The sperm acrosome reaction and fertilization in the guinea-pig: a study *in vivo*

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Summary. Female guinea-pigs were naturally or artificially inseminated before or after ovulation and the distribution of spermatozoa in the oviducts and the time of sperm penetration into the eggs were determined. When animals were inseminated before ovulation, the spermatozoa stayed in the distal half of the oviduct until about the time of ovulation. Only a very few spermatozoa were present in the proximal half of the oviduct at the time of ovulation, but these were sufficient to effect fertilization. When animals were inseminated after ovulation, the spermatozoa ascended the oviduct faster than when animals were inseminated before ovulation, and fertilization commenced in 4 hr. Regardless of the time of insemination, the spermatozoa participating in fertilization appeared to undergo the acrosome reaction after they reached the proximal part of the oviduct or when they were very near the eggs.

Introduction

When guinea-pig spermatozoa are incubated in a bicarbonate-buffered Krebs–Ringer solution containing pyruvate, lactate, glucose and albumin, they undergo the acrosome reaction and gain the ability to penetrate eggs *in vitro* (Yanagimachi, 1972). In this solution, a very few spermatozoa show the acrosome reaction in 2–5 hr; the majority do not react until they have been incubated for 10 hr or more (Yanagimachi, 1972; Yanagimachi & Usui, 1974). Barros (1974) found that the majority of guinea-pig spermatozoa undergo the acrosome reaction in 1–4 hr when they are placed in a simple salt solution containing only sodium chloride, calcium chloride, sodium bicarbonate, pyruvate and lactate. This apparent contradiction was resolved by Rogers & Yanagimachi (1975) who found that the type of sperm energy source in the medium greatly influences or regulates the timing of the acrosome reaction. When glucose is present either as the sole energy source or with pyruvate and lactate, the acrosome reaction occurs much later than when pyruvate and lactate are the only energy sources. An absence or deficiency of calcium ions in the medium inhibits the initiation of the acrosome reaction (Yanagimachi & Usui, 1974). The present study was initiated to determine how quickly guinea-pig spermatozoa undergo the acrosome reaction and effect fertilization *in vivo*. It is hoped that the data obtained can be used for evaluating the results of in-vitro studies on the acrosome reaction and fertilization in the guinea-pig.

Materials and Methods

Females used in this study were either (1) non-pregnant and cyclic, or (2) post-parturient. Adult females with 16- to 17-day oestrous cycles were checked daily. Those showing early signs of perforation of the vaginal membrane (Stockard & Papanicolaou, 1919) were checked for oestrus by manual stroking of the back and vulvar regions. Some of the females which showed lordosis in response to manual stroking during the test period (20.00–23.00 hours) were allowed to mate with fertile males for $\frac{1}{2}$ hr or artificially inseminated during this period. Others were artificially inseminated the next morning (08.00 to 11.00 hours). Pregnant females with widely separated pelvic bones (an indication of the terminal stage of pregnancy) were closely watched and the time of parturition was recorded. Most

of the post-partum females showed oestrus when examined between 2 and 7 hr after parturition. Some females were naturally or artificially inseminated during this oestrus. Others were artificially inseminated 20–24 hr *post partum*.

Artificial insemination was performed during ether anaesthesia by exposing the uterus through a ventral incision, and injecting a sperm suspension directly into the lumen of each uterine horn through a 23-gauge needle. The sperm suspension was prepared by suspending spermatozoa from the cauda epididymidis in Tyrode's solution containing 0.1% bovine serum albumin (Fraction V; Reheis Chemical, Chicago, Illinois). The volume of the sperm suspension injected into each uterine horn was 0.4 ml containing 8×10^7 – 9×10^7 spermatozoa. Before the incision was sutured, the ovaries of each animal were examined for the presence of ruptured follicles.

It was found that during oestrus the ovaries had no ruptured follicles, whereas 12–24 hr after oestrus was detected the ovaries always contained ruptured follicles. It is known that the length of oestrus in guinea-pigs averages 8.6 hr in cyclic females and 3.5 hr in post-partum females (Asdell, 1964), and ovulation occurs between 10 and 12 hr after the onset of oestrus (Blandau & Young, 1939; Deanesly, 1966).

Females were killed at various times after insemination, and the oviducts were excised from a point slightly proximal to the uterotubal junction. The fat surrounding the oviduct was removed, each oviduct was bisected, and each half was washed separately with 0.9% NaCl. The proximal halves of the right and left oviducts of each female were flushed with prewarmed (30–38°C) BWW medium (Biggers *et al.*, 1971) into a small dish. The distal halves of the right and left oviducts were handled in the same manner, but the flushings were collected into another dish. The oviducal flushings were immediately examined at $\times 100$ for the presence of spermatozoa. When motile spermatozoa were found, they were examined to determine the condition of the acrosomes and whether or not they displayed 'activated movement'. Spermatozoa which had lost the acrosomal cap while retaining progressive motility were recorded 'acrosome reacted'. 'Activated movement' refers to the extremely vigorous movement of spermatozoa characterized by whiplash flexing of the flagella (Yanagimachi & Usui, 1974). The number of spermatozoa in the flushing was determined either by directly counting

Text-fig. 1. Diagrammatic representation of the stages of fertilization in the guinea-pig. I, Unfertilized; II, unfertilized, spermatozoa attached to or within the zona pellucida; III, penetrated by spermatozoa, most of the cortical granules have disappeared; IV, sperm head has started to swell, egg nucleus in the anaphase of the second meiotic division; V–VI, swelling of sperm head is advancing, egg nucleus in early and late telophase; VII, early sperm and egg pronuclei; VIII, well developed sperm and egg pronuclei with distinct nucleoli. cg, Cortical granule; ep, egg pronucleus; p1, first polar body; p2, second polar body; ps, perivitelline space; sp, sperm pronucleus; z, zona pellucida.

the spermatozoa in the dish (when only a small number of spermatozoa was found) or by using a haemocytometer after diluting the flushing with 0.9% NaCl containing formalin (for large numbers of spermatozoa). The number of spermatozoa thus determined was divided by two to represent the mean number of spermatozoa in the proximal or distal portions of the oviduct. Eggs were found in the flushings of only the proximal halves of oviducts. They were rinsed with BWB medium and any cumulus cells surrounding the eggs were removed by treatment for 15 min (25°C) with 0.1% bovine testicular hyaluronidase (300 N.F. units/mg; ICN Pharmaceuticals, Cleveland, Ohio). After the eggs were rinsed with BWB medium, they were mounted between a slide and coverslip supported by four dots of a Vaseline-paraffin mixture, pressed moderately under the coverslip and examined with a phase-contrast microscope for the gross morphology of the eggs and the presence of spermatozoa on/in the zona pellucida and in the perivitelline space. The eggs were then fixed with acetic alcohol and stained with aceto-lacmoid for study of their cytological details (Yanagimachi, 1972). The stages of fertilization were classified as illustrated in Text-fig. 1.

Results

Table 1 summarizes the results obtained. No spermatozoa were obtained from the proximal parts of the oviducts of GP1 and GP2. About 80% of the spermatozoa flushed from the distal parts of the oviducts of these females were motile, and all the motile spermatozoa had intact acrosomes. Females GP3 and GP4 had eggs in the proximal parts of the oviducts and 50% of the eggs had been penetrated by spermatozoa. Although few spermatozoa were recovered from the proximal parts of the oviducts of these females, all of them were highly motile and most displayed 'activated movement' and had no acrosomal caps (acrosome reaction completed). About 95% of the spermatozoa recovered from the distal oviducts were motile, but only a very few (0.4%) were 'acrosome-reacted'.

When females were inseminated after ovulation (GP5 to GP15), sperm penetration into the eggs began about 4 hr after insemination and most of the eggs were penetrated within the next 3 hr. All the eggs penetrated were monospermic. The number of spermatozoa flushed from the distal half of each oviduct was constant, ranging from 1100 to 1880. About 20% of these spermatozoa were immotile (presumably dead) and had lost their acrosomal caps. The incidence of the acrosome reaction in live spermatozoa recovered from the distal halves of the oviducts was nil (GP5, GP7, GP10, and GP14) or very low (GP11). With one exception (GP14), the number of spermatozoa flushed from the proximal oviduct was much lower than that recovered from the distal half of the same oviduct. Most of the live spermatozoa flushed from the proximal oviducts had intact acrosomes and were found stacked together head to head (in rouleaux; Martan & Shepherd, 1973), but there were some spermatozoa (0.6–21%) that had undergone the acrosome reaction and were freely swimming or attached to the zonae pellucidae of the eggs. These acrosome-reacted spermatozoa were displaying 'activated movement'.

Discussion

According to Blandau (1969), one ejaculate of the guinea-pig contains approximately 80×10^6 spermatozoa. Many spermatozoa are trapped in the vaginal plug and eventually shed from the vagina, but some are quickly transported through the uterus to the oviduct (Blandau, 1969). The slow ascent of spermatozoa to the proximal half of the oviduct observed in the present study was unexpected, since Leuckart (1853) reported that guinea-pig spermatozoa are transported to the middle of the oviduct within 15 min after coitus. We observed (not shown in Table 1) that some of the spermatozoa deposited in the uterus during oestrus migrated to the distal tenth of the oviduct (including the uterotubal junction) within 1 hr. Some spermatozoa must migrate proximally with time, but none of them is likely to reach the proximal oviduct within a few hours. The spermatozoa probably remain in the distal oviduct until about the time of ovulation and then migrate quickly when ovulation commences.

Table 1. Sperm distribution and fertilization stages of eggs in guinea-pig oviducts at various times after insemination

Female	Time between insemination and autopsy (hr)	No. of eggs recovered from oviducts	No. of eggs at fertilization stage								Mean no. (% acrosome reaction*) of sperm. flushed from oviduct			
			I	II	III	IV	V	VI	VII	VIII	Proximal	Distal		
Inseminated (N or A) before ovulation														
GP1 Cyclic (A)	7	0	—	—	—	—	—	—	—	—	—	0	2160 (0)	
GP2 Post-partum (N)	10	0	—	—	—	—	—	—	—	—	—	0	1100 (0)	
GP3 Cyclic (N)	12	4	1	1†	—	—	—	2	—	—	—	2 (100)	N.D.	
GP4 Post-partum (A)	12	4	1	1†	—	—	1	—	1	—	—	4 (63.3)	4300 (0.4)	
Inseminated (A) after ovulation														
GP5 Post-partum	3	5	5	—	—	—	—	—	—	—	—	1 (0)	1200 (0)	
GP6 Cyclic	3	5	5	—	—	—	—	—	—	—	—	0	N.D.	
GP7 Post-partum	4	4	2	1†	1	—	—	—	—	—	—	10 (0)	1375 (0)	
GP8 Cyclic	5	4	1	1†	1	—	—	1	—	—	—	N.D.	N.D.	
GP9 Post-partum	5	5	2	2†	—	—	—	—	1	—	—	660 (0.6)	N.D.	
GP10 Cyclic	6	3	1	—	1	—	—	—	—	1	—	385 (21.2)	1880 (0)	
GP11 Cyclic	6	4	1	—	—	—	1	2	—	—	—	470 (3.1)	1830 (1.0)	
GP12 Cyclic	7	4	2	—	—	—	—	1	1	—	—	N.D.	N.D.	
GP13 Post-partum	7	3	—	—	1	—	—	—	—	—	—	330 (2.8)	N.D.	
GP14 Cyclic	7	4	—	—	—	—	—	1	—	1	2	5850† (1.0)	1100 (0)	
GP15 Cyclic	9	4	—	—	1	—	—	—	1	—	2	N.D.	N.D.	

N, natural mating; A, artificial insemination; N.D. no data

* No. of motile spermatozoa that had undergone the acrosome reaction $\times 100$ /total no. of motile spermatozoa examined.

† Spermatozoa found on and in the zona pellucida of these eggs had no acrosomal caps, indicating that they had completed the acrosome reaction.

‡ An unexpectedly large number of spermatozoa was collected from this proximal oviduct.

When spermatozoa were deposited in the uterus after ovulation, sperm transport to the proximal oviduct occurred faster than when spermatozoa were placed in the uterus before ovulation (compare GP1 with GP13 and GP14). Faster sperm transport in postovulatory than in preovulatory females has been noted in other species, e.g. rat (Braden & Austin, 1954), hamster (Yanagimachi & Chang, 1963) and rabbit (Turnbull, 1966). The reason for this is unknown, but it may be the shift in hormonal state of the animal, which influences the muscular and ciliary activity of the oviduct.

The number of spermatozoa reaching the site of fertilization ("ampullary portion" of the oviduct) is reported to be 25–50 in the guinea-pig (Blandau, 1969), but it could vary depending on the time of insemination in relation to ovulation and also how long after insemination the count is made. In any case, the number of spermatozoa present at the site and time of fertilization must be extremely low, yet these few spermatozoa seem to be sufficient for effecting fertilization of all the eggs. The length of time the fertilizing spermatozoa remain in the oviduct before they begin penetrating the eggs obviously depends on when the animals are inseminated, i.e. 10–12 hr in the present study when the animals were inseminated at or shortly after the onset of oestrus, but 4–7 hr when they were inseminated after ovulation (Table 1).

The fertilizing spermatozoa apparently undergo the acrosome reaction in the proximal part of the oviduct or when they are very near the eggs. Some spermatozoa may undergo the acrosome reaction in the distal oviduct (e.g. GP4 and GP11) or even in the uterus (Austin & Bishop, 1958), but it is unlikely that these spermatozoa can participate in fertilization. The factor(s) that triggers the acrosome reaction of fertilizing spermatozoa at the site of fertilization is unknown, but it could be the secretory products of the proximal oviduct or substances associated with the eggs (e.g. the matrix of the cumulus oophorus and follicular fluid). The high incidence of acrosome-reacted spermatozoa in the proximal oviduct compared with that in the distal oviduct suggests that the environment in the former is more favourable for initiation of the reaction. This could be partly due to more optimal concentrations of calcium ions and/or sperm energy sources (such as glucose, lactate and pyruvate), factors which are known to greatly influence the initiation of the acrosome reaction of guinea-pig spermatozoa (Yanagimachi & Usui, 1974; Rogers & Yanagimachi, 1976).

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