DECAPACITATION AND RECAPACITATION OF
RABBIT SPERMATOZOA TREATED WITH MEMBRANE
VESICLES FROM SEMINAL PLASMA

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(Received 11th May 1974)

Two classes of membrane vesicles have been isolated from rabbit seminal
plasma (Davis, 1973a, b). The less dense vesicle fraction (Fr. II) was shown to
inhibit fertilization at concentrations between 20 and 72 µg protein/ml, after
oviducal insemination of does with treated spermatozoa capacitated in utero
(Davis, 1971). When these sperm cells were exposed to the dense vesicle fraction
(Fr. I), at concentrations up to 160 µg protein/ml, their fertilizing capacity
was unaffected. Both vesicle fractions, however, blocked fertilization of rat
eggs in vitro at relatively low concentrations (Davis & Niwa, 1974). This result
demonstrated that both classes of vesicles have decapacitation activity.

The present experiments were undertaken to establish if Fr. I can decapacitate
rabbit spermatozoa at higher concentrations than those used previously.
A second objective was to show that spermatozoa decapacitated by treatment
with these vesicle fractions can recover their ability to fertilize. Sperm decapacita-
tion by seminal plasma is reversible (Chang, 1957) and, consequently,
the inhibitory action of active fractions from seminal plasma would also be
expected to be reversible.

Semen was obtained from fertile bucks with an artificial vagina and sedi-
mented in a desk-top centrifuge at 1000 g for 30 min. The seminal plasma
(1 to 2 ml) was aspirated and layered on a discontinuous density gradient with
14 ml of 20% (w/v), 12 ml of 40% and 5 ml of 60% sucrose zones in Krebs-
Ringer phosphate buffer, pH 7.0, and fractionated by ultracentrifugation as
described previously (Davis, 1973b). Isolated fractions containing membrane
vesicles were then resedimented and dialysed against Hanks solution (Difco)
supplemented with 100 µg Ca⁺⁺/ml. The effect of the seminal plasma vesicle
fractions on sperm fertilizing capacity was determined from the fertilization
rates achieved following insemination with treated and control sperm cell
preparations. Capacitated sperm cells used in this assay were obtained by
flushing the uterus of a doe with 4 ml Hanks solution containing 150 µg
Ca⁺⁺/ml, usually 12 to 13 hr after mating. The recovered sperm cell suspension
was subsequently used for oviducal insemination of does that had been induced
to ovulate with 90 i.u. HCG about 2 hr before sperm deposition. These does
were killed on the following day to permit egg recovery (see Davis, 1971).

The fertilization rates obtained following insemination of does, about 2 hr
Table 1. Effect of seminal plasma vesicle fractions and epididymal fluid on the fertilizing capacity of rabbit spermatozoa

<table>
<thead>
<tr>
<th>Addition to sperm suspension</th>
<th>Concentration (mg protein/ml)</th>
<th>Time before (−) or after (+) ovulation (hr)</th>
<th>Site</th>
<th>No. of sperm.</th>
<th>Control oviduct</th>
<th>Treated oviduct</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Total</td>
<td>No. (%) fertilized</td>
</tr>
<tr>
<td>Fr. I</td>
<td>0.5 to 1.0</td>
<td>+ 2 to 3</td>
<td>Oviduct</td>
<td>110,000 to 415,000</td>
<td>66</td>
<td>64 (97)</td>
</tr>
<tr>
<td>Fr. I + Fr. II</td>
<td>1.0</td>
<td>− 6 to 5</td>
<td>Uterus</td>
<td>84,000 to 280,000</td>
<td>27</td>
<td>5 (19)</td>
</tr>
<tr>
<td>Fr. I + Fr. II†</td>
<td>1.0</td>
<td>− 6 to 5</td>
<td>Uterus</td>
<td>126,000 to 220,000</td>
<td>15</td>
<td>12 (80)</td>
</tr>
<tr>
<td>Epididymal fluid</td>
<td>†</td>
<td>+ 2 to 3</td>
<td>Oviduct</td>
<td>30,000 to 40,000</td>
<td>19</td>
<td>13 (68)</td>
</tr>
</tbody>
</table>

* Significant (P < 0.05), χ² difference; N.S., not significant.
† Spermatozoa obtained only 7 hr after mating; in the other experimental groups, spermatozoa were flushed from the uterus 12 to 13 hr after mating.
‡ Fluid from the cauda epididymidis diluted 1:3 with Ca⁺⁺-enriched Hanks solution.
Schlieren patterns of fluid from rabbit cauda epididymidis (lower), diluted fourfold with modified Hanks solution, and seminal plasma (upper) after centrifugation at 81,310 g for 3 min at 20°C in an An D rotor with standard and wedge 4° sector cells; bar angle 65°.

(Facing p. 242)
after ovulation, revealed that 0.5 to 1 mg Fr. I/ml impaired the fertilization
capacity of rabbit spermatozoa (Table 1). To discount the possible presence
of Fr. II vesicles, control spermatozoa were suspended in dialysed pooled
fractions from the 20% and 40% sucrose interfaces, which corresponded to
the presumptive Fr. II region in the density gradients employed to isolate Fr. I
(Davis, 1973b). As shown in Table 1, 97% of the recovered eggs (64/66)
were fertilized in oviducts in which control spermatozoa were deposited,
whereas only 13% of eggs (7/52) were fertilized with spermatozoa exposed to
0.5 to 1.0 mg protein/ml Fr. I; six of the seven eggs fertilized by spermatozoa
treated with Fr. I were from a single doe. To minimize the effects of variation
between animals on the fertilization rates observed, control spermatozoa were
placed in the left oviduct and treated spermatozoa in the right oviduct. Variation
from this source was, however, relatively small in these experiments.

The results given in Table 1 also demonstrate that epididymal fluid inhibited
the fertilizing ability of rabbit spermatozoa capacitated in utero. Epididymal
fluid used in this experiment was flushed from an excised cauda epididymidis
of a fertile buck with 0.5 ml modified Hanks solution and sedimented at 1000 g
for 30 min to remove sperm cells.

The decapacitation activity of epididymal fluid (Weinman & Williams,
1964) and the need to capacitate epididymal spermatozoa before fertilization
(Yanagimachi & Chang, 1963; Toyoda, Yokoyama & Hosi, 1971) can evidently
be explained by the high content of Fr. I-type vesicles in the epididymis. The
presence of these rapidly sedimenting vesicle components in rabbit epididymal
fluid and seminal plasma may be established from the Schlieren patterns
developed during centrifugation in an analytical ultracentrifuge. As can be
seen in Plate 1, seminal plasma formed two boundaries corresponding to Fr. I
and Fr. II (Davis, 1971) that were calculated to have sedimentation coefficients
of 156S and 57S, respectively. Epididymal fluid (diluted fourfold with modified
Hanks solution) displayed only one boundary that sedimented at approximately
224S which was close to the sedimentation coefficient of 221S obtained for
Fr. I in buffer. The retarded sedimentation of Fr. I (156S versus 221S) in
seminal plasma compared with that in buffer can be attributed to the higher
viscosity of the former fluid.

The recovery of fertilizing capacity by decapacitated spermatozoa was
studied with sperm cells flushed from the uterus of does that had been mated
either 7 or 13 hr before autopsy. When spermatozoa capacitated in utero,
obtained 7 hr after mating, were decapacitated with a vesicle suspension con-
taining 1.0 mg protein/ml (0.5 mg protein/ml of Fr. I and Fr. II) and were then
deposited into the uterus approximately 6 hr before ovulation, it was possible
to demonstrate sperm recapacitation. Table 1 shows that 68% (19/28) fertiliza-
tion was achieved in eggs from oviducts receiving spermatozoa exposed to
seminal plasma vesicle-containing fractions (Fr. I + Fr. II) and this was not
significantly lower than the 80% (12/15) fertilization rate obtained with un-
treated sperm cells. Spermatozoa obtained 13 hr after mating displayed poor
fertilization capacity when they were inseminated 6 hr before ovulation (Table
1). Their relatively advanced age (19 to 20 hr) at the time of ovulation could
have been responsible. Insemination into the uterus, rather than into the ovi-
duct, was employed for recapacitation of spermatozoa in the present experiments because capacitation seems to proceed more efficiently with the former method (Adams & Chang, 1962).

The fact that seminal plasma from intact and vasectomized bucks and epididymal fluid can decapacitate spermatozoa is consistent with both types of vesicles having inhibitory activity. Sperm decapacitation can perhaps be achieved in different ways, since two superficially different factors have been shown to induce this state. Both active factors were identified as vesicles (Davis, 1973b), however, and this may give some clue to their mechanism of action.

The skilful assistance of Mr John Whitney is gratefully acknowledged. Financial support was received from N.I.H. Contract No. 1-HD-3-2781.

REFERENCES


