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BRIEF COMMUNICATION

CORONA CELL DISPERSING PROPERTIES OF RABBIT TUBAL FLUID

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Summary. Exposure to tubal fluid *in vitro* causes loosening of the corona radiata of recently ovulated ova, pretreated with hyaluronidase. Incubation of ova in 0·05 ml of fluid results in denudation of ova to the zona pellucida if mechanical agitation is provided by shaking with admixed sand. Control ova in Krebs–Ringer phosphate solution, Gey's solution, hyaluronidase or rabbit serum are unaltered under similar conditions. Both oestrous and postovulatory fluid are effective. The responsible factor is dialysable, heat-stable and survives lyophilization.

Motile spermatozoa, a suspension of killed spermatozoa, or sperm free fluid from the vas deferens cause dispersion *in vitro* of the cumulus cells of recently ovulated rabbit ova (Pincus & Enzman, 1932). Swyer (1947) has produced rapid dispersion of the cumulus with a suspension of washed spermatozoa, the supernatant from dilute, centrifuged semen or hyaluronidase, but the inner layers of cumulus cells, the corona radiata, remain undisturbed, even after continued exposure for as long as 30 hr. When ova, so treated, are transferred to the oviduct of the oestrous rabbit, they are completely denuded within 3 hr. Since extracts of Fallopian tube failed to bring about dissociation of the corona radiata *in vitro*, Swyer concluded that mechanical, not chemical, factors were responsible for denudation of ova *in vitro*. The following experiments were designed to assess the influence of tubal secretions on the corona radiata.

Ova were flushed from the oviducts of adult New Zealand White rabbits with Krebs–Ringer phosphate solution 13½ hr after an intravenous injection 500 i.u. of chorionic gonadotropin (generously supplied as A.P.L. by the Ayerst Company). The cumulus mass was dispersed by exposure to hyaluronidase for 15 to 20 min in a depression slide. Individual ova with their corona cells undisturbed were transferred to a parafilm-lined centre well of a small Warburg flask, two to three to each flask. The wells contained 0·2 ml of rabbit tubal fluid, obtained by a means of a continuous collecting system (Clew & Mastroianni, 1960) or calcium-free Krebs–Ringer phosphate solution with pH adjusted to that of fluid (8·2 to 8·4.). Incubation was carried out in air at 37° C for 4 hr. Ova were then transferred to a depression slide for study with the phase-

contrast microscope and photography. In preliminary experiments an occasional ovum was denuded in tubal fluid. More often, the corona cells appeared loosened but were not separated from the zona pellucida. Ova in Krebs–Ringer phosphate solution were almost invariably unaltered. In an attempt to establish a more discrete end point, 30 mg of fine sand were added to some flasks to provide a 'mechanical' factor. Flasks without sand were run simultaneously (Table 1, Series A). A few ova in the flasks containing sand were denuded, but this effect was inconstant. Control ova in Krebs–Ringer phosphate solution with sand were essentially unchanged. The corona cells of the undenuded ova in tubal fluid often appeared loosened, but this change was difficult to evaluate objectively.

TABLE 1

IN-VITRO EFFECT OF TUBAL FLUID ON THE CORONA RADIATA

*Series**	*No. experiments*	*No. ova*											
		Fluid with sand				*Fluid without sand*				*Control with sand*			
		d	pd	l	u	d	pd	l	u	d	pd	l	u
A	12	7	4	7	12	0	2	5	5	1	0	1	17
B	8	15	0	–	3	7	3	–	8	1	2	–	15
C	46	115	7	–	13					1	1	–	111
D	10	20	1	–	4					0	0	–	20
E	4	10	0	–	0					0	0	–	8
F	6	9	6	–	2					0	1	–	22

d=denuded; pd=partially denuded; l=loose; u=unchanged

* A—0·2 ml oestrous fluid, collected by continuous cannulation method. Control: Krebs–Ringer phosphate. B—0·05 ml oestrous tubal fluid collected by ligation. Control: Krebs–Ringer phosphate. C—0·05 ml postovulatory fluid collected by ligation. Control: Gey's solution. D—0·05 ml phostovulatory fluid collected by ligation, boiled for 2 min. Control: Gey's solution. E—0·05 ml of oestrous fluid collected by ligation, heated for 30 min at 98° C, reconstituted to volume with distilled water. Control: Gey's solution. F—0·05 ml fluid, lyophilized and reconstituted to volume with distilled water. Control: Gey's solution.

Because the tubal fluid used in the above experiments had been collected in an external chamber over many hours, the possibility of alterations in the quality of the fluid either from bacterial contamination or from prolonged storage could not be excluded. In all subsequent experiments, fluid was collected by ligating the oviduct just proximal to the fimbria and at the uterotubal junction. The fluid which accumulated between the ligatures was aspirated from the oviduct at laparotomy 24 to 36 hr later. At the 0·2 ml volume, it appeared that ova floated on the surface of the fluid and were not always exposed to the mechanical effect of the sand. Accordingly, the amount of fluid in each flask was reduced to 0·05 ml. Gey's solution, being a more completely balanced tissue culture medium, was substituted for Krebs–Ringer phosphate solution as a control, the pH being adjusted to that of tubal fluid for each experiment. A standard incubation time of 2 hr was used. The other experimental conditions were unaltered. At

the completion of each experiment, eggs were described as denuded, partially denuded, or unchanged. Ova were characterized as unchanged unless at least some of the zona was entirely free of corona cells. These modifications of the experimental design resulted in denudation of the majority of ova incubated with sand in oestrous tubal fluid (Series B) and in fluid from animals ovulated 36 hr previously with intravenous chorionic gonadotropin (Series C). In the absence of sand, denudation was obtained less consistently. Ova in the control flasks, with rare exceptions, remained unchanged.

Boiling for a few moments (Series D) or heating for 30 min at 98° C (Series E) did not materially affect the ability of the fluid to disperse corona cells. Activity was retained after lyophilization and reconstitution to volume with distilled water (Series F). To investigate the possibility that the denudation phenomenon might have been brought about by traces of hyaluronidase transferred initially with the ova, incubation was carried out in Gey's solution containing hyaluronidase, 300 i.u./ml. In five experiments, all eleven ova used remained unaltered after 4 hr of incubation in flasks containing 0·05 ml of hyaluronidase solution with sand. Fluid was dialysed against normal saline for 2 hr and ova were incubated with sand in both solute and dialysate. In twenty-one separate experiments, thirty-eight of sixty ova were denuded in the solute and thirty-eight of fifty-seven in the dialysate. In six experiments, fifteen ova were incubated in flasks containing 0·05 ml of fresh serum from the donor animals and sand; in thirteen, the corona radiata remained unaltered, while eleven of eleven ova from the same donors, incubated simultaneously in tubal fluid, were denuded.

Tubal fluid is capable of loosening the densely arranged corona radiata cells which surround recently ovulated ova in the rabbit. Denudation of ova to the zona pellucida can then be brought about by mechanical action. The activity of the fluid is not destroyed by heating or lyophilization and is observed in both solute and dialysate following dialysis against saline.

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