regeneration of more green plants. Further, the proplastids present in pollen cell[15], were possibly provided with better environment by starch for their proper development.

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Changes in the follicular plasminogen activator during ovulation after steroidal treatments in rat

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Activity of plasminogen activator (PA) in the ovaries of adult rats was localized by the fibrin slide method during ovulation. PA activity was detected only in the Graafian follicles before and immediately after their rupture and this was significantly increased by treatment of rats with progesterone (0.5 mg/rat) and estradiol-17β (10 μg/rat) on the morning of proestrous. The nonsteroidal estrogen antagonist, tamoxifen, caused inhibitory effects on the follicular PA activity compared to control and estradiol-17β treatment. These studies confirm the role of PA in ovulation and show a positive relationship between steroids and PA during the follicular rupture during ovulation.

A specific biochemical mechanism for the proteolytic decomposition of connective tissue in the apex of the follicle wall[1] during ovulation was earlier proposed[2] which revealed that plasminogen activator (PA) was responsible for disruption of the follicle wall. PA acts on its substrate plasminogen to produce plasmin which is a serine protease shown to be capable of reducing the tensile strength of the follicle wall strips *in vitro*[3]. An increase in PA secretion by rat granulosa cells was related to the approach of ovulation[4,5]. PA activity in rat granulosa cells was stimulated by LH, FSH, cAMP derivatives and prostaglandins E_1 and E_2[2,4]. Reich *et al.*[6,7] revealed that 80–90% of the total follicular PA activity was contributed by granulosa cells and it is retained within the follicles for its localized action during ovulation. Like PA, the level of steroids[8–10] increases in the hours preceding ovulation, but so far no causal link has been worked out between the two. The present study is an attempt to understand the effects of estradiol-17β and progesterone on follicular PA activity during ovulation.

Thirty adult female albino rats, aged 2–3 months, weighing 150–200 g, housed in groups of two to three per cage were maintained under controlled conditions of light, temperature and humidity and provided with pelleted food and water *ad libitum*. Vaginal smears were examined daily and rats showing two or more cycles of four days length were used. Five rats each were given i.m. injections of progesterone (0.5 mg/rat), estradiol-17β (10 μg/rat) and tamoxifen (0.4 μg/rat) on the morning of proestrous between 11.00 a.m. and 12.00 noon. An equal number of rats was maintained as untreated controls and others treated with olive oil and propane-1,2 diol, which were used as vehicles for steroids and tamoxifen respectively. The time of ovulation was 2.30 a.m.[11], when all the rats were sacrificed. Both the ovaries and the oviducts were dissected out in cold normal saline. The ovaries were cleaned from fat and adhering tissues and processed for the localization of PA by fibrinolytic assay.

For the assay of PA, the fibrin slide method of Todd[12] modified by Kwann and Astrup[13] was used. The ovaries were sectioned at 30°C in a cryostat. The 10 μm thick sections were collected on clean dust and grease-free microscope glass slides, over-layered with plasminogen-rich fibrin and thrombin and incubated in a moist chamber at 37°C for 30 min. The slides were then fixed in 10% formalin and stained with Harris Alum Haematoxylin so that the cells immediately associated

with the clear lysis zone can be identified. Maximum and minimum lengths of these lysed zones were measured with an ocular micrometer to study the effects of various treatments. Data represented as mean ± S.E. were statistically analysed by Student's t test.

Fibrinolytic activity was observed only in intact preovulatory Graafian follicles and the freshly ruptured follicles. Progesterone treatment significantly increased this activity in both intact as well as ruptured Graafian follicles (Table 1, Figure 1). This increase was double when compared with enzyme activity in the ovarian follicles of control and olive oil-treated rats and the change was statistically significant ($P<0.05$) (Table 1, Figures 1, 2).

Estradiol-17 β treatment also caused an increase in the follicular fibrinolytic activity (Table 1, Figure 1). This increase was smaller as compared to that of progesterone treatment but was statistically significant ($P<0.05$) when compared with control and olive oil treatments (Table 1, Figures 1, 2). Tamoxifen exerted a negative effect on the follicular fibrinolytic activity when compared with estradiol-17 β, control and propane-1, 2 diol treatments. This difference was also statistically significant ($P<0.05$) (Table 1, Figure 2).

The fact that PA activity occurs exclusively in the Graafian follicles of the ovary in the present studies confirms its role in follicular disruption by localized action in follicular wall[2,6,7,11,14,15]. The stimulatory

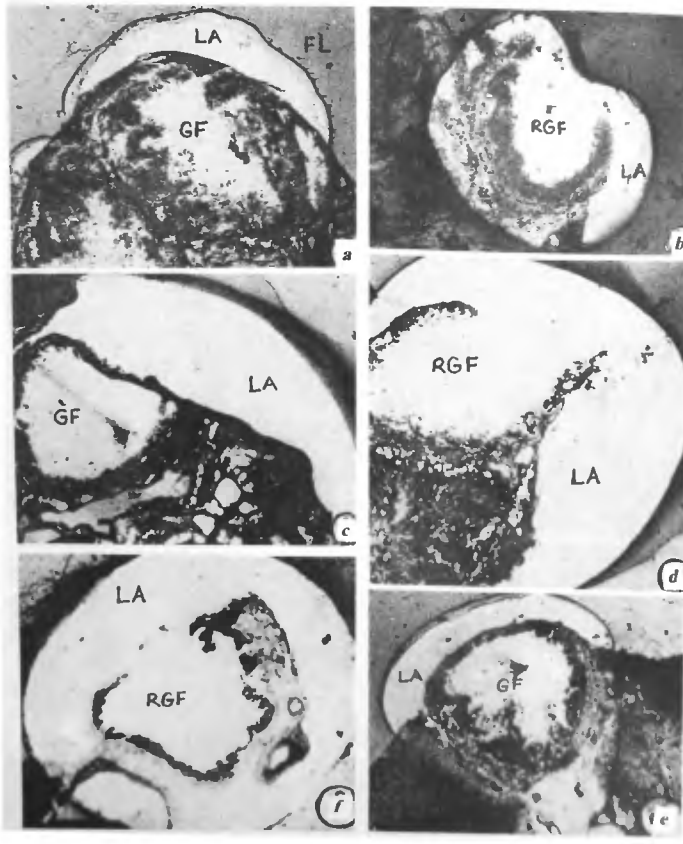

Figure 1. A Graafian follicle (GF) and ruptured Graafian follicle (RGF) respectively with lysed areas (LA) on a fibrin layer (FL) *a, b,* in olive oil-treated rat; *c, d,* in progesterone-treated rat; *e, f,* in estradiol-17β-treated rat. ×5.

Table 1. Effect of progesterone, estradiol-17β and tamoxifen on rat follicular plasminogen activator (PA) during ovulation

Treatment	No. of rats	PA activity (lysed area in mm^2 follicle) Mean ± S.E.
Control	5	0.110 ± 0.01
Progesterone	5	0.203 ± 0.02*
Estradiol-17β	5	0.139 ± 0.01*
Olive oil	5	0.088 ± 0.01
Tamoxifen	5	0.060 ± 0.01*
Propane-1, 2 diol	5	0.086 ± 0.01*

*$p < 0.05$

effects induced by progesterone and estradiol-17 β treatments on follicular PA activity, support the positive role of these steroids in ovulation[16,17].

The specific role of estrogen and other steroids in ovulation is unclear[18] since both inhibitory and stimulatory effects of estradiol-17 β on follicular PA activity may be direct or indirect via LH as LH, FSH, cAMP derivatives and prostaglandins E_1 and E_2 also stimulated PA activity in rat granulosa cells[2,4,15,19]. The antiestrogenic effects of estrogen antagonist,

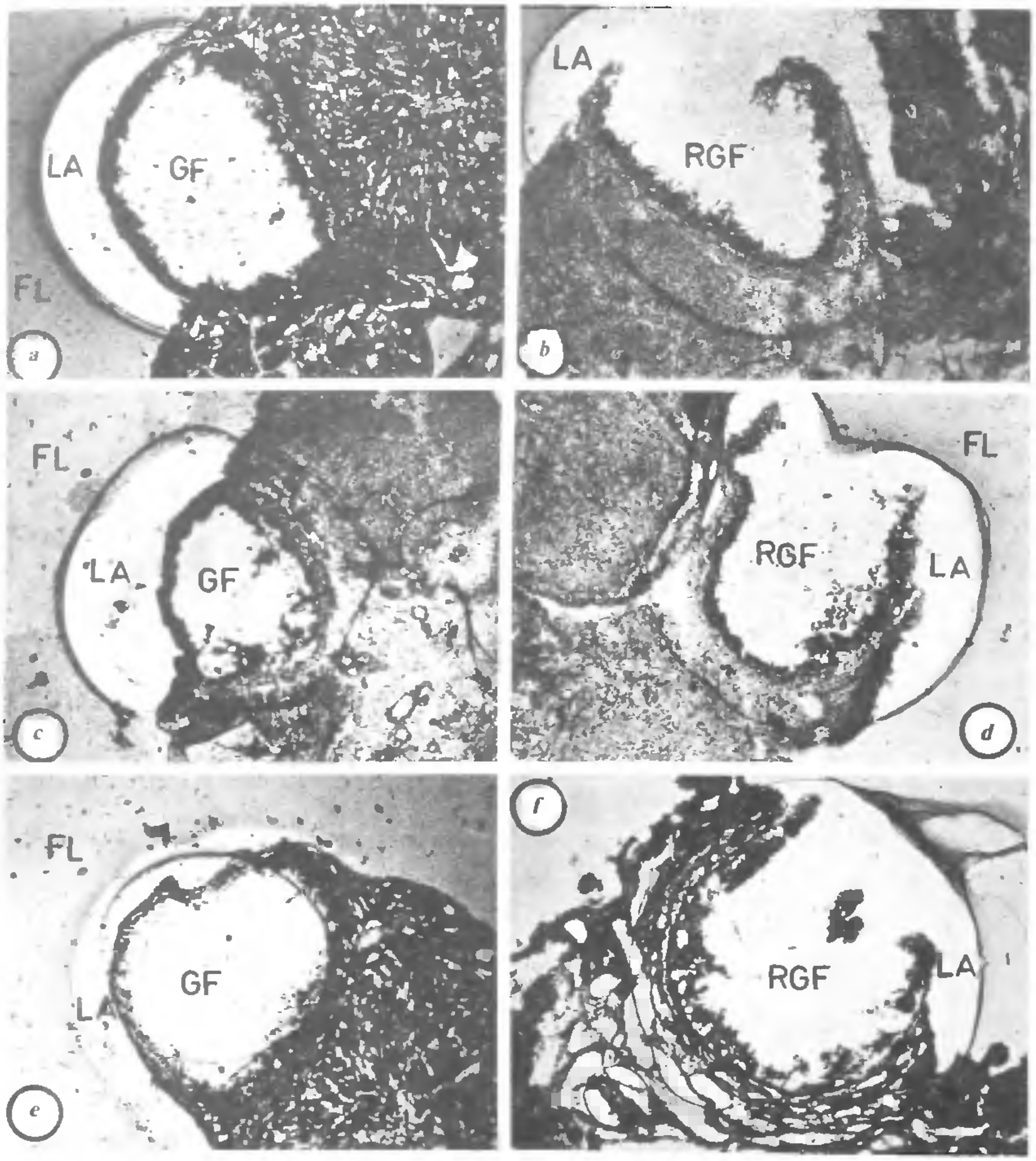

Figure 2. A Graafian follicle (GF) and ruptured Graafian follicle (RGF) respectively with lysed areas (LA) on a fibrin layer, *a*, *b*, in control rat, *c*, *d*, in propane 1,2-diol-treated rat, *e*, *f*, in tamoxifen-treated rats. 65 ×.

tamoxifen, further support such action via LH at the level of pituitary[20]. A correlation between the preovulatory increase in progesterone and the periovulatory increase in follicular PA activities in immature rat ovaries primed with PMSG and hCG was earlier observed by Ohno and Mori[21] who suggested that PA in rat ovaries is synthesized *de novo* and progesterone may be responsible for increase of PA in rat ovaries during the periovulatory period perhaps due to its direct effect on the ovaries[22]. The present observations suggest a similar situation for adult cyclic rat ovaries.

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