	3.0	3.0	3.0	3.0	3.5	3.5	2.0	2.0	3.0	3.0	3.0	4.0	3.0
	3.0	3.0	3.0	3.0	3.0	3.5	3.0	3.0	2.5	3.0	2.5	3.0	4.0	3.0
		2.0	3.0	3.0	3.0	4.0	3.0	3.0	2.5	3.0	2.5	3.0	4.0	2.5
Total	11.0	13.0	15.0	14.0	15.5	19.0	15.5	12.0	12.0	15.0	14.0	15.5	19.0	14.5

VITA

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