Table 2

	Plants observed	L. fruits examined	Normal seeds	Deformed seeds	Sterile seeds	Total	R. fruits examined	Normal seeds	Deformed seeds	Sterile seeds	Total
FSL-plants											
Total	21	115	2104	181	773	3058	297	4979	410	2338	7727
Mean			18.30	1.57	6.72	26.59		16.76	1.38	7.87	26.01
FSR-plants											
Total	30	426	7192	542	2792	10526	196	3766	278	1172	5216
Mean			16.88	1.27	6.55	24.71		19.21	1.42	5.98	26.61

FSR, foliar spiral right; FSL, foliar spiral left.

data using a parallel-sample chi-square test. The comparison between the number of different types of fruits for left-twisting and right-twisting flowers of left-spiralled plants yielded a chi-square value of 26.70 (d.f. = 2) which is significant at the 5% level. Corresponding chi-square value for the other group is 35.24 (d.f. = 2), which is also significant at the 5% level. These tests thus support the conclusions drawn.

Uterine fluid from progesterone treated rabbits contains subcellular membranes

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Worcester Foundation for Experimental Biology, Shrewsbury (Massachusetts 01545, USA), 12 July 1977

Summary. Uterine fluid from progesterone treated rabbits was shown to be rich in subcellular membrane components consisting of vesicles and cilia-like fragments. In contrast, uterine fluid from untreated does lacked subcellular membranes. Thus, they arise when uterine sperm capacitation ability is suppressed.

Administration of progesterone to female rabbits inhibits intra-uterine sperm capacitation[2], which is an essential pre-condition for fertilization in this species and other mammals[3]. Likewise, rabbit spermatozoa fail to achieve fertilizing capacity during incubation in the progesterone-dominated uterus of a pseudopregnant doe[4,5]. The cause of this anti-fertility action by the steroid is unknown.

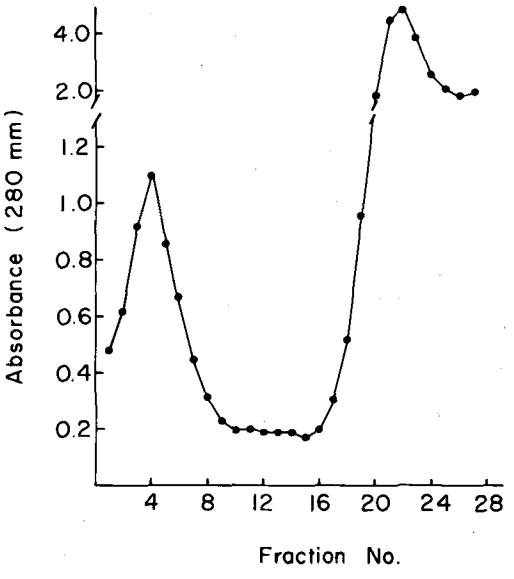

Fig. 1. Shows the OD profile at 280 nm of uterine fluid from progesterone treated rabbits after ultracentrifugation on a sucrose density gradient. The uterine fluid (6.0 ml) flushed with saline from 2 does was layered above zones of 17 ml 40 (w/v) % and 5 ml 60 (w/v) % sucrose, and centrifuged at 110,000 × g for 16 h. A sedimentable fraction, which appeared cloudy, was arrested by the 60% sucrose zone. Nonsedimentable material at the top of the gradient was clear indicating large rapidly sedimenting components had been removed.

In a series of experiments undertaken in this laboratory, it has been shown that membrane vesicles occurring in seminal plasma reversibly inhibit the fertilizing potential of uterine capacitated rabbit spermatozoa[6–9]. This communication now shows that subcellular membranes occur in rabbit uterine fluid following progesterone treatment.

Mature female rabbits, New Zealand strain, with proven fertility were obtained from a local breeder. These animals weighed from 4.0 to 5.0 kg. To facilitate collection of uterine fluid, each uterine horn was ligated at its cervical and oviductal ends. During ligation the does were anesthetized by i.v. injecting 30 mg of sodium pentobarbital/kg b.wt. Progesterone (Sigma), suspended in sesame oil, was s.c. injected at a dose of 25 mg/day for 14 days. Control animals were injected with sesame oil alone. 1 day before autopsy, the does received 75 IU of human chorionic gonadotrophin (Squibb) by i.v. injection. Each uterine horn was flushed with 3 ml of isotonic saline from a 10 ml glass syringe with a blunt No. 16 needle, which was inserted into the uterus through the cervix. The fluid obtained was centrifuged at 1000 × g for 30 min to remove any cells. The resulting supernatant was placed on a sucrose density gradient and centrifuged at 110,000 × g for 16 h in an SW27 rotor (Beckman) at 4 °C. Following centrifugation the cellulose nitrate tube containing the sucrose gradient preparation was punctured with a needle

1 The assistance of Mr R. Byrne is gratefully acknowledged. Mr K. Bedigian skilfully prepared the electron micrographs. Financial support was received from N.I.H. grant HD10206-01.

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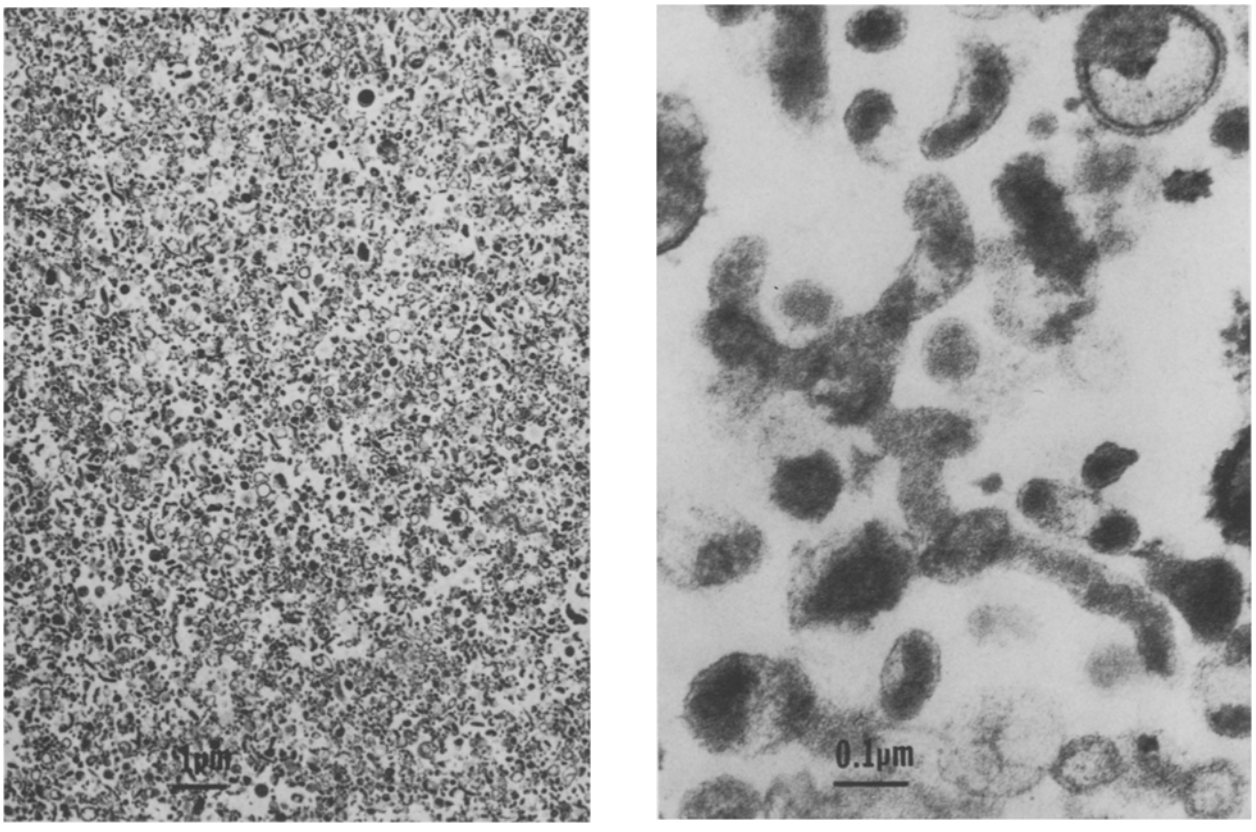

A B

Fig. 2. Electron micrographs showing a rapidly sedimenting fraction from uterine fluid of progesterone administered does. *A* Low magnification, *B* high magnification.

at its base. Fractions of 1 ml were collected dropwise from the bottom of the tube and then assessed for OD at 280 nm. The fractions that corresponded to a peak of absorbance were pooled, dialyzed in Visking tubing for about 12 h against 1000 volumes buffer (0.1M KCl, 0.01M Tris, pH 7.4), and subsequently sedimented at 110,000 × g in a titanium 50 rotor for 4 h. The pellet obtained was fixed with 2% OsO_4, and stained with 20% uranylacetate and 0.5% lead citrate. Thin sections from the Epon (Ladd) embedded pellet were prepared with a glass blade microtome and examined under an electron microscope (Zeiss, model EM9S2).

Uterine fluid from progesterone treated does was cloudy in appearance suggesting that it contained large, light scattering particles. In contrast, untreated does had clear fluid. Ultracentrifugation on a sucrose density gradient revealed the presence of a rapidly sedimenting fraction in uterine fluid from does given progesterone. Figure 1 shows the OD profile obtained after centrifugation of uterine fluid from steroid treated animals on a discontinuous sucrose gradient. The sedimented fraction in this density gradient contained 11.7 mg protein, as determined by the method of Lowry[10]. This represented $^1/_5$ of the total protein. Since the fluid placed on this gradient was obtained by flushing the uteri of 2 does, there were 2.9 mg of sedimentable protein per uterine horn. After centrifugation on a linear 20 to 60 (w/v)% sucrose gradient at 110,000 × g for 16 h at 4 °C, a broad peak was observed with a modal density of 1.2 g/cm³. In other experiments, a membrane fraction with lower density (about 1.15 g/cm³) has also been observed after sedimentation of uterine fluid from progesterone administered does. Uterine fluid from untreated does showed no evidence of containing a rapidly sedimenting fraction.

An electron microscopic examination of thin sections through a pellet of the rapidly sedimenting fraction in uterine fluid of progesterone administered rabbits, demonstrated that it was rich in subcellular membranes (figure 2A). It can be seen from figure 2A that they are mainly spherical vesicles or tubular, cilia-like entities. Inspection of these rabbit uterine fluid components at high magnification (figure 2B) reveals they are bounded by a single membrane showing a typical tri-laminar staining pattern that appears 80 to 90 nm thick. Most of these membrane fragments can be seen to exceed 1000 nm in size. They are therefore larger than previously described membrane vesicles occurring in rabbit seminal plasma[7].

In view of the inhibitory effect of membrane vesicles in seminal plasma on sperm fertilizing capacity[6–9], it seems possible that subcellular membrane components present in uterine fluid after progesterone treatment may also impede sperm capacitation. This would provide a simple explanation for the inability of the progesterone-dominated uterus to promote expression of sperm fertilizing capacity, and it therefore deserves further investigation.

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