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J. C. Kile, Jr.

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# AN IMPROVED METHOD FOR THE ARTIFICIAL INSEMINATION OF MICE

J. C. Kile, Jr.

The purpose of this investigation was to determine a convenient and productive method for the large scale artificial insemination of mice. The success of artificial insemination is dependent both on the introduction of the sperm suspension at the proper time interval from ovulation and on the concomitant induction of the neurohumoral reaction necessary to elicit the progestational response of the uterus. Snell, Fekete and Hummel ('40) have shown the modal time for the onset of estrus in cyclic females to be from 4 to 6 hours following darkness. Ovulation occurred, on the average, from 2 to 3 hours following the onset of estrus. The onset of estrus was observed by them to be correlated with the diurnal cycle rather than with the specific hour of day. Mating the inseminated female with a vasectomized male has been used frequently as a reliable method for effecting implantation.

The techniques employed for the artificial insemination of mice have differed chiefly in one or both of two details, these being the estrus used and the avenue by which the spermatozoa were injected. The methods used by earlier investigators are given with those of the present work in table 1.

The techniques for the artificial insemination of mice at the postweaning estrus are described by Snell, Hummel and Abelmann ('43, '44). The approach of the first postweaning estrus was ascertained by them by examining daily the vaginal smears of females from which offspring about 3 weeks old had just been removed. On the day the vaginal smear indicated estrus, the females were inseminated per vagina about 6½ hours following the onset of darkness. They were then permitted to mate with vasectomized males. Ninety-six vaginal plugs were observed following 153 inseminations. The sperm suspension leaked out following the insemination of 13 of the 96 females in which plugs were later observed. Fifty-one of the inseminations

Table 1

| | ESTRUS USED | AVENUE OF SPERM INJECTION | INVESTIGATOR |
|---|---|---|---|
| EARLIER WORK | Postweaning | vagina | Snell, et al. |
| | Postweaning, following sterile mating | wall of uterine horn | Snell, et al. |
| | Postpartum | ovarian capsule | Runner |
| | Cyclic | ovarian capsule | Runner |
| PRESENT WORK | Postpartum, 12 to 18 hours | vagina | Logan, Kile |
| | Postpartum, following sterile mating | vagina after plug removed | Kile |
| | Cyclic, following sterile mating | vagina after plug removed | Kile |
| | Cyclic, following sterile mating | wall of uterine horn | Kile |

proved fertile. Snell, Hummel and Abelmann ('44) also described a method whereby females were inseminated by surgical procedure at the postweaning estrus immediately following mating with a vasectomized male. The sperm suspension was introduced directly into the uterus by an operative technique developed by Fekete and Little ('42). The fertility obtained by this method was "disappointingly low, varying from 10 to 44%."

It has been known for many years that, in mice, parturition is closely followed by ovulation. As noted in table 1, this postpartum ovulation has been used for artificial insemination techniques. The recent work of Runner and Ladman (in press) has greatly clarified the confusing time relationship between parturition and the postpartum ovulation. They observed that the postpartum ovulation tends to take place nocturnally, irrespective of the time of day when delivery occurs. The interval between parturition and the postpartum ovulation varies between 12 and 18 hours, depending on the hour of delivery. Runner ('47) stated that litters were obtained from successful inseminations at the postpartum estrus. Since the time of ovulation at the postpartum estrus could be closely predicted according to Runner's data, we first attempted to inseminate females at this estrus. However, as will be shown, the results obtained by such inseminations were quite unsatisfactory.

In an attempt to find a more productive method, our efforts were directed toward the cyclic estrus. The convenience of using cyclic females compared to postpartum ones can be readily appreciated, as the time consumed in the development of the first pregnancy is eliminated as well as the inconvenient waiting period of 12 to 18 hours between delivery and insemination. An additional 3 weeks for rearing the young were required when inseminations were made at the postweaning estrus. Two techniques involving the use of cyclic mice were tried in our present work. We obtained good results with the second of these techniques: the injection of

sperm suspension through the wall of each uterine horn following sterile mating.

## Methods and Results

Postpartum estrus. The work at the postpartum ovulation was done with females of two inbred strains. The animals were kept in a room in which the lights were operated automatically by an electric timing clock. The lights were turned off at 9 A.M. and on at 3 P.M. The cycle was thus controlled so that inseminations could be made during the normal working day. The time of delivery was observed or estimated, and the females were inseminated from 12 to 18 hours later at the time predicted for the occurrence of ovulation. Approximately 0.1 cc of the sperm suspension was introduced into each horn per vagina by a technique slightly modified from that of Snell, Hummel and Abelmann ('43). The vaginal speculum was made of glass and was held in a fixed position by a clamp on a ring stand. The shafts of the hypodermic needles used to inject the sperm suspension were bent 90° to give an unobstructed view of the os cervix with the aid of light reflected from an ophthalmoscope. Two 20-gauge needles with blunted points beveled in opposite directions were used, one for each horn. After insemination, females were placed with vasectomized males and kept under observation for the presence of plugs. Results obtained previously at this laboratory from similar work by R. E. Logan are given with the present results in table 2.

It seemed possible that inaccurate estimation of the ovulation time was responsible for the poor results obtained. Our next approach, therefore, was to use the appearance of a vaginal plug from a natural mating as a convenient indicator of the onset of estrus from which the time of ovulation might be more accurately approximated. The necessity for observing the exact delivery time is then eliminated. Pregnant females of C57 brown strain were placed with vasectomized C57 black males prior to delivery. Mating occurred from 9 to 14 hours following

**Table 2**

**Results from inseminating postpartum females**

| | Logan | Kile |
|---|---|---|
| Females inseminated | 110 | 44 |
| Plugs observed | 73 | 35 |
| Number of litters | 22 | 9 |
| Mean litter size | -- | 3.7 ± .56 |

delivery. From 2 to 3 hours after mating, the plug was removed from the vagina with a pair of small blunt tweezers and the sperm suspension was introduced through the cervix, after which the plug was replaced. The sperm were collected by stripping the vasa deferentia of a freshly killed C57 brown male with watchmaker's forceps. (This combination of males was used throughout our work as the paternity of the offspring could then be easily determined by the disposition of pigment in the eyes at birth as well as by the coat color a few days later.) The results obtained were little better than by the other method. Of 34 females inseminated, nine delivered litters.

Cyclic estrus. Because of the advantages, already enumerated, of using the cyclic estrus, the plug indicator method of estimating the onset of estrus was next attempted on cyclic females. It was found that in cyclic females the plug was attached so firmly to the vaginal wall that it was extremely difficult to remove mechanically without extensive injury to the vaginal mucosa and the uterine ligaments. For this reason, the introduction of the sperm suspension directly into the uterus by operative procedure was tried.

C57 brown females were maintained in a room in which the daily light cycle was controlled by an electric timing switch. As a matter of convenience, the room was kept dark in the daytime, as the onset of estrus in cyclic females is known to occur nocturnally. The lights were turned off at 7 A.M. and on at 3 P.M. The females were kept with vasectomized males and examined regularly for plugs at 8:45 and 10:45 A.M. and at 12:45 P.M., and sometimes later if the behavior of the mice indicated the female was nearing estrus. The time of mating could be ascertained within 2 hours in this manner. In addition, a closer approximation of the mating time could be made from the consistency of the plug. (If the mating is quite recent the seminal fluid is still in a viscous, liquid state. This fluid soon hardens to form a hyaline appearing plug which then becomes increasingly opaque.)

The females were inseminated at from 1½ to 4½ hours following mating. From one to three females were inseminated with the sperm from one male. For the first 59 inseminations, the sperm from each male was suspended in from 0.25 cc to 0.5 cc of a 0.9% sodium chloride solution. For the remaining 44, a solution containing 0.9% sodium chloride, 0.042% potassium chloride and 0.72% glucose was used. Quantities of the suspension varying from 0.02 to 0.10 cc were injected with a 28-gauge hypodermic needle into each of the uterine horns, which were alternately lifted from the abdominal cavity with a small glass hook following mid-ventral laparotomy one centimeter anterior to the clitoris. The abdominal incision was closed with two layers of silk sutures. The surgical procedure was carried out under ether anesthesia.

Of 103 inseminations performed, 74 (71.8%) were fertile. Six of these females were unable to deliver their litters, possibly because of operative damage incurred at the time of insemination. One other female which was observed to start delivery ate all her young prior to the next observation. The difference between columns 3 and 5 in table 3 is attributable to these seven females. The mean litter size of the delivered young was 5.97. It is quite possible that some of the newborn were eaten prior to counting, as cannibalism was not uncommon in this group. As is shown in table 3, the results obtained with the two types of sperm media were not significantly different.

Many of the females were inseminated two or more times. One female, which was inseminated five times, delivered three litters following two failures. Two other females were successfully inseminated three times each. Complete successive insemination data are given in table 4.

## Discussion

In the present work, border cases such as questionable mating times were not excluded from insemination. It is thus possible that the percentage of fertility

Table 3

Insemination of cyclic mice following sterile matings

| SPERM MEDIA | NO. INSEM. | NO. FERTILE | PER CENT FERTILE | NO. LITTERS | MEAN SIZE OF LITTERS DEL. |
|---|---|---|---|---|---|
| NaCl | 59 | 42 | 71.2 | 38 | 6.34 ± .44 |
| Salt-Dex. | 44 | 32 | 72.7 | 29 | 5.48 ± .46 |
| Totals | 103 | 74 | 71.8 | 67 | 5.97 ± .32 |

**Table 4**

**Successive inseminations**

| INSEMINATION PER FEMALE | FERTILE INSEMINATIONS PER FEMALE | NO. OF FEMALES | PER CENT FERTILE INSEMINATIONS |
|---|---|---|---|
| 5 | 3 | 1 | 60 |
| 3 | 3 | 2 | 76.2 |
| | 2 | 5 | |
| 2 | 2 | 9 | 70.6 |
| | 1 | 6 | |
| | 0 | 2 | |
| 1 | 1 | 31 | 72.1 |
| | 0 | 12 | |
| | Total | 68 | 71.8 |

can be increased somewhat if inseminations are made only under optimum conditions. However, the success obtained here may already approach that occurring from natural matings in this inbred strain.

The data presented by Snell, et al. ('44) for the uterine wall technique are inadequate for comparison; but, from the percentages given, the results obtained at this laboratory appear much better. The exact cause of the difference in results is not readily apparent. The major differences in the techniques were the anesthesia used and the interval between the sterile mating and the sperm injection. (It is assumed that "just mated," in Snell's description, meant not longer than one hour.) The latter difference would be important only if sperm in artificial medium do not retain viability sufficiently long to effect semination when injected at a considerable interval preceding ovulation. It is suggested, however, that the use of barbiturate anesthesia by Snell, et al., was the cause of their failure to obtain good results with this method.

In comparing the results of the other of Snell, et al.'s methods (per vaginal insemination at the postweaning estrus, which in their experience was more successful) with the one developed in the present experiment, it is obvious that the latter is more productive when calculation of the percentage of success is based on the total number of inseminations performed (71.8% versus their 33%, t = 6.0). Snell, et al., in presenting their data, indicate that the percentage of success obtained by them is 61.4 , a figure not differing significantly from our 71.8% (t = 1.5). However, their figure is based only on the number of females retaining the sperm suspension and mating with vasectomized males, thus excluding 46% of the inseminations performed. These must be regarded as wasted effort in a consideration of the productivity of the method.

It is apparent that the time for performing successful artificial insemination can be approximated more accurately by observing the time of a sterile mating than

from the examination of vaginal smears. In addition to avoiding the general fallibility of the smear technique, individual differences between animals are automatically allowed for, which is not the case when a mean value must be used for estimation of ovulation time.

## Summary

Several methods for the artificial insemination of mice were tested, of which the following proved to be most satisfactory and more successful than any reported by other workers. Females were inseminated during the cyclic estrus at from 1½ to 4½ hours following mating with a vasectomized male. After mid-ventral laparotomy, the sperm suspension was introduced through the wall of each uterine horn with a 28-gauge hypodermic needle. Of 103 inseminations made within the C57 brown strain, 74 (71.8%) were fertile. The mean litter size was 5.97.

## Acknowledgment

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