EFFECT OF AN INTRAUTERINE DEVICE ON MENSTRUAL CYCLICITY AND LUTEAL FUNCTION IN THE BABOON

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(Received 20th May 1971, accepted 12th August 1971)

Summary. In the baboon, the presence of an IUD slightly shortens the length of the menstrual cycle and prolongs that of menstruation. Sexual skin changes showed that the part of the cycle shortened was the detumescent (= secretory) phase, the tumescent (= proliferative) phase remaining unaffected. Plasma progesterone levels during the latter half of the cycle were not affected by the IUD, but the onset of menstruation in experimental animals was associated with a higher blood concentration of progesterone than that present in controls. It is concluded that an IUD does not interfere with luteal function in the baboon.

INTRODUCTION

The mode of action of IUDs remains unknown in both women and other mammals. Most of the present evidence indicates that they act primarily at the uterine level (cf. Eckstein, 1970). Some preliminary findings by Faucher, Ellegood, Mahesh & Greenblatt (1969) suggest, however, that in women the luteal phase is shorter and urinary pregnanediol excretion is reduced after insertion of an IUD. Marginal shortening of the cycle was also found in rhesus monkeys fitted with IUDs (Eckstein, Kelly & Marston, 1969), but neither the morphology of the luteal cells nor the responsiveness of the endometrium to decidualizing stimuli appeared to be affected by the presence of a device (Kelly, Marston & Eckstein, 1969a, b).

In the present study, baboons were used. Unlike the highly tortuous cervical canal of rhesus monkeys, baboons possess a relatively straight one, which greatly facilitates the insertion and removal of IUDs. A second advantage is that their sexual skin undergoes conspicuous periodic changes which are synchronous with the phases of the endometrial cycle, swelling or 'tumescence' of the sexual skin occurring during the proliferative phase and shrinkage ('detumescence') during the progestational part. Since exogenous progesterone has been shown to induce collapse of the sexual skin in baboons (Gillman, 1940), it is assumed that normal detumescence is correlated with, and may be used as a sensitive indicator of, the hormonal activity of the corpus luteum in

this species. The onset of detumescence does not, however, coincide precisely with, and probably follows a few days after, ovulation (cf. Hendrickx & Kraemer, 1969; present study).

This paper reports the effects of a plastic IUD on the overall length of the menstrual cycle and that of the tumescent and detumescent stages of the sexual skin cycle, as well as on the plasma concentration of progesterone during the luteal phase.

MATERIALS AND METHODS

Animals and recording of sexual skin activity

A total of eight adult, probably parous, baboons, *Papio cynocephalus*, were used and kept under conditions similar to those described for rhesus monkeys (Eckstein *et al.*, 1969). After an initial quarantine period of about 6 weeks, a daily record of their sexual skin development and menstruation was kept. The degree of sexual skin activity was recorded as follows: 0 = quiescence; 1 to 2+ = progressive tumescence; 3 or 3+ = maximum tumescence; 2+ to 0 = progressive detumescence. Thus the first day of detumescence was taken as the day when the sexual skin showed noticeable shrinkage compared with its condition on the previous day. The last day during the detumescent phase before the onset of macroscopical bleeding was taken as the end of the cycle and the first day of menstruation as the beginning of the next cycle.

Collection of blood

The animals were bled after receiving an intramuscular injection of 0.8 to 1.0 ml phencyclidine hydrochloride (Sernylan, Parke Davis & Co.; 20 mg/ml). This resulted in temporary sedation during which 5 ml blood were withdrawn from a superficial vein. Blood was taken three times a week in this way. The samples were centrifuged immediately at about 3000 rev/min in heparinized tubes and the resulting plasma pipetted off and stored at -10°C until required for assay.

Insertion and removal of IUDs

The devices used were shortened Margulies spirals (15 to 20 mm in diameter and 2 mm thick). In seven of the eight baboons, insertion of the device into the uterus was performed transcervically during sedation with phencyclidine, using a human IUD inserter. In the remaining female, insertion was by hysterotomy. Subsequent X-ray photography, and in some cases laparotomy, confirmed that all IUDs were correctly placed and had fully re-coiled within the uterine cavity. The IUDs of five of the animals in this study were later removed by means of a Paterson's forceps introduced through the cervix.

Progesterone assay

Plasma progesterone was determined by the competitive protein-binding method of Johansson (1969), with the following minor modifications. Plasma containing binding protein was obtained from a normal young woman who had taken 50 μg ethinyl oestradiol daily for 21 days. The plasma was stored in

1-ml aliquots at -15°C . The protein-binding solution used in the assay contained 0.1% of this plasma mixed with [1,2- ^3H]corticosterone (Radiochemical Centre, Amersham) with a specific activity of 41.5 Ci/mmol.

Petroleum ether (British Drug Houses; boiling range 30 to 40°C) was washed with sulphuric acid and redistilled twice before use. Estimations on each batch showed extractions of 85 to 90% of progesterone and of only 10 to 12% of 17α -hydroxyprogesterone. Plasma (0.5 ml) was extracted with 5 ml petroleum ether, and 4 ml of supernatant was withdrawn and evaporated to dryness under nitrogen in a 35°C water bath.

Recoveries were $0.99 \text{ ng} \pm 0.047$ (S.E.) from an added amount of 1 ng of progesterone, and $1.98 \text{ ng} \pm 0.066$ (S.E.) from an added 2 ng of progesterone (sixteen duplicate estimations). The error in repeated determinations was found to be $\pm 0.1 \text{ ng}$ (S.E.) for a mean value of 4.81 ng (based on a series of forty-five duplicate estimations) and $\pm 0.03 \text{ ng}$ (S.E.) for a mean value of 0.48 ng (thirty-five duplicates).

RESULTS

Effect of an IUD on the phases of the menstrual cycle and on cycle length

All cycles observed in the eight baboons have been included in the analysis unless they lasted more than 49 days. Cycles exceeding this length were rare (three out of 132, all in animals without IUDs), and may have been due to menstruation being either very brief or microscopic and therefore remaining undetected. If an IUD was inserted or removed during any particular cycle, this was excluded from the analysis. Data on cycle length in animals that had been in the main colony for less than 6 weeks were not included. After this time, no apparent change in the length of the cycle occurred.

Table 1 shows that in each of the eight baboons, the mean cycle length was slightly shorter when the IUD was present. Statistical analysis (paired t test) indicated that the difference between the means, 1.15 days, is significant ($P < 0.05$). This also emerged from an analysis of the pooled data obtained in all eight animals ($P < 0.05$; Table 2).

The device had no consistent effect on the tumescent phase of the sexual skin cycle, but the detumescent phase was shortened in seven of the eight females studied (Table 1). An analysis of the group means by paired t test ($P < 0.05$) and of the pooled data from all eight females ($P < 0.002$; Table 2) showed that the reduction in the length of the detumescent phase in females fitted with the device was significant.

The length of the cycle in the baboons without IUDs was slightly longer than the mean for 200 cycles considered to be ovulatory in the series of Stevens, Sparks & Powell (1970), but similar to the means reported by Hendrickx & Kraemer (1969) and Eckstein & Zuckerman (1956).

The greater length of the tumescent compared with the detumescent phase of the cycle in females without a device (Tables 1 and 2) is consistent with previous observations in several species of baboon (see Eckstein & Zuckerman, 1956). It contrasts with the findings of Hendrickx & Kraemer (1969) who reported no differences in length between the two phases in females isolated from

TABLE 1
THE EFFECT OF AN IUD ON THE LENGTH OF THE MENSTRUAL AND SEXUAL SKIN CYCLES AND ON THE DURATION OF MENSTRUATION IN EIGHT BABOONS

No. of animal	Menstrual cycle		Sexual skin				Menstrual flow	
	Menstrual cycle		Tumescient phase		Detumescient phase		Menstrual flow	
			Without IUD	With IUD	Without IUD	With IUD		
2055	36.45 ± 1.20 (11)	36.25 ± 1.03 (4)	24.18 ± 1.24 (11)	25.00 ± 0.91 (4)	12.27 ± 0.85 (11)	11.25 ± 0.85 (4)	3.07 ± 0.29 (14)	4.17 ± 0.16 (6)
2057	34.44 ± 0.56 (9)	33.54 ± 0.62 (11)	19.66 ± 0.53 (9)	19.72 ± 0.75 (11)	14.67 ± 0.23 (9)	13.82 ± 0.32 (11)	3.80 ± 0.25 (10)	4.69 ± 0.40 (13)
2062	39.45 ± 1.25 (11)	36.17 ± 0.97 (6)	26.00 ± 1.05 (11)	22.33 ± 1.05 (6)	13.36 ± 0.92 (11)	13.83 ± 0.54 (6)	3.09 ± 0.39 (11)	4.20 ± 0.58 (5)
2063	34.53 ± 0.74 (13)	34.42 ± 0.72 (7)	19.00 ± 0.41 (13)	20.43 ± 0.92 (7)	15.46 ± 0.53 (13)	14.14 ± 0.40 (7)	4.15 ± 0.32 (13)	5.57 ± 0.20 (17)
2064	31.30 ± 0.80 (10)	29.50 ± 0.52 (10)	18.90 ± 0.66 (10)	18.80 ± 0.49 (10)	12.40 ± 0.79 (10)	10.70 ± 0.26 (10)	4.17 ± 0.38 (12)	4.60 ± 0.54 (10)
2106	33.55 ± 0.67 (9)	33.33 ± 1.20 (3)	19.11 ± 0.75 (9)	21.33 ± 0.33 (3)	14.44 ± 0.47 (9)	12.00 ± 1.15 (3)	3.33 ± 0.35 (15)	4.75 ± 0.85 (4)
2108	32.83 ± 0.83 (6)	32.44 ± 0.63 (9)	19.33 ± 1.02 (6)	19.22 ± 0.72 (9)	13.50 ± 0.34 (6)	13.22 ± 0.57 (9)	4.00 ± 0.49 (7)	4.82 ± 0.26 (11)
2109	30.73 ± 0.72 (11)	28.50 ± 0.50 (2)	16.54 ± 0.28 (11)	18.50 ± 0.50 (2)	14.06 ± 0.39 (11)	10.00 ± 1.00 (2)	2.87 ± 0.20 (16)	6.00 ± 0.58 (3)

Values expressed as mean length in days ± S.E. Number of observations in parentheses.

TABLE 2
EFFECT OF AN IUD ON THE MENSTRUAL AND SEXUAL SKIN CYCLE OF EIGHT BABOONS

	Days (mean $\pm$ S.E.)		d*	P
	Without IUD	With IUD		
Cycle length	34.30 $\pm$ 0.43 (80)	33.00 $\pm$ 0.41 (52)	2.11	0.05 to 0.02
Menstrual flow	3.50 $\pm$ 0.12 (98)	4.78 $\pm$ 0.16 (59)	6.46	< 0.001
Tumescence phase	20.45 $\pm$ 0.43 (80)	20.31 $\pm$ 0.39 (52)	0.24	> 0.05
Detumescence phase	13.81 $\pm$ 0.23 (80)	12.71 $\pm$ 0.26 (52)	3.11	0.002 to 0.001

Number of observations in parentheses.

* d value calculated according to Bailey (1959); n > 30.

males. Their method of recording sex changes, however, differed somewhat from ours (Hendrickx & Kraemer 1971).

Effect of an IUD on length of menstruation

The findings listed in Table 1 also show that, in all eight baboons, the duration of menstruation was prolonged when an IUD was present. The differences between the group means, when analysed by paired *t* test ($P < 0.01$) and between the pooled data ($P < 0.001$; Table 2) are significant. No such difference was found in rhesus monkeys (Eckstein *et al.*, 1969).

Effect of an IUD on plasma progesterone levels

The results are shown in Table 3 and Text-fig. 1. They are based on observations in only seven baboons owing to the elimination of one female following the spontaneous expulsion of her IUD during the experimental cycle. Each baboon

TABLE 3
PROGESTERONE LEVELS IN PERIPHERAL BLOOD IN SEVEN BABOONS WITH OR WITHOUT IUDS

Days before menstruation	Without IUD		With IUD	
	No. of samples assayed	Progesterone (ng/ml)	No. of samples assayed	Progesterone (ng/ml)
0 and 1	5	0.60 $\pm$ 0.06	6	1.51 $\pm$ 0.39
2 and 3	5	2.92 $\pm$ 1.01	7	4.61 $\pm$ 1.15
4 and 5	7	6.01 $\pm$ 1.00	5	7.33 $\pm$ 0.87
6 and 7	4	8.19 $\pm$ 0.57	7	8.31 $\pm$ 1.11
8 and 9	7	8.56 $\pm$ 1.13	6	9.96 $\pm$ 1.52
10 and 11	7	8.17 $\pm$ 1.25	6	7.15 $\pm$ 0.98
12 and 13	4	4.56 $\pm$ 0.97	4	4.00 $\pm$ 1.18
14 and 15	7	3.83 $\pm$ 0.24	7	3.63 $\pm$ 0.62
16 and 17	7	1.69 $\pm$ 0.39	7	1.50 $\pm$ 0.36
18 and 19	7	0.81 $\pm$ 0.22	5	0.76 $\pm$ 0.27
20 and 21	5	0.35 $\pm$ 0.15	5	0.37 $\pm$ 0.20
22 and 23	5	0.47 $\pm$ 0.06	5	0.65 $\pm$ 0.29
24 and 25	6	0.54 $\pm$ 0.16	5	0.44 $\pm$ 0.21
26 and 27	3	0.43 $\pm$ 0.05	4	0.55 $\pm$ 0.32

Progesterone levels expressed as means $\pm$ S.E.

TEXT-FIG. 1 Sexual skin development (○) and concentration of progesterone in peripheral plasma (●) in seven baboons with (—) and without (---) IUD. For scale of left-hand ordinate, see p. 250.

was studied for one complete cycle with the device present and one without it. The cycles ranged from 28 to 36 days. Since luteal development and secretion of progesterone occurs mainly during the second half of the normal cycle, the recorded progesterone levels (in ng/ml plasma) have been expressed in terms of days before menstruation (Table 3).

It is clear from the Table and Text-fig. 1 that the concentration of progesterone in the peripheral blood during most of the luteal phase was not affected by the presence of a device in the uterus ($P > 0.05$). In both the IUD and non-IUD group of baboons, a marked rise took place about Day 16 before menstruation and continued for about 7 days, after which the concentration declined steadily until menstruation. The onset of bleeding, however, was found to be associated with a higher plasma level of progesterone in the presence of the device (Table 3). Therefore, after completion of the main experiment, three additional samples of blood were obtained at the onset of menstruation and analysed with the eleven results listed in Table 3. The respective concentrations on Day 0 were 0.52 ± 0.07 ng/ml (four samples) without IUD and 1.11 ± 0.29 ng/ml (four samples) with IUD ($P = 0.10$ to 0.05), and on Day 1, 0.56 ± 0.11 (three samples) and 2.18 ± 0.54 ng/ml (three samples) ($P = 0.05$ to 0.02).

Relationship between sexual skin and progesterone levels

Text-figure 1 shows that the presence of an IUD does not interfere with the full expression of sexual skin activities and that, in animals both with and without the device, the concentration of progesterone starts to rise 2 or 3 days before the sexual skin subsides. Once detumescence has begun, it proceeds rapidly, coincident with a progressive increase in plasma progesterone. In the group of baboons studied, however, the sexual skin showed no sign of renewed activity after the concentration of progesterone had fallen to a barely detectable level; it usually began to swell shortly after the onset of menstruation.

DISCUSSION

Our study has shown that in the baboon an IUD causes a slight, though significant, reduction in the length of the menstrual cycle, but does not appear to interfere with luteal function, as assessed by the plasma concentration of progesterone.

The reduction in the overall length of the cycle seems to be due to shortening of the detumescent phase of the sexual skin (Tables 1 and 2). Detumescence starts about 2 days after the beginning of the rise in blood progesterone, irrespective of the presence of a device (see Text-fig. 1). These findings, together with the outcome of timed matings just before sexual skin subsidence (Hendrickx & Kraemer, 1969), suggest that, in normal mature baboons, ovulation precedes the collapse of the sexual skin by about 2 to 3 days. The precise relationship between it and ovulation can, however, only be finally settled by simultaneously determining an ovulatory peak in plasma LH (Stevens *et al.*, 1970) and recovering tubal eggs.

The competitive protein-binding method used in this investigation measures

primarily plasma progesterone. No chromatographic separation is used, but the major steroids extracted by petroleum ether are progesterone, 20 α - and 17 α -hydroxyprogesterone (Johansson, Neill & Knobil, 1968). There is no significant competition of 20 α -hydroxyprogesterone with progesterone for binding sites and only 10 to 12% of the 17 α -compound is extracted.

The occurrence of normal blood progesterone concentrations during the luteal phase and the full development of the sexual skin during the proliferative half of the cycle in animals fitted with an IUD indicate that, in baboons, the device does not interfere with the ovarian secretion of either progesterone or oestrogen and exerts no luteolytic effect. This finding differs from the provisional conclusion of Faucher *et al.* (1969), who reported lower pregnanediol levels in patients following insertion of an IUD. The discrepancy may be due to the small number of women and cycles examined by these authors. Instead, our results support the view of Kelly *et al.* (1969a, b), based on histological observations on the corpora lutea and endometrium, that luteal function in rhesus monkeys is unaffected by the presence of an IUD. In species such as the ewe (Nalbandov, Moore & Norton, 1955; Ginther, Pope & Casida, 1966; Bhalla & Casida, 1970), cow (Ginther, Wood, Janakiraman & Casida, 1966) and guinea-pig (Bland & Donovan, 1966; Ginther, Mahajan & Casida, 1966), intrauterine foreign bodies exert a marked luteolytic effect. In these and some other species, however, hysterectomy prolongs the functional life of the corpora lutea, thus implying the existence of a uterine luteolytic factor upon which an IUD might act. By contrast, hysterectomy does not appear to extend the lifespan and hormonal function of the corpus luteum in primates (e.g. Neill, Johansson & Knobil, 1969; Doyle, Barclay, Duncan & Kirton, 1971).

An interesting additional finding in the present study is the fact that, in the baboons with IUDs, menstruation started with higher blood levels of progesterone than in females without the devices. This could account for the shorter length of the menstrual cycle and of the detumescent phase in the animals with IUDs. It is possible that an IUD may alter the sensitivity of the endometrium to endogenous progesterone; such a conclusion has already been drawn from studies in rats (Psychoyos, 1966; Chaudhury & Sethi, 1970) and mice (Martin & Finn, 1970).

The contraceptive action of IUDs in sub-human primates has so far only been established in rhesus monkeys (Kelly *et al.*, 1969a). While this remains to be confirmed for the baboon, it can be concluded that the devices do not alter ovarian function in this species, but may affect uterine responsiveness to progesterone.

ACKNOWLEDGMENTS

We wish to thank Mrs A. Fraser and Mr R. Morris for skilled technical assistance, and Mr A. Smith, Mr F. James and Mr G. Tonks for care of the animals. The financial support of the Ford Foundation is gratefully acknowledged.

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