INITIATION OF MENSTRUAL CYCLICITY AND DECIDUOMAL REACTION IN THE BONNET MONKEY (*MACACA RADIATA*)

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(Communicated by M.R.N. Prasad, F.N.A.)

(*Received 14 January 1974*)

Decidualization has been induced in immature and acyclic mature bonnet monkeys by following a 30-day estrogen-progesterone combined therapy, traumatization being achieved on day 16 of treatment. This treatment led to an increase in the weight and glycogen content of the uterus. Histological examination of the uterus of traumatized animals revealed typical decidual cell pockets resembling a plaque reaction.

Regular menstrual cyclicity has been induced in bonnet monkeys, which tend to show an amenorrheic behaviour soon after capture and transfer to the laboratory, by following a combination of estrogen and progresterone therapy.

INTRODUCTION

Extensive work has been carried out on endometrial morphology and its response to intra-uterine devices and trauma in the rhesus monkey (*Macaca mulatta*) (Marston and Kelly 1969; Kelly, Marston and Eckstein 1969; Kar, Chowdhury and Kamboj 1965,1967; Eckstein and Kelly 1969 and Marston and Kelly 1967). A deciduomal reaction confined to the epithelial and sub-epithelial layers of the uterus has been described in this species by Rossman (1940). However, very little is known about the menstrual cyclicity and uterine physiology of a closely related species, the bonnet monkey (*Macaca radiata*). The bonnet monkey on transfer to the laboratory most often ceases to cycle and appears to exhibit protracted amenorrhia. The present communication describes our attempts o induce cyclicity and to analyse the nature of deciduomal reaction in this pecies.

MATERIALS AND METHODS

Mature as well as immature female monkeys were caught around Bangalore city nd quarantined for a minimum of 2–4 weeks before using them for experiments.

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They were housed in individual cages with adequate food consisting of bread, vitamin supplements, fresh vegetables, bananas and clean water.

Induction of synchronised cyclicity

The mature female monkeys caught in the wild and transferred to the animal quarters were observed regularly for menstrual bleeding. Since these monkeys appeared amenorrheic and did not show manstrual bleeding over a period of 3 months, menstruation was initiated using a composite steroid therapy as described by Eckstein *et al.* (1969). This consisted in injecting intramuscularly 100μg of estradiol 17 β daily for 7 days, followed by 2 mg of progesterone daily for 5 days. Within 3–5 days of discontinuation of treatment withdrawal bleeding ensued. Considering day 1 of the cycle as the day menstrual bleeding started, several cycles were followed for each monkey.

Induction of decidualization

Attempts to induce deciduomal reaction in both mature non-cycling and immature monkeys were made using the following experimental schedule :

Days	1–3	4–10	11–15	16	16–30	32
	50 μg of estradiol-17β	75 μg of estradiol-17β; 5 mg progesterone	10 mg progesterone	Traumatization	10 mg progesterone	Autopsy

Traumatization was achieved by placing 3 silk sutures into the fundus of the uterus, two traversing laterally and one dorso-ventrally. The monkeys were autopsied or gonadectomised and hysterectomised 15 days after traumatization and reproductive organs taken for histological examination and glycogen estimation. Control monkeys were maintained to account for or exclude the effects of vehicle or steroids.

All surgical procedures were carried out taking full aseptic precautions. Chloroform, ether (1:2 v/v) mixture was administered by open-drop inhalation method as anaesthetic. At the end of the surgery topical application of "Nebasulf" (oxytetracycline sulfa combination Cyanamid) provided the necessary antibiotic protection. Tissues were prepared for examination by fixing in Bouin's fluid for 24 hr, embedded in paraffin, sectioned at 8μ thickness and stained with haematoxilin-eosin.

A portion of the uterus was used for glycogen estimation by the method of Seifter *et al.* (1950). Briefly this consisted in specific precipitation of glycogen with alcohol, hydrolysis of glycogen to glucose with concentrated sulphuric acid and estimating the glucose using the anthrone reagent; glycogen was expressed as glucose units.

RESULTS

Induction of menstrual cyclicity in bonnet monkeys

Mature females of this species caught all round the year were found not to menstruate even when observed for three consecutive months in captivity. However,

menstrual bleeding could be induced upon composite steroid thereapy and such monkeys showed normal cyclicity over a period of time, average length being 28 days. The results of our studies on 10 monkeys are summarized in Fig. 1.

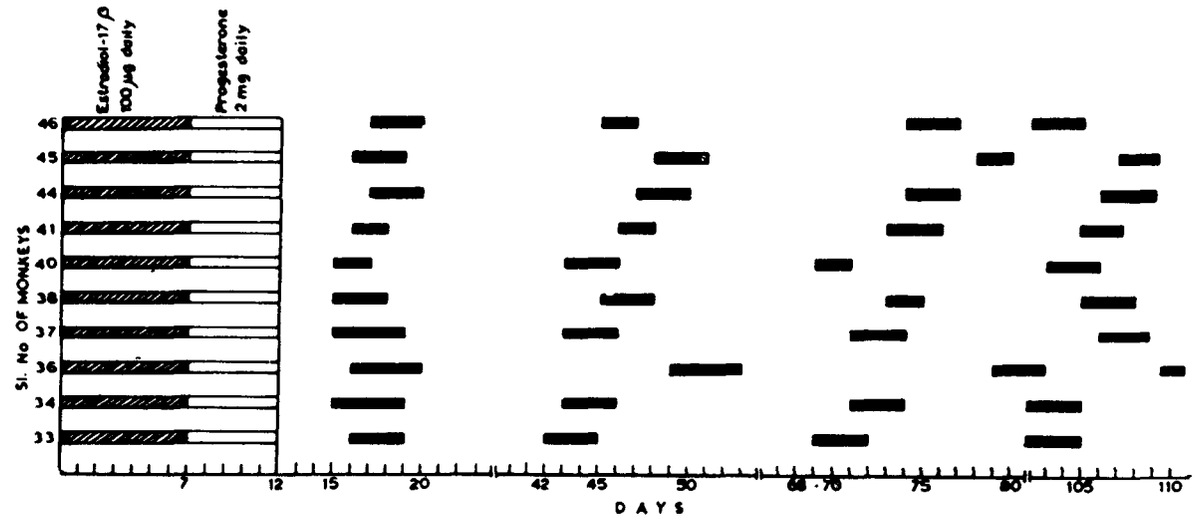


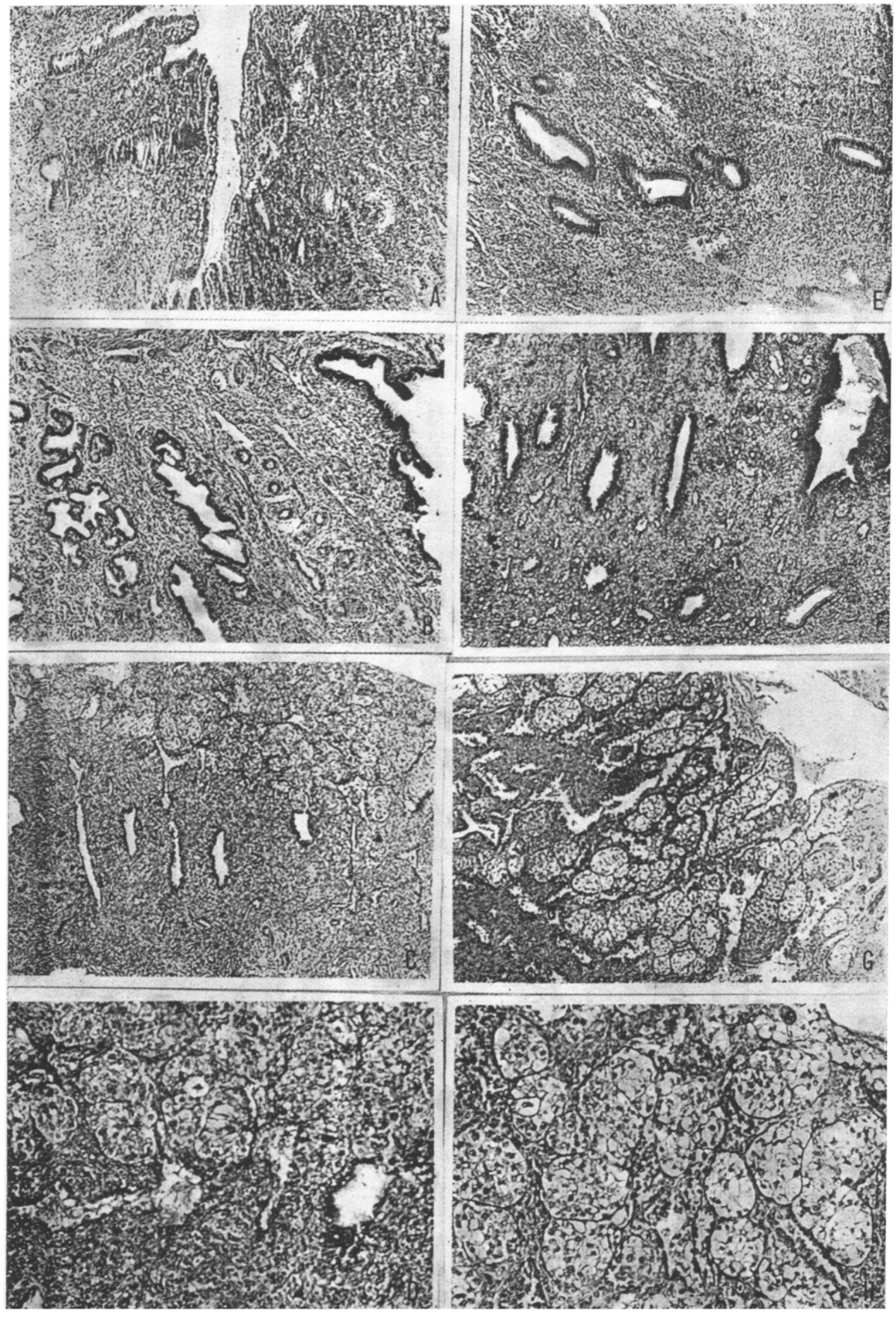
FIG. 1. Initiation of menstrual cyclicity in 10 bonnet macaques (*Macaca radiata*) by using composite steroid therapy. Menstrual bleeding was of 2–4 days, day of start of bleeding was considered as day 1 of cycle, withdrawal bleeding ensued within 5 days of discontinuation of steroid treatment. [Sl. No. = Serial number of monkeys, Horizontal black bars = duration of menstrual bleeding. The first bar indicating the withdrawal bleeding, Bars with crossed lines = Estradiol 17β 100 µg, Bars (open) = 2 mg progesterone]

TABLE I

Induction of decidualization and its effect on the uterine weight, total glycogen in mature and immature female bonnet monkeys (Macaca radiata)

Group	n	Treatment	Body weight at autopsy(kg)	Gross uterine weight (g)	Uterine weight/kg body weight Mean ±S.D.(g)	Total glycogen content Mean±S.D.(g)
Immature						
I	2	Oil control	1.85	0.525	285 ± 2.4	211.0± 10.0
II	2	Steroids only	2.07	1.960	1040±25.0	2000.0± 180.0
III	5	Steroids+thread (Trauma)	2.60	2.910	1187±23.0	4012.0±2019.0*
Mature						
IV	2	Oil control	3.12	2.43	680±16.0	104.0± 17.0
V	2	Steroids only	4.0	5.80	1480±13.4	1111.0± 25.0
VI	5	Steroids+thread (Trauma)	3.80	7.72	2255±23.0	1490.0± 255.0*

*All values are significant when compared to their respective controls ($p<0.1$) excepting those marked with an asterisk,



FIGS. 2 A-H. Histology of uterus of immature (Figs. A-D) and mature (Figs. E-H) monkeys receiving different treatments. A, Endometrium of control monkey (immature), × 80; B, Endometrium of steroid treated monkey (immature). ×80; C, Endometrium showing deciduomal reaction in traumatized monkey with steroid treatment (immature). × 80; D, Endometrium showing deciduomal reaction in traumatized monkey with steroid treatment (immature). × 200; E, Endometrium of control monkey (mature), × 80; F, Endometrium of steroid-treated monkey (mature) × 80; G, Endometrium showing deciduomal reaction in traumatized monkey with steroid treatment (mature). × 80; H, Endometrium showing deciduomal reaction in traumatized monkey with steroid treatment (mature). × 200.

Induction of decidualization

Generally, both mature and immature monkeys reacted to steroids and trauma in a similar fashion. There was a significant increase in uterine weight with steroid treatment, and this further increased upon traumatization (Table I). The effect of steroid treatment was also reflected by a significant increase (about 10 fold) in total uterine glycogen. However, on traumatization the glycogen increase over steroid controls was not significant (Table I).

Steroid treatment brought about, as seen in histology, endometrial oedema and hypertrophy of the endometrial glands (compare Fig. 2 A and E with B and F). Decidual reaction was clearly noticeable in the traumatized groups of both immature and mature monkeys (Fig. 2 C, D, G and H). The decidual reaction was mostly confined to the subepithelial region beneath the uterine lumen. In general, this reaction was localized in the areas surrounding the contact points with the thread. This was a characterized by hyperplasia of cells resulting in large cell pockets just beneath the epithelium.

DISCUSSION

The present study confirms the possibility of inducing synchronised cycles in a large number of monkeys by using steroid withdrawal regimen. Some of these cycling monkeys have since been mated and have become pregnant in captivity, an indication of having induced ovulatory cycles.

This method is useful for undertaking large-scale mating experiments and obtaining a number of pregnant animals of the same stage at a time. It is, however, necessary to caution that menstrual bleeding pattern itself does not necessarily mean that all animals are going through ovulatory cycles. In our experience a few of these monkeys though showing normal menstrual bleeding pattern, actually exhibited nonovulatory cycles, the number of such cases, however, being small.

It is evident from the above study that it is possible to induce decidualization in both mature and immature bonnet monkeys following artificial steroid regimen detailed above. The decidual reaction in this species, similar to that observed by Rossman (1940) for the rhesus monkey, seems mostly confined to the epethelium and a few endometrial glands and does not extend to the stromal cells. The observation that this reaction was restricted to contact areas (portions through which threads were drawn) may indicate its similarity to the epithelial 'plaque' reaction found during implantation.

The increase in uterine glycogen content observed in the present case seems to be more of a consequence of progesterone treatment, traumatization not having any additional effect on it. Perhaps the reason for not observing an increase in glycogen content with decidualization, an event which has been shown to be true for the rat (Shelesnyak 1967) may be due to the fact that decidualization in the monkey is only confined to contact points and as such its contribution to the overall increase of uterine glycogen may not be significant. Since the histological changes accompanying decidualization in the monkey appears very similar to that observed at implantation, it may be worthwhile to use the above experimental set-up, after suitable modifications as a model system to study biochemical and hormonal changes occurring at or close to implantation.

ACKNOWLEDGEMENTS

This work was supported by a grant-in-aid from the Ford Foundation, New York and the Ministry of Health and Family Planning, Government of India. We thank Messrs. B. Shanmugavelu and P. Pasupathy, for technical assistance.

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