Effect of Sex Steroids on Immunological Distribution of IgG and Specific Protein of Uterine Fluid to Oviduct, Uterus and Gamates in the Rat

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Effect of Sex Steroids on Immunological Distribution of IgG and Specific Protein of Uterine Fluid to Oviduct, Uterus and Gamates in the Rat

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Summary

Effect of sex steroid on distribution of the anti-IgG conjugate and specific protein to reproductive tract was performed with the ovariectomized rat, gamates and embryos.

In the ovariectomized rats treated with ovarian hormones, much grain of fluorescence of anti-IgG was distributed to the uterine endometrium, especially in the stroma of the rats treated with estrogen.

On the other hand, uterine endometrium in the rats with progesterone alone produced moderate staining of IgG conjugate, although the staining was not observed in the non-treated rats.

It was found that the specific protein appeared to the uterine secretion of the rats, when they were injected with estrogen. However, the staining of fluorescence in the non-treated rats was stronger than that in the progesterone treated ones.

In one cell ova and blastocysts, peripheral region (zona pellucida) and boundary of vitelline membrane were stained with fluorescences of three conjugates.

Spermatozoa collected from the uterine horn at 30 minutes post coitus had strong fluorescences of three conjugated in their surface membrane and the staining decreased as the time progressed to 4 hrs after mating.

Possible roles of the specific protein, IgG and serum protein on the reproductive tract, gamates and embryos during early reproductive process were discussed.

Information on the biological and biochemical role of the secretion from genital tracts in many species were gathered (1 ~5). We had been studing the qualitative and quantitative alternation of the biochemical components from uterine fluid during the estrus cycle and early pregnancy in the rats (6 ~10).

In a previous paper, we have reported the immunological survey of the

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distribution of IgG and specific protein of uterine fluid in the rats during the estrus cycle and early pregnancy (11).

The experiments were undertaken to elucidate the relationship between the appearance of specific proteins and their localization under various hormonal conditions.

Materials and Methods

Experimental animals:

Wistar virgin rats maintained in our laboratory were used for the experiments. Vaginal smears of the rats were daily, at least, for two weeks and the animals having regular 4 days cycle were selected for the study. The rats were ovariectomized under ether anesthesia in metestrus and devided in two groups.

Ovarian hormone treatment:

The animals of group 1 were daily injected with 0.2 ml sesame oil alone for seven days subcutaneously (s.c.). The group 2 was daily treated with the injection of 4 mg progesterone in 0.2 sesame oil. All animals in group 2 were given 10 μg of estrone at seven days from the injection of progestrone.

Sampling of materials:

The rats in each group were killed at 0, 12, 24, 36 and 48 hours after the estrone treatment. Uterine weight and protein contents of each rat were recorded.

Uterine and oviduct tissues were fixed for the immunological observation. The preparation of the tissues for immunological study was performed by the method reported by us previously (11).

Spermatozoa were collected by aspiration from the uterus at 0, 0.5, 1, 2 and 4 hours post coitus.

Unfertilized and fertilized ova at the 1-cell stage and blastocysts were collected from the ampulla on the morning of estrus and from the uterus on the 4 or 5th days of pregnancy, respectevilly.

Results and discussion

The uterine weights and protein contents in uterine fluid of the rats ovariectomized following the treatment of ovarian hormones are shown in the Fig. 1.

Immunohistological survey was performed in rats non-treated and pretreated with progesterone at 0 and 24 hours after estrogen injection.

Fluorescent staining by anti-IgG conjugate is shown in Plate 1.

The uterine endometrium in ovariectomized rats, which were not injected with ovarian hormones, had faint fluorescence in stroma and lumen epithelial cells (Plate 1, Fig. 1). In the rats treated with estrone, stromal cells were stained strongly with

fluorescence, although uterine gland luminal epithelial cells, and material in uterine cavity were not stained by anti-IgG conjugate (Plate 1, Fig. 2).

In the uterus of rat treated with progesterone, fluorescent staining was strong in the stroma and weak staining was observed in the apical part of epithelial cells.

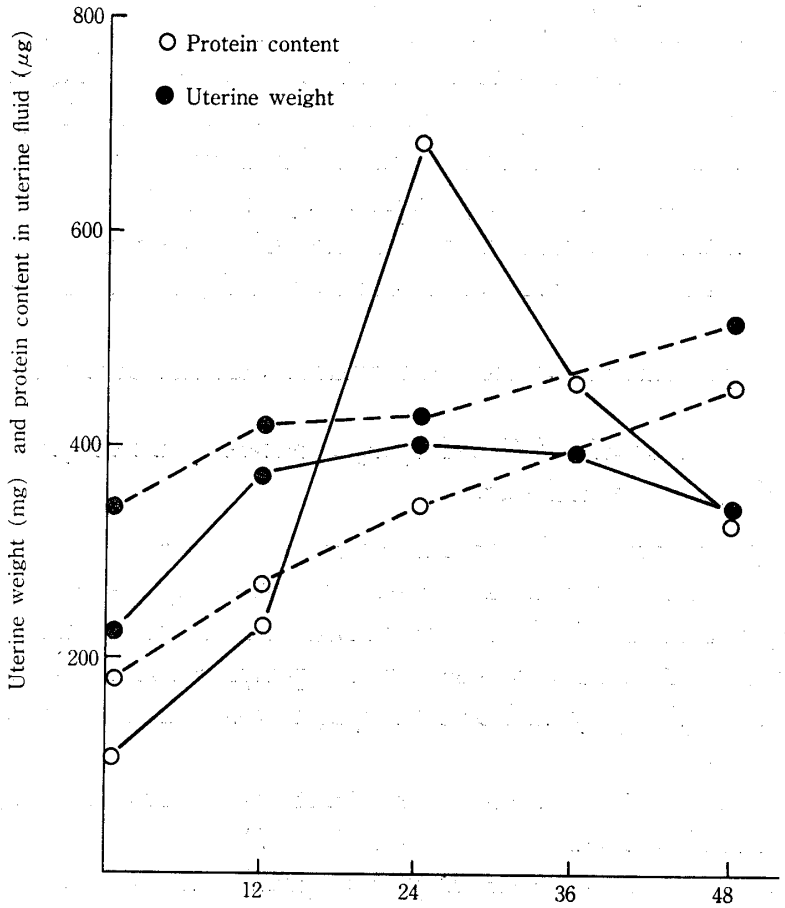

FIG. 1. Uterine weight and protein content in uterine fluid after 10 μg estrone injection in progesterone non-treated (—) and pretreated rat (- - - -)

A marked difference in the fluorescence staining between stroma cell and, uterine gland and luminal epithelial cells was observed in the rat primed with progesterone followed by an injection of estrone. e.g. the staining with anti-IgG conjugate was strong in stroma, but the conjugate did not distribute to each epitheleal cell.

In the uterine sections stained by anti-UFSP conjugate, fluorescent staining was not observed except for scant staining of the luminal epithelium in the rats non-treated by estrogen (Plate 1, Fig. 5, 6). It was shown that the fluorescent staining of the uterine gland and luminal epithelial cells were stronger in the rat treated by estrongen alone, than in the rat pretreated with progesterone and estrogen (Plate 1, Fig. 6, 8).

Distribution of serum protein, IgG and specific protein of uterine fluid to oviduct tissue:

Serum proteins were distributed in part of the oviduct sections and fluorescent staining was observed especially in the epithelial cells in the estrus stage. IgG was observed in the connective and subepithelial tissues, but not in the epithelial cells and muscular layer. Specific protein of uterine fluid was found in the oviductal epithelial cells, especially in the ampulla epithelial cells.

At 2 and 54 hours after fertilization (assumed time of fertilization was at 4:00 in estrus) oviduct tissues were sectioned serially. Preliminary observations on the interaction of IgG and specific protein in uterine fluid were performed in the oviduct tissue and fertilized ova. The fertilized ova in the oviduct at both stages mentioned above were strongly stained by anti-UFSP conjugate and the granullosa cells at 2 hours after fertilization also had very strong fluorescence.

On the other hand, the weak ova fluorescence in the oviduct stained by anti-IgG conjugate.

The specific proteins in ova from the oviduct were not observed.

Distribution of serum protein, IgG and specific protein of uterine fluid in gamates collected from the female genital tract:

Preliminary experiments for protein components in ova were performed in unfertilized and fertilized ova at the one-cell and blastocyst stages.

In the unfertilized and fertilized ova treated with anti-rat serum and anti-IgG conjugates, fluorescent staining was seen in the peripheral region (zona pellucida) and moreover the staining by anti-UFSP conjugate was limited to the boundary of vitelline membrane.

A difference in the localization of serum and specific proteins between unfertilized and fertilized ova was not observed (Plate 2).

The blastocysts collected from Day 5 pregnant rats were stained by three conjugates. Fluorescent stainings in each treatment were almost like-those in the one-cell ova, although the fluorescence of the conjugate was not observed in the zona free blastocysts treated with anti-IgG conjugate. The blastocysts obtained from uterus treated with anti-UFSP conjugate had a moderate fluorescence in zona pellucida, and the zona pellucida removed from the blastocysts retained their fluorescence.

Spermatozoa aspirated from uterus at 0, 0.5, 1, 2 and 4 hours post coitus were stained by three antibodies labelled with FITC.

Fluorescent bright by anti-rat serum and anti-IgG was strong in the spermatozoa at 30 min post coitus, but progressively diminished and almost diappeared by 4 hrs post coitus. On the other hand, specific protein in the uterine fluid was still present on the sperm surface at 4 hours post coitus. From these observations, it was suggested that the surface of the spermatozoa was covered by serum protein components which were lost in the female genital tract. In the

opposite direction, spermatozoa were covered by specific protein in uterine fluid (Plate 3).

Because, the protein composition of specific protein and immunoglobulins in genital tract are very complex, it was very difficult to make clear the physiological significances in the reproductive process. However, the macromolecular components would have some roles for gamates in the process of fertilization to implantation stages.

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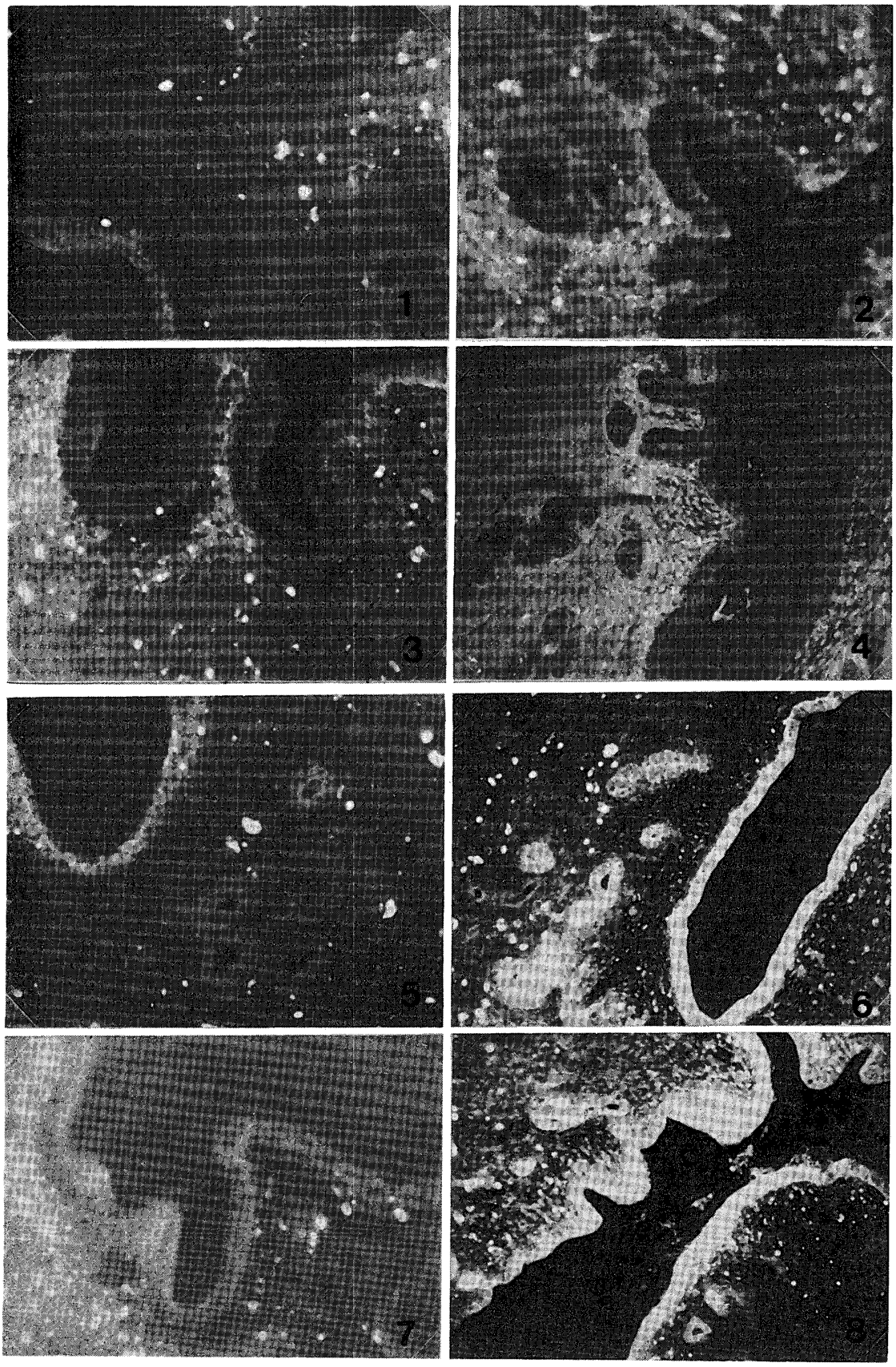
PLATE 1

FIG. 1~4. Sections of endometrium of ovariectomized rats injected with ovarian hormone, treated with anti-rabbit globulins labeled with FITC for the localization of specific protein of uterine fluid.
1, 2, 3, and 4 were similar status to Fig. 5.
Fig. 1. Note weak staining in epithelial cells.
Fig. 2. Note strong staining in epithelial and uterine gland cells.
Fig. 3. Note scant staining in epithelial cells.
Fig. 4. Note moderate staining in epithelial and uterine gland cells.

FIG. 5~8. Sections of endometrium of ovariectomized rats injected with ovarian hormone, treated with anti-rabbit globulins laveled with FITC for the localization of IgG.
FIG. 5. Sections of endometrium from ovariectomized rats receiving no hormone. Note moderate staining in stroma and moderate staining at basal and apical parts of epithelial cells.
FIG. 6. Sections of endometrium from ovarictomized rats at 24 hrs after 10 μg estrone single injections. Note strong staining in stroma and faint staining in epithelial and uterine gland cells.
FIG. 7. Sections of endometrium from ovarictomized rats following of progestrone injection for 7 days. Note strong staining.
FIG. 8. Section of ampulla in oviduct stained by anti-specific protein of uterine fluid antisera labeled with FITC.
Note strong staining in epithelial cells.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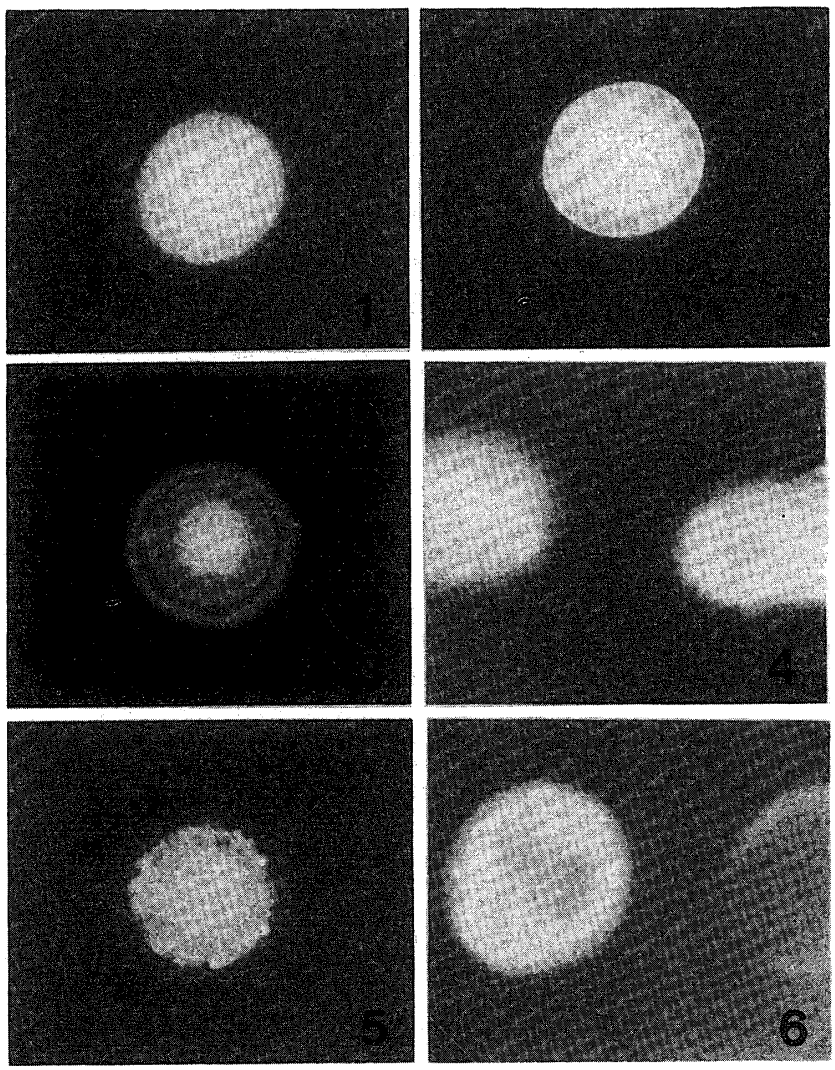

Plate 2

Fig. 1~6. One-cell ova and blastocysts treated with anti-rabbit globulins labeled with FITC.

Fig. 1. One-cell ovum stained by normal rabbit gloublins labeled with FITC, showing no staining.

Fig. 2. One-cell ovum stained by anti-rat serum antisera labeled with FITC. Note faint staining in zona pellucida.

Fig. 3. One-cell ovum stained by anti-rat IgG antisera labeled with FITC. Note strong staining in zona pellucida and weak staining in porlar body.

Fig. 4. Blastocysts stained by anti-rat IgG antisera labeled with FITC. Note strong staining in zona pellucida and no staining in zona free blastocyst.

Fig. 5. One-cell ovum stained by anti-specific protein of uterine fluid antisera labeled with FITC. Note weak staining in zona pellucida and moderate staining inner layer of ova.

Fig. 6. Blastocyst stained by anti-specific protein of tuerine fluid antisera labeled with FITC. Note strong staining in zona pellucida and weak staining inner part of blastocyst, and strong staining in empty zona pellucida.

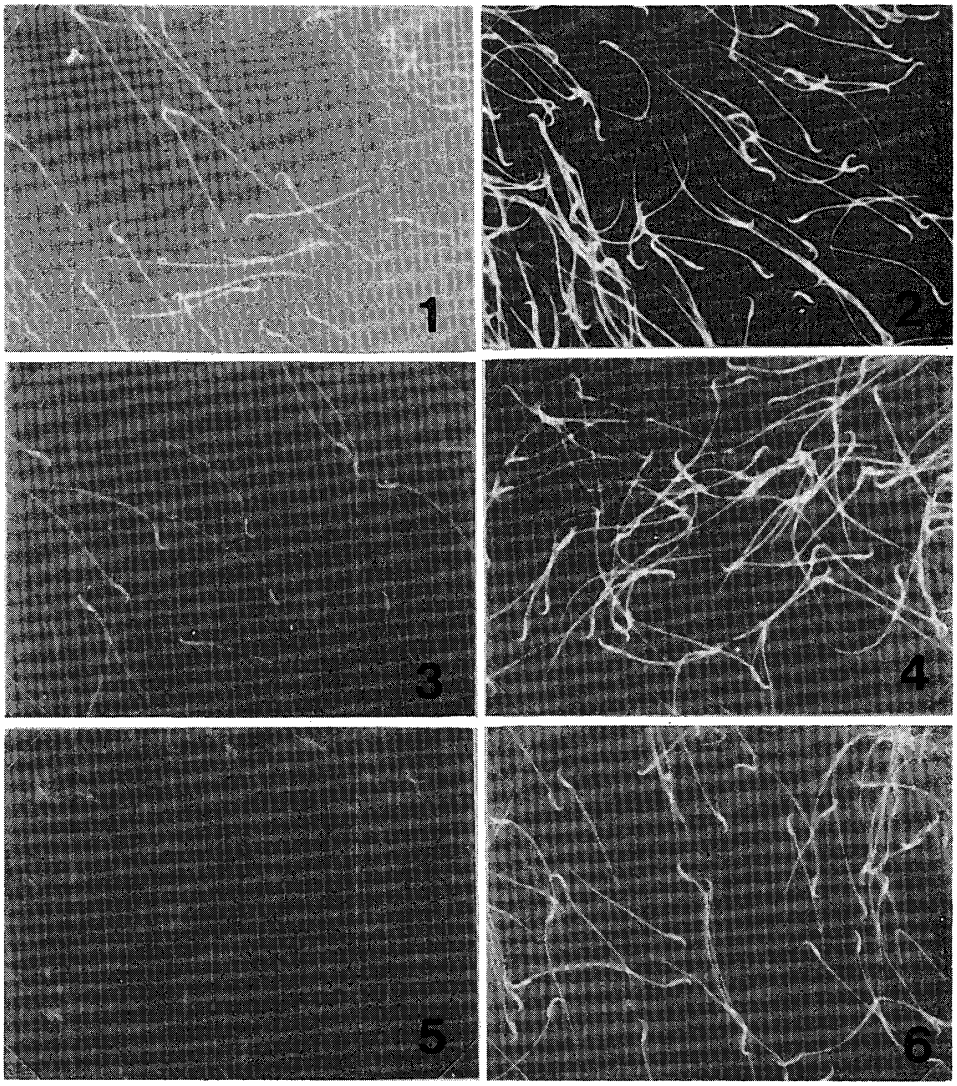

PLATE 3

FIG. 1~6. Sperms in uterine lumen at various post coitus time treated with anti-rabbit globulins labeled with FITC for the interaction of sperm and IgG and specific protein of uterine fluid.

FIG. 1, 3 and 5. Sperms at 30 min, 2 hrs and 4 hrs postcoitus respectively stained by anti-rat IgG antisera labeled with FITC. Note moderate staing at 30 min, scant staining at 2 hrs and no staining at 4 hrs post coitus.

FIG. 2, 4 and 6. Sperms at 30 min, 2 hrs and 4 hrs post coitus respectively stained by anti-specific protein of uterine fluid antisera labeled with FITC. Note strong staining at 30 min and 2 hrs and moderate staining at 4 hrs post coitus.