Sperm recovery from oviducts of turkeys at known intervals after insemination and oviposition

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Summary. To determine whether there is a relationship between oviposition time and the release of spermatozoa from the uterovaginal junction–sperm-host glands (UVJ–SHG), oviducal segments from turkey hens were isolated and washed immediately or 8–12 and 18–22 h after oviposition on Days 1, 2, 3 and 10–13 after artificial insemination. Spermatozoa were found in nearly all segments at all times examined, indicating that sperm release from the UVJ–SHG of the turkey is probably continuous or episodic and not exclusively associated with oviposition and ovulation. Alternatively, spermatozoa may be released at specific times of the daily ovulatory cycle and ascend variable distances between the UVJ and infundibulum. The finding that greater numbers of spermatozoa were recovered from the distal infundibulum and proximal magnum than from other segments supports suggestions that these regions act as sperm storage sites. Significantly greater numbers of spermatozoa were recovered from oviducts without an ovum than from oviducts containing an ovum, indicating that an oviducal ovum either sequesters luminal spermatozoa or slightly inhibits sperm release from the UVJ–SHG.

Introduction

The uterovaginal junction (UVJ)–sperm-host glands (SHG) are regarded as the primary storage site of spermatozoa in the fowl and turkey after copulation or intravaginal artificial insemination (AI). However, the mechanisms of sperm acceptance, survival, and release from the UVJ–SHG are not known. Bobr, Lorenz & Ogasawara (1964) and Van Krey (1964) successfully recovered oviducal spermatozoa only around the time of oviposition and ovulation (ovulation in the fowl follows oviposition by 30–60 min) and consequently suggested that spermatozoa are released from the UVJ–SHG in concert with these events. Verma & Chermis (1966) recovered significantly more spermatozoa from uteri removed from turkey hens shortly after oviposition than from other oviducal segments and concluded that sperm release from the UVJ–SHG might be enhanced by ovulation. Another possibility is that spermatozoa are released from the UVJ–SHG continuously throughout the daily ovulatory cycle. This was first suggested by Burke (1968) and Burke & Ogasawara (1969) after they found spermatozoa in expressed vaginal and UVJ fluid before and after ovulation and at the infundibulum in the absence of ovulation.

The present study was conducted to determine whether sperm release from the UVJ–SHG in the turkey is associated with oviposition and ovulation or whether spermatozoa are released continuously from these storage sites.

Materials and Methods

Mature Small White turkeys were caged individually in a light-proof building with lights on from 01:00 to 15:00 h. Food and water were available continuously. Eggs laid were recorded hourly between 08:00 and 15:00 h. For artificial insemination, semen was collected from Small White turkey males, pooled and diluted with Beltsville Poultry Semen Extender (Sexton, 1977). Hens were inseminated 4 times with about 300×10^6 spermatozoa per insemination, at 3–4 day intervals during their first 2 weeks of egg production. Thereafter, hens laying fertile eggs (as determined by candling after 7 days incubation) were inseminated twice, 3 days apart, every 12–16 days. The hens were killed by overdose of pentobarbitone sodium at 1, 2, 3 and 10–13 days after the second insemination of each AI episode. On each of these days after AI, the hens were killed 2–10 min after oviposition (0 h) and at 8–12 and 18–22 h after oviposition. The oviduct was removed and then ligated at the following points: the base of the fimbria, the infundibulum–magnum junction (IMJ) (infundibulum), 10 cm distal to the IMJ (proximal magnum), 20 cm distal to the IMJ (middle magnum), isthmus–magnum junction (distal magnum), isthmus–uterine pouch junction (isthmus), and uterovaginal junction (uterus/UVJ). The distal vagina was ligated before it was dissected free of connective tissue. An estimate of the number of spermatozoa contained in the infundibulum, the proximal, middle and distal magnum, the isthmus, the uterus/UVJ and the vagina was obtained with the procedure described by Hawk & Cooper (1978).

The method used for sperm recovery was as follows. Isoterge II (Curtin Matheson Scientific Inc., Beltsville, Maryland), a dilute detergent used for cleaning glassware, was injected into each ligated segment until it was distended (about 5–15 ml). The segment was gently rocked to ensure distribution of the detergent between the mucosal folds. The cranial end of the segment was then freed, and its contents poured into a 100 ml beaker. The segment was cut longitudinally, placed in the beaker with the mucosal surface up, and covered with additional detergent (20–30 ml). The detergent plus tissue were kept at 5°C for 18–24 h with intermittent agitation. After soaking, the beaker and contents were gently shaken, and the detergent was decanted into a 50 ml centrifuge tube. The beaker and oviducal segment were then rinsed with enough detergent to bring the total volume in the centrifuge tube to 40 ml. The fluid was centrifuged at 1100 *g* for 10 min. The supernatant was carefully removed until only 3 ml remained. The pellet was re-suspended and the spermatozoa counted with a haemocytometer.

Data were statistically analysed with Duncan's Multiple Range Test according to the General Linear Models Procedure (SAS) (Barr, Goodnight, Sall & Helwig, 1976).

Results

A total of 428 oviducal segments from 63 hens was examined. Three hens, killed 10–13 days after AI, had spermatozoa in all 7 segments, while no spermatozoa were recovered from the oviducal segments of 2 hens killed at 1 day and 2 killed 3 days after AI. The mean number of segments in which spermatozoa were found was 2.4 at 1 day, 2.7 at 2 and 3 days and 4.2 at 10–13 days.

When grouped without regard to time after AI, each segment at all times after oviposition contained spermatozoa (Table 1), but larger numbers were present in the infundibulum and proximal magnum particularly at 8–12 h. The infundibulum and proximal magnum contained more spermatozoa than did the other segments at all times after oviposition at all times after AI except at 10–13 days (Table 2) when the vagina contained the most spermatozoa at 0 h and 18–22 h after oviposition.

There was little statistical variation in the number of spermatozoa contained in the same segment at each time after oviposition and AI (Table 2). For sperm numbers at different times

Table 1. Numbers of spermatozoa ($\times 10^2$) recovered from different segments of the oviducts of turkeys at different times after oviposition and on all days after AI

Segment	Time of sperm recovery after oviposition			Mean s.e.
	0 h	8–12 h	18–22 h	
Infundibulum	(16) 400 ^{ax}	(23) 794 ^{ax}	(20) 859 ^{ax}	± 297
Proximal magnum	(14) 217 ^{abx}	(23) 722 ^{ax}	(20) 157 ^{ax}	± 193
Middle magnum	(18) 69 ^{bx}	(23) 135 ^{bx}	(21) 69 ^{abx}	± 44
Distal magnum	(18) 33 ^{bx}	(23) 48 ^{bx}	(22) 9 ^{by}	± 12
Isthmus	(18) 19 ^{bx}	(23) 26 ^{bx}	(22) 14 ^{bx}	± 10
Uterus-uterovaginal junction	(17) 41 ^{bx}	(23) 29 ^{bx}	(21) 43 ^{abx}	± 19
Vagina	(18) 139 ^{bx}	(23) 80 ^{bx}	(22) 79 ^{abx}	± 55
Mean s.e.	± 88	± 186	± 41	

Values are the means for the number of observations in parentheses.

^{ab} Values within each column without a common superscript letter differ significantly ($P < 0.05$).

^{xy} Values within each row without a common superscript letter differ significantly ($P < 0.05$).

Table 2. Numbers of spermatozoa ($\times 10^2$) recovered from different segments of the turkey oviduct in relation to times after oviposition and AI

Days after AI	Time (h) after oviposition	Segment							Mean s.e.
		Infundibulum	Magnum			Isthmus	Uterus/UVJ	Vagina	
			Proximal	Middle	Distal				
1	0	(3) 267 ^{ax}	(3) 166 ^{abx}	(4) 50 ^{bxx}	(4) 50 ^{bxx}	(4) 0 ^{cx}	(4) 25 ^{bxx}	(4) 49 ^{bxx}	± 45
	8–12	1300 ^{ax}	300 ^{ax}	91 ^{ax}	0 ^{ax}	0 ^{ax}	37 ^{ax}	25 ^{ax}	± 410
	18–22	(5) 50 ^{abx}	(5) 470 ^{ax}	133 ^{abx}	16 ^{bx}	0 ^{bx}	58 ^{abx}	66 ^{abx}	± 135
	Mean s.e.	± 821	± 348	± 133	± 19	± 0	± 42	± 34	
2	0	(5) 190 ^{abx}	(4) 437 ^{ax}	149 ^{abx}	42 ^{bx}	25 ^{bx}	(5) 90 ^{bx}	42 ^{bx}	± 92
	8–12	175 ^{ax}	191 ^a	42 ^{abx}	33 ^{abx}	0 ^x	0 ^x	25 ^{abx}	± 54
	18–22	66 ^{ax}	75 ^{ax}	50 ^{ax}	8 ^{ax}	25 ^{ax}	25 ^{ax}	25 ^{ax}	± 25
	Mean s.e.	± 91	± 133	± 45	± 27	± 17	± 34	± 16	
3	0	(5) 720 ^{ax}	(4) 100 ^{xy}	(5) 10 ^{ax}	(5) 0 ^{ax}	(5) 20 ^{ax}	(5) 20 ^{ax}	(5) 30 ^{ax}	± 253
	8–12	233 ^{bx}	508 ^{ax}	150 ^{bx}	58 ^{bx}	75 ^{bx}	8 ^{bx}	91 ^{bx}	± 82
	18–22	93 ^{ax}	(5) 10 ^{xy}	42 ^{ax}	0 ^{ax}	8 ^{ax}	60 ^{ax}	58 ^{ax}	± 32
	Mean s.e.	± 332	± 113	± 58	± 15	± 32	± 25	± 34	
10–13	0	(3) 350 ^{ax}	(3) 133 ^{ax}	(3) 33 ^{ax}	(3) 50 ^{axy}	(3) 33 ^{ax}	(3) 17 ^{ax}	(3) 634 ^{ax}	± 240
	8–12	(5) 1601 ^{ax}	(5) 2120 ^{ax}	(5) 280 ^{ax}	(5) 110 ^{ax}	(5) 31 ^{ax}	(5) 80 ^{ax}	(5) 200 ^{ax}	± 669
	18–22	(3) 167 ^{ax}	(4) 75 ^{ax}	(3) 33 ^{ax}	(3) 13 ^{ay}	(4) 25 ^{ax}	(4) 25 ^{ax}	(4) 212 ^{ax}	± 75
	Mean s.e.	± 1165	± 812	± 115	± 31	± 19	± 44	± 294	

Values are the means for 6 observations except as indicated in parentheses.

^{abc} Values within each row without a common superscript letter differ significantly ($P < 0.05$).

^{xy} Values within each column without a common superscript letter differ significantly ($P < 0.05$).

after oviposition on the days after AI the values in the proximal magnum at 3 days and the distal magnum at 10–13 days after AI were significantly different. When the mean number of spermatozoa contained in each segment was considered in relation to the day after AI, each segment except the isthmus contained more spermatozoa at 10–13 days than at other times (Table 3).

Table 3. Numbers of spermatozoa ($\times 10^2$) recovered from turkey oviduct segments on the different days after AI

Segment	Day of sperm recovery after AI				Mean s.e.
	1	2	3	10-13	
Infundibulum	(14) 623 ^a	(17) 141 ^a	(17) 363 ^a	(11) 869 ^a	± 344
Proximal magnum	(14) 332 ^{ab}	(16) 209 ^b	(15) 233 ^b	(12) 942 ^a	± 220
Middle magnum	(16) 97 ^a	(18) 80 ^a	(17) 71 ^a	(11) 145 ^a	± 51
Distal magnum	(16) 19 ^b	(18) 28 ^{ab}	(17) 21 ^b	(12) 63 ^a	± 13
Isthmus	(16) 0 ^a	(18) 17 ^a	(17) 39 ^a	(12) 29 ^a	± 12
Uterus/uterovaginal junction	(16) 42 ^a	(17) 35 ^a	(16) 28 ^a	(12) 46 ^a	± 23
Vagina	(16) 47 ^b	(18) 31 ^b	(17) 62 ^b	(12) 313 ^a	± 64

Values are means for the number of observations in parentheses.

Values within each row without a common superscript letter differ significantly ($P < 0.05$).

When all oviducts and oviducts without and with an ovum were grouped by segments without regard to time after oviposition and AI more spermatozoa were recovered from the infundibulum and proximal magnum than from the remaining segments (Table 4, see columns). Sperm numbers in all segments except the isthmus were greater when no ovum was present in the oviduct at the time of killing (Table 4, see rows). The total number of spermatozoa recovered was significantly greater ($P < 0.05$) in oviducts without an ovum (30 300 ($n = 154$)) than in oviducts with an ovum (7200 ($n = 274$)).

Table 4. Numbers of spermatozoa ($\times 10^2$) recovered from each segment from all oviducts and from oviducts without and with an ovum

Segment	Spermatozoa recovered			Mean s.e.
	All oviducts	No ovum in oviduct	Ovum in oviduct	
Infundibulum	(59) 447 ^a	(22) 957 ^{ax}	(37) 143 ^{aby}	± 230
Proximal magnum	(57) 400 ^a	(22) 773 ^{ax}	(35) 166 ^{ay}	± 168
Middle magnum	(62) 93 ^b	(22) 141 ^{bx}	(40) 67 ^{bcx}	± 38
Distal magnum	(63) 30 ^b	(22) 50 ^{bx}	(41) 20 ^{cy}	± 9
Isthmus	(63) 19 ^b	(22) 14 ^{bx}	(41) 23 ^{cx}	± 9
Uterus/uterovaginal junction	(61) 37 ^b	(22) 41 ^{bx}	(39) 35 ^{cx}	± 17
Vagina	(63) 97 ^b	(22) 143 ^{bx}	(41) 72 ^{bcx}	± 46
Mean s.e.	± 72	± 202	± 28	

Values are means for the number of observations in parentheses.

^{abc} Values within each column without a common superscript letter differ significantly ($P < 0.05$).

^{xy} Values within each row without a common superscript letter differ significantly ($P < 0.05$).

Discussion

Spermatozoa were found in the oviducal segments at all times examined. The variability in the number of spermatozoa recovered per segment may have been due to the sperm recovery technique or it may have been a reflection of the variability in the number of oviducal spermatozoa present in individual hens during the daily ovulatory cycle. The total number of spermatozoa recovered per oviduct was low when compared to the number inseminated. Howarth (1971) reported that 57.6% of turkey spermatozoa were excreted within 15 min after insemination. In addition, it is doubtful that the sperm recovery procedure employed in this

study dislodged spermatozoa residing in the UVJ-SHG considering that oviposition (and concomitant distension of the UVJ) has a minimal effect on sperm release from the UVJ-SHG (Compton & Van Krey, 1979). Furthermore, although there is a slight tendency for the number of spermatozoa in the oviduct to increase with each successive insemination, it was not a significant factor in the numbers of spermatozoa recovered. However, as the interval between successive inseminations increases the number of spermatozoa recovered from the oviducts also increases due to: (1) daily increments in the population of residual spermatozoa (spermatozoa released from the UVJ-SHG and not sequestered by the ovum), and (2) aged or dead spermatozoa leaving the UVJ-SHG and infundibulum and proximal magnum. Nevertheless, these data appear to support the suggestion (see 'Introduction') that sperm release from the UVJ-SHG is probably continuous or episodic and is not associated exclusively with oviposition and ovulation. Another possibility is that spermatozoa are released at a specific time of the daily ovulatory cycle, ascend the oviduct at variable rates and subsequently are present in each segment throughout the daily ovulatory cycle. An additional source of luminal spermatozoa in the turkey may be the infundibulum and proximal magnum which appear to serve as secondary sperm storage sites. Whether spermatozoa in these segments are transported in an abovarian direction and thus contribute to the population of spermatozoa in the more distal segments is not known.

The high numbers of spermatozoa recovered from the infundibulum and proximal magnum support previous suggestions (Van Drimmelen, 1946; Fujii & Tamura, 1963) that spermatozoa can be stored in these segments in fowls. Fujii & Tamura (1963) found spermatozoa in the "... undifferentiated albumen secretory glands ... situated at the beginning of the magnum." It is suggested that the decrease in sperm numbers in the infundibulum and proximal magnum after passage of an ovum is directly related to the fertilization process, and that in order to maintain high fertility a substantial population of spermatozoa must reside in the infundibulum and proximal magnum. These segments would be continually supplied with spermatozoa, from the UVJ-SHG. Assuming that spermatozoa randomly interact with the surface of an ovum (Bobr *et al.*, 1964), a high population of spermatozoa at the site of fertilization would increase the chances of spermatozoa penetrating the animal pole. Benoff & Renden (1978) showed that fertility of the C_1 (first egg in a clutch) and C_2 (second egg in a clutch) eggs were the same when hens were grouped by the number of eggs laid from the time of insemination to when C_1 was laid; they concluded that factors other than oviposition are involved in the release of spermatozoa from the UVJ-SHG. However, when fertility from all hens was considered, the percentage of infertile eggs was higher for the C_2 eggs (10.0%) than for the C_1 eggs (6.2%). Benoff & Renden (1978) suggested that the lowered fertility with the C_2 egg was due to the increased time between insemination and ovulation of the C_2 egg, but our results suggest that the difference may be due to a decreased sperm population at the infundibulum and proximal magnum after the passage of the C_1 ovum. The C_1 ovum had the advantage of a rest day(s) in which the sperm population increased at the infundibulum and proximal magnum, and the chance of fertilization was thus increased. Wall & Jones (1977) found that hens that laid higher numbers of eggs required higher numbers of spermatozoa to maintain optimal fertility. Therefore, the more frequently a hen ovulates the more depleted the sperm population at the infundibulum and proximal magnum becomes, and the greater the need for more spermatozoa to maintain maximal fertility.

Although a substantial population of spermatozoa at the infundibulum and proximal magnum may be necessary to maintain optimal fertility, an excess of spermatozoa in these segments is inimical to embryo survival (Van Krey, Ogasawara & Lorenz, 1966; Lorenz & Ogasawara, 1968). Large numbers of spermatozoa are found at the infundibulum after intramaginal insemination (Van Krey *et al.*, 1966). Possible explanations for the high embryonic death rate observed during the first week after intramaginal insemination include pathological polyspermy, fertilization by spermatozoa that after intravaginal insemination would normally be excluded by sperm-selective mechanisms at the UVJ, and/or the inability of the infundibular

storage sites to maintain large numbers of spermatozoa (Van Krey *et al.*, 1966; Lorenz & Ogasawara, 1968).

Higher numbers of spermatozoa were in oviducts without an ovum than in those with an ovum. Luminal spermatozoa could be sequestered by the ovum in transit or an ovum (particularly when in the uterus) could inhibit the release of spermatozoa from the UVJ-SHG. That residual spermatozoa do remain in the segments after passage of an ovum was particularly evident in hens killed 10–13 days after AI which had high numbers of spermatozoa in the oviducal segments. Other explanations for the high numbers of oviducal spermatozoa at this time, particularly in the vagina, may relate to ageing of the spermatozoa in the UVJ-SHG. Aged or dead spermatozoa leaving the UVJ-SHG may be disposed of by expulsion through the vagina.

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