Rapid transport of seminal immunoglobulin to distal parts of the female reproductive tract in rabbits

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Summary. Bucks immunized with the bacteriophage-cloning vector fd-tet display strong humoral seminal immunity of 1.5×10^6 fd-tet neutralizing units (nu) ml^{-1} . We used this model to study the distribution of seminal immunoglobulin in the female reproductive tract after copulation and artificial insemination. In contrast to artificial insemination, significant ($P < 0.01$) amounts (147 nu ml^{-1} , i.e. about 1.25×10^{-4} input ejaculate) of neutralizing activity reached the uterus within 20 min of a single copulation, which is evidence for rapid transport of seminal plasma to distal parts of the tract. Oviducts were also quickly impregnated with antibodies to fd-tet, which persist in the distal compartments for at least 24 h.

Keywords: semen; immunoglobulin; rabbit

Introduction

Rapid sperm transport occurs in mammalian species, but its biological significance is poorly understood. As it is usually not directly involved in fertilization, it might be viewed as an epiphenomenon of pericoital events occurring in the female tract. However, Overstreet (1983) suggested that rapidly transported spermatozoa could act as local messengers to the female reproductive tract in preparing it for sperm migration, fertilization and implantation. Pandya & Cohen (1985) showed that women, like rabbits, respond to cervical deposition of spermatozoa by leukocytosis within 15 min.

It is uncertain whether seminal plasma participates in rapid transport in humans and rabbits. Resolution of this question is essential to an appraisal of possible factors that could 'prime' the female tract to perform its reproductive functions. Early experiments using dyes, particles and radio-opaque media as markers of seminal fluid gave inconsistent results. Krehbiel & Carstens (1939) observed mass movement of contrast medium through the cervix into the uterus of supine-restrained does, but Noyes *et al.* (1958) obtained different results. In an attempt to reconcile these findings, Akester & Inkster (1961) described two types of entry of radio-opaque medium into the uterus: the type of Krehbiel & Carstens, which takes place within 2 min of vulval stimulation, could be ascribed to abnormal conditions of animal restraint whereas minute projections of opaque material through the cervixes lasting for several hours could be observed after mating or vulval stimulation of unrestrained does. Asch *et al.* (1977) noticed the failure of seminal plasma to enter the uterus and oviducts of rabbit does artificially inseminated with radiolabelled semen containing unlabelled spermatozoa.

In this study, we investigated whether, after copulation, neutralizing immunoglobulin G (IgG) from seminal fluid of bucks hyperimmune to bacteriophage fd-tet is rapidly transported to the uterus and oviducts. This investigation might be relevant to understanding the role of seminal antibodies in transmission of diseases such as acquired immune deficiency syndrome (AIDS).

Materials and Methods

Neutralization titration

Fd-tet is a bacteriophage-cloning vector which confers tetracycline resistance to infected hosts (Zacher III *et al.*, 1980). The methods of its propagation and purification have been described elsewhere (Smith, 1988). The neutralizing titre of the sample was scored as the dilution that caused 50% inhibition of a standard amount of about 50 tetracycline-resistance-transducing units (tu) of fd-tet; 50 µl of serial twofold sample dilutions were mixed with 50 µl of phosphate-buffered saline with 1% bovine serum albumin (PBS/BSA) containing 250 tu fd-tet. After incubation for 2 h at room temperature, a drop of indicator *Escherichia coli* K91 was added and 25 µl aliquots were poured onto tetracycline agar in 24-well plastic Costar plates. Each sample was assayed in quadruplicate.

Animals and treatments

Three adult Big Chinchilla (a commercial breed of rabbit introduced to Czechoslovakia in 1922 from Germany and distributed by VELAZ Prague) bucks were immunized with CsCl-gradient purified fd-tet as follows: first, 5 µg in 250 µl PBS intravenously (i.v.), then two doses of 10 mg in 5 ml PBS intraperitoneally (i.p.) at intervals of 14 days. After this treatment, the seminal fluid titre reached a basal plateau of 5×10^5 nu ml⁻¹, which could be boosted to 1.5×10^6 nu ml⁻¹ with 10 mg fd-tet i.p. This maximum titre persisted from day 10 to day 23 after the booster injection. At this time the bucks were mated once to virgin adult does or used for ejaculate collection with artificial vaginas (Adams, 1972); 0.8 ml of pooled fresh ejaculates were used for artificial insemination (Adams, 1972) of receptive females. No leakage was observed. Seminal fluid was obtained for immunological analysis by clarifying ejaculates at 14 000 g for 3 min. Immunoglobulin-class determination was achieved by fractionation on Ultrogel AcA-34 (LKB) (Peri *et al.*, 1982). Females were killed by an overdose of thiopental (Spofa) i.v. at intervals of 20 min, 4.5 h and 24 h after insemination, using three specimens for each interval and type of insemination.

Immediately after opening the abdomen, clamps were placed at both ends of the cervixes, the uterotubal junctions and the ends of the oviducts. All precautions (Overstreet & Cooper, 1978) to prevent contamination and other artefacts of handling were carefully observed. The uterine and cervical lumina were flushed with 1 ml PBS/BSA and the oviducts with 0.5 ml of the same solution. Samples were centrifuged for 3 min at 14 000 g, the supernatant was titrated for neutralization and the sediment inspected for presence of spermatozoa.

Results

The ejaculates contained $(1-5) \times 10^8$ spermatozoa ml⁻¹ of which 30-40% were nonmotile. The seminal fd-tet-neutralizing response is associated with IgG and not with the cellular fraction of the semen (Table 1). In contrast to artificial insemination, significant ($P < 0.01$) amounts of this immunity reached distal parts of the female reproductive tract within 20 min of copulation and remained there for at least 24 h (Table 2). In control does inseminated with nonimmune semen, the fd-tet neutralizing activity was below the detection limit of 20 nu ml⁻¹ throughout the tract.

Table 1. Fd-tet neutralizing properties of ejaculate of hyperimmune rabbit bucks

Maximum neutralizing titre of the fluid fraction (neutralizing units (nu) ml ⁻¹) consisting of:	$1.52 \times 10^6 \pm 3.7 \times 10^5$
IgG fraction (%)	> 99.93
IgA fraction (%)	< 0.07
Titre associated with the cellular† fraction (nu ml ⁻¹)	$6.2 \times 10^3 \pm 2 \times 10^3$ i.e. $\leq 0.53\%$ of total

Ig: immunoglobulin.

†Sediment obtained after clarification of ejaculate at 14 000 g for 3 min.

Table 2. Regional distribution of seminal fd-tet neutralizing antibodies in the reproductive tract of female rabbits at various intervals after copulation or artificial insemination (nu ml⁻¹)

Region	Insemination	Time after insemination		
		20 min	4.5 h	24 h
Cervix	Natural	5120 ± 1280	3840 ± 1280	1492 ± 368
	Artificial*	747 ± 184	747 ± 184	320 ± 68
Uterus	Natural	147 ± 23	187 ± 115	67 ± 24
	Artificial*	< 20	< 20	< 20
Oviducts	Natural	54 ± 24	60 ± 20	< 20
	Artificial*	< 20	< 20	< 20

*0.8 ml of fresh immune ejaculate of titre 1.5×10^6 nu ml⁻¹ was used for artificial insemination of receptive does.

Ten days after copulation *ad libitum* (3–5 times), 160 nu ml⁻¹ was still present in the fluid aspirated from the cranial end of the vagina. The distribution of spermatozoa in the flushed compartments was assessed at the level of orders of magnitude and no significant difference between mating and artificial insemination was observed. The occurrence of rapid sperm transport after both types of insemination was confirmed by the recovery of up to 300 spermatozoa from the uterus and oviducts after 20 min. At 4.5 h the uteri contained about 10⁶ spermatozoa. However, the methods of sample collection were not designed for accurate determination of numbers of spermatozoa but for maximum antibody elution.

Discussion

We have shown that, in contrast to artificial insemination, about 1.0×10^{-4} ejaculated seminal fluid is rapidly transported to the uterus after copulation of rabbits.

Artefacts of handling of the reproductive tract are therefore not a likely source of the positive result. It will also not be due to transport of IgG adherent to spermatozoa, as the increase in their number in the uterus is not concomitant with a rise in neutralizing activity.

The negative outcome of the experiment of Asch *et al.* (1977) was based on a detection limit of 1.5×10^{-4} ml of seminal fluid in the distal reproductive tract. Our approach offers a higher sensitivity, as 2.5×10^{-5} ml of seminal fluid could be traced. The contrasting positive result can be best explained by the different efficiency of transport of seminal plasma after copulation or AI. Similar results with both techniques were obtained by increasing the neutralizing titre of the artificial inseminate at least 50 times by admixture of immune IgG isolated from serum. This shows that seminal fluid may reach distal parts of the reproductive tract even after artificial insemination, although in much smaller amounts. These observations are inconsistent with the similarity between the uterine and oviductal sperm counts after copulation and artificial insemination. We assume that this discrepancy is due to physiological, rather than technical, factors since Overstreet & Cooper (1978) also failed to observe a substantial difference between these parameters.

In rabbits, the cervix functions as a valve with only a potential space between its apposing mucosal folds, the numerous overlapping cilia of which are vigorously beating towards the vagina (Blandau, 1977). The initial transport of ejaculate through the cervix is mediated by contractions of the reproductive tract (Fuchs, 1972). In this complex rheological system, what holds for the particulate cellular components of semen need not hold for seminal plasma. There is evidence that the rapid delivery of spermatozoa into the upper reproductive tract is a quantized process. Overstreet & Tom (1982), inseminated rabbit does with whole semen and with killed spermatozoa suspended in seminal plasma at roughly similar sperm inputs; 15 min later, the cervixes contained

2.1×10^6 spermatozoa in the former case, but only 7.3×10^4 in the latter. The efficiency of sperm recovery from the oviducts was nevertheless the same in both cases. Another example of quantization of rapid sperm transport is its asymmetry (Overstreet & Cooper, 1978). These phenomena are probably due to the spatial and hydrodynamic characteristics of the interaction of sperm particles with the bottlenecks of the distal tract (Katz *et al.*, 1989). In contrast to the complex behaviour of spermatozoa, small amounts of seminal fluid may simply be permeated through the cervical sluice during the initial phase of transport. This leakage may be proportional to the receptive pericoital activity of the female reproductive tract. We understand it to be more pronounced during copulation, as the mere presence of a buck can elicit uterine contractions (Fuchs, 1972) – a factor which is obviously missing at AI.

The salient fact revealed by this study is that the transit of seminal plasma into distal parts of the female reproductive tract is confined to the rapid phase of transport only.

Our finding of transport of seminal fluid markers in rabbits is in agreement with similar results in other species: cows (Rowson, 1955), gilts (Einarsson *et al.*, 1980) and man (De Boer, 1972). However, in contrast to the methods used in the previous reports on this matter, our approach does not interfere with the natural course of insemination. It can also be successfully applied to other animals. We found much more pronounced impregnation of the uterus with inseminated antibodies in mice than in rabbits (D. Jappel, unpublished).

As Barratt *et al.* (1990) describe the presence of 10^6 leucocytes (T cells, monocyte-macrophages and others) ml^{-1} semen, at least three factors may be considered as chemical signals along the entire female reproductive tract: spermatozoa, other seminal cellular components and constituents of seminal plasma.

The possibility that small quantities of immunocompetent molecules and cells reach distal parts of the female tract may relate to the mechanisms of sexual transmission of viral infections. Complex patterns of humoral and local secretory immunity to HIV have been observed in semen of sero-positive individuals (Belec *et al.*, 1989) and low concentrations of specific antibodies have been shown to enhance HIV infectivity (Takeda *et al.*, 1988).

Our study shows that the relative concentrations of inseminated components vary dramatically along different compartments of the female reproductive tract, which may strongly influence their role in establishment of infection.

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