In vitro fertilization of frozen-thawed mouse and hamster eggs

Animal	Final temperature	No. of eggs frozen	No. of normal eggs after thawing (%)	No. of normal eggs inseminated	No. of eggs penetrated (%)	No. of eggs undergoing fertilization or fertilized (%)	No. of eggs penetrated by more than one sperm	
							Total (%)	Polyspermic (%)
Mouse	−70 to −79 °C	569	79 (14)	75	19 (25)	10 (13)	8 (42)	0
	−196 (liquid N_2)	227	27 (12)	25	1 (4)	1 (4)	0	0
Hamster	−70 to −79 °C	126	119 (94)	66	65 (98)	65 (98)	65 (100)	65 (100)
	−196 (liquid N_2)	188	164 (87)	88	79 (90)	78 (89)	79 (100)	78 (99)

From the results presented in the Table, it is clear that after freezing and thawing only 12–14% of the unfertilized mouse eggs appeared to be intact and normal (Figure a) and only 4–13% of the apparently normal mouse eggs can be fertilized in vitro (Figure b). In contrast, 87–94% of the unfertilized hamster eggs appeared to be normal after freezing and thawing and 89–98% of these eggs can be fertilized (Figure c), but polyspermic fertilization was high since most eggs were penetrated by more than 5 spermatozoa. One might speculate whether the high proportion of hamster eggs fertilized after freezing and thawing is correlated with the survival of hamsters exposed to low temperatures[13].

Although the recovery rate of normal appearing hamster eggs was high after freezing, the high incidence of polyspermy suggested that only a small percentage might be capable of normal cleavage. No attempt to study further cleavage was made, since successful methods have not yet been developed for culturing hamster eggs beyond the 2-cell stage. Some normal appearing frozen-thawed mouse eggs fertilized in vitro have been successfully cultured beyond the 2-cell stage, but only one egg developed to a blastocyst. Studies are currently underway to improve techniques for freezing mouse eggs so that larger numbers of normal eggs can be fertilized and cultured to obtain blastocysts for transfer to pseudopregnant females. Only after achieving term fetuses or newborn will the potential of freezing unfertilized mammalian eggs be fully realized.

[13] A. U. SMITH, Proc. R. Soc. B147, 533 (1957).

Deciduoma Formation in Rats with Cornified Vagina

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Summary. In female rats given a single injection of 1.25 mg 5α-dihydrotestosterone during neonatal life, reactivity of the vaginal mucosa to hormones was permanently altered, so that the rats, when adult, could show vaginal cornification under the hormonic conditions supportive of development of deciduomata in the uterus.

It seems to be generally accepted that in female rats placed under hormone conditions which support development and maintenance of deciduomata, cornification of the vaginal epithelium cannot take place. While studying deciduoma formation in response to uterine trauma in adult rats treated neonatally with sex steroids, we found that, in those which had been ovariectomized and treated with 5α-dihydrotestosterone (DHT) during neonatal life, deciduoma formation in the uterus and cornification of the epithelium in the vagina could be induced simultaneously by administration of progesterone (P) plus oestradiol-17β (OD) combined with traumatization of the endometrium.

Eight T-strain rats ovariectomized on day 4 of postnatal life (the day of birth = day 1), under cold anaesthesia, received a single s.c. injection of 1.25 mg DHT (Sigma Chemical Co., St. Louis, Mo., USA) in 0.05 ml sesame oil on day 5 (OX-DHT-rats), while 8 other rats likewise ovariectomized on day 4 were not injected with DHT (OX-rats). From day 60 on, both groups of rats were given s.c. injections of 0.2 μg OD for 3 consecutive days, followed by combined injections of 2 mg P and 0.2 μg OD in 0.15 ml oil over a period of 7 days commencing 2 days after deprivation of OD. On the 4th day of P-OD injections, the antimesometrial wall of the right uterine horn of each animal was scratched longitudinally along its entire length with a hooked needle inserted into its lumen[2]. The contralateral uterine horn was left untouched.

In 4 of the 8 OX-DHT-rats, the vagina was patent on day 50. Vaginal smears remained leucocytic until day 60 when OD priming was started. Either on the day of, or following the last injection, all rats had the vaginal aperture giving smears dominated by cornified cells. On the first 1 or 2 days of the 7-day period of P-OD injections, vaginal smears were of the dioestrous type, but thereafter

[1] We wish to thank Prof. T. KIMOTO of Kawasaki Medical College for valuable advice and information.

[2] K. TAKEWAKI, Annotns. zool. japon. *42*, 126 (1969).

smears typical of pro-oestrus or oestrus occurred in 7 of the 8 rats and remained predominantly cornified until the day of the last injection in 3 of the 7. In the other 4, vaginal smears became leucocytic again toward the end of the injection period.

Histological studies revealed that, in 6 rats, the vaginal mucosa was largely lined with a stratified squamous non-cornifying epithelium. PAS-positive mucous cells were encountered only in 1 rat in the parts of the epithelium lining the depths of the mucosal folds. In 2 rats in which vaginal smears remained cornified until the day of the last P-OD injection, the greater part of the vaginal epithelium was of the stratified squamous cornifying type (Figure 1). In some other parts, the epithelium was infiltrated with leucocytes and cornified cells had been desquamated. In the vaginal mucosa, lymphocytes and plasma cells appeared in great numbers. Ovoid masses of the cells were embedded in the lamina propria in 1 of the 2 rats. The vaginal lumen was filled with degenerating mucous cells, leucocytes and/or detached cornified cells. All animals elicited deciduomata in response to uterine trauma (Figure 2), the mean weight of the traumatized horns being 333.5 $\pm$ 49.4 mg, in contrast to the contralateral intact horns averaging 68.9 $\pm$ 3.4 mg.

In only 1 of the 8 OX-rats, the vagina was open on day 60. However, within 2 days following the 3-day priming with OD, the vagina became patent in all animals, showing predominantly cornified smears. From the next day on, smears remained leucocytic until the end of the P-OD injection period. Histological examinations showed that the vaginal epithelium was mucified to varying degrees. In 4 rats, some areas of the epithelium were stratified and squamous but neither cornified nor mucified. Marked infiltration of lymphocytes and plasma cells was never observed. The 8 rats invariably responded to uterine trauma by formation of deciduomata. The mean

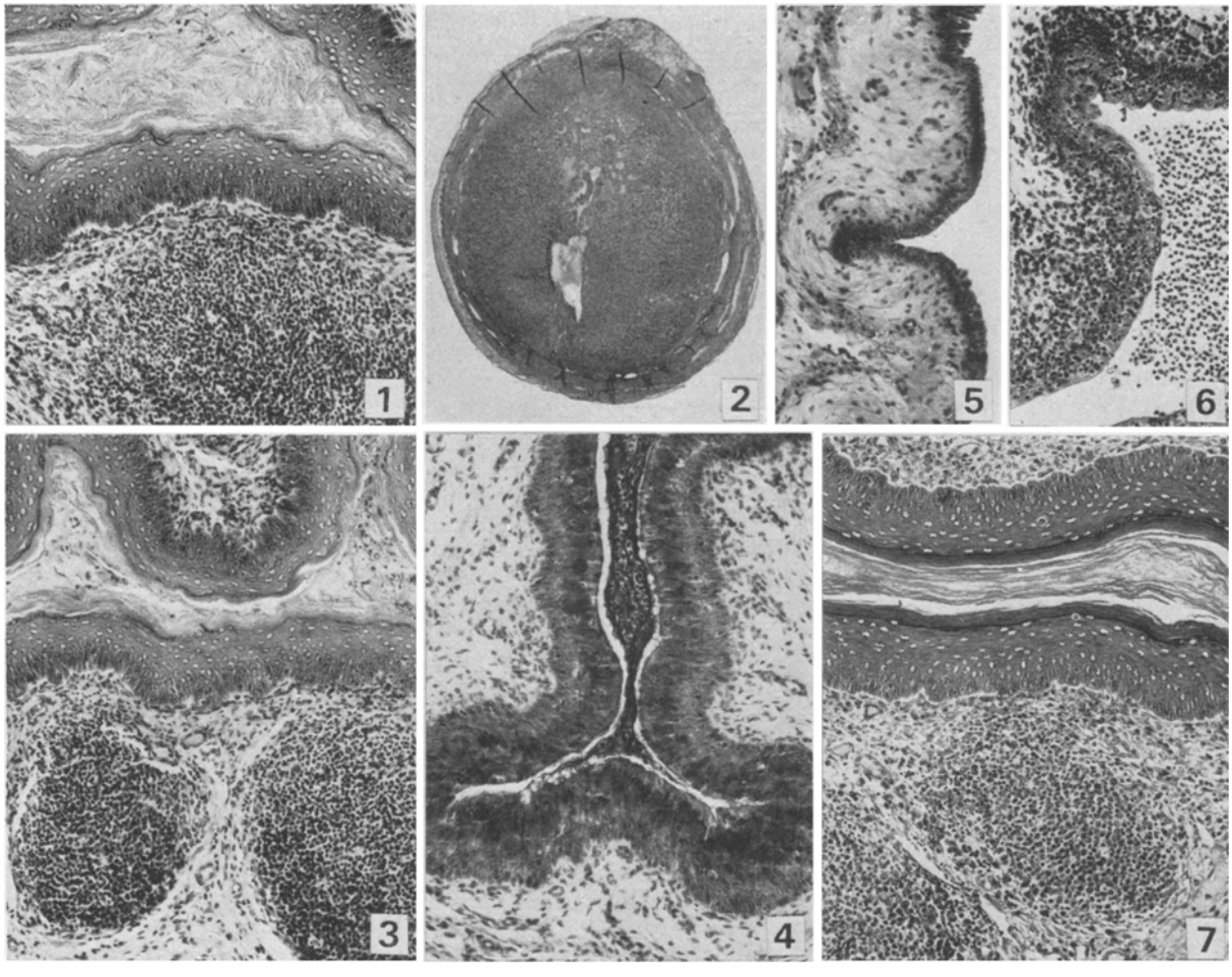

Fig. 1. Vaginal cornification in a rat ovariectomized and given DHT neonatally and 7 daily injections of P-OD following priming with OD in adulthood. A mass of lymphocytes and plasma cells are visible in lamina propria. × 130.
Fig. 2. Section through uterus of the same rat bearing deciduomata. × 13.
Fig. 3. Vaginal cornification in a rat ovariectomized and given DHT neonatally and 5 daily injections of P-OD following priming with OD in adulthood. Masses of lymphocytes and plasma cells are visible in lamina propria. × 130.
Fig. 4. Vaginal mucification in a rat ovariectomized neonatally and given 5 daily injections of P-OD following priming with OD in adulthood. × 130.
Fig. 5. Atrophic vagina of a 60-day-old rat ovariectomized neonatally. × 130.
Fig. 6. Thickened vaginal epithelium in a 60-day-old rat ovariectomized and given DHT neonatally. Diffuse infiltration of lymphocytes and plasma cells is occurring in lamina propria, and numerous leucocytes are present in vaginal lumen. × 130.
Fig. 7. Vaginal cornification in a rat ovariectomized and given DHT neonatally and 3 daily injections of OD in adulthoood. Masses of lymphocytes and plasma cells are visible in lamina propria. × 130.

weight of the traumatized horns was 366.4 ± 29.2 mg, and that of the intact horns, 72.1 ± 3.9 mg.

Since, in a majority of these 2 groups of rats, the superficial layers of the vaginal epithelium appeared to have been exfoliated into the lumen before autopsy, in the next series of experiments, 5 OX-DHT-rats and 5 OX-rats were placed on the daily P-OD injection schedule for 5 days, instead of 7 days, after being primed with OD for 3 days from day 60. Uteri were not traumatized.

In 3 of the 5 OX-DHT-rats, vaginal smears were dominated by cornified cells throughout the P-OD injection period. Vaginal cornification on the first 1 or 2 days of the period was undoubtedly due to the residual effects of the OD-priming. Sections through vaginae of these rats, sacrificed on the day following the last P-OD injection, clearly demonstrated a stratified squamous cornifying epithelium and nodular masses consisting largely of lymphocytes and plasma cells embedded in the lamina propria (Figure 3). In the remaining 2 rats which gave leucocytic vaginal smears for the last 2 or 3 days of P-OD injections, the greater part of the vaginal epithelium was stratified squamous but not cornified. Small patches of mucified epithelium were found in the depths of the mucosal folds.

Five OX-rats gave predominantly cornified vaginal smears for the first few days of P-OD injections due to the residual effects of OD-priming, followed by leucocytic smears for the rest of the injection period. At autopsy, the vaginal epithelium was invariably mucified, as evidenced by the positive PAS reaction (Figure 4). Different amounts of amorphous material, not necessarily PAS-positive, and some leucocytes were present in the lumen.

To compare the initial state of the vaginal epithelium, 6 OX-DHT-rats and 5 OX-rats were killed on day 60. At the time of sacrifice, the OX-DHT-rats invariably had the vaginal orifice showing leucocytic vaginal smears, while the OX-rats were still without the aperture. Histological studies revealed a marked difference in structure of the vaginal mucosa between the 2 groups. In OX-rats, the vaginal epithelium consisted of only 2 to 3 layers of small cells (Figure 5). The vaginal lumen was almost empty. By contrast, in the OX-DHT-rats, the vaginal epithelium was decidedly thickened, consisting of 2–6 layers of much larger cells, as compared with that in the OX-rats (Figure 6). Occasionally, areas infiltrated with leucocytes occurred in the epithelium. The vaginal lumen was distended, being filled with numerous leucocytes. The lamina propria was diffusely infiltrated with lymphocytes and plasma cells.

In both OX- and OX-DHT-rats killed on the day following injections of 0.2 µg OD for 3 days from day 60, the vaginal epithelium was always of the stratified squamous cornifying type. The vaginal lumen contained varying amounts of desquamated nucleated and cornified cells. Lymphocytes and plasma cells were not numerous in the lamina propria of the vaginal mucosa in the OX-DHT-rats, except in one animal in which many ovoid masses of the cells were found (Figure 7). In the OX-rats, these types of cells were few, if any, in the lamina propria.

It seems evident that treatment of female rats with DHT during early postnatal life induces a permanent or at least long-lasting alterations in reactivity of the vaginal mucosa to hormones, so that vaginal cornification can occur under hormone conditions which support development of deciduomata. However, it is not presently known whether the effects are specific to DHT.

Possible effects of lymphocytes and plasma cells on the reaction of the vaginal mucosa to hormones also need to be studied. Cyclical changes in accumulation of plasma cells in the lamina propria of the hamster vaginal mucosa in relation to the blood level of oestrogen have been reported[3].

[3] C. E. ROIG DE VARGAS-LINARES, J. Reprod. Fertil. *15*, 389 (1968).

Intrauterine Device Increases the Fibrinolytic Activity of the Rat Endometrium at Deciduation, a New Aspect of its Contraceptive Effect

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Summary. The fibrinolytic activity of the endometrium of the two uterine horns in the rat was histochemically determined. One of the horns contained a plastic-IUD, the other served as control. The endometrium of the control horn was almost inactive in contrast with that of the IUD-horn in which fibrinolytic activity was apparent. The fibrinolytic activity induced by the IUD might be involved in the prevention of ova implantation.

The mechanism of the contraceptive effect of intrauterine devices (IUDs) is not yet properly understood. Insertion of foreign material into the uterus causes biochemical and cellular reactions to dispose of the provocative agent[2]. According to GREENWALD[3], the presence of a plastic IUD in the rat uterus causes a local increase in the number of the neutrophils with reduction of fertility as a result. PARR[4] thought that the contraceptive effect might be due to products of cellular decomposition.

It has recently been shown that fertilized rat ova possess a high fibrinolytic activity during their passage through the tubes that on implantation of the ova the activity disappears and that the fibrinolytically active endometrium changes into an almost fibrinolytic inactive decidua[5]. Absence of fibrinolytic activity thus seems to be a necessary pre-requisite for implantation of the ova[5]. But IUDs have recently been shown to increase the fibrinolytic activity of the endometrium[6,7]. We thus thought that the increased fibrinolytic activity at the time of implantation and deciduation might be one of the reasons why IUDs have a contraceptive effect. We therefore studied the effect of a plastic device on the fibrinolytic activity of the rat endometrium at the time of implantation.

Materials and methods. 13 Sprague-Dawley female rats, weighing 180–220 g were used. They were fed a standard diet and maintained in an artificially illuminated room. Surgery was performed under ether anaesthesia and under