MAXIMUM genetic progress by artificial insemination depends on progeny testing sires at the youngest possible age and then exploiting those that prove to be genetically superior. Martig and Almquist (1969) found that fertility for 17 Angus and Hereford bulls was not influenced by ejaculation frequencies of one, three or six times weekly between 1 and 2 years of age. Fertility for these bulls was sufficiently high at puberty to initiate progeny testing; collection at the higher frequencies extended the possible number of matings (Cunningham et al., 1967). Weekly sperm output of beef bulls can be greatly increased by more frequent semen collection (Hupp et al., 1962; Almquist and Cunningham, 1967). O'Dell, Almquist and Amann (1959) reported that successive collection of 10 rather than two ejaculates per week from dairy bulls yielded about 2.5 times as many ampules of frozen semen and that time could be saved in semen processing and freezing.

This report considers the freezability and fertilizing capacity of sperm in successive ejaculates from beef bulls. Changes in sperm output, semen characteristics and sexual behavior of these bulls have been presented by Foster, Almquist and Martig (1970).

Experimental Procedure

In the first of two trials, seven successive ejaculates were collected by artificial vagina from 10 Angus bulls. Two methods of sexual preparation were compared: (a) one false mount followed by 2-min. of restraint and two false mounts (3FM) and (b) one false mount followed by 5-min. of restraint (5R). For each method, there was an initial test and a recovery test 7 days later. At least 30 days of sexual rest preceded each initial test. In Trial II, seven successive ejaculates were collected from each of six Angus and four Hereford bulls at 21-day intervals by (a) electroejaculation (EE), (b) artificial vagina (AV) after 5R and (c) AV without sexual preparation (0FM). Each bull was sexually rested at least 21 days before the first test.

Only ejaculates containing at least 40% progressively motile sperm and 840 x 10^6 total motile sperm were frozen. Each acceptable ejaculate was diluted initially to 60 x 10^6 motile sperm per ml in heated skim milk diluent containing antibiotics and cooled to 5 C over a 4-hr. period. In both trials ejaculates were assigned to the following classes: (a) a portion of ejaculate 1, (b) a portion of ejaculate 2, (c) equal portions of ejaculates 1 and 2, (d) ejaculate 3 or 4 or both, and (e) ejaculate 5, 6 or 7 or any combination. The final diluted semen sample consisted of 30 x 10^6 motile sperm per ml, 11% glycerol, 1.25% fructose, 1,000 units of penicillin per ml and 1 mg of dihydrostreptomycin sulfate per milliliter. During the first 2 hr. of the 18±1 hr. equilibration period, 1.0-ml ampules were filled, sealed and placed on 6-ampule racks. The ampules were cooled in a Linde BF-3-2 automatic liquid nitrogen freezer at a within-ampule rate of 1 C per min. from 5 C to the start of crystallization, 4 C per min. from the end of crystallization to --60 C, and about 60 C per min. to --100 C. After reaching --100 C, the ampules were plunged into liquid nitrogen for storage.

After storage for 3 weeks, ampules of frozen semen were thawed in ice water and evaluated for the percentage of progressively motile sperm. The recorded value for post-thaw motility was the mean of independent estimates made by two observers each using a different ampule from the same sample. To be accepta-
ble for fertility testing, ampules had to contain at least 10 x 10⁶ motile sperm. However, in a few cases, if one of the five classes for a particular test was acceptable, then any of the other four classes which contained at least 7.5 x 10⁶ motile sperm per ampule also was sent to the field for testing.

Technicians were instructed to use the semen as soon as possible after thawing the ampule for 8 rain. in ice water and to record the semen collection number and bull code on the breeding receipt. Each sample used in the fertility trial was distributed to at least two technicians. Breeding receipt information for first and second inseminations was recorded on punch cards and all second services were matched to first services by ear tag or registration number. Receipts with conflicting bull codes and semen collection numbers were discarded.

For statistical analysis, technician differences were ignored and nonreturn percentages were determined for each bull-treatment class on the basis of first services. Analysis of variance and regression analysis were used for statistical interpretation of the data.

Results

The percentages of ejaculates acceptable for freezing for ejaculates 1 through 7 in Trial I were 91, 81, 69, 43, 38, 36 and 32, respectively. In Trial II the percentages were 70, 70, 40, 60, 33, 30 and 17, respectively. There was no significant change in freezability among acceptable ejaculates in Trial I (table 1). A higher (P<.05) percentage of sperm survived freezing and thawing when 3FM was used before each ejaculation rather than 5R. Interactions of bull x ejaculate and bull x collection method were significant (P<.01).

In Trial II sperm freezability varied (P<.05) among ejaculate classes (table 1). Means for ejaculate classes 1, 2 and 1–2 were higher (P<.05) than those for class 5–6–7. Differences in sperm freezability varied among bulls (P<.01) but not among collection methods.

The 60- to 90-day nonreturn percentages are presented in table 2 for 4,390 first services to semen from the 10 Angus bulls in Trial I. The nonreturn rates varied (P<.01) significantly among bulls (range 62.9 to 81.7% 60- to 90-day nonreturns) but not among ejaculate classes. There were 502 first services to seven bulls from ampules containing between 7.5 x 10⁶ to 9.6 x 10⁶ motile sperm after storage for 3 weeks in liquid nitrogen. These services were represented in all ejaculate classes and averaged 72% 60- to 90-day nonreturns.

Discussion

The finding that pooling of first and second ejaculates from beef bulls is not harmful to freezability agrees with that of O'Dell et al. (1959) for dairy bulls.

The general conclusion from these data is that neither freezability nor fertility differed among the seven successively collected ejaculates. However, only selected ejaculates which met minimum standards were tested. Consequently, some of the 3–4 and 5–6–7 ejaculate classes actually contained only one acceptable ejaculate and represented relatively small percentages of the ejaculates collected. Thus, collection of more than three or four ejaculates is not an efficient procedure even though the fertilizing capacity of subsequent ejaculates is satisfactory.

No difference in freezability was found for sperm obtained from the same bulls by electroejaculation and artificial vagina. This finding is in agreement with a report for Angus bulls by Colleary and Ehlers (1964).

<table>
<thead>
<tr>
<th>Method of collection</th>
<th>Ejaculate class</th>
<th>1</th>
<th>2</th>
<th>1-2</th>
<th>3-4</th>
<th>5-6-7</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>AV–3FM</td>
<td>37.8</td>
<td>40.8</td>
<td>37.3</td>
<td>32.9</td>
<td>29.9</td>
<td>35.6</td>
<td></td>
</tr>
<tr>
<td>AV–5R</td>
<td>28.9</td>
<td>30.4</td>
<td>30.0</td>
<td>28.5</td>
<td>23.9</td>
<td>28.4</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>33.2</td>
<td>35.5</td>
<td>33.6</td>
<td>30.7</td>
<td>27.1</td>
<td>.......</td>
<td></td>
</tr>
<tr>
<td>EE–0</td>
<td>38.1</td>
<td>33.5</td>
<td>35.0</td>
<td>25.1</td>
<td>20.5</td>
<td>32.3</td>
<td></td>
</tr>
<tr>
<td>AV–0FIM</td>
<td>35.6</td>
<td>43.6</td>
<td>41.9</td>
<td>29.0</td>
<td>30.6</td>
<td>35.8</td>
<td></td>
</tr>
<tr>
<td>AV–5R</td>
<td>34.3</td>
<td>38.1</td>
<td>37.2</td>
<td>33.8</td>
<td>28.1</td>
<td>34.3</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>36.1*</td>
<td>39.0*</td>
<td>38.0*</td>
<td>30.1</td>
<td>27.7</td>
<td>.......</td>
<td></td>
</tr>
</tbody>
</table>

* AV=artificial vagina; FM=false mount; R=restraint; EE=electroejaculation.

b Significantly (P<.05) higher than AV–5R.

e Significantly (P<.05) higher than ejaculate class 5–6–7.
TABLE 2. FERTILITY OF SEVEN SUCCESSIVE EJACULATES COLLECTED FROM 10 ANGUS BULLS

<table>
<thead>
<tr>
<th>Item</th>
<th>1</th>
<th>2</th>
<th>1-2</th>
<th>3-4</th>
<th>5-6-7</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent 60- to 90-day nonreturns *</td>
<td>67.8</td>
<td>68.2</td>
<td>70.0</td>
<td>74.6</td>
<td>74.0</td>
<td>70.8</td>
</tr>
<tr>
<td>No. 1st serv.</td>
<td>1,028</td>
<td>965</td>
<td>372</td>
<td>1,057</td>
<td>988</td>
<td>4,390</td>
</tr>
</tbody>
</table>

* Bulls weighted equally.

Differences among treatments may have been masked in the fertility test by relatively high minimal standards established for acceptable samples: 30 x 10^6 motile sperm per ml at prefreeze and 10 x 10^6 motile sperm per ml at 3-weeks post-freeze. However, Pickett et al. (1964) showed that fertility was the same for Holstein semen diluted to either 30 x 10^6 or 20 x 10^6 motile sperm per ml before freezing. In the present study, based on 502 first services, fertility was not reduced when samples containing as few as 7.5 x 10^6 motile sperm at 3-weeks post-freeze were used for insemination.

Foster et al. (1970) found that an average of 11 x 10^6 sperm were collected in the first two successive ejaculates, while 10 x 10^6 sperm were collected in the last five ejaculates from these beef bulls. Our study shows that by prefreeze selection and post-freeze culling an acceptable level of fertility can be obtained. Although the collection of seven successive ejaculates probably would not become routine at a bull stud, it may be useful for bulls of relatively low sexual activity. Once such bulls are sexually stimulated it may be advisable to collect three or four successive ejaculates rather than only one or two and, thus, increase the number of sperm available for freezing. This procedure also could be used advantageously in custom collecting and freezing operations.

Summary

Sperm freezability and fertility were determined for seven ejaculates collected in succession from beef bulls. Sperm was collected by artificial vagina with and without sexual preparation prior to each ejaculation and by electroejaculation. Only ejaculates showing initial motility of 40% or more and at least 840 x 10^6 motile sperm were frozen. The proportion of culled ejaculates generally increased between the first and seventh ejaculates.

There was no difference in freezability among the first four ejaculates when portions of ejaculates 1 and 2 were frozen singly and pooled and ejaculates 3 and 4 were frozen after pooling. In the second of two trials, freezability of pooled ejaculates 5, 6 and 7 was lower (P<.05) than that for ejaculates 1, 2 and pooled 1 and 2. Sexual preparation involving one false mount and 5 min. of restraint prior to each ejaculation did not improve sperm freezability. No significant difference in freezability was found for sperm obtained by electroejaculation and artificial vagina. Pooling of first and second ejaculates did not affect sperm survival.

Fertility based on 4,390 first services from 10 Angus bulls did not differ among seven successive ejaculates. Fertility was not affected adversely for 502 first services representing samples containing as few as 7.5 x 10^6 motile sperm per ampule instead of the usual minimum of 10 x 10^6 per ampule.

Literature Cited


