*Evaluation of Frozen Bovine Semen

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The best measure of fertility is the percent pregnancies or the number of inseminations per live calf. The larger the number of cows bred to that ejaculate of semen or to that bull, the more reliable the estimate of fertility. At least 20 cows should be bred to an individual ejaculate or the variation in fertility can be so large that the possibility of making an error in judgment with respect to fertility is greatly increased (2). Fertility varies among bulls and ejaculates within bulls. In fact, when an experiment is conducted utilizing semen from bulls even of the same breed, the largest source of variation is almost always due to bulls.

Since breeding cows to obtain an estimate of fertility of a semen sample is too time consuming and expensive, some laboratory test or tests must be applied to portions of the semen to obtain an estimate of fertility. Numerous laboratory methods of evaluating semen have been developed; and an excellent review of this subject, with respect to liquid semen, has been published (10). The ideal method should be rapid, inexpensive, simple and objective; no ideal test has been developed to date and it is highly unlikely that one will be developed in the near future that fulfills all these requirements. Even the best laboratory methods available are not entirely satisfactory.

Fertility of beef herds should rise slowly as the use of A.I. increases, because the good bulls of proven fertility will be used more heavily. The big problem comes from the young or new bull and from bulls presented for "custom freezing." The "custom bull," is generally presented to the A.I. organization to be frozen with only limited information, if any, about his fertility. The semen is frozen without benefit of prior knowledge, the semen is shipped at the owner's discretion, and fertility information is rarely obtained. Even if fertility data is available, little is generally known about the other factors affecting fertility, such as conditions under which the cows were bred, semen handling procedures, etc. Consequently, even the data available may be of very limited value. If a very high percentage of the cows settled, at least the semen was satisfactory. However, it must be remembered that some clients are satisfied with a pregnancy rate of 50% while others complain about 75%.

The need for a reliable test for quality of frozen semen is clearly evident, and numerous studies have been conducted to develop a test in which the results were highly correlated with fertility (3,4,6,8,9). In all of these tests, motility was the criterion used, following the imposition of some treatment on the semen such as freeze-thaw cycles, exposure to elevated temperatures, or storage or incubation at various temperatures over time. In most cases, correlations of these stress tests with fertility were reasonably high. However, the tests are time consuming, highly subjective and require considerable experience and equipment; thus, they are used routinely in few, if any, laboratories.

Many times criticisms are leveled at frozen semen which was evaluated in the field by inexperienced persons. In many cases the evaluator was expecting too much, particularly from beef bulls. For example, the mean pre-freeze motility of 132 semen samples from 43 bulls of six breeds averaged 62% and 44% post-freeze. These bulls were all selected for fertility and were predominately Holstein (7). In a recent study (12) involving three ejaculates from each of 22 unselected Angus bulls, the mean pre- and post-freeze motility estimates were 43 and 15.2%, respectively. In most laboratories, pre-freeze motility is reduced by 50 to 66% during freezing before the semen is discarded. Elliott (1) has shown definite differences in post-freeze motility

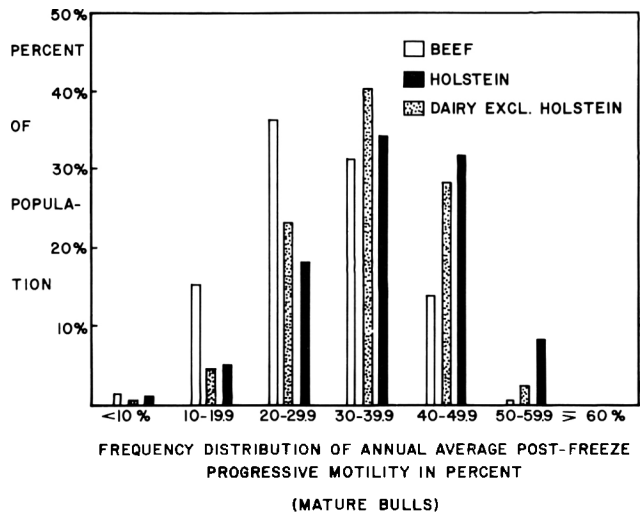

Figure 1. Comparison of percent post-freeze motility among beef, Holstein and dairy exclusive of Holstein bulls. (Elliott, F. I. Proc. 20th Ann. N.A.A.B. Conv. 1967.)

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among beef, dairy other than Holsteins, and Holstein bulls. It is evident from the data presented in Figure 1 that the ranges among the groups are approximately the same. However, the average post-freeze motilities for the beef, dairy, and Holstein bulls were 29.3, 34.7, and 36.6%, respectively. Complaints have been received from farm owners, veterinarians, etc., that frozen semen contained less than 50% motile cells upon receipt. It is difficult to convince these clients that less than 10% of the semen samples, regardless of breed, have post-freeze motility that exceeds 50%.

With respect to percent abnormal spermatozoa (Figure 2), approximately 70% of the Holsteins

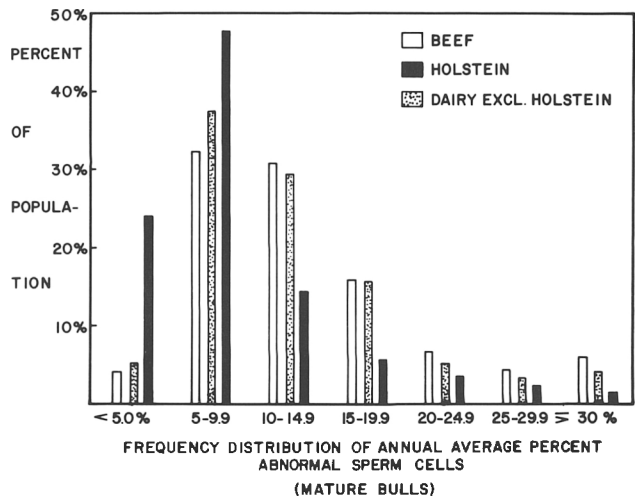

Figure 2. Comparison of percent abnormal spermatozoa among beef, Holstein and dairy exclusive of Holstein bulls. (Elliott, F. I. Proc. 20th Ann. N.A.A.B. Conv. 1967.)

had fewer than 10% abnormal spermatozoa while approximately 60% of the beef bulls had more than 10% abnormal sperm per ejaculate. The averages were 13.8, 12.7, and 9.1% for the beef, dairy, and Holstein, respectively.

Fertility is affected by many factors, and one that is essential to measure is number of sperm per insemination dose. To properly evaluate an ampule of frozen semen about which the evaluator has no prior information, it is essential to count the spermatozoa and calculate the sperm per dose and motile sperm per dose. Once this is done it is still impossible to know if sufficient sperm are present or if too many sperm are present for *maximum reproductive efficiency* (11). Breed and fertility level of the bull are necessary for proper evaluation. To further complicate matters, there are differences among semen-freezing organizations in inseminating dose and inseminating equipment which can influence the volume and number of sperm deposited in the female.

Sullivan (11) conducted a study in which 57,130 cows were bred to Holstein bulls. The 60- to 90-day %NR was significantly lower for insemination doses of 5 million motile spermatozoa post-thaw, compared to doses of 10 and 15 million motile cells. These data were further examined on the basis of the %NR of the bulls used in the study. The bulls were divided into three groups, low (72.6% NR), medium (75.4% NR) and high (77.9% NR). When the concentrations of progressively motile spermatozoa were compared, there was an increase in fertility of each group when the spermatozoa were increased from 5 to 10 million. However, when the number was raised from 10 to 15 million, the %NR increased 1.7 percentage units in the low fertility group, 0.9% NR in the medium group, and *decreased* 1.5 percentage units in the high group (Figure 3). Similar data were collected

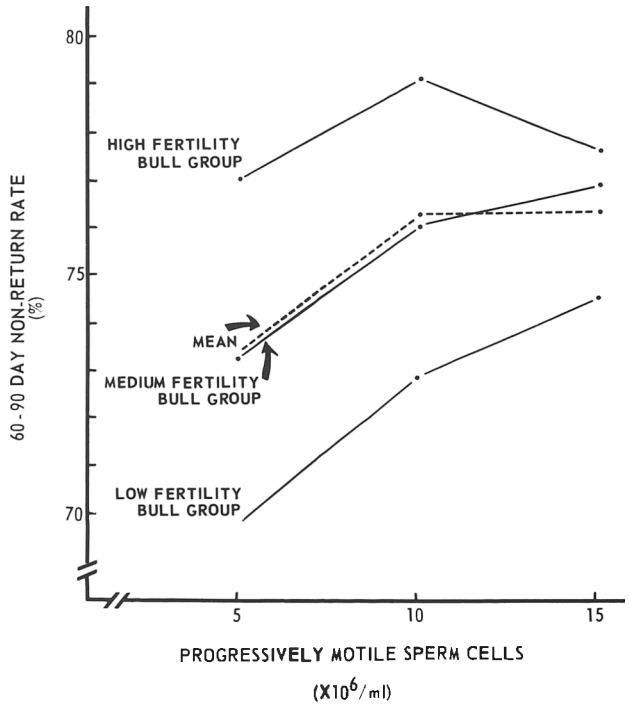

Figure 3. Non-return rate as affected by motile spermatozoa concentration and fertility level of Holstein bulls. (Sullivan, J. J. Proc. Third N.A.A.B. Tech. Conf. on Artif. Insem. and Reprod. 1970.)

on 5,230 Angus and Hereford services to dairy cows. The %NR was higher with 10 million motile sperm post-thaw than with 5 or 15 million sperm (Figure 4). There is obviously a relationship between the number of sperm necessary for maximum reproductive efficiency and fertility level of the bull, and fertility cannot be raised in most bulls by increasing the sperm beyond an optimal number.

In most reputable semen-freezing laboratories, semen volume, sperm concentration and percent progressive motility are determined on each

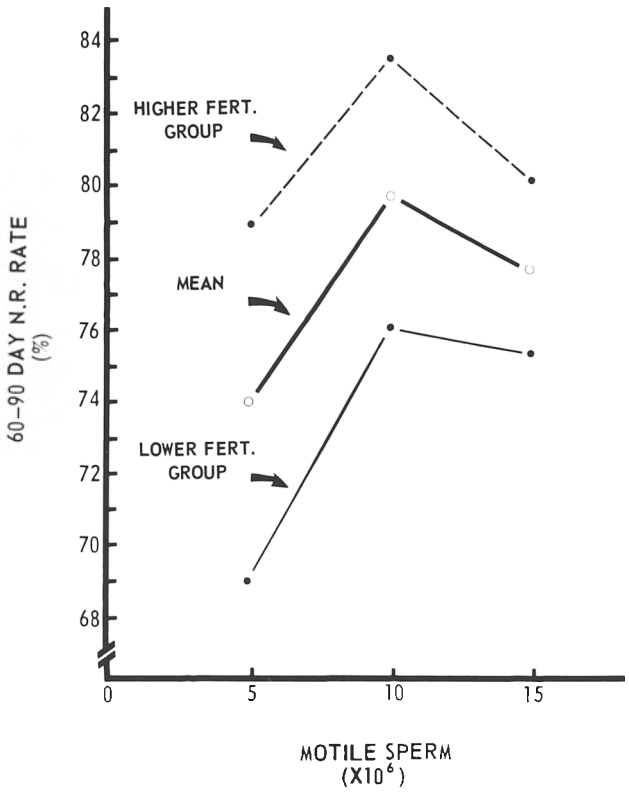

Figure 4. Non-return rate of dairy cows as affected by motile spermatozoa concentration and fertility level of semen from Angus and Hereford bulls. (Sullivan, J. J. Proc. Third N.A.A.B. Tech. Conf. on Artif. Insem. and Reprod. 1970.) Please note that the description of the numbers on the horizontal plane (abscissa) of this figure were not placed on the plate and must be included as indicated above.

ejaculate immediately following collection. Spermatozoon morphology is also determined on selected samples at regular intervals. The results of these measurements determine the number of spermatozoa that will be put into each ampule or insemination dose. If the fertility level of the bull and the number of his sperm normally surviving the freezing process is known, these may also be taken into account when the semen is extended prior to freezing. After processing and freezing, a pre- and post-freeze motility estimate is made on each ejaculate. Then a post-freeze estimation is made on the rate of motility, i.e., the relative vigor of motility. From these measurements, the number of vigorous, motile spermatozoa is determined and from this the decision to retain or discard the semen is made.

The method of evaluating motility in our laboratory is as follows:

1. The equipment, i.e., the thawing bath, slide warmer, stage incubator, ampule holder, phase-contrast microscope, etc. (Figure 5) are checked for cleanliness, adjustment, and proper temperature. *Note:* All material that comes into contact with semen should be maintained at 38°C, except the thawing bath, which should be approximately 0°C.

2. The ampule is removed from the LN container and placed in the thawing bath (Figures 6 and 7). The recommended procedure for thawing of frozen semen has been previously described (5).

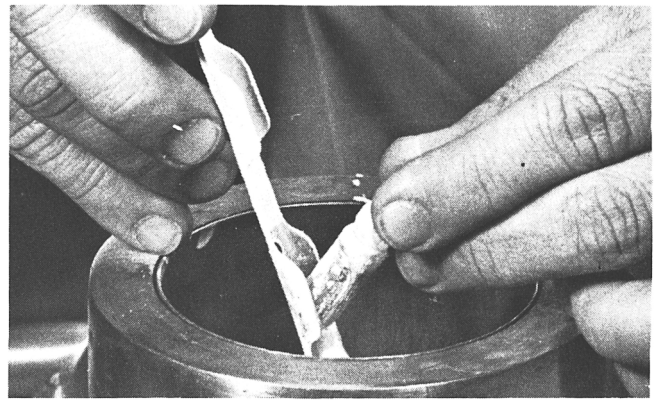

Figure 6. Ampule of frozen semen being removed from a cane. Note that precautions are being taken to expose only the ampule being removed.

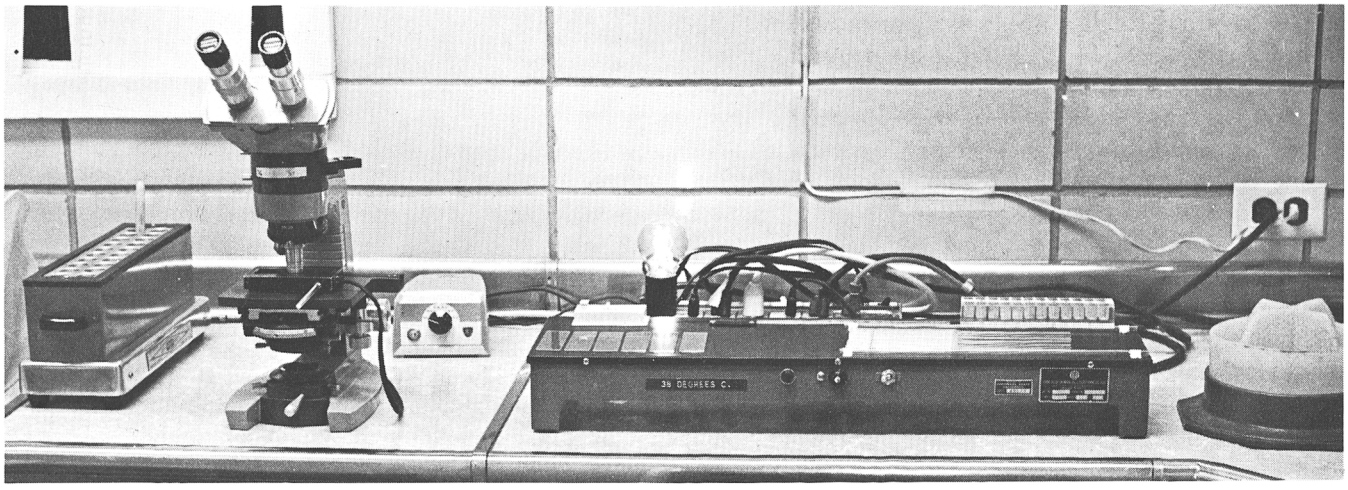

Figure 5. Equipment needed for evaluation of frozen semen.

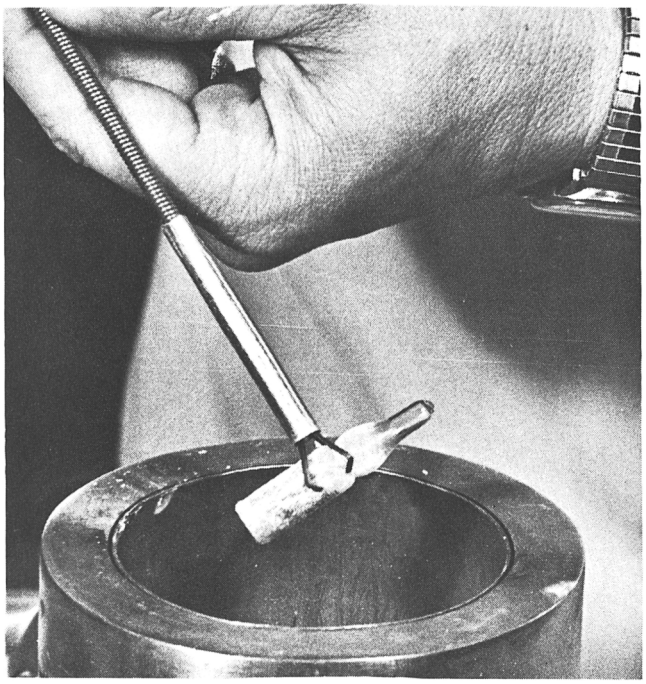

Figure 7. Ampule of frozen semen being removed from bulk storage.

3. The ampule is thoroughly but gently dried after thawing (Figure 8).

4. The dried ampule is scored with a metal scribe, unless pre-scored ampules are used and the top removed (Figure 9).

5. The semen is thoroughly mixed with a pre-warmed pasteur pipette (Figure 10).

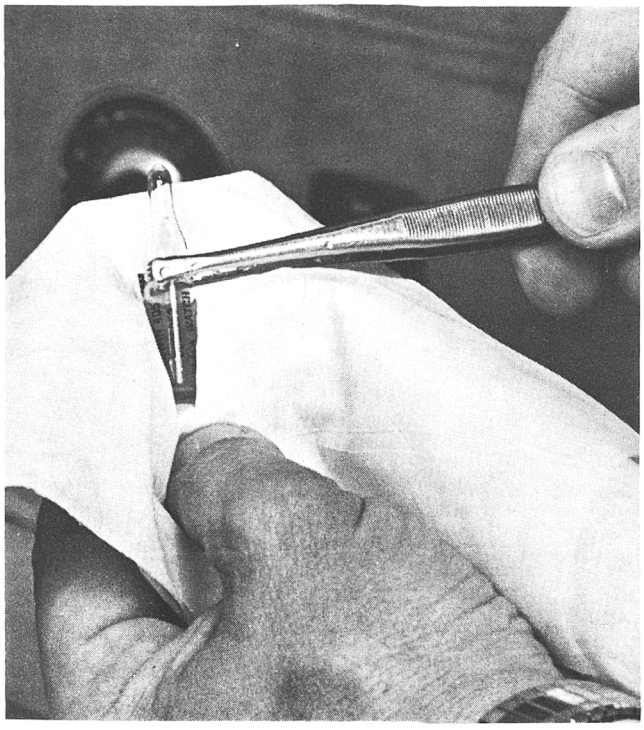

Figure 8. Water is spermicidal. Dry the ampule carefully before opening.

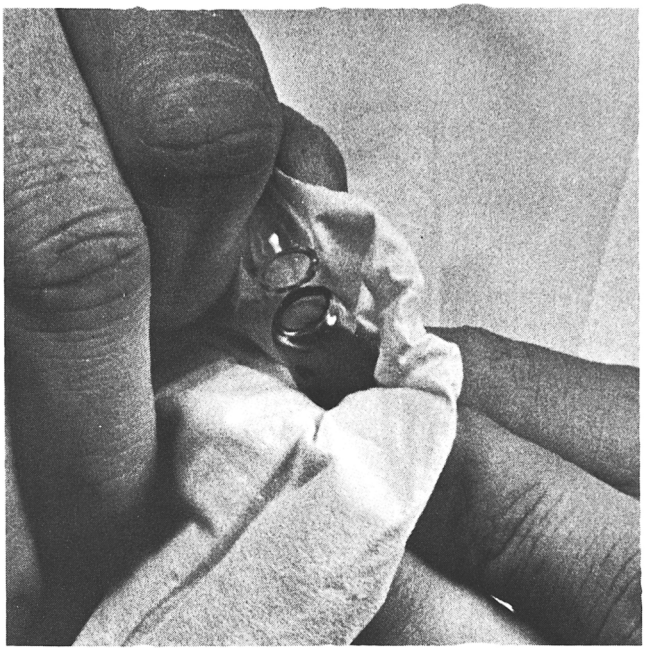

Figure 9. Open the ampule in the upright position.

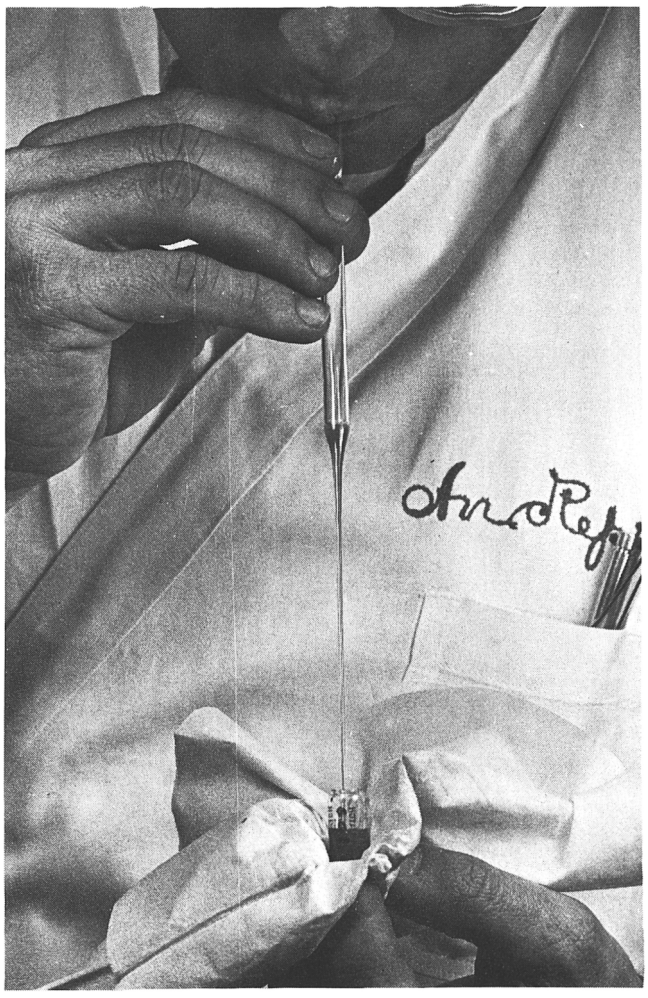

Figure 10. For proper sampling, the semen must be thoroughly mixed.

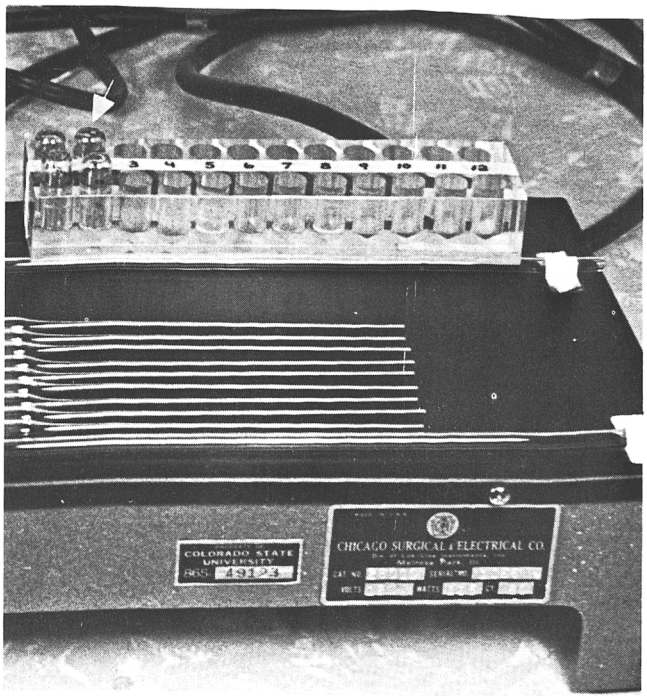

Figure 11. A lucite or metal block should be used to hold the ampules after they have been opened.

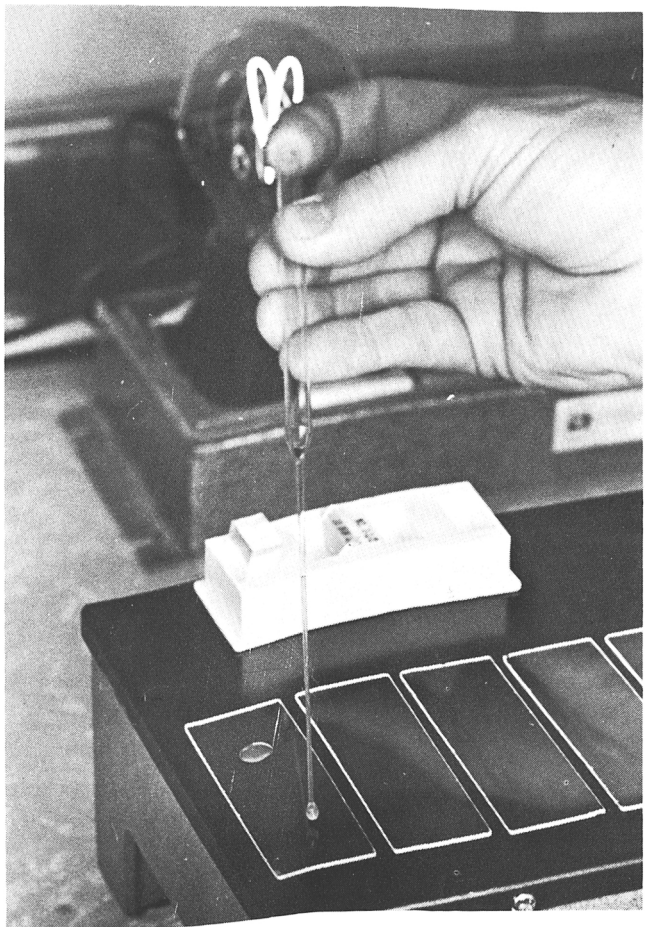

Figure 12. Proper drop size will insure good observation of the spermatozoa.

6. The ampule of semen is placed into a warmed lucite block for further sampling, if needed (Figure 11).

7. A small drop of semen is placed on each end of a microscopic slide and covered with a cover slip in a manner that prevents air bubbles from being trapped under the cover slip (Figures 12 and 13).

8. The slide is removed from the warming table and placed in a stage incubator maintained at 38°C (Figure 14).

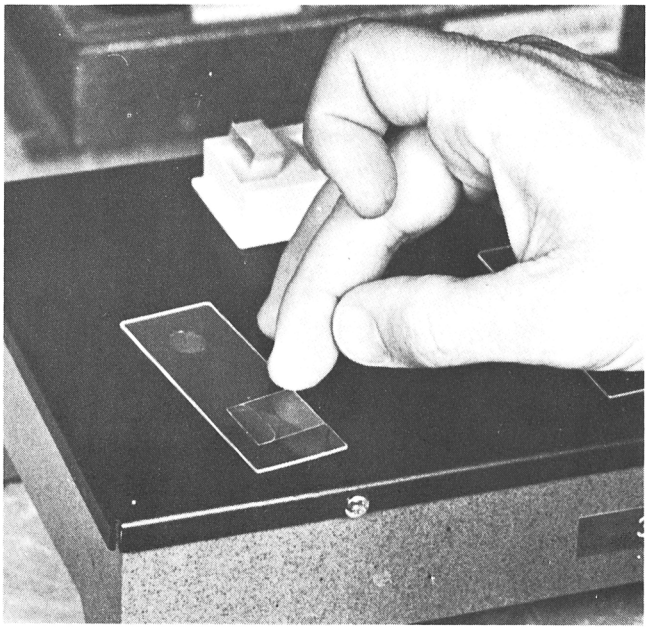

Figure 13. The coverslip is carefully placed on each drop of semen.

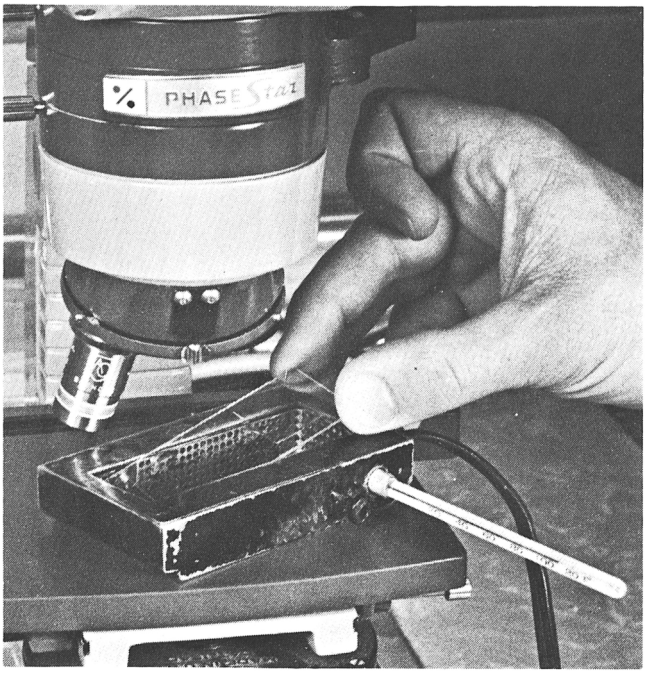

Figure 14. A thermostatically controlled stage incubator is an indispensable item of equipment.

9. Both samples are viewed by phase-contrast microscopy at 480 magnifications (Figure 15).

Figure 15. An adequate microscope for semen evaluation.

10. At least two ampules of each ejaculate are evaluated; if there is a discrepancy of ten percentage points or more, a third ampule is evaluated. Motility is recorded to the nearest 5%, and rate of motility recorded as 0, 1, 2, 3, 4, or 5.

Total cost of the necessary equipment for an adequate semen evaluation is between $1,500 and $2,000.

In summary, the *proper* evaluation of frozen semen requires:

1. Adequate equipment.
2. Experienced, highly trained, technical personnel.
3. Information on motility of the sample immediately after collection and processing.
4. Knowledge of the relative fertility level of the bulls used.
5. Knowledge of the processing methods employed.
6. Knowledge of how the semen had been handled and how long it had been frozen.

It is highly unlikely that anyone in the field could meet all these requirements. Thus, semen should be processed and distributed by a reputable organization, and do not evaluate the semen under inadequate conditions. In instances where the owner or veterinarian would like an evaluation of the semen, samples should be submitted to the original semen processor who should be obligated to, with the aid of previous records, properly evaluate the submitted samples.

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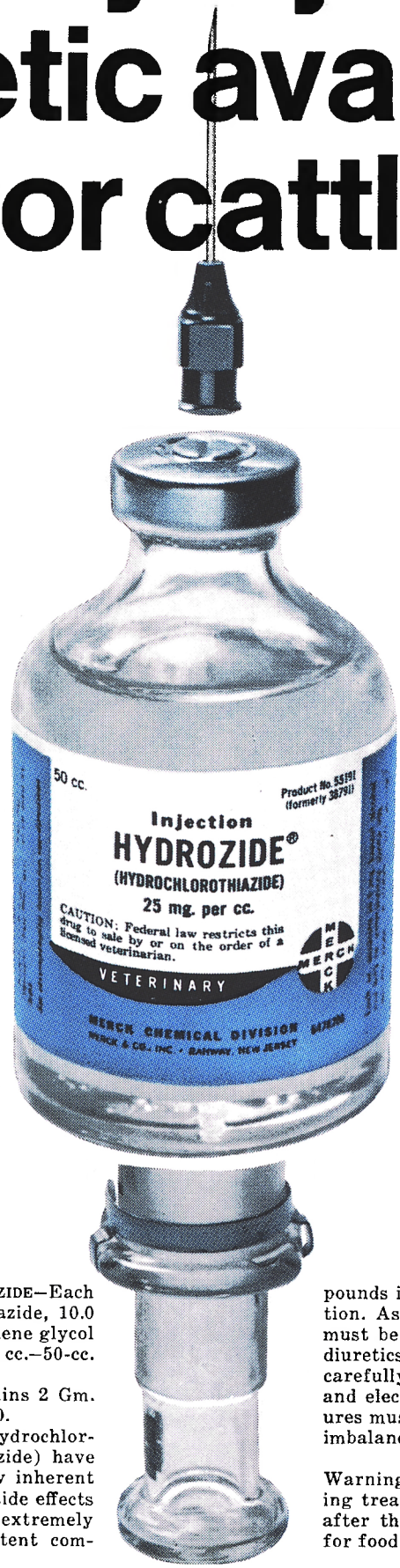

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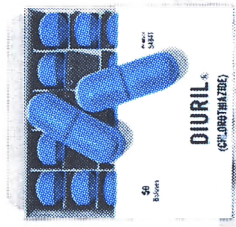

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