

Effect of ovulation on sperm transport in the hamster oviduct

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Summary. When hamsters mate shortly after the onset of oestrus (4.5–6 h before the onset of ovulation), spermatozoa are stored in the caudal isthmus of the oviduct until near the time of ovulation. At this time, a few spermatozoa ascend to the ampulla to fertilize the eggs. Superovulation resulted in a significant increase in the number of spermatozoa in the caudal isthmus at 6 h post coitus (p.c.) and in the ampulla and bursal cavity at 12 h p.c. Precocious ovulation resulted in a highly significant reduction in the total number of spermatozoa in the oviduct at 3 and 6 h p.c. This effect was completely overcome by intrauterine artificial insemination, suggesting lack of cervical patency as the block to sperm transport in precociously ovulated animals. Ligation of the ampulla–infundibulum junction in naturally ovulating hamsters resulted in significantly fewer spermatozoa in the caudal isthmus and ampulla at 12 h p.c. Preclusion of ovulation also resulted in fewer spermatozoa in the caudal isthmus and ampulla at 12 h p.c., suggesting that the products of ovulation stimulate sperm transport in the oviduct.

Keywords: sperm transport; superovulation; precocious ovulation; oocyte-cumulus complex; hamster

Introduction

In many species in which there is a delay between the onset of oestrus and ovulation, the caudal isthmus of the oviduct functions as a site for sperm storage during the preovulatory period. This is the case for hamster (Yanagimachi & Chang, 1963; Smith *et al.*, 1987), mouse (Olds, 1970; Nicol & McLaren, 1974), rabbit (Harper, 1973; Overstreet & Cooper, 1978), cow (Thibault *et al.*, 1975; Hunter & Wilmut, 1984), guinea-pig (Yanagimachi & Mahi, 1976), rat (Shalgi & Kracier, 1978), ewe (Hunter *et al.*, 1980) and sow (Hunter, 1981). In these species, large numbers of spermatozoa are stored in the caudal isthmus of the oviduct until near the time of ovulation, when a few then ascend to the ampulla to effect fertilization. The nature of the mechanisms controlling the highly synchronized rendezvous of sperm and eggs has not been determined. Several explanations have been put forward including ovulation-associated changes in oviducal contractions, hyperactive movement of spermatozoa and chemotactic attraction between sperm and egg (for review, see Overstreet, 1983; Hunter, 1988a). In the present study, we used superovulation, precocious ovulation, preclusion of ovulation and prevention of the ovulatory products from entering the oviduct to determine the effect of ovulation on the transport of spermatozoa in the hamster oviduct.

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Materials and Methods

Animals. Mature (2–3-month-old females; 4–6-month-old males) golden hamsters were housed in an air-conditioned room having light from 05:00 to 19:00 h. Under these conditions, mature females ovulated between 00:30–02:00 h of every fourth day. Each morning, for 4 consecutive days, females were checked for the presence of a postovulatory vaginal discharge (Orcini, 1961). The day of ovulation (the day of the discharge) was designated Day 1 of the oestrous cycle. After the day of ovulation had been determined, females were individually housed. Mature males were housed together in sibling pairs.

Mating, artificial insemination and euthanasia. Hamsters were mated by placing an oestrous female in a cage containing a pair of males of proven fertility that had been rested for at least 4 days between matings. Mating began within 5 min. A female was allowed to remain in the male's cage for 30 min. At this time, the female was removed and a vaginal smear was examined for the presence of spermatozoa to confirm that at least one ejaculation had occurred during the 30 min.

In some experiments, females were also artificially inseminated. To obtain semen, donor females were mated as described above. At 30 min after the onset of mating, the females were killed and semen was expressed from both uterine cornua into a watchglass and then drawn up into a 1 ml syringe (Air-Tite, Virginia Beach, VA, USA). Since hamster uteri contain virtually no fluid during oestrus, the spermatozoa recovered in this way were suspended in almost pure seminal plasma (Smith & Yanagimachi, 1989). Each female to be inseminated was anaesthetized with ether and the right uterine cornua was exposed through a small abdominal incision. A volume of 0.15 ml of semen was injected through a 20-gauge needle into the right cornua. The abdominal incision was closed and the inseminated female was allowed to recover from surgery. At various times after mating and artificial insemination, females were killed by cervical dislocation under ether anaesthesia.

Effect of superovulation on sperm transport. Females were induced to superovulate by injecting 30 iu of pregnant mares' serum gonadotrophin (PMSG) (Calbiochem, La Jolla, CA, USA) intramuscularly at 10:00 h on Day 1. Using this method, PMSG-treated females came into heat in the early evening of Day 4 (Fleming & Yanagimachi, 1980) and ovulated 30–50 oocytes between 00:30 and 02:00 h on Day 1. PMSG-treated females were mated at 20:00 h on Day 4 (4.5–6 h before superovulation) and killed at 23:00 h on Day 4 or at 02:00 or 08:00 h on Day 1 (3, 6 or 12 h after mating, respectively).

Effect of precocious ovulation on sperm transport. Females were induced to ovulate precociously by injecting 25 iu of human chorionic gonadotrophin (hCG) (Organon, West Orange, NJ, USA) intramuscularly at 14:00 h on Day 3. Ovulation occurred ~12 h after hCG injection at 02:00 h on Day 4 (24 h earlier than natural ovulation). One group of hCG-treated females was mated at 20:00 h on Day 3 (~6 h before precocious ovulation) and then killed at 23:00 h on Day 3 or 02:00 h on Day 4 (3 or 6 h after mating, respectively). Another group of hCG-treated females was mated at 20:00 h on Day 3 and then, in addition to mating, was artificially inseminated with 0.15 ml of ejaculate into the right uterine cornua at 20:30 h on Day 3. These females were killed at 23:00 h on Day 3 (3 h after mating, 2.5 h after artificial insemination).

Effect of ovulatory products on sperm transport. The products of ovulation (oocytes, cumulus oophorus, follicular fluid) were prevented from entering the ampulla by ligating the upper region of the oviduct. The right oviduct of an anaesthetized female was exposed through a dorso-lateral incision and the ampulla–infundibular junction was ligated with a 7-0 Dexon-S suture (Davis & Geck, Manatti, Puerto Rico) at 23:00 h on Day 4. In another group of females, ovulatory products were prevented from entering the oviduct by injecting resin (Technovit, grade 7134; Kulzer and Co., German Federal Republic) into the ovarian bursa to block physically the rupture of follicles (preclusion of ovulation). Technovit is a two-part (powder and liquid) cold-curing resin that works on a methyl methacrylate basis. Coagulation time depends on the powder:liquid ratio. For these experiments, 0.3 mg of powder was dissolved in 0.4 ml of liquid; this mixture coagulated in 2–3 min. Oestrous females were mated at 20:00 h on Day 4. Ovulation was blocked by injecting 0.05–0.1 ml of Technovit through a 23-gauge needle into the right ovarian bursa at 23:00 h on Day 4 (1.5 h before the anticipated onset of ovulation). The left side was left untouched and served as a control. Females were killed at 08:00 h on Day 1 (12 h after mating). As an additional control, to determine the effect in the ovarian bursa on sperm transport, Technovit was injected into the right ovarian bursa of 4 mated females at 03:00 h on Day 1 (1 h after the end of ovulation). These females were killed at 08:00 h on Day 1 (12 h after mating).

Determination of the number and distribution of spermatozoa in the oviduct. Immediately after the females were killed, oviducts, ovaries with surrounding bursa and the uppermost 5 mm of the uterine apex were removed and fixed in AFA fixative (Orcini, 1962). Fixed specimens were embedded in paraffin, serially sectioned (10 μ m), stained by Schiff's reagent and counterstained with Fast Green FCF. Spermatozoa in the various segments of the oviduct and the bursal cavity were counted using the criteria of Smith *et al.*, 1987.

Statistics. The unpaired Student's *t* test was used for all comparisons. A probability of less than or equal to 0.05 ($P \leq 0.05$) was considered significant.

Results

Superovulation, induced by PMSG treatment, had the overall effect of stimulating sperm transport in the oviduct. There were significantly more spermatozoa in the caudal isthmus of the superovulated females at 6 h after mating than in the controls (Table 1). By 12 h after mating, a much higher number of spermatozoa had ascended to the cephalic isthmus, ampulla and bursal cavity in superovulated animals than in the control animals.

Table 1. Mean number (range) and distribution of spermatozoa in the oviduct and bursal cavity of hamsters after induction of superovulation with pregnant mares' serum gonadotrophin (PMSG) by injection at 10:00 h on Day 1. Superovulation began at 00:30–02:00 h on Day 1; ($n = 4-6$ oviducts); control and treated animals mated at 20:00 h on Day 4

Treatment	Hours after mating	Segments of the oviduct				Total
		Intramural isthmus	Caudal isthmus	Cephalic isthmus	Ampulla and bursa	
PMSG	3	12 459 (91–28 534)	6548 (245–8467)	1.7 (1–7)	0	19 009 (337–35 064)
Control	3	7360 (2322–11 754)	3346 (1839–4605)	0	0	10 706 (6255–16 256)
PMSG	6	15 704 (7336–25 210)	19 640* (7517–27 477)	2.2 (0–4)	7.2 (1–28)	35 352 (16 597–49 531)
Control	6	8745 (3896–12 237)	10 323 (6762–15 343)	1.6 (0–3)	0.8 (0–2)	19 070 (15 157–22 635)
PMSG	12	4095 (3660–6178)	5427 (3968–11 966)	141* (42–236)	1820* (183–4244)	11 484 (6422–15 954)
Control	12	2034 (181–5239)	4807 (629–7484)	7.8 (2–18)	9.5 (8–14)	6860 (818–9804)

*Significantly different from control ($P \leq 0.05$).

Precocious ovulation, induced by injection of hCG on Day 3, had the overall effect of inhibiting sperm transport in the oviduct. Significantly fewer spermatozoa entered the oviducts of precociously ovulated females than in the controls (Table 2). When natural mating of hCG-treated females was augmented with artificial insemination into the right uterine cornua, the number of spermatozoa in the oviduct on the side receiving artificial uterine insemination was much greater than in the other side (i.e. naturally mated only) and was not significantly different from that in control oviducts after natural mating (Table 2).

Blocking the products of ovulation from entering the oviduct by ligation resulted in significantly fewer spermatozoa reaching the caudal isthmus and ampulla than in the control (Table 3). Blocking ovulation by injection of Technovit also resulted in significantly fewer spermatozoa reaching the caudal isthmus and ampulla, but, when Technovit was injected into the bursal cavity after ovulation, there was no significant difference between the number of spermatozoa in the oviducts of Technovit-injected and control females at 12 h p.c.

Discussion

Our results show that manipulation of ovulation affects both the number and distribution of spermatozoa in the hamster oviduct. When females were superovulated, more spermatozoa entered the oviduct and more spermatozoa ascended to the ampulla than in control females (Table 1). This

Table 2. Mean number (range) and distribution of spermatozoa in the oviduct of hamsters following mating or mating plus artificial insemination (AI) after induction of precocious ovulation with human chorionic gonadotrophin (hCG). Precocious ovulation began 14:00 h on Day 4. ($n = 4-6$ oviducts)

Treatment	Hours after mating	Segments of the oviduct				Total
		Intramural isthmus	Caudal isthmus	Cephalic isthmus	Ampulla	
hCG 14:00 h on Day 3 and mated 20:00 h on Day 3	3	0.3 (0-1)	0.3 (0-1)	0	0	0.7* (1-2)
Mated 20:00 h on Day 4 (control)	3	7360 (2322-11 754)	3346 (1839-4605)	0	0.3 (0-1)	10 706 (6255-16 256)
hCG 14:00 h on Day 3 and mated 20:00 h on Day 3	6	205 (0-691)	1003 (0-4021)	0.7 (0-1)	0	1208* (0-4722)
Mated 20:00 h on Day 4 (control)	6	8745 (3896-12 237)	10 323 (6762-15 343)	1.6 (0-3)	0.8 (0-2)	19 070 (15 905-22 635)
hCG 14:00 h on Day 3 and mated 20:00 h on Day 3 and AI 20:30 h on Day 3	6	6884 (455-17 122)	10 174 (3902-14 522)	3.5 (1-8)	4.3 (0-13)	17 065* (9497-22 751)
hCG 14:00 h on Day 3 and mated 20:00 h on Day 3 (control)	6	0 (0-1)	0.5 (0-1)	0.5 (0-2)	0	1.0

*Significantly different from control ($P \leq 0.05$).

Table 3. Mean number (range) and distribution of spermatozoa in the oviduct of hamsters after ampullary ligation or Technovit injection into the bursal cavity. Ovulation began 00:30-02:00 h on Day 1. ($n = 3-4$ oviducts). All were mated at 20:00 h on Day 4

Treatment	Hours after mating	Segments of the oviduct				Total
		Intramural isthmus	Caudal isthmus	Cephalic isthmus	Ampulla	
Ligation 23:00 h on Day 4	12	2539 (564-4719)	43* (12-75)	0	0*	2582 (639-4731)
Control	12	2913 (2197-6765)	2026 (1064-2828)	34 (15-58)	200 (63-354)	6158 (3643-9686)
Technovit 23:00 h on Day 4	12	2388 (1993-2954)	2913* (1248-5097)	11 (3-26)	8.7* (2-16)	5324 (4399-7357)
Control	12	6040 (4544-7369)	8601 (5850-11 243)	18 (9-33)	25 (16-38)	14 686 (12 009-16 147)
Technovit 03:00 h on Day 1	12	3129 (1138-5280)	4029 (2308-6914)	19 (4-48)	59 (21-163)	6988 (4510-12 232)
Control	12	2509 (461-6988)	4714 (1311-10 692)	36 (12-59)	84 (28-185)	7342 (2006-17 767)

*Significantly different from control ($P \leq 0.05$).

is surprising because superovulation has been reported to result in poor or asynchronous sperm transport within the female tract of ewes (Evans & Armstrong, 1984; Hawk *et al.*, 1987) and cows (Lineweaver *et al.*, 1970; Hunter, 1988b). However, in these experimental studies, females received additional treatments to synchronize oestrus (progesterone sponges, prostaglandin) and/or induce ovulation (hCG). The poor sperm transport reported in these studies may not have been due to superovulation *per se*, but, rather, due to the ancillary treatments used to synchronize oestrus and/or induce ovulation. Superovulation does not appear to inhibit sperm transport in rodents. In agreement with the findings of the present study in hamsters, Shalgi & Phillips (1988) reported 10 times more spermatozoa in the ampulla of rat oviduct following superovulation.

We found significantly fewer sperm reaching the oviduct in precociously ovulated females (Table 2). Since this inhibition of sperm transport into the oviduct was completely overcome by intrauterine insemination (i.e. bypassing the cervix), it seems likely that poor sperm transport through the cervix is responsible for this phenomenon. We confirmed the presence of spermatozoa in the vagina after mating during precocious oestrus, but at autopsy the highly visible bolus of semen usually present in each uterus shortly after mating was notably absent in these females. Since, under natural conditions, spermatozoa are transported rapidly through the hamster cervix at ejaculation (Chang & Sheaffer, 1957), it seems likely that the cervical canal is occluded during precocious oestrus. A high plasma concentration of relaxin has been associated with cervical patency in hamsters (O'Byrne *et al.*, 1976). It is possible that, under the conditions of precocious ovulation, the plasma concentration of relaxin or the number of relaxin receptors was not adequate to induce cervical patency. Inadequate cervical dilation could explain why semen was prevented from entering the uterus and, hence, the subsequent reduction in the number of spermatozoa in the oviducts of these females.

When the products of ovulation (oocytes, cumulus oophorus, follicular fluid) were prevented from entering the oviduct, the number and distribution of spermatozoa were altered significantly (Table 3). Ligation of the ampulla–infundibulum junction resulted in substantially fewer spermatozoa in the caudal isthmus and a complete absence of spermatozoa from the ampulla, indicating that ovulatory products have a direct effect on sperm transport in the oviduct. However, these results should be interpreted with caution, as some of the observed effect may have been due to the ligation interfering with oviducal physiology. When follicular rupture was prevented by injection of resin into the ovarian bursa and, consequently, no products of ovulation were released, there was a less pronounced, but significant, inhibitory effect on sperm transport. The injection of resin into the bursal cavity immediately after ovulation apparently did not interfere with oviducal physiology or sperm transport, which suggests that some factor in the ovulatory products has an effect on sperm transport in the oviduct.

There is evidence for the stimulatory role of ovulatory products on sperm transport in rabbits (Harper, 1973). Harper reported that a stimulatory factor was present in rabbit and rat oocyte–cumulus complexes and that the effect on sperm transport was eliminated by introducing frozen–thawed, oocyte–cumulus complexes into the oviduct. Since the physiological integrity of oocytes and cumulus cells was required for the effect on sperm transport, a secretory product is probably involved. The stimulatory effect may be mediated through a chemotactic attraction between spermatozoa and egg. However, although chemotaxis between gametes has been demonstrated convincingly in invertebrates (Miller, 1977), there is no convincing evidence for this phenomenon in mammals (Yanagimachi, 1981). Alternatively, some factor in the ovulatory products may stimulate sperm motility and thereby facilitate sperm ascent to the ampulla. Such a possibility is supported by the finding that hamster spermatozoa in the isthmic reservoir do not initiate hyperactive movement until ovulatory products are present in the oviduct (Smith & Yanagimachi, 1989). However, it seems unlikely that a stimulatory factor is transported within the oviducal lumen from the ampulla to the isthmus when enhanced adovarian oviducal contractions occur (Battalia & Yanagimachi, 1979). Therefore, it is probably the oviduct, and not the spermatozoa, that is stimulated by a factor in the products of ovulation.

Hunter *et al.* (1983) suggested that periovulatory oviducal contractions in pigs were a result of local delivery of ovarian steroids and prostaglandins to the spermatozoa. The delivery of these substances depends on a specialized anatomical arrangement in pigs and other animals in which venous and arterial vessels lie adjacent to each other facilitating a counter-current transfer. In hamsters this specialized anatomical arrangement has not been demonstrated, so the stimulatory factor may be delivered directly to the oviduct in the form of the products of ovulation. Rat cumulus cells secrete progesterone and prostaglandins of both the E and F series (Schuetz & Dubin, 1981). Prostaglandin F-2 α is a potent stimulator of smooth-muscle activity in the oviduct (Spilman & Harper, 1975). Therefore, it seems likely that prostaglandin F-2 α secreted by the cumulus oophorus shortly after ovulation stimulates the oviducal musculature, which, in turn, enhances sperm transport in the oviduct.

Such a hypothesis would explain our finding that sperm transport in the oviduct is inhibited by blocking the entry of ovulatory products into the oviduct and enhanced when a larger oocyte-cumulus complex enters the ampulla following superovulation. It is also possible that the increase in systemic ovarian steroids, which accompanies superovulation, could be responsible for increased sperm transport in the oviduct, as suggested by Shalgi & Phillips (1988). The relative amounts of systemic oestrogen and progesterone have been shown to have a distinct effect on rate and direction of contractions of the hamster oviducal musculature (Battalia & Yanagimachi, 1980). Further support for the involvement of steroids in the observed increase in oviducal sperm transport following superovulation lies in our observation that an intracardiac injection of 0.2 μ g of oestradiol at mating gave a similar increase in the number of spermatozoa transported to the ampulla at 3 and 6 h after mating (data not shown). However, the highly significant difference between superovulated and control females in the number of spermatozoa in the ampulla at 12 h after mating (cf. Table 1), when the products of ovulation are present in the oviduct, could not be duplicated by oestradiol treatment. These data tend to support the notion that the products of ovulation have a direct effect on sperm transport in the oviduct, but the mechanisms by which ovulation and/or ovulatory products affect number of spermatozoa and their distribution in the oviduct remain to be determined.

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