THE NUMBER OF SPERMS ABOUT THE EGGS IN MAMMALS AND ITS SIGNIFICANCE FOR NORMAL FERTILIZATION

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Summary

Figures are given for the numbers of sperms at the site of fertilization in normally mated rats, mice, and sheep, and also in rats and rabbits mated at the time of ovulation or later. For the sheep, data are also given on the distribution of sperms throughout the female genital tract.

In the four species investigated, the degree of restriction exercised by the female genital tract on sperm passage varied between species in such a way that the number of sperms at the site of fertilization tended to be inversely related to the size of the site. The concentration of sperms at the site would therefore presumably vary relatively little between species.

It is shown that, in normally mated rats, higher concentrations of sperms at the site of fertilization were significantly associated with the presence of larger numbers of sperms within the eggs.

Experimental evidence thus supports the concept that the usual concentration of sperms at the site is high enough to give all the eggs a good chance of being penetrated before the end of their fertile life, but not so high that there is much risk of a disturbance of fertilization through the entry of excess sperms.

The frequency of successful sperm-egg collisions was estimated to be one every 2 min for the rabbit and one every 10 min for the rat.

The restrictive action of the female genital tract in the rat is evidently relaxed towards the end of oestrus, for sperm passage is then facilitated.

I. Introduction

Special significance attaches to the number of sperms that gather after coitus at the site of fertilization, for the magnitude of this number will influence not only the chances for the meeting of egg and sperm, but also the chances for the entry of an excess of sperms into the eggs. The chances of an egg being penetrated by an excessive number of sperms will, of course, also depend upon the changes undergone by the zona pellucida and the surface of the vitellus in consequence of sperm penetration; these are discussed elsewhere (Austin and Braden 1953*a*, 1953*b*; Braden, Austin, and David 1954).

Data on the number of sperms at the site of fertilization in the rat, rabbit, and ferret were reviewed in a recent communication (Austin and Braden 1952); the numbers reported were all relatively small and it was suggested that an important function of the female genital tract was to restrict the number of sperms that accumulate in the vicinity of the eggs. More detailed figures for the rabbit were supplied by Braden (1953) and these further supported the idea that the female tract has a restrictive function.

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Information is now available on two other species of mammals, the mouse and the sheep, and a better understanding has also been gained of the mechanism of the restrictive function in the rat, rabbit, and sheep, and of the manner in which it varies in relation to the time of coitus and ovulation.

II. Methods

Adult albino and "hooded" rats were allowed to copulate at certain periods before, during, or after ovulation. The animals were slaughtered 1-20 hr after coitus and the number of sperms in the distended portion of the ampulla was counted by the method previously described (Austin 1952). Female albino mice were run continuously with males and those with copulation plugs in the morning were killed during the course of the day. The number of sperms in the distended part of the ampulla was determined by a similar method to that used for the rats.

Ovulation was induced in cross-bred rabbits by the intravenous injection of 40 I.U. of "Gonan,"* and, 10 hr later, the animals were each allowed to copulate with two males. Sperm counts were made by the method employed by Braden (1953).

Merino ewes were run continuously with a raddled ram and inspected for marking each morning and evening. Animals that were marked were killed 24-48 hr after the estimated time of coitus. The numbers of sperms in the various parts of the genital tract were estimated by a technique similar to that used for the rabbit.

Table 1

NUMBER OF SPERMS AT THE SITE OF FERTILIZATION IN RATS KILLED AFTER NORMAL MATING

Time of Killing (Hr. after Ovulation)	Number of Rats	Number of Sperms per Tube	
		Mean	Range
0	46	24	0-134
8	30	44	0-164
12	30	45	4-160

III. Observations

(*a*) *Number of Sperms at the Site of Fertilization*

(i) *Rats.*—The number of sperms in the distended part of the fallopian tube was determined in 105 rats that had been permitted to mate freely under normal colony conditions. Generally, copulation occurred in the late afternoon and was seldom observed later than 8 p.m.; ovulation took place between midnight and 4 a.m. (Austin and Braden 1954). In 48 rats with copulation

* A preparation of chorionic gonadotrophin supplied by British Drug Houses Ltd.

plugs, killed between 1 a.m. and 4 a.m. on the day of ovulation (day 1), a mean number of 24 sperms was found in individual fallopian tubes (Table 1). Another group of rats was killed between 11 a.m. and noon of day 1, and in these animals the mean number of sperms in each fallopian tube was 44. There is a significant difference ($P < 0{\cdot}01$) between these two means. A third group of rats was killed between 3 and 4 p.m. on day 1, and the mean number of sperms in these was 45 (excluding one abnormal count of 332).

TABLE 2

NUMBER OF SPERMS AT THE SITE OF FERTILIZATION IN RATS MATED DURING AND AFTER OVULATION

Time of Mating		Hours after Mating							
		1	2	2½	3	4	5	6	7
3 a.m.	Number of rats	—	15	—	10	—	10	—	10
	Number of sperms per tube — Mean:	—	27	—	57	—	47	—	63
	Number of sperms per tube — Range:	—	0-131	—	0-197	—	8-149	—	9-188
7 a.m.	Number of rats	10	15	10	10	10	10	10	—
	Number of sperms per tube — Mean*:	7	21	33	32	50	55	60	—
	Number of sperms per tube — Range:	0-23	0-81	4-107	4-131	0-152	0-267	15-167	—
11 a.m.	Number of rats	—	10	10	10	—	10	—	10
	Number of sperms per tube — Mean:	—	53	92	115	—	94	—	79
	Number of sperms per tube — Range:	—	5-152	3-317	6-345	—	0-312	—	5-188

* The mean figures for the 7 a.m. mating have already been reported (Austin 1952).

Determinations were also made of the sperm numbers at the site of fertilization in rats that were not permitted to mate until the time of ovulation or later. Oestrous female rats were selected by examination of the vaginal smear and placed with males for half an hour from 3 a.m., 7 a.m., or 11 a.m. Those that mated were recognized by the presence of copulation plugs. Coitus generally occurred in the first 15 min of the period; for convenience, therefore, the mating times are referred to as 3 a.m., 7 a.m., and 11 a.m. respectively. Altogether, 170 mated rats were obtained in this way. The results are set out in Table 2. In the rats mated at 3 a.m. the mean number of sperms per fallopian tube was found to increase from 27 at 2 hr to 57 at 3 hr; thereafter there was no significant change. The mean number of sperms in the tubes of rats mated at 7 a.m. increased from 7 at 1 hr to 50 at 4 hr. The mean values for 5 and 6 hr were similar to those obtained at 4 hr. In the rats mated at 11 a.m. the mean number of sperms per tube was 53 at 2 hr and 92 at 2½ hr after mating; after 2½ hr the mean values did not change significantly. Thus the mean number

of sperms per tube increased for 2½-4 hr after delayed mating, and higher figures were observed after mating at 11 a.m. than at 3 a.m. or 7 a.m.

(ii) *Mice.*—As in the rat, fertilization takes place in a distension of the ampulla (Sobotta 1895). Counts of the number of sperms at this location about 10-15 hr after ovulation were made in 15 mice. The mean number was 16·9 sperms per tube (range 2-75).

TABLE 3

NUMBER OF SPERMS IN THE FALLOPIAN TUBES OF RABBITS KILLED AFTER COITUS PERFORMED AT THE TIME OF OVULATION

Hr after Coitus	Number of Rabbits	Number of Sperms in Tubes		
		Entire Tubes	Ovarian 2/3	Ovarian 1/3
4	7	1775 (520-2592)*	132 (0-444)	20 (0-64)
5	4	2335 (1450-3296)	451 (138-1136)	77 (0-200)
6	6	5387 (1616-11,282)	1166 (68-4,010)	228 (0-550)

* Mean value (per rabbit) and range of values.

(iii) *Rabbits.*—Estimates of the number of sperms in the fallopian tubes of untreated rabbits at intervals after mating have already been reported (Braden 1953). Similar counts have now been made for rabbits allowed to copulate 10 hr after the injection of chorionic gonadotrophin, i.e. at about the time of induced ovulation (Table 3). The mean number of sperms per rabbit in the entire tubes was found to increase from 1775 at 4 hr to 5387 at 6 hr after mating. These values are very similar to those found in untreated animals killed 4 and 6 hr after mating (Braden 1953). In the ovarian two-thirds of the tubes, which includes the site of fertilization, the mean number of sperms increased from 132 at 4 hr to 1166 at 6 hr after coitus.

(iv) *Sheep.*—The distribution of sperms in the genital tracts of six ewes was examined 24-48 hr after coitus. The mean number of sperms per animal in the entire fallopian tubes was 15,075 (range 1,500-23,700 sperms) and in the ovarian two-thirds of the tubes, the approximate extent of the ampulla, it was 673 (range 72-2700 sperms). For the ovarian third alone, the mean number present was 184 (range 40-524 sperms). In the caudal and cranial halves of the uterine horns, mean numbers of 254,000 (range 40,000-1,170,000) and 171,000 (range 5,000-560,000) sperms, respectively, were found. The mean number recovered from the cranial half of the vagina was $1{\cdot}81 \times 10^6$ sperms (range $0{\cdot}2 \times 10^6$ to $6{\cdot}0 \times 10^6$).

(*b*) *Number of Sperms that Penetrate the Eggs in Rats*

Sixty-eight female rats mated under normal colony conditions, and killed between 11 a.m. and 3 p.m. on the day of ovulation, yielded 627 eggs. There

were 614 penetrated eggs, of which 133 (21·7 per cent.) contained more than one sperm. Examination of the data revealed that relatively large numbers of sperms at the site of fertilization were often associated with increased numbers of sperms within the eggs. The information is best considered in terms of individual fallopian tubes rather than entire rats, because the two tubes in any one rat represent two different sets of circumstances and often vary in the results they yield. From the 68 rats killed, five tubes were recovered that contained one or more unpenetrated eggs; the eggs in the remaining 131 tubes were all penetrated. Among the 131 tubes, there were 50 in which the eggs contained only one sperm each and in these tubes the mean number of sperms about the eggs was 35·7. In each of another 35 tubes, one of the eggs had two sperms within the zona pellucida; there was thus one sperm in excess of requirements in the eggs in each tube. The mean number of sperms about the eggs was 39·4. The data were sorted in the same way for tubes in which there were 2, 3, 4-5, 6-8, and more than 8 extra penetrating sperms. The results are in Table 4. Most of the sperms that have been referred to as being in excess of requirements were seen in the perivitelline space of the eggs; 10 eggs, however, had one extra sperm in the vitellus and were therefore polyspermic (dispermic).

TABLE 4

NUMBER OF EXTRA SPERMS IN THE EGGS OF ANY ONE FALLOPIAN TUBE AND ITS RELATION TO THE NUMBER OF SPERMS ABOUT THE EGGS, AND THE NUMBER OF POLYSPERMIC (DISPERMIC) EGGS

Number of Extra Sperms per Tube	Number of Tubes	Number of Sperms About the Eggs		Total Number Penetrated Eggs	Dispermic Eggs	
		Total	Mean per Tube		Total	Per cent.
0	50	1785	35·7	211	0	—
1	35	1379	39·4	174	3	1·7
2	17	749	44·1	80	2	1·5
3	10	563	56·3	42	1	2·4
4-5	11	720	65·5	59		
6-8	6	399	66·5	30 } 107	3	2·8
>8	3	408	136	18		

A regression analysis (Table 5) was carried out of $y = \log y^1$ against x, where $y^1 =$ the number of sperms at the site of fertilization and $x =$ the total number of extra sperms in the eggs of any one tube. A highly significant association ($P < 0{\cdot}01$) was found between the two values.

There appears to be a positive association between the incidence of polyspermy and the presence of higher numbers of extra sperms within the eggs of any one tube. The data (Table 4), however, are insufficient for any definite conclusion to be reached.

IV. Discussion

It is generally conceded that the site of fertilization in mammals lies in the ampulla of the fallopian tube. The size of the site varies, however, between species; in some it is restricted to a small distension of the ampulla, e.g. the rat (Tafani 1889); the mouse (Sobotta 1895); the guinea pig (Lams 1913); and the elephant shrew (Van der Horst and Gillman 1941); while in others it may involve the whole ampulla, as in the rabbit (Braden 1953). Only for a few mammals has it been suggested that fertilization takes place elsewhere than in the ampulla. In the Tenrecidae — *Hemicentetes* (Bluntschli 1938), *Ericulus* (Strauss 1938), and *Centetes* (Strauss 1950) — fertilization is reported to occur in the follicle. Pearson (1944) considered the same to be true for the short-tailed shrew; Brambell (1935), however, stated that in the common shrew the ampulla is the site of fertilization. In the ferret, Hammond and Walton (1934) thought that the eggs were fertilized in the ovarian capsule, but this seems unlikely, for Enders (1952), who worked with another member of Mustelidae, the mink, observed that the eggs were penetrated by sperms only after they entered the ampulla.

Table 5

REGRESSION ANALYSIS OF THE NUMBER OF SPERMS AT THE SITE OF FERTILIZATION AGAINST THE NUMBER OF EXTRA SPERMS IN THE EGGS

Source of Variation	Mean Square	Degrees of Freedom	*F*
(1) Slope	2625	1	15·19*
(2) Array of means about regression	0·082	6	—
(3) Residual	0·173	120	

* $P<0·01$.

The numbers of sperms that were found at the site of fertilization in four species of mammals differed widely. In rats killed at the time of ovulation the mean number was 24 sperms per tube, and in those killed 8-12 hr after ovulation the mean number was observed to be 45 sperms. These results are consistent with an earlier report of 43 sperms per tube in rats killed after ovulation (Austin 1948), and differ little from the findings of Blandau and Odor (1949), who noted means of 12 and 30 sperms in rats killed 12 and 24 hr, respectively, after mating. The mean number of sperms in the mice used in the present investigation was 17 per tube. In strong contrast to the figures for rats and mice are those for rabbits (Braden 1953) and sheep, namely, about 500 and 340 sperms per tube, respectively.

The number of sperms at the site of fertilization in all these animals is very small compared with the number introduced into the female tract at coitus. As has already been pointed out (Austin and Braden 1952), this reflects a restrictive action of the female genital tract on the passage of sperms to the site of

fertilization. The data obtained on the distribution of sperms in the genital tract of the ewe further illustrate the relative importance of various parts of the tract in this restrictive mechanism. In the sheep, the influence of the cervix far surpasses that of other structures: the normal ram ejaculate contains about 3000×10^6 sperms (Terrill 1937), but less than a million of these pass the cervix. In the rabbit (Braden 1953), on the other hand, the utero-tubal junction is the most efficient part. The cervix of the ewe is structurally well adapted for this function: it is about 4 cm long and the lumen is practically closed by mucosal folds (Sisson and Grossman 1940). The numbers of sperms found in the uterus and tubes of ewes in the present study are somewhat higher than figures published by Warbritton *et al.* (1937). They found a mean number of 26,350 (range 130-91,300 sperms) in the uteri of seven ewes killed 11 hr after mating, and a mean number of 6860 (range 8-22,500 sperms) for the entire tubes. The difference between these and the present results (425,000 and 15,075 sperms, respectively) is probably due to variations in the technique used for estimating sperm number.

The magnitude of the number of sperms at the site of fertilization is of importance, for it influences the chances of meeting of egg and sperm, and thus also the chances of sperm penetration into the egg. Other variables also affect the chances of meeting of sperm and egg but these are not easy to measure. Nevertheless, an approximate assessment of the frequency of sperm-egg collisions in the rabbit and rat may be made in the following way. The zona pellucida of the rabbit does not become less penetrable after the entry of the first sperm (Braden, Austin, and David 1954), so that the rate at which sperms accumulate within the bounds of the zona will give an indication of the collision frequency. Rabbits that were killed 13 hr after normal mating yielded eggs containing a mean number of 18 sperms each (Braden, Austin, and David 1954). As ovulation begins about 9½ hr after mating and the mean number of eggs recovered at 13 hr was 7·5, it follows that effective sperm-egg collisions must have occurred at an average rate for the 3½ hr of about one every 2 min. The rate was probably somewhat slower than this when penetration was just beginning, for the data indicate a progressive increase in the rate of effective collisions up to 6 hr after ovulation.

The same method of calculation cannot be used for the rat, for in this species the zona loses its penetrability to sperms within 1½-2 hr after the entry of the first sperm (Braden, Austin, and David 1954). However, it has been shown that, in rats mated at 7 a.m., the penetration of all the eggs in any one tube took about 1 hr (Austin and Braden 1954). When 40-60 per cent. of the eggs in any one tube had been penetrated, the penetrated eggs contained an average of 1·3 sperms (unpublished data). As the mean number of eggs was 4·8, it follows that about three successful sperm-egg collisions must have occurred during the first ½ hr after penetration had begun, so that the collision rate may be stated as about one every 10 min. Thus, sperm-egg collisions occurred five times more frequently in rabbits than in rats. Since the rabbit egg has roughly four times the surface area of the rat egg, the collision frequency per unit of surface area would be about the same. Other things being

equal, this would mean that the sperm concentration was of the same order in the two species. Evidently the effect of the greater sperm number in the rabbit compensated for the larger size of the site of fertilization. Consistent is the fact that in the mouse a small number of sperms is associated with a small site, and in the sheep a large number with a large site.

These considerations suggest that the restrictive action of the female genital tract on sperm passage varies between species in such a way as to bring the frequency of sperm-egg collisions within a certain range, in spite of the size differences of the site of fertilization. This range is such that all eggs will have good chances of being fertilized, while at the same time there is little danger of the entry of an excess of sperms into the eggs. The data on the rat show that, even under normal circumstances, large numbers of sperms at the site of fertilization are associated with an increased frequency of sperm-egg collisions, resulting in larger numbers of sperms in the perivitelline space, and possibly also in an increased incidence of polyspermy. Both the presence of numerous sperms in the perivitelline space and polyspermy could be pathological. In the rat and the pocket gopher, eggs that had an unusually large number of sperms in the perivitelline space were clearly degenerate (Braden, Austin, and David 1954; Mossman and Hisaw 1940). Polyspermy probably leads to polyploidy in the embryo (Austin and Braden 1953*b*) and polyploid embryos, as far as is known, seldom go to term (Beatty 1951).

As already mentioned, the number of sperms at the site of fertilization in normally mated rats was found to be significantly higher 8 and 12 hr after ovulation (44 and 45 sperms per tube) than at the time of ovulation (24 sperms per tube). Furthermore, in rats mated during the period of ovulation (at 3 a.m.) and a few hours after ovulation (at 7 a.m.) the mean sperm number reached a level of 50-60 at 3-4 hr after mating. Even higher mean values, up to 115 sperms per tube, were seen when the rats were mated at 11 a.m. These observations indicate that the restriction on the passage of sperms through the female genital tract of the rat is relaxed towards the end of oestrus. On the other hand, in the rabbit no such relaxation was detected, for the numbers of sperms in the fallopian tubes of animals mated at the time of ovulation were very similar to those already reported (Braden 1953) for normally mated rabbits.

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