Short Communication

THE DISTRIBUTION OF DIPLOID RABBIT SPERMATOZOA IN THE FEMALE TRACT AFTER ARTIFICIAL INSEMINATION

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ABSTRACT


The distribution of diploid rabbit spermatozoa in the female tract following artificial insemination is generally similar to that after natural mating, except that the incidence of diploid spermatozoa in the female tract is generally greater after A.I. than after coitus, despite a lower proportion of diploids in the semen used for A.I. Therefore the chances of the formation of triploid zygotes due to fertilization of normal eggs by diploid spermatozoa may be increased following A.I., although the frequency of such zygotes would still be very low.

INTRODUCTION

In an earlier paper (Mortimer, 1977 a) a large and highly significant reduction in the incidence of diploid rabbit spermatozoa between the ejaculate and the oviducts post coitum was described. Such a reduction greatly reduces the chances of, although not actually prevents, diploid spermatozoa contributing to the production of embryos which would be triploid, and therefore non-viable. The reduction was attributed to a combination of selectivity of sperm transport dependent on sperm motility, and reduced survival of diploid spermatozoa in the female tract. There was also some evidence for the existence of a brief phase of rapid sperm transport associated with coitus which was considered to be dependent on the buck’s coital performance.

The present experiments were carried out to elucidate the effects of artificial insemination upon the distribution of abnormal spermatozoa within the female tract. The use of A.I. as the method of semen deposition in the female tract would also provide some indication of the significance of coital stimulation in the induction of rapid sperm transport (see reviews by Blandau, 1969; Hafez, 1975).
MATERIALS AND METHODS

The buck used in this series of four experiments, chosen for the high proportion of diploid spermatozoa in his ejaculates, was the same one as in the earlier series (see Mortimer, 1977a and b). In each experiment semen was collected by artificial vagina, diluted with an equal volume of artificial seminal plasma (O'Donnell, 1969), and inseminated artificially into the cranial region of the doe's vagina. An aliquot of the inseminate was retained, preserved with formol citrate (Dott and Foster, 1975) and used for making nigrosin-eosin smears (Hancock, 1951). Spermatozoa were recovered from the female tract and treated as in the earlier series of experiments using natural mating (Mortimer, 1977a). Ten thousand spermatozoa (2000 from each of five slides) from the inseminate and each of the vaginal, cervical and uterine levels of the female tract, and also all the spermatozoa recovered from the oviducts, were scored at a magnification of ×500 for the percentage diploid. Diploid spermatozoa were identified by their larger and more pear-shaped heads (see Beatty and Fechheimer, 1972; Carothers and Beatty, 1975). For the inseminate sample the eosinophilic nature of each diploid spermatozoon was noted in the count of 10,000 cells. The proportion of live haploids was determined in a separate sampling of 1000 cells (200 from each of five slides). Estimations of live and dead spermatozoa after recovery from the female tract were made on separate samplings of 400 cells (200 from each of two slides) for haploids and the same number of diploids as had been scored among the sample of 10,000 spermatozoa.

RESULTS

The results of the four experiments, summarized in Table I(a), show that at 6 h after insemination there is a reduction in the percentage of diploid spermatozoa in the cervix and uterus compared to that in the inseminate. This difference was found to be significant ($P < 0.05$). There was no significant decrease in the proportion of diploids across the uterotubal junction. A $2 \times 2$ heterogeneity $\chi^2$ test on the numbers of diploid and haploid spermatozoa scored from the inseminates (664 and 39,336 respectively) and from the oviducts (3 and 572) showed that the difference in the incidence of diploid spermatozoa between the sites of insemination and fertilization was significant ($0.05 > P > 0.02$).

After scoring the proportions of live and dead cells in nigrosin-eosin preparations of spermatozoa from the inseminate and in those recovered from the female tract, the values for the incidence of diploids amongst live spermatozoa only were calculated for all sources (Table I(b)) except the oviducts (because these flushings had at one stage been treated with distilled water to burst contaminating red blood cells). The results indicate that the survival of diploids in the female tract is impaired compared to that of haploids.

Observations on spermatozoa with two tails (the incidence and degrees of
<table>
<thead>
<tr>
<th></th>
<th>Inseminate</th>
<th>Vagina</th>
<th>Cervices</th>
<th>Uteri</th>
<th>Oviducts</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Diploid spermatozoa (%)</td>
<td>$1.660 \pm 0.063$</td>
<td>$0.893 \pm 0.110$</td>
<td>$1.055 \pm 0.093$</td>
<td>$0.968 \pm 0.178$</td>
<td>$0.5425 (3/572) \pm 0.302$</td>
</tr>
<tr>
<td>(b) Live diploid spermatozoa (as % of all live sperm.)</td>
<td>$1.338 \pm 0.039$</td>
<td>$0.737 \pm 0.132$</td>
<td>$0.756 \pm 0.126$</td>
<td>$0.703 \pm 0.133$</td>
<td>not determined</td>
</tr>
<tr>
<td>(c) Double-tailed spermatozoa (as % of all sperm.)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Haploid</td>
<td>$0.168 \pm 0.017$</td>
<td>$0.025 \pm 0.012$</td>
<td>$0.020 \pm 0.007$</td>
<td>$0.0$</td>
<td>$0.0$</td>
</tr>
<tr>
<td>Diploid</td>
<td>$0.603 \pm 0.044$</td>
<td>$0.125 \pm 0.032$</td>
<td>$0.110 \pm 0.007$</td>
<td>$0.003 \pm 0.003$</td>
<td>$0.0$</td>
</tr>
</tbody>
</table>

Values are means of four experiments with standard errors based on discrepancies between experiments, except for diploid spermatozoa in the oviducts, the S.E. of which is based on the expectation of a Poisson distribution for data summed over all experiments.
TABLE II

Incidence of diploids in spermatozoa from the uterus relative to those from the cervix for the series of four experiments

<table>
<thead>
<tr>
<th>Experiment</th>
<th>% 2N in uterus</th>
<th>% 2N in cervix</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.972</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1.167</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.684</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.769</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>0.898</td>
<td>± S.E. 0.108</td>
</tr>
</tbody>
</table>

fusion between the two tails being variable) showed that such spermatozoa were not very successful in traversing, or even penetrating, the cervix (Table I (c)), most probably due to their impaired motility compared to those with single tails.

The results presented in Table II show the incidence of diploid spermatozoa in the uterus, relative to that in the cervix, in the four experiments. This ratio is usually < 1.0, as is the mean value for the series.

DISCUSSION

The overall pattern of distribution of diploid spermatozoa in the female tract after A.I. is similar to that found after natural mating (see Mortimer, 1977 a). This finding supports the earlier conclusion that the significant reduction in the incidence of diploid spermatozoa between the semen deposited in the vagina and the population of spermatozoa reaching the site of fertilization is due to a combination of selectivity of sperm transport dependent on sperm motility, and reduced survival of diploids in the female tract (Mortimer, 1977 a). That the motility of diploid rabbit spermatozoa is impaired compared to that of haploids has been shown using in vitro systems where the selection of sperm populations can only occur on the basis of differential motility (Mortimer, 1977 b, 1979 a).

However, in spite of a lower incidence of diploids in the semen used for A.I. than in that inseminated at coitus in the earlier study, the proportions of diploid spermatozoa in the female tract are apparently higher. Analyses of variance on the data from the two series of experiments have shown that, at least for the incidence of diploids in the cervix, the two series are significantly different ($P < 0.05$, see Mortimer, 1977 b). Therefore, a significantly greater proportion of the diploid spermatozoa in the inseminate penetrate the cervix after A.I. than following coitus. Whether this is due to the forcing of spermatozoa into the cervix by the insemination technique, and/or to improved conditions in the vagina due to the larger volume of buffer in which the spermatozoa are
suspended at the time of deposition has not been determined. Since available evidence suggests that diploid rabbit spermatozoa are probably capable of fertilizing eggs (Mortimer, 1979 b), and there is direct evidence that diploid mouse spermatozoa are fertile (Maudlin and Fraser, 1977), the implication of the present results is clearly that the chances of the production of triploid zygotes due to the fertilization of normal eggs by diploid spermatozoa is likely to be increased after A.I., although still only to a level which is appreciably lower than the simple incidence of diploid spermatozoa in the semen.

If a brief phase of rapid sperm transport associated with coitus had occurred, then it would have resulted in the transfer to the uterus of a small population of ejaculate spermatozoa with a high incidence of diploids. This population would then have been diluted over the following hours by the large numbers of spermatozoa, with a low incidence of diploids, entering the uterus having swum through the cervix during the period of slow transport. Therefore, following a period of rapid transport the ratios of the proportions of diploids in the uterus compared to the cervix would be expected to be > 1.0. Since in the present experiments this ratio was < 1.0 (in contrast to the situation after natural mating, see Mortimer, 1977 a), it is considered that rapid transport is absent when insemination is by artificial means. Therefore the available information suggests that coital stimulation plays a significant role in the induction of rapid sperm transport.

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REFERENCES


