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The Physiological Uterine Secretion of Rats and its Activity in Dispersing Ovum Cumulus Cells *in vitro* *

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The quantity of physiological uterine fluid secreted by rats exhibiting a regular four-day sexual cycle was measured at every stage and the freshly ovulated rat ova, surrounded by their cumulus cells, were incubated at 37° C in this secretion sample. The results showed that the fluid began to be secreted on the morning of vaginal pro-oestrus and increased steadily for about 24 h. Two declines in quantity are observed, a slight one at the end of pro-oestrus and a marked one about 4 hours after the start of vaginal oestrus which coincided with ovulation. No secretion is obtained either at metaoestrus or at dioestrus.

Only the secretion at the oestrus stages caused total dispersion of the corona cells *in vitro*. This property is retained after freezing, heating to 60° C and lyophilization.

A correlation with plasma sexual hormone measurement and some fertility factor contained in uterine secretion was discussed in relation to this dispersing activity.

In most mammals the postovulatory ovum is surrounded by several thousand cumulus cells, relatively separated from one another and embedded in a gelatinous matrix (7, 26).

When the ovum transits the oviduct, this matrix is dissolved, the cumulus cells and corona radiata dispersed and the ova liberated. Some workers showed that the tubal fluid from intact oestrus rabbits (24, 13) and also from women (22) is responsible for this biological phenomenon. It was subsequently demonstrated *in vivo* that uterine fluid, accumulated from the spayed estradiol- primed rats (16) and from the intact rats in pro-oestrus and more particularly in the oestrus phase of the sexual cycle, presented the same activity (17). Those observations suggested that the uterine secretion might have a factor responsible for this property similar to that in the oviduct secretion.

Since our first results were obtained from accumulated secretion in closed uterine horns, the investigation described

* Parts of these results were reported and discussed at scientific meetings.

in this paper was carried out to determine in detail the quantity of fluid secreted in normal uteri in every phase of the rat sexual cycle and the measurement of the dispersing effects of the cumulus cells, in an effort to shed some light on the physiology of the mammalian genital tract secretions.

Materials and Methods

The experiments were performed on intact adult rats, about 200 g with a regular four-day oestrus cycle and kept under standardized conditions of temperature, relative humidity, and light. They were fed a standard laboratory diet and given water *ad libitum*.

Measurement of uterine secretion during the sexual cycle. Vaginal smears were examined by the exfoliative-cytology technique and stained with carbol-fuchsin and methylene blue to determine the stage of the sexual cycle according to EMMENS (8) classification and intermediate stages (pre- pro-oestrus [p.p.o.] and early oestrus [e.o.]. The rats in dioestrus were segregated, and in these animals the further evolution of the sexual cycle was followed during the whole pro-oestrus, oestrus, metaoestrus and dioestrus stages, using the same method.

Simultaneously, groups of 5 rats in similar phases were sacrificed every hour; uteri and oviducts were removed. The presence of ova in the ampulla was verified.

The secretion from the uterus of each animal was collected separately. The sterility of the fluid was tested by bacteriological cultures. The quantity of secretion produced was calculated from the difference in uterine weight before and after aspiration of the fluid. The relation between the quantity of uterine fluid, the stage of the cycle as determined from the vaginal smear and time of ovulation were chartered graphically.

Uterus-secretion activity on cumulus cells dispersion. Ova surrounded by cumulus cells from rats having recently ovulated were placed separately in small chambers containing uterine secretion extracted from each rat sacrificed every hour along the sexual cycle and incubated for 6 h at 37° C.

Immediately thereafter and then every two hours the projected surface areas of the ova surrounded by the cumulus cells were measured planimetrically and the differences between the areas before and after 6 h incubation were calculated. As a supplement to these measurements, the evolution of the ova was checked by phase contrast microscopy and some photographs were taken (16).

Results

Quantity of uterine secretion (fig. 1 *b*). In normal rats, no fluid was collected in the lumen of the uterus during metaoestrus and dioestrus. It began to be produced at the start of pro-oestrus (3.00-4.00 a.m.), and thereafter the amount of secretion increased steadily to about 200 mg. In the last hours of pro-oestrus, between 6.00 and 8.00 a.m., secretion diminished, within the limits of significance, and some uteri were found to be fairly empty.

During early vaginal oestrus, there was a marked increase of secretion into the uterus, and in the first few hours of the day of oestrus the amount secreted increased to about 400 mg. Afterwards and, prior to ovulation, the quantity of fluid diminished abruptly. The ova were discharged into the ampulla of the oviduct approximately 4 h after the vagina had become completely cornified (oestrus phase). After ovulation, the majority of uteri were empty and only very small quantities of secretion could be obtained.

Cumulus cells dispersion activity. Increase in surface area of ova with corona

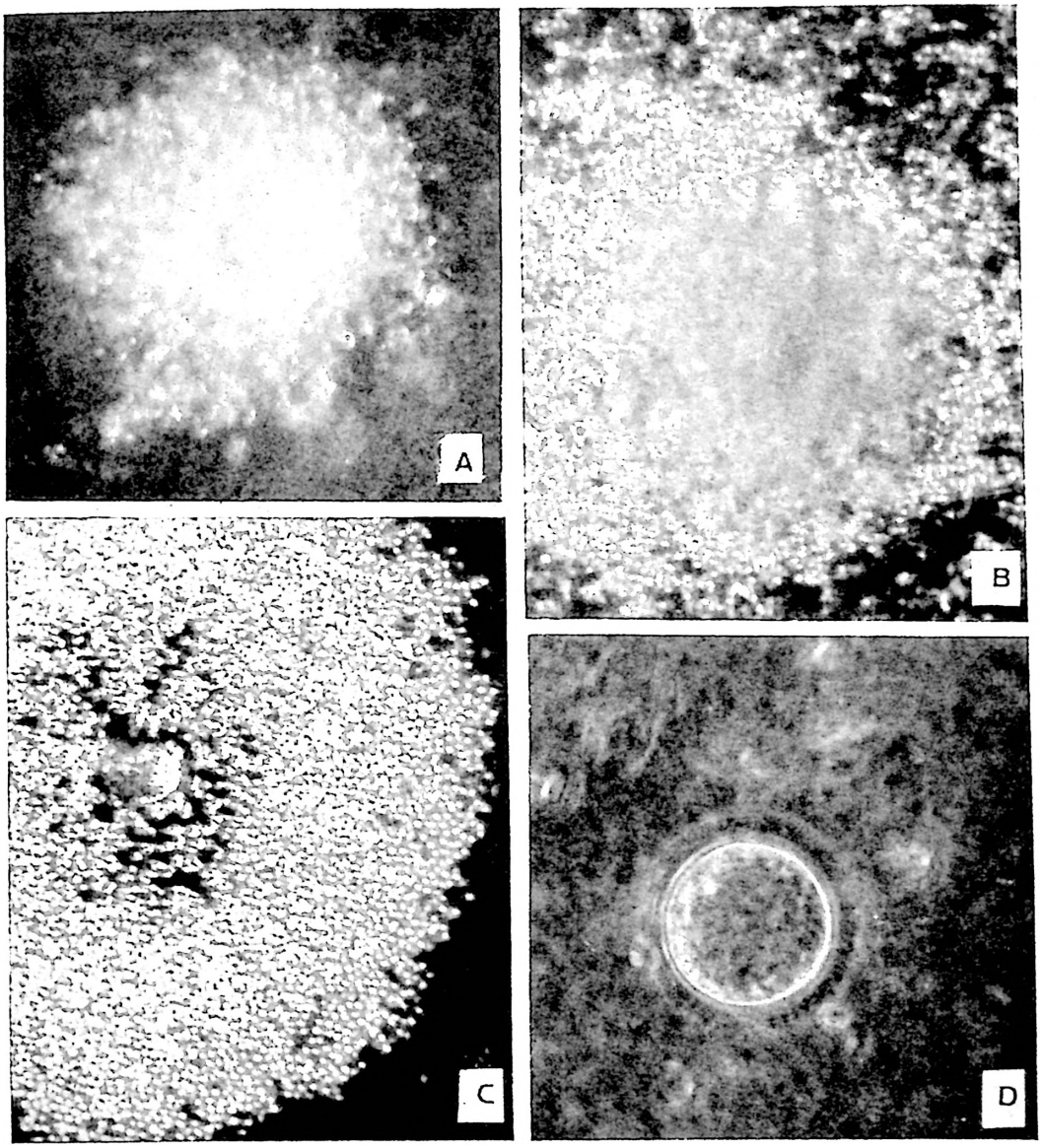

Fig. 2. *Rat ovum surrounded by corona cells.*
A) Intact. *B*) After 6 h incubation in uterine secretion from rats in pro-oestrus the ovum is still surrounded by corona cells. *C*) After 4 h incubation in uterine secretion from rats in oestrus the corona cells are completely dispersed. *D*) After 6 h incubation in uterine secretion from rats in oestrus the ovum is denuded and free of the corona radiata (same ovum as shown in *C*) (*A*, *B* and *C* ×125. *D* ×320).

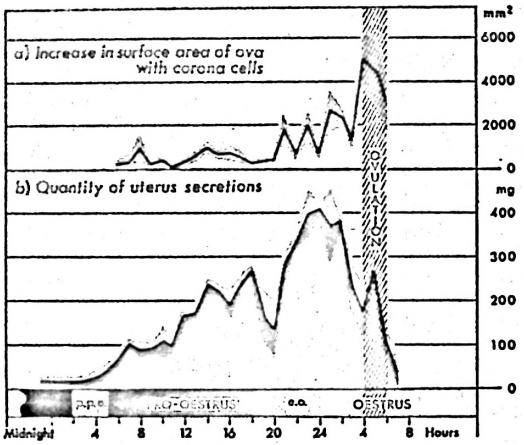

Fig. 1. *Relationship between the physiological quantity of uterine secretion in the normal rat (b) and its capacity to disperse the corona cells of ova* in vitro *(a).*
Time-correlation with vaginal smears, sexual cycle and ovulation. The shading around the curve represents the standard error on the means of more than two individual values.

cells (fig. 1 *a*). The corona cells dispersion property seems to be less active in uterine secretion from rats in pro-oestrus, the surface area of the ova after 6 h incubation in fluid collected during these phases increased to less than double the initial area, and the ova were still surrounded by corona cells (fig. 2 *a* and *b*).

In contrast, when the ova were treated with uterine secretion from rats in early oestrus and particularly in oestrus, after 2 to 4 h incubation, the surface area had increased 2 to 3 fold (fig. 2 *c*) and after 6 h the corona cells were completely dispersed and the ova denuded (fig. 2 *d*). This activity was completely marked at the time around the ovulation, when the quantity of secretion started to diminish.

In the first few hours after ovulation, however, although the quantity of secretion was greatly reduced, the degree of corona cells dispersion was as high as before ovulation.

This property of oestrus-uterine secretion was retained after freezing, heating to 60° C and lyophylization.

Discussion

From the results above described, it is evident that the secretion of uterine fluid in the normal rat follows a cyclic pattern regulated by a hormonal mechanism, like other peripheral responses.

In spayed rats the discharge of the fluid into uterine lumen can be induced experimentally both in intact (20) and in hypophysectomized animals (15) by estrogen treatment.

In normal rats, this secretion is already found at the beginning of pro-oestrus but the highest quantitative peak is observed at the start of oestrus. It could be a delayed response to the release of estrogen by the ovary about 12 h earlier, as established in blood up to 15.00 h on the day of dioestrus, i.e. the day prior to pro-oestrus (4).

ASTWOOD (2) found that the uterus becomes fully distended during the period of regression of uterine tissue water. In this case an increase in the water content of the uterine tissue constitutes one of the first responses to ovarian estrogens, preceding the production of lumen fluid.

On the other hand, in the measurement curve of uterine secretion, two declines are observed, a slight one occurring at the end of pro-oestrus (7.00-8.00 p.m.) and a marked one some hours after the onset of vaginal oestrus (3.00-4.00 a.m.). Since the quantity of secretion accumulating in the uterine lumen is reduced by progesterone treatment (14), these declines could well be a result of the peaks of plasma progesterone measured at about 5.00-6.00 and 12 p.m. respectively on the day of pro-oestrus (3, 18, 21).

Cessation of the secretion in the uterine lumen and expulsion of the ova into the ampulla of the oviduct almost coincide,

the former preceding the latter by one hour or less.

Some workers (9, 18) have found that the increase in blood levels of LH (luteinizing hormone) during the sexual cycle in the rat occurs 10 to 12 hours prior to ovulation, i.e. roughly the same length of time before the reduction in uterine secretion. It is interesting to note that at the time when these two phenomena occur (i.e. approximately 2-6 a.m. on the day of vaginal oestrus) the levels of ovarian and pituitary hormones in rat serum are diminished (3, 5, 9).

All this correlation helps to deduce that the physiological secretion observed in the lumen of the uterus is a late biological peripheral response to the hormonal activity.

The maximum degree of the corona cells dispersion of ova incubated *in vitro* in uterine secretion from the normal rat did not coincide with the period of greater production of uterine fluid, but with the complete cornification of vaginal epithelium (vaginal oestrus). This time-correlation suggests that the dispersion factor detected in uterine secretion could be a product of the endometrial epithelium activated by estrogen simultaneously with the vaginal epithelium and a concentration of this factor dissolved in uterine secretion is produced at the moment when the fluid is diminished.

On the other hand, information on the chemical composition of the cumulus matrix is limited, but hyaluronic acid is considered to be a major component (19). Experimentally, hyaluronidase (7) and sperm hyaluronidase (10) is used to produce lysis of the matrix and liberate the cumulus cells *in vitro*.

At the moment, it is difficult to determine if some endometrial enzyme, such as estradiol-17 dehydrogenase (12) or some factor with stimulated conversion enzyme activity sperm pro-acrosin to acrosin detected in uterine secretion, such as glycosamino-glycan (25) or some other, like a capacitation factor (6), luteolytic factor (11), bicarbonate ion (23), etc., could be responsible for this biological property in the secretion of the genital tract at the oestrus time. Our next step, the chemical identification, may be helpful in this way.

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Resumen

Se investiga en ratas adultas normales la cantidad de fluido uterino secretado fisiológicamente a lo largo de cuatro días del ciclo sexual determinado por frotis vaginal. Además, con el fin de precisar la propiedad que posee la secreción uterina de disolver, *in vitro*, el cemento biológico que une las células de la corona y del cúmulus ooforus, se incuba en cada una de las muestras obtenidas un óvulo recientemente ovulado, rodeado de su cúmulus ooforus, a 37° C, durante 6 horas. Los resultados muestran que el fluido uterino comienza a ser secretado en la mañana del dia del proestro, aumentando progresivamente durante 24 horas, hasta obtener valores de 200 mg por rata, al comienzo del estro. La curva muestra dos disminuciones, una pequeña, al final del proestro, y otra marcada 4 horas después de comenzar el estro, coincidiendo con la ovulación. No se obtiene secreción posible de medir durante el metaestro ni el diestro.

La mayor actividad de disolver el cemento biológico, *in vitro*, se observa en el fluido obtenido en el momento del estro, especialmente alrededor de la ovulación. Esta propiedad se mantiene después de la congelación, calentamiento hasta 80° C y liofilización de la secreción. La secreción del útero y su propiedad de lisis son respuestas periféricas a la acción estrogénica ovárica.

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