Histochemical and Ultrastructural Effects of Enovid E on the Endometrium of the Baboon

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Summary. Endometrium from a group of baboons treated with the oral contraceptive, Enovid E, was studied histologically, histochemically and ultrastructurally, and compared to endometrium from normally cycling animals. All endometria were obtained by transcervical uterine biopsy between 10 and 14 days of the treatment cycle or the normal menstrual cycle. Histologically, no discernible differences between the control and experimental endometria were apparent. While observable differences were not evident between the endometrial alkaline phosphatase and succinate dehydrogenase activities of control and experimental animals, there was an increased acid phosphatase activity in the Enovid E-treated baboon endometrium. Ultrastructurally, the glandular cells of treated animals appeared to be more physiologically advanced than did those from the control endometria. These advances were evident from the prominent Golgi complex, increased development of the endoplasmic reticulum and increases in the size, number and complexity of mitochondria. The functional correlates of these morphological and histochemical observations are discussed and compared to human endometrial studies.

Key words: Endometrium – Baboon – Oral contraceptive – Histochemistry – Ultrastructure.

The baboon, because of its close phylogenetic relationship to man, is used frequently in medical research. However, information regarding the structure and function of the reproductive system of this animal remains fragmentary. The results of studies conducted on baboon endometrium throughout its menstrual cycle

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(MacLennan and Wynn, 1971; MacLennan et al., 1971; Dollar et al., 1976; Kraemer et al., 1977) correlate closely with the endometrial changes occurring in the human menstrual cycle. However, before the baboon can become widely accepted as a surrogate in the study of human reproductive problems, more information concerning its reproductive system must be obtained. This new information must not only involve the normal functioning of the system, but must also include studies on the response of the reproductive organs to either abnormal or exogenous stimuli. The present research was undertaken in order to study baboon endometrial response to a widely accepted and frequently used oral contraceptive agent, Enovid E.

Materials and Methods

Five normally cycling female baboons, *Papio anubis*, were given Enovid E (UpJohn Co., Kalamazoo, Michigan) (norethynodrel, 2.5 mg; mestranol, 0.1 mg) for nine months. The oral contraceptive was administered in food (fruit), beginning on day one of the cycle and continuing for 30 days. Two normally cycling animals were used as controls. The animals were maintained at a constant temperature (24°C) on an alternating light-dark cycle (12h light, 12h dark). Food and water was provided ad libitum. Throughout these experiments daily records of sex skin turgescence and menses were kept for each animal.

At three, six and nine months, the endometrium was sampled by transcervical uterine biopsy with a Novak curette. These biopsies were all obtained during the late pre-ovulatory phase (day 10–14) in the control baboons and between 10 and 14 days of the treatment cycles. The tissue was divided into three portions. One piece was fixed in cold (4°C) neutral buffered formalin, embedded in paraffin and sectioned at six micrometers. These sections were mounted on glass slides and stained either with hematoxylin and eosin or by the PAS method. Some sections, prior to their reaction by the PAS method, were treated with diastase (1 h at 37°C). These slides were then compared to ones stained only by the PAS method in order to determine glycogen content. A second portion of the endometrial biopsy was rapidly frozen on dry ice and sectioned at ten micrometers with a cryostat. This tissue was then incubated for the determination of acid and alkaline phosphatase activities (Pearse, 1972) and of the succinate dehydrogenase activity (SDH) (Nachlas, 1957).

The remaining endometrial portion was immersed in a mixture of equal parts of cold (4°C) osmium tetroxide (2%) and paraformaldehyde (6%) buffered with sodium cacodylate (0.2 M, pH 7.4) for 12 h (tissues were minced during the first hour). These tissues were then transferred to distilled water, where after several rinses for a total of 30 min, they were placed in 0.5 % uranyl acetate for 24 h at 4°C. The tissues were then dehydrated at 4°C in absolute alcohol and brought to room temperature before transfer to propylene oxide (Shino et al., 1972). Tissue was then embedded in Epon 812 and sectioned

![Fig. 1](image1.jpg) The endometrium of a baboon treated with Enovid E. Note the two endometrial glands with subnuclear vacuolization (arrows) and a mitotic figure (arrowhead). (Hematoxylin and eosin; ×870)

![Fig. 2](image2.jpg) Enovid E-treated baboon endometrium. Carbohydrate is conspicuous only within the epithelium where it is more prominent within the subnuclear region. (PAS; ×1000)

![Fig. 3](image3.jpg) Control baboon endometrium during the late pre-ovulatory phase that has been incubated for acid phosphatase. Activity is very scarce and is demonstrable as small black granules (arrowheads) predominantly within the epithelium (×725)

![Fig. 4](image4.jpg) Acid phosphatase activity of endometrium of Enovid E-treated baboons. Note the heavy activity in the apical cytoplasm of the glandular cells (arrowheads). (×550)