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# Studies on the Function of the Epididymis[1]

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In their reviews of the male accessory organs, Mann and Lutwack-Mann, 1951; Maneely, 1959; Risley, 1963, stated that the function of these organs was not very well known and that the epididymis, in particular, had received very little attention. In 1961, Bishop could state that "the intrinsic changes within the maturing sperm and the interrelations between the gametes and the various segments of the male duct system are only just beginning to be appreciated." In recent years progress has been made but many questions remain to be answered. The difficulty lies in the multifarious function of the epididymis: besides the obvious role of transport, conveying spermatozoa from the testis to the ejaculatory duct, spermatozoa mature while passing through the epididymis, are stored therein, and very probably are resorbed also under certain circumstances.

### *When Do Sperm Become Mature?*

The first function of sperm transport has been successfully investigated in different species by the use of radioactive isotopes. For example: in the rabbit, the transit time has been estimated as from 9 to 12 days (Koefoed-Johnsen, 1961; Amann, Koefoed-Johnsen, and Levi, 1963; Swierstra and Foote, 1965; Orgebin-Crist, 1965). The maturation function of the epididymis is more complex. Several morphological, physiological, or biochemical features of the spermatozoa change during passage through the epididymis and these have all been interpreted as being part of the ripening process undergone by the sperm. The end result of this ripening is the ability of the sperm to fertilize an ovum. Young, in 1931, had observed that the pregnancy rate after insemination with guinea pig sperm taken from the proximal cauda was slightly lower than after insemination with sperm from the distal cauda. Nishikawa and Waide, 1952, extended this observation and showed that six rabbits inseminated with testicular sperm did not become pregnant; only one out of six did when inseminated with sperm from the caput, while four out of seven delivered after insemination with sperm from the ductus deferens. These observations indicated that sperm gradually attained their full fertilizing ability while going through the epididymis. This was confirmed in the rat by Blandau and Rumery, 1964; they showed that only 8% of the ova exposed to sperm suspensions from the caput were fertilized, while 93% of the ova exposed to sperm suspensions from the cauda epididymidis were fertilized. A step further was made when it was demonstrated in the rabbit that the fertilizing ability is attained when spermatozoa reach the lower half of the corpus epididymidis (Bedford, 1966, Fulka and Koefoed-Johnsen, 1966, Orgebin-Crist, 1967a). After intrauterine insemination of spermatozoa from the lower corpus, 63.3% of 226 ova are fertilized (Text Fig. 1); there is no correlation between the number of spermatozoa inseminated and the percentage of ova fertilized in each fe-

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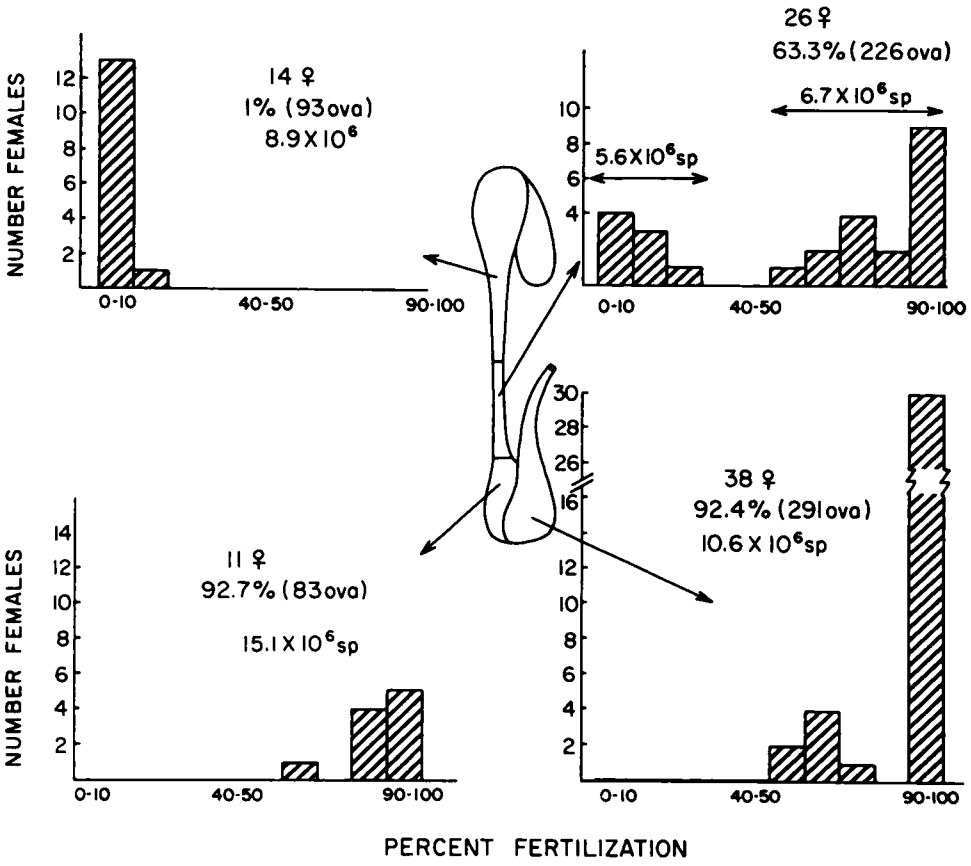



Text FIG. 1. Fertilizing ability of sperm taken from different epididymal regions.

male. After tubal insemination, 97% of the ova are fertilized (Bedford, 1966). But in both cases contact with the eggs was established by significantly less sperm than after insemination with distal cauda sperm: This suggests that some sperm become mature enough in the lower corpus to bring about fertilization but that the majority become fully mature more distally. Ejaculated spermatozoa are even more fully mature than distal cauda spermatozoa, since more sperm ($55.1 \pm 3.1$ vs. $28.6 \pm 2.2$) attach to the eggs (Orgebin-Crist, 1968).

### *Are the Sperm That Have Just Become Mature Fully Competent to Induce Normal Egg Development?*

Abnormally fertilized polyploid ova ranging from 5 to 11.6% were seen after exposure to epididymal spermatozoa from the corpus or the cauda region (Bedford, 1966; Orgebin-Crist, 1967a). None were found after insemination of ejaculated spermatozoa (Austin and Braden, 1953; Austin, 1960; Adams and Chang, 1962; Thibault, 1959, 1967; Orgebin-Crist, 1967a, 1968). Another abnormality observed was the delayed fertilization by spermatozoa from the lower corpus epididymidis and to a lesser extent by spermatozoa from the cauda. Sixteen hours after HCG, fertilization is still in process in eggs exposed to sperm from the corpus region, whereas in control ova fertilized with ejaculated spermatozoa, pronuclei are already formed. As a result, ova fertilized by corpus spermatozoa are delayed in their development 25, 27 or 28 hrs after injection of HCG. Twenty-five hours after HCG, 51.3 vs. 7.2% of the fer-

tilized ova are still undivided after insemination with lower corpus spermatozoa or after normal mating. Twenty-seven hours after HCG, 18.1% are still undivided, whereas the normal percentage lies around 1.8; and only 11.3% of the ova have reached the four-cell stage, whereas after normal mating 67.2% of the ova are already four-cell (Orgebin-Crist, 1967a, 1968). From Table 1 it can be seen that, although ova fertilized by sperm from the corpus region were recovered later (27 hr 50 min after HCG) than those from the mated does (27 hr 31 min after HCG), more ova are still at the two-cell stage (68.0 vs. 46.1%) and less are at the four-cell stage (29.8 vs. 51.9%).

What is the ulterior development of these ova? It has been reported that while only 8 young were obtained from 11 does inseminated surgically with lower corpus spermatozoa, 36 and 55 young were obtained, respectively, from 9 does inseminated surgically with cauda and 9 with ejaculated sperm. The suggestion was therefore made, comparing the fertilization rates normally brought about by just mature sperm with the implantation rates obtained in this experiment, that these ova, some of which are polyploid and/or delayed, were lost somehow during the preimplantation period (Orgebin-Crist, 1967a). The embryonic mortality induced by distal cauda sperm is rather puzzling, since sperm from the cauda

TABLE 1

SEGMENTATION OF RABBIT OVA AFTER INTRAUTERINE INSEMINATION OF EPIDIDYMAL SPERMATOZOA

| | Sperm suspension from | | |
|---|---|---|---|
| | Lower corpus | Distal cauda | Normal mating |
| Time after HCG (range) | 27 hr 50 min (26 hr 55 min–28 hr 55 min) | 27 hr 32 min (26 hr 45 min–28 hr 05 min) | 27 hr 31 min (26 hr 39 min–28 hr 35 min) |
| Number animals | 7 | 6 | 12 |
| Number ova | 62 | 54 | 113 |
| Ova fertilized | 47 | 50 | 104 |
| Percentage undivided | 0 | 2.0 | 0 |
| Percentage 2-cell | 68.0 | 50.0 | 46.1 |
| Percentage 3-cell | 2.2 | 4.0 | 1.9 |
| Percentage 4-cell | 29.8 | 44.0 | 51.9 |

TABLE 2

EMBRYONIC MORTALITY AFTER SURGICAL INTRAUTERINE INSEMINATION WITH EPIDIDYMAL OR EJACULATED SPERMATOZOA

| | Sperm suspension from | | |
|---|---|---|---|
| | Lower corpus | Distal cauda | Ejaculate |
| Number females | 22[a] | 20 | 18 |
| Number pregnant females Day 10 | 13 (59%) | 18 (90%) | 17 (94%) |
| Number implant / Number corpora lutea Day 10 | 62/247 (25%)[b] | 97/210 (46%)[c] | 116/191 (61%) |
| Number fetuses / Number implant Day 28 | 36/52 (69%) | 76/97 (78%) | 85/116 (73%) |

[a] One died between Days 10 and 28.

[b] $p < .001$.

[c] $0.1 < p < 0.2$.

as well as from the ductus deferens form the ejaculate. Thus, the observation has been extended, and Table 2 summarizes the data obtained on 60 does inseminated surgically with sperm from the lower corpus, the cauda or the ejaculate. Sperm suspensions were made in either 0.9% sodium chloride or Hanks' balanced salt solution. The embryonic mortality does not depend on the type of diluent used (Table 3) but depends rather on the type of sperm suspension inseminated (Table 2). The day after insemination of lower corpus spermatozoa, only 7.6% of 26 does were not pregnant (Orgebin-Crist, 1967a). At Day 10, 41% of 22 does were not pregnant; only 25% of the ova ovulated implant in the lower corpus group in contrast to 61% in the control group ($p < .001$). In the distal cauda group fewer ova ovulated implant than in the control (46 vs. 61%) although the difference is not statistically significant. However all ova ovulated are not necessarily fertilized, especially in the lower corpus group.

To dissociate completely the failure of fertilization from the embryonic mortality, the problem was reinvestigated by transferring into recipient does only cleaved ova fertilized by epididymal or ejaculated sperm. Epididymal sperm suspensions were surgically inseminated in utero, control does were mated, sham operated and their uteri manipulated. Ova were recovered from the donors 27-29 hr after HCG and transferred into recipient does injected with HCG at the same time as the

TABLE 3
INFLUENCE OF THE SPERM DILUENT ON THE NUMBER OF IMPLANTATION SITES PER NUMBER OF CORPORA LUTEA (DAY 10)

| | Sperm suspension from | |
|---|---|---|
| | Lower corpus | Distal cauda |
| 0.9% Sodium chloride | 16/49 | 22/42 |
| | 32.6% | 52.3% |
| Hanks' solution | 23/70 | 35/67 |
| | 32.8% | 52.2% |

TABLE 4
EMBRYONIC MORTALITY AFTER TRANSFER OF OVA FERTILIZED BY EPIDIDYMAL SPERM

| | Sperm suspension from | | | |
|---|---|---|---|---|
| | Lower corpus | Control | Distal cauda | Control |
| Number implant | | | | |
| Number cleaved ova Day 10 | 10/35 (28.5%) | 15/29 (51.7%) | 14/50 (28.0%) | 15/32 (46.8%) |
| Number fetuses | | | | |
| Number implant Day 28 | 5/10 (50%) | 11/14[a] (78.5%) | 10/14 (71.4%) | 8/11[a] (72.7%) |
| Total embryonic loss | 30/35 (85.7%) | 17/28 (60.7%) | 40/50 (80.0%) | 20/28 (71.4%) |

[a] One female died between Days 10 and 28.

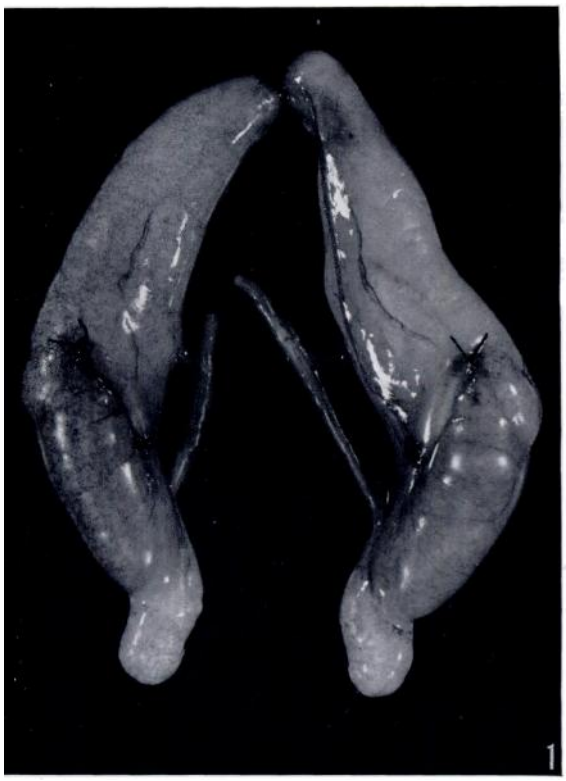
TABLE 5
COMPARISON OF THE EMBRYONIC MORTALITY OBSERVED NORMALLY OR AFTER OVUM TRANSFER

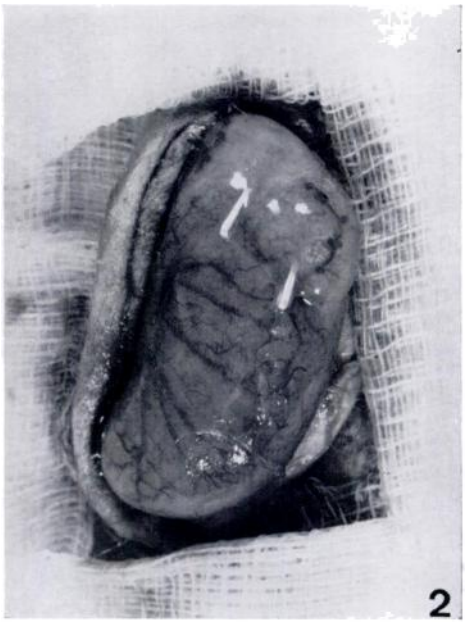
| | Lower corpus | | Distal cauda | | Control | |
|---|---|---|---|---|---|---|
| | No transfer | Transfer | No transfer | Transfer | No transfer | Transfer |
| Preimplantation losses Day 10 | 60.3[a] | 71.5[b] | 50.0 | 72.0[c] | 36.0 | 49.1 |
| Post implantation losses Day 28 | 30.8 | 50.0 | 21.7 | 28.6 | 26.8 | 24.0 |
| Total embryonic loss | 75.4 | 85.7 | 60.9 | 80.0 | 53.1 | 66.0 |

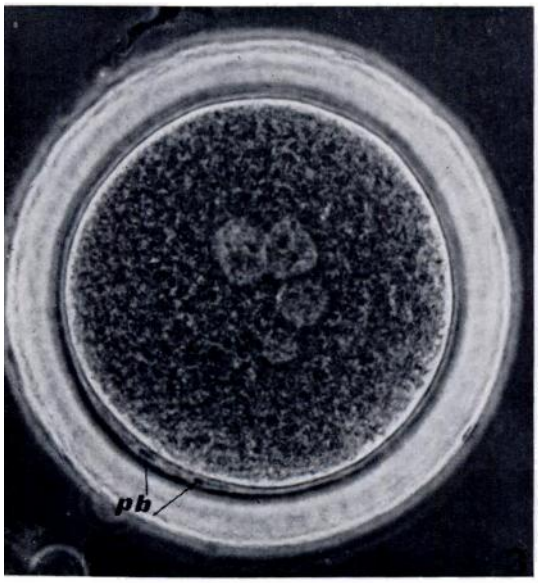
[a] The preimplantation losses amount to 75%, since only 63.3% of the ova are expected to be fertilized, the true preimplantation losses are 60.3%.

[b] $p < .05$.

[c] $p = .10$.







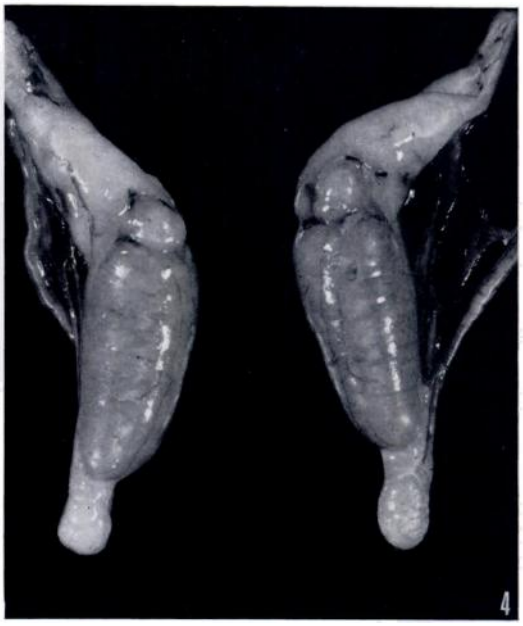



PLATE 1

FIG. 1. Silk ligatures isolating the proximal corpus epididymidis.

FIG. 2. Silver clips ligatures isolating the distal caput epididymidis (ductuli efferentes not severed).

FIG. 3. Ovum with 4 pronuclei and 2 polar bodies fertilized by sperm retained in the distal caput for 8 days.

FIG. 4. Silk ligatures isolating the proximal and the distal caput epididymidis (ductuli efferentes severed). Note the swollen testes.

TABLE 6
MEAN CONTENT OF FEULGEN-DNA AND UV-DNA IN SPERMATOZOA RECOVERED FROM DIFFERENT EPIDIDYMAL SEGMENT BEFORE AND AFTER CAPACITATION
(Esnault, Orgebin-Crist, Ortavant; 1968)

| Origin of spermatozoa | Rabbit No. | Feulgen-DNA (560 mμ) | | | UV-DNA (265 mμ) | | |
|---|---|---|---|---|---|---|---|
| | | Uncapacitated | Capacitated | *In vitro* incubated | Uncapacitated | Capacitated | *In vitro* incubated |
| Caput | 1 | 6.26 ± 0.076 | 5.14 ± 0.107 | — | — | — | — |
| | 2 | 4.94 ± 0.102 | 3.35 ± 0.123 | — | — | — | — |
| | 3 | 3.25 ± 0.049 | 3.04 ± 0.088 | 0 | 8.49 ± 0.116 | 8.69 ± 0.111 | 8.72 ± 0.080 |
| | 4 | 5.28 ± 0.071 | 3.02 ± 0.066 | 2.67 ± 0.026 | 7.07 ± 0.073 | 7.70 ± 0.083 | 8.37 ± 0.087 |
| Corpus 1 | 1 | 5.77 ± 0.067 | 5.34 ± 0.083 | — | — | — | — |
| | 2 | 4.11 ± 0.109 | 3.68 ± 0.091 | — | — | — | — |
| | 3 | 3.15 ± 0.034 | — | 0 | 8.88 ± 0.146 | — | 8.43 ± 0.205 |
| | 4 | 4.08 ± 0.037 | 3.67 ± 0.080 | 3.63 ± 0.073 | 6.64 ± 0.079 | 7.86 ± 0.059 | 7.73 ± 0.081 |
| Corpus 2 | 1 | 5.89 ± 0.063 | 5.11 ± 0.052 | — | — | — | — |
| | 2 | 5.36 ± 0.152 | 3.80 ± 0.155 | — | — | — | — |
| | 3 | 3.71 ± 0.034 | — | 0 | 8.72 ± 0.048 | — | 7.81 ± 0.143 |
| | 4 | 3.77 ± 0.089 | 3.12 ± 0.117 | 3.63 ± 0.044 | 7.17 ± 0.091 | 8.12 ± 0.083 | 8.43 ± 0.089 |
| Cauda (distal) | 1 | 5.91 ± 0.056 | 4.64 ± 0.046 | — | — | — | — |
| | 2 | 3.66 ± 0.063 | 2.86 ± 0.102 | — | — | — | — |
| | 3 | 3.00 ± 0.045 | — | 2.46 ± 0.031 | 8.78 ± 0.105 | 8.64 ± 0.102 | 8.57 ± 0.114 |
| | 4 | 4.27 ± 0.059 | 3.53 ± 0.085 | 5.09 ± 0.033 | 6.65 ± 0.050 | 7.10 ± 0.086 | 8.42 ± 0.088 |
| Ejaculate no. 1 | 1 | 4.37 ± 0.083 | 4.81 ± 0.060 | — | — | — | — |
| | 2 | 3.22 ± 0.070 | 2.13 ± 0.111 | — | — | — | — |
| | 3 | 2.39 ± 0.070 | 3.07 ± 0.049 | 1.77 ± 0.043 | 7.99 ± 0.130 | 8.66 ± 0.122 | 8.77 ± 0.114 |
| | 4 | 3.32 ± 0.063 | 4.06 ± 0.094 | 1.38 ± 0.043 | 7.77 ± 0.092 | 7.28 ± 0.062 | 7.41 ± 0.082 |
| Ejaculate no. 2 | 3 | 2.94 ± 0.037 | 3.23 ± 0.069 | 1.89 ± 0.050 | 8.94 ± 0.130 | 8.30 ± 0.136 | 8.37 ± 0.133 |
| | 4 | 2.70 ± 0.045 | 4.09 ± 0.056 | 3.19 ± 0.049 | 7.52 ± 0.075 | 7.54 ± 0.047 | 9.43 ± 0.082 |

donors. Ova fertilized by lower corpus or distal cauda spermatozoa were placed in the oviduct via the fimbria, the contralateral oviduct receiving ova fertilized after normal mating. Ova were transferred in numbers corresponding to the number of ovulation points on the ovary of the recipient does. Only 28.5% of the ova fertilized by lower corpus sperm implant in contrast to 51.7% in the control series (Table 4). The difference (23.2) is of the same magnitude as in the preceding series (24.3) after the data were corrected to compensate for the lack of fertilization (Table 5). Fewer ova fertilized by distal cauda sperm implant than in the control series (28.0 vs. 46.8%) (Table 4). The dif-

PLATE 2

FIG. 1. Normal proximal caput epididymidis. ×164.

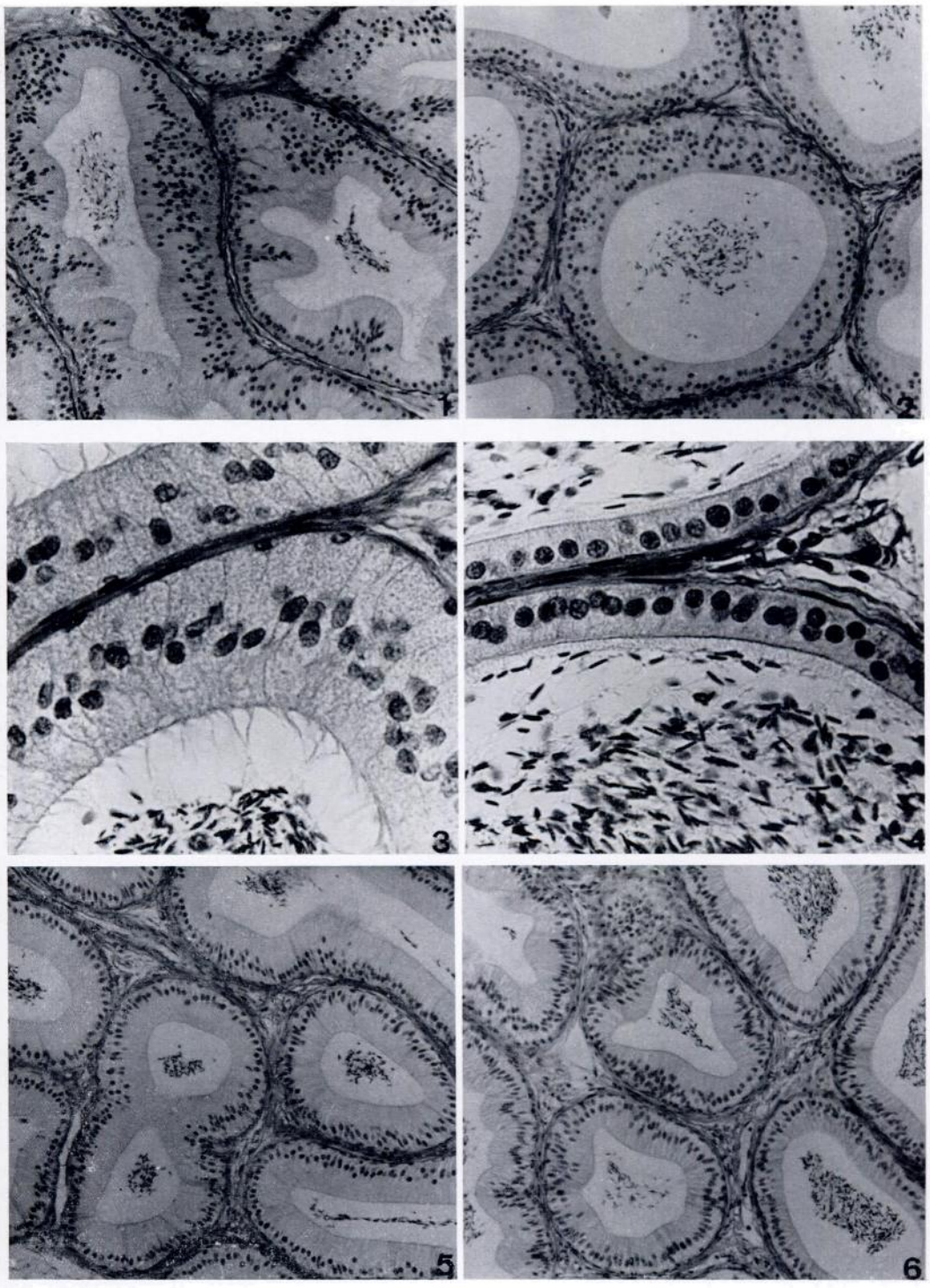
FIG. 2. Proximal caput epididymidis 3 days after the ductuli efferentes were severed and a silk ligature was placed at the caput flexure. Note the reduction of epithelial height. ×164.

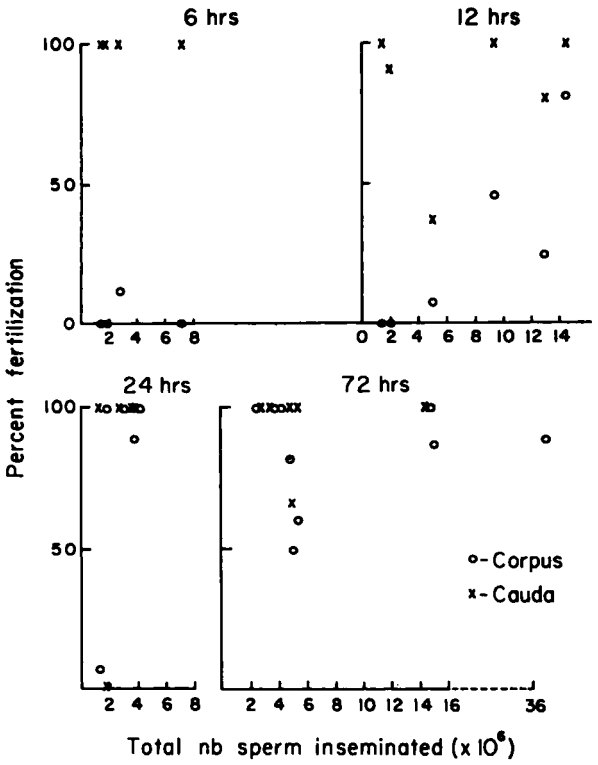
FIG. 3. Normal distal caput epididymidis. ×410.

FIG. 4. Distal caput epididymidis isolated for 3 days by two ligatures. Note the reduction in epithelial height. ×410.

FIG. 5. Normal corpus epididymidis. ×164.

FIG. 6. Corpus epididymidis isolated for 24 hr by two ligatures. Note the reduction in epithelial height. ×164.






Text FIG. 2. Fertilizing ability of sperm retained by double ligation for 6, 12, 24, and 72 hr in the proximal corpus epididymidis.

ference (18.8) corresponds to the one observed in the preceding experiment (14.0) (Table 5). While the difference in preimplantation mortality between the lower corpus and control groups is statistically significant ($p < .05$), the difference between the distal cauda and the control group does not reach the level of statistical significance ($p = .1$) Table 5). Differences in embryonic mortality after implantation are not statistically significant between the different groups studied in both series. These data confirm those already published (Orgebin-Crist, 1967*a*): Embryos developing after insemination of spermatozoa from the lower corpus epididymidis tend to die and disappear before implantation. The embryonic mortality after insemination of spermatozoa from the distal cauda may be more questionable since it is close to but does not reach the level of statistical significance.

What triggers the sperm fertilizing ability? What is the cause of the increased embryonic mortality observed after insemination of epi-

PLATE 3

FIG. 1. Columnar cells of the proximal corpus epididymidis. N: nuclei, D: dense bodies, G: Golgi apparatus, ER: vesicular endoplasmic reticulum. Fixative: Phosphate buffered 2% $OsO_4$ solution, pH 7.2-7.4 osmolality of about 368 milliosmoles. Stain: uranyl acetate and lead citrate. ×8,100.

FIG. 2. Columnar cells of the proximal corpus epididymidis isolated for 3 days by two ligatures. Note the changes in the apical cytoplasm and the stereocilia, the collapsed endoplasmic reticulum (ER) especially in the supranuclear cytoplasm. Osmium tetroxide, uranyl acetate and lead citrate. ×7,650.

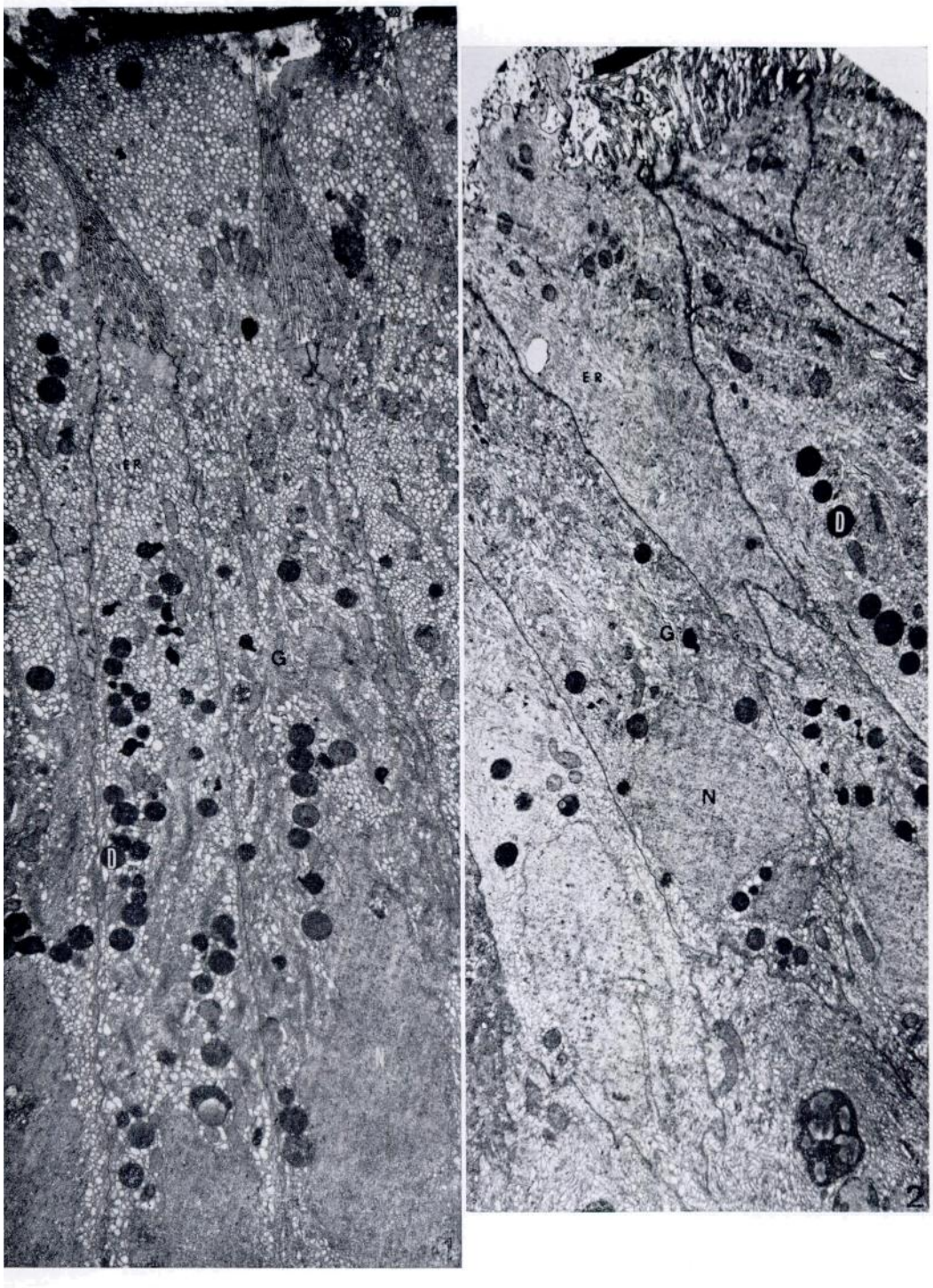


TABLE 7

SEGMENTATION OF RABBIT OVA AFTER INSEMINATION OF EPIDIDYMAL SPERMATOZOA RETAINED BY LIGATION IN PROXIMAL REGIONS OF THE EPIDIDYMIS (25 hr after HCG)

| | Sperm suspension | | |
|---|---|---|---|
| | Corpus | Distal cauda[a] | |
| | Uterine insemination | Uterine insemination | Tubal insemination |
| Number fertilized ova | 132 | 272 | 33 |
| Number pronuclear ova | 11 ( 8.3%) | 37 (13.6) | 13 (39.3) |
| Abnormal pronuclear ova | 2 (18.1%) | 12 (32.4) | 2 (15.3) |
| Number 2-cell ova | 105 (79.5%) | 204 (75.0) | 20 (60.6) |
| Number 3-cell ova | 1 ( 0.7%) | 8 ( 2.9) | |
| Number 4-cell ova | 14 (10.6%) | 23 ( 8.4) | |
| Number 6-cell ova | 1 ( 0.7%) | | |

[a] Distal to ligation.

didymal spermatozoa? As spermatozoa pass from the testis through the epididymis they undergo many changes. The most marked of the morphological changes are the position of the cytoplasmic droplet (Lagerlof, 1934; Branton and Salisbury, 1947; Nicander, 1957; Orgebin-Crist, 1967a), size, shape, and internal structure of the acrosome (Fawcet and Hollenberg, 1963; Bedford, 1963, 1965), structural organization of the mitochondria of the sperm middle piece (Anberg, 1957; Nicander and Hellstrom, 1968) membrane characteristics (Ortavant, 1953; Glover, 1962; Amann and Almquist, 1962a; Bedford, 1963, 1965), and percentage of abnormal spermatozoa (Glover, 1962; Amann and Almquist, 1962a). Capacity for motility increases as the spermatozoa pass through the epididymis (Tournade, 1913; Yochem, 1930; Gaddum, 1968) and a progressive loss of water occurs as well as a corresponding increase in the specific gravity and the light-reflec tion power of sperm (Lindahl and Kihlstrom, 1952; Lindahl and Thunquist, 1965; Lavon, Volcani, Amir and Danon, 1966). Variations in Feulgen DNA content (Gledhill, Gledhill, Rigler and Ringertz, 1966; Bouters, Esnault, Ortavant, Salisbury 1967) and in resistance to cold shock (Lasley and Bogart, 1944; Bialy and Smith, 1959; White and Wales, 1961), and to variations in pH (Yochem, 1930) have been observed as well as possible distinct metabolic differences between epididymal and ejaculated spermatozoa (Henle and Zittle, 1952; Lardy, Gosh and Plaut, 1949; Lardy and Gosh, 1952; White and Wales, 1961; Salisbury and Lodge, 1962; Salisbury and Graves, 1963). Since in the rabbit the fertilizing ability is acquired in a very definite segment of the epididymis, it

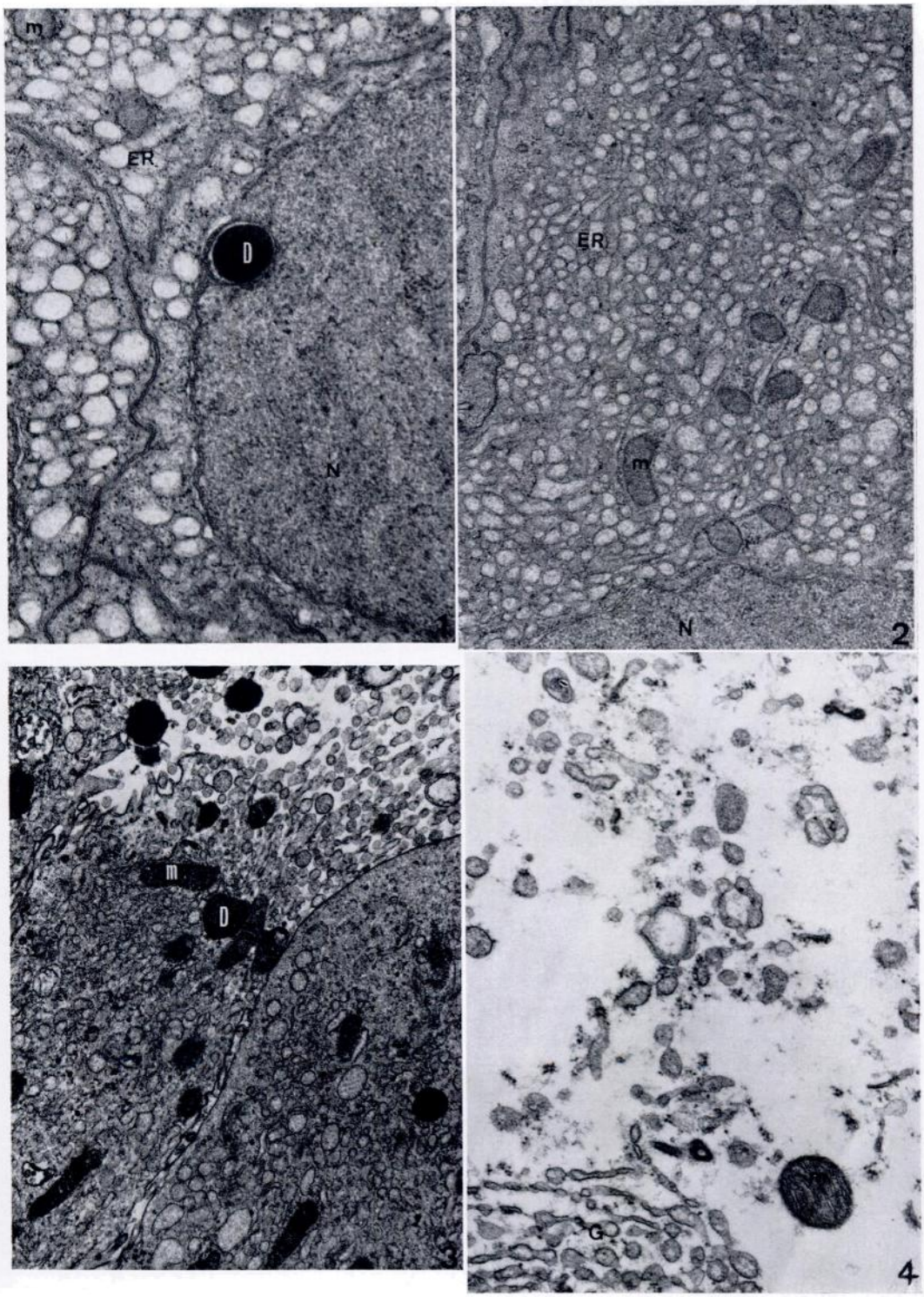
PLATE 4

FIG. 1. Perinuclear cytoplasm of a columnar cell of a normal distal caput epididymidis. Endoplasmic reticulum (ER), free ribosomes (r), mitochondria (m). Note a dense body (D) located in a nuclear invagination. ×39,600.

FIG. 2. Perinuclear cytoplasme of a distal caput epididymidis isolated for 12 days by two ligations. Note the less drastic changes in the endoplasmic reticulum as compared to these in the apical cytoplasm (see Plate 3). Osmium tetroxide, uranyl acetate and lead citrate. × 38,250.

FIG. 3. Apical part of distal caput epididymidis cells isolated for 12 days by two ligatures. Note the ruptured cell membrane and the cell contents (mitochondria (m), dense bodies (D)) escaping in the lumen. Osmium tetroxide, uranyl acetate and lead citrate. ×27,450.

FIG. 4. Cell organelles (Golgi apparatus (G), mitochondria (m)) free in the lumen of a proximal corpus epididymidis isolated for 3 days by two ligatures. Osmium tetroxide, uranyl acetate and lead citrate. ×38,700.



would be logical to think that any changes correlated directly with the fertilizing ability will be found between the upper corpus and the lower corpus epididymidis. There is no difference in the number of sperm without cytoplasmic droplets between the two regions (Orgebin-Crist, 1967a). The reduction in size and shape of the acrosome occurs in the proximal region of the caput (Bedford, 1965). Changes in sperm swimming movement from a circular to a progressive forward fashion occur gradually and become significant only in the proximal cauda (Orgebin-Crist, 1967a; Gaddum, 1968). These are only negative pieces of evidence; in the rabbit it would be extremely interesting to concentrate biochemical and metabolic studies on the epididymal segment where the first fertile sperm are found. Much may be learned about how sperm become able to fertilize.

The mechanism by which epididymal sperm induce several abnormalities at the time of fertilization and during the preimplantation period can only be hypothetical at this time. Epididymal sperm may require a longer time for capacitation in the female genital tract or to migrate to the utero-tubal junction into the oviduct, or both. In either case, the fertilization will be delayed. Delayed fertilization leads to spontaneous polyploidy (Austin and Braden, 1953; Austin 1960, Piko, 1958; Adams and Chang, 1962; Thibault, 1967) and this could explain the frequency of polyploid ova observed after insemination with epididymal spermatozoa. However, if the fertilization by lower corpus sperm is delayed by approximately 3 hr as seen by the delay in ovum development at 16, 25, 27, and 28 hr post HCG, the delay when cauda sperm is inseminated is very small, if any, and certainly no more than an hour. Yet, 5-10% of the ova are polyploid (Bedford, 1966; Orgebin-Crist, 1967a). Moreover, the main abnormality caused by ovum aging is the suppression of the second polar body, resulting in digynic ova (Thibault, 1967; Chang and Hunt, 1968), whereas most ova fertilized by epididymal sperm had two distinct polar bodies, which seems to indicate that they were polyspermic. The embryonic mortality is probably due to the combination of anomalies acquired at the time of fertilization. Polyploid ova can segment normally at least up to the eight-cell stage without showing noticeable abnormalities (Austin and Braden, 1953), but later they are retarded in their development and die around mid-gestation (Bomsel-Helmreich, 1967). Fast cleaving ova are more viable than slow cleaving ova as judged by the implantation rate after storage of the 2-day blastocyst at 10 C. for 48 hr (Hafez, 1962).

The root of all these anomalies may lie at the time of capacitation although in a still unknown manner. The Feulgen reactivity of sperm nuclear DNA was studied on epididymal or ejaculated sperm, fresh, after 9 hr *in vitro* in Hanks' solution at 37° C or after 9 hr *in vivo* in the uterus of an estrous doe. Results indicate that all 12 epididymal sperm suspensions, after 9 hr incubation in the uterus showed a reduction in the Feulgen reactivity of nuclear DNA ranging from 6 to 43% ($m = -19\%$). Five of six ejaculated sperm suspensions from the same rabbits and incubated in the same conditions showed an increase in the Feulgen DNA sperm content ranging from 9 to 41% ($m = +25\%$). A similar increase in an unspecified range was also observed by Chang and Thornsteinsson 1959. The nuclear 2650 Å absorption remained unchanged (Table 6). During spermiogenesis several cytochemical changes occur in the deoxyribonucleoprotein complex; one of them is the reduction in stainability of DNA by the Feulgen reaction (Gledhill *et al.*, 1966a, b). This has been interpreted as being indicative of a change in the deoxyribonucleoprotein toward a more stable form during the course of sperm maturation. The fact that the deoxyribonucleoprotein only of ejaculated sperm shows a reactivation toward a more unstable form during capacitation may be of signifi-

cance when related to the abnormalities observed after fertilization with epididymal sperm.

*What Are the Interrelations between the Sperm and the Various Segments of the Male Duct System?*

More specifically, are the factors governing the maturation process intrinsic to the sperm or do they reside in the epididymal secretions? The second belief was commonly held (Tournade, 1913) until Young, in 1931, challenged it after observing that guinea pig sperm retained by ligation in proximal regions of the epididymis became motile and that the fertility seemed to be enhanced. This development of sperm motility following ligation has since been confirmed (Nishikawa and Waide, 1952; Gaddum and Glover, 1965; Fulka and Koefoed-Johnsen, 1966; Bedford, 1967; Orgebin-Crist, 1967b). The development of sperm fertilizing ability can be very easily studied in the rabbit since both the time for normal sperm passage through the epididymis is known, as well as the exact segment where the fertilizing ability is acquired. Sperm can be retained by ligations in a segment, from which sperm normally do not fertilize, and checked at different time intervals after ligation for their fertilizing ability.

In a first series of experiments, ligations were made using a silk thread (No. 000) passed around the epididymis to avoid as much as possible damage to the vascular system (Plate 1, Figs. 1 and 3). Since it was felt that such ligature, especially at the caput level, could be very traumatic for the tissue, in a second series, the sperm flow was arrested by simply clamping two or three convolutions of the epididymal canal with a McKenzie brain silver clip (4 mm) (Plate 1, Fig. 2). Histological evidence of the efficacy of this type of ligation was first obtained. Epididymides of 25 males of known fertility were ligated at the mid-corpus and at the junction of the caput and the corpus thus isolating the proximal corpus region. They were killed at later intervals and the sperm of the ligated segment assessed for fertility by surgical insemination in utero as previously described (Orgebin-Crist, 1967a). Although 6 hr is the normal transit time from the upper to the lower corpus, sperm retained by ligation for that period are not fertile (Text—Fig. 2). After 12 hrs a few become able to fertilize, the higher fertilization rate being obtained when more than 8 million sperm were inseminated. After 24 or 72 hr, 74.5 and 87.5% of all ova are fertilized and the number of accessory spermatozoa ($11.0 \pm 1.5$, $11.1 \pm 1.2$) is in the same range as after insemination of sperm from normal lower corpus ($8.7 \pm 2.2$, $9.0 \pm 1.5$). Ova are not as delayed in their development as after insemination of normal lower corpus spermatozoa. This would suggest that after 24-72 hr these sperm have attained at least the same stage of maturity as sperm normally do after 6-12 hr. Abnormal fertilized ova were also observed: One pronuclear ovum

TABLE 8

EMBRYONIC MORTALITY AFTER UTERINE INSEMINATION OF UPPER CORPUS SPERMATOZOA RETAINED BY LIGATION FOR 24 HR

| | Corpus | Cauda[d] |
|---|---|---|
| Number females | 8[a,c] | 10[b] |
| Number sperm inseminated $\times 10^6$ | $15.1 \pm 4.5$ | $12.0 \pm 4.1$ |
| Number implant / Number ovulation Day 10 | 45/95 (47.3%) | 60/111 (54.0%) |
| Number implant / Number fetuses Day 28 | 25/45 (55.5%) | 41/45 (91.1%) |
| Total embryonic loss | 73.7% | 57.3% |

[a] Four females inseminated with $0.6 \times 10^6$ were not pregnant. Although the control females inseminated with the same amount were pregnant, it was feared that the failure was due to a lack of fertilization and they are not included.

[b] One female (15 ovulation points) died betweeen Days 10 and 28.

[c] Two females did not ovulate.

[d] Distal cauda distal to ligation.

TABLE 9
FERTILIZING ABILITY OF SPERMATOZOA RETAINED BY TWO LIGATIONS IN THE DISTAL REGION OF THE CAPUT EPIDIDYMIDIS

| | Uterine insemination | | Tubal insemination | |
|---|---|---|---|---|
| | No. sperm inseminated ($\times 10^6$) | Fertilization (%) | No. sperm inseminated ($\times 10^3$) | Fertilization (%) |
| No ligation | 15.1 ± 1.7 | 2.1 (1[a]/47) | — | — |
| 1-Day ligation | 21.1 ± 7.9 | 5.0 (1[a]/20) | — | — |
| 3-Days ligation | 5.8 ± 3.5 | 0 (0/57) | — | — |
| 8-Days ligation | 19.3 ± 7.5 | 7.1 (3[b]/42) | — | — |
| 12 Days ligation | 17.3 ± 5.8 | 0.7 (1[a]/133) | 181.2 ± 41.4 | 0 (0/74) |

[a] Two-cell ovum
[b] Pronuclear ova, one of which had four pronuclei (Plate 1, Fig. 4).

TABLE 10
FERTILIZING ABILITY OF SPERMATOZOA RETAINED IN THE DISTAL REGION OF THE CAPUT EPIDIDYMIS FOR 12 DAYS BY ONE LIGATION ON THE CORPUS (tubal insemination)

| | Distal caput | | |
|---|---|---|---|
| | High ligation | Low ligation | Cauda[a] |
| Number sperm inseminated | $2.3 \times 10^6$ | $0.8 \times 10^6$ | $4.6 \times 10^6$ |
| Number ova | 39 | 121 | 55 |
| Percentage fertilization | 0 | 0 | 60 |

[a] Distal to ligation.

had a single pronucleus with 3 normal sized polar bodies and a smaller one, another ovum had 3 pronuclei and 2 polar bodies. In all, 18.1% of 11 pronuclear ova were abnormal and 10.7% of the 121 cleaved ova were irregularly cleaved. Ova fertilized by sperm taken from the distal cauda distal to the ligation had the same segmentation rate 25 hr after HCG as normal cauda sperm but the incidence of abnormal pronuclear ova was much higher: 28% of 50 pronuclear ova (8 ova with 3 pronuclei, 2 with 4 pronuclei, 4 with multiple pronuclei ranging from 6 to 9 nuclei,) all 14 ova had 2 polar bodies (Table 7). The

PLATE 5

FIG. 1. Sagittal section of a sperm head in the lumen of a proximal corpus epididymidis ligated for 3 days. Note the separation of the plasma membrane and the acrosome (A) from the nucleus (N). Space is filled with a flocculent material and vesicular structures as in cytoplasmic droplet. The plasma membrane is broken (arrow). Osmium tetroxide, uranyl acetate and lead citrate. ×60,750.

FIG. 2. Sagittal section of the apex of a sperm head in the lumen of a proximal corpus ligated for 3 days. The acrosome has disappeared exposing patches of acrosomal material (A). The plasma membrane (1) outer acrosome membrane (2) and inner acrosome membrane (3) are visible. The broken plasma membrane is shown (arrows). Osmium tetroxide, uranyl acetate and lead citrate. ×58,950.

FIG. 3. Sagittal section through the apex of a sperm head from a proximal corpus epididymidis ligated for 3 days. Note the separation of the plasma membrane and the acrosome from the nucleus and vesicular structures in between. Osmium tetroxide, uranyl acetate and lead citrate. ×54,900.

FIG. 4. Transverse section through sperm head in the lumen of a proximal corpus epididymidis ligated for 3 days. Note the thin acrosome, its separation from the nucleus and the vesicular structures between the acrosome and the plasma membrane. Osmium tetroxide, uranyl acetate and lead citrate. ×14,850.

FIG. 5. As Fig. 4. ×13,950.

FIG. 6. Section through the lumen of a tubule in a normal mid-corpus epididymidis. Note the absence of plasma membrane swelling or detachment of the acrosome from the nucleus (one exception at the bottom left). Part of an epithelial cell shed in the lumen with nucleus (N) and surrounding cytoplasm. Osmium tetroxide, uranyl acetate and lead citrate. ×9,450.

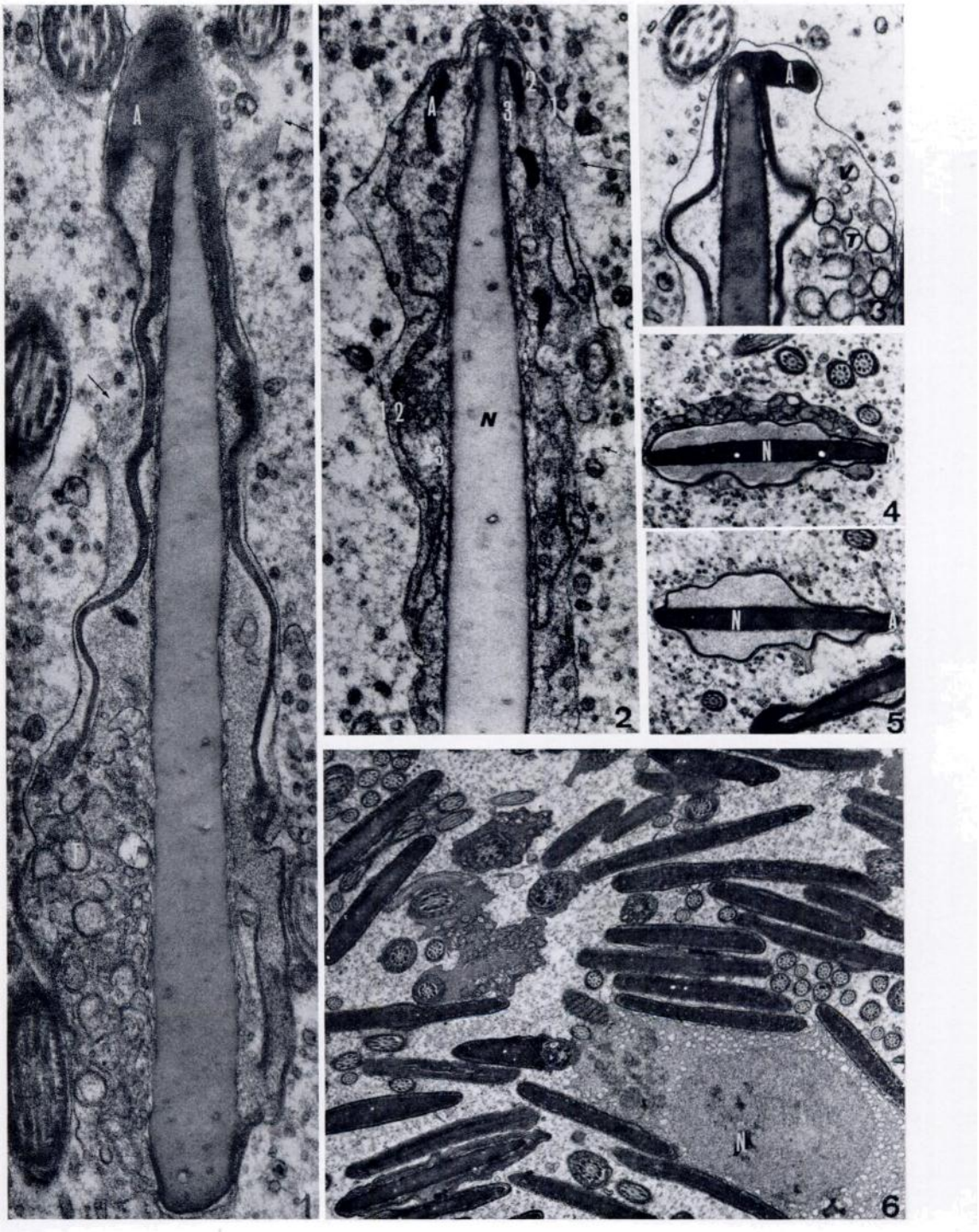


TABLE 11

FERTILIZING ABILITY OF SPERMATOZOA RETAINED BY LIGATION IN THE PROXIMAL REGION OF THE CAPUT EPIDIDYMIDIS (intrauterine insemination)

| | Ductuli efferentes (severed) | | Ductuli efferentes (intact) | |
|---|---|---|---|---|
| | No. sperm inseminated ($\times 10^6$) | Fertilization (%) | No. sperm inseminated ($\times 10^6$) | Fertilization (%) |
| 3 Days ligation | 23.1 ± 5.2 | 0 (26[a]) | — | — |
| 8 Days ligation | 15.8 ± 3.0 | 0 (34[a]) | — | — |
| 12 Days ligation | 37.6 ± 8.3 | 0 (55[a]) | 26.2 ± 4.7 | 0 (45[a]) |

[a] Total number of ova.

embryonic development of the ova fertilized by upper corpus sperm retained by ligation for 24 hr was studied as previously described (Orgebin-Crist, 1967a). The total embryonic loss after insemination of upper corpus sperm retained by ligation (73.7%) or caudal sperm (57.3%) corresponds to the losses found after insemination of lower corpus sperm (75.4%) or caudal sperm (60.9%) (Table 8).

The experiment was pursued by retaining sperm in the proximal and distal region of the caput of 17 males of known fertility. Regardless of the route of insemination, the position of the ligatures or the length of time for which spermatozoa were retained, very few spermatozoa became fertile in the segment of the caput epididymis shown on Plate 1, Figs. 2, 4 (all fertilized ova were found in females inseminated with 16, 29, and 40 $\times$ $10^6$ sperm, Tables 9 and 10). Nor were any fertilizations obtained after insemination of spermatozoa from the proximal caput even when the ductuli efferentes were severed so as to prevent the continuous flow of newly formed spermatozoa (Table 11). Similarly, Nishikawa and Waide, 1952; Fulka and Koefoed-Johnsen, 1966; Bedford, 1967, have also reported improved fertilization rates when spermatozoa are retained by ligation in the corpus region. Paufler and Foote, 1968 have reported no fertilization when spermatozoa are retained in the whole caput or the proximal caput. Some fertilization with distal caput sperm has been reported (Fulka and Koefoed-Johnsen, 1966; Bedford, 1967), this probably comes from a difference in tissue sampling since our distal ligature was placed rather high. No fertilization has been reported with proximal caput sperm (Bedford 1967). It can be concluded that by aging alone the development of full

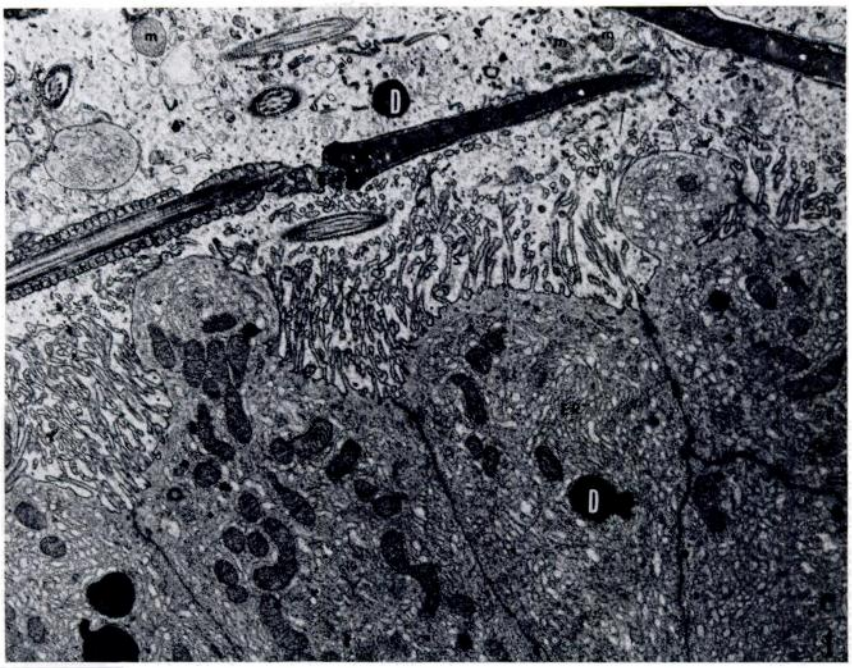
PLATE 6

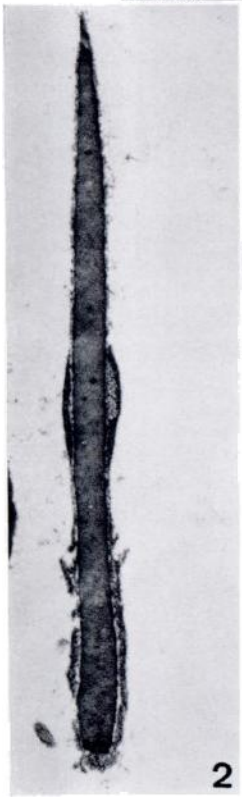
FIG. 1. Apical part of columnar cells of a normal distal caput epididymidis. Note the regular stereocilia (5), the well-developed endoplasmic reticulum (ER), the free mitochondria (m) and dense bodies (D) in the lumen, the sagittal section of a sperm head with only remnants of acrosome and vesicles resulting probably from the fusion of the plasma and outer acrosome membranes (arrows). Osmium tetroxide, uranyl acetate and lead citrate. ×11,250.

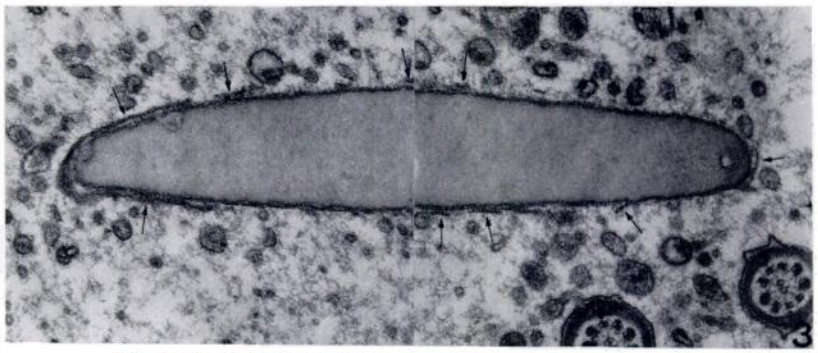
FIG. 2. Sagittal section through a sperm head in a distal caput epididymidis isolated for 12 days by two ligatures. Note the disappearance of both the acrosome and most of the membranes. Osmium tetroxide, uranyl acetate and lead citrate. ×31,950.

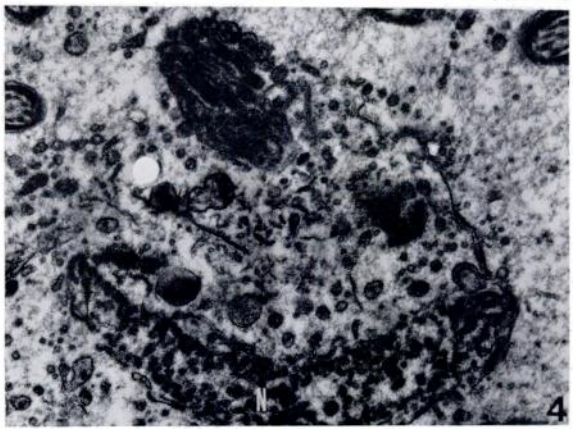
FIG. 3. Posterior and anterior portions of a transverse section through a sperm head in a distal caput epididymidis isolated for 12 days by two ligatures. Note the remnants of plasma membrane (arrows). Osmium tetroxide, uranyl acetate, lead citrate. ×63,900.

FIG. 4. Conglomerate fund in the lumen of a proximal corpus epididymidis isolated for 3 days by two ligatures in which degenerated sperm or spermatid nucleus and tail can be recognized. Osmium tetroxide, uranyl acetate and lead citrate. ×37,350.



motility of the sperm is possible regardless of their position in the male genital tract. This is not true for the sperm fertilizing ability: Extrinsic factors are needed. Before implying that the epididymis and more specifically the corpus, is responsible for this in some way, the effect of ligation on the epididymal environment must be studied and first of all its effect on the integrity of the epididymal epithelium and of the spermatozoa.

A preliminary study indicates that after ligation there is an enlargement of the lumen, a reduction in height of the epididymal epithelium (Plate 2) and reduction of the endoplasmic reticulum specially in the supra nuclear region (Plates 3 and 4, Figs. 1 and 2). Stereocilia undergo regressive changes, cytoplasm protrudes in the lumen of the epididymal canal, in many instances the cell membrane ruptures and the cells organelles flow into the lumen (Plate 3, Figs. 1 and 2; Plate 4, Figs. 3 and 4). Spermatozoa undergo degenerative changes as early as 3 days after ligation of the upper corpus. From the many degenerative figures seen, a pattern of degeneration can be established: (1) the acrosome does not lie in close apposition to the nucleus and the space in between is filled with an amorphous material where components resembling those of the cytoplasmic droplet (vesicles) can be recognized; the plasma membrane breaks in several places (Plate 5, Figs 1, 3, 4, 5); (2) the acrosome disappears leaving some patches of acrosomal materials (Plate 5, Fig. 2) and at the end of the process a row of vesicles probably derived from fused plasma and acrome membranes (Plate 6, Fig. 1); (3) the nucleus then lies bare and degenerates beyond recognition (Plate 6, Figs. 2-4). After 3 days ligation of the upper corpus epididymidis approximately half of the spermatozoa observed presented some type of degenerative changes. After 2 or 4 weeks isolation of the caput, severe degeneration recognizable with light microscopy and disappearance of sperm occurs (Gaddum and Glover, 1965; Paufler and Foote, 1968). It thus appears that after ligation, the epididymal duct is distended by sperm or fluid, or both, especially above the distal ligature, the epithelium border is disrupted, cell content passing into the lumen and sperm degenerate. It would be tempting to see a direct causative relation in this sequence. Amann and Almquist, 1962b, studying the rate of sperm resorption in normal and ligated bull epididymides also had postulated that the rate of sperm resorption was dependent upon the number of spermatozoa present in the duct. The initial segment is able to resorb fluids and particles (Van Wagenen, 1924a; Toothill and Young, 1931; Young, 1933; Clubb, 1951; Mason and Shaver, 1952; Burgos, 1964; Nicander, 1965). The caput epididymidis has therefore both dissolutive and absorptive properties.

In conclusion, while it is fairly well established that intrinsic factors alone (as postulated by Young, 1931) are not sufficient for sperm maturation and that the epididymal environment is needed for the sperm to become fully mature (that is able to fertilize) it is not possible, due to the regressive changes in the epididymal epithelium after ligation, to assign to any specific epididymal segment the function of sperm maturation. Furthermore, the process of sperm maturation is not fully completed in the epididymis since distal cauda and ejaculated spermatozoa are different where polyploidy, reaction to capacitation and probably embryonic mortality are concerned.

The epididymis is a dynamic organ apparently able (1) to convey the sperm flow from the testis to the ejaculatory duct, (2) to play a role in sperm maturation, (3) to store sperm formed, (4) to foster the dissolution of aging sperm probably in proportion to the fullness of the epididymis depending on the frequency of collection, (5) to resorb both fluids and products of sperm breakdown.

Production of sperm able to fertilize and to develop normal progeny is therefore a result of an equilibrium between these different functions. After isolation of the cauda the

incidence of abnormal pronuclear ova was very high (Table 7) and fertility was maintained for only 4 weeks in contrast to 12 weeks after a single ligation of the ductus deferens indicating that in the latter case younger sperm were responsible for the fertility observed (Paufler and Foote, 1968). It seems quite probable that the life-span during which sperm are able to fertilize normally is rather short; the next step should therefore be to try to determine this optimal life-span.

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