THE USE OF TRANSABDOMINAL PALPATION TO DETERMINE THE COURSE OF PREGNANCY IN THE MARMOSET (*CALLITHRIX JACCHUS*)

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Summary. Transabdominal palpation was used to determine maturity, course of pregnancy and post-partum changes in marmosets throughout seventy-four full-term pregnancies. The accuracy of the technique is compared with that of other methods of studying the course of pregnancy in this species.

INTRODUCTION

Several workers have reported the use of the marmosets (*F. Callithricidae*) in biomedical research (Levy & Artecona, 1964; Hampton, Hampton & Landwehr, 1966; Deinhardt, Devine, Passovoy, Pohlman & Deinhardt, 1967; Gengozian, 1969; Eppele, 1970; Poswillo, Hamilton & Sopher, 1972). The members of this family are unusual in that they exhibit neither the menstrual cycle of other primates, nor the obvious oestrous cycle of other mammals. Preslock, Hampton & Hampton (1973) demonstrated a mean reproductive cycle of 15.5 ± 1.5 days in *Saguinus oedipus*. Hearn & Lunn (1975) determined a mean cycle of 16.4 ± 1.7 days for *Callithrix jacchus*. The exact day during the reproductive cycle on which ovulation occurs has not been determined.

Under optimum conditions of husbandry and nutrition, marmosets are exceptionally prolific breeders with no sign of a breeding season in captivity (Grist, 1975; Phillips, 1975). They are best maintained as monogamous pairs for breeding purposes. Although the female marmoset has a simplex uterus, the vast majority of conceptions result in twins, with occasional triplets or singletons. The literature relating to the precise duration of pregnancy in *C. jacchus* is confusing. The most reliable information would appear to be the three dated conceptions quoted by Hampton, Levy & Sweet (1969) of 143, 146 and 147 days. Hearn & Lunn (1974) gave 148 ± 4.3 days as the gestation period. In this laboratory, we have observed four accurately dated pregnancies of 150, 150, 151 and 153 days.

Hampton *et al.* (1969) estimated the levels of chorionic gonadotrophin in marmoset urine using bioassay methods. A detectable gonadotrophin is excreted from about Day 25 of pregnancy to near term. Hampton *et al.* (1969), however, observed a number of false positive results in non-pregnant animals and for this

reason concluded that a single assay on an animal less than 6 to 8 weeks pregnant could lead to an erroneous diagnosis of pregnancy.

Hearn & Lunn (1974) measured plasma progesterone, oestradiol and gonadotrophin levels by radioimmunoassay which enabled them to determine pregnancy 14 to 20 days after conception. Pregnancies were subsequently confirmed by weekly measurement of uterine size by palpation.

The possibility of using physical palpation as a means of detecting pregnancy in the marmoset was first discussed by Poswillo *et al.* (1972) and S. Jones and S. Mitchell (personal communication). The present paper elaborates the techniques of transabdominal palpation in marmosets and comments on the accuracy of the method.

MATERIALS AND METHODS

Animals

Transabdominal palpation was performed on primiparous and multiparous marmosets (*Callithrix jacchus*). The parent females were quarantined feral animals and the F₁ females were born in captivity into a closed colony. All the females were paired monogamously with a male of the same generation either from 14 months of age (F₁ females) or at the time of reception if imported. The pairs remained together during pregnancy, parturition and lactation and throughout their economic breeding life.

The data presented here were based on the observation of seventy-four normal pregnancies which proceeded to full term. Observations were also made on fourteen pregnancies that terminated in spontaneous abortion and on sixty-seven pregnancies terminated by sequential hysterotomy at selected stages of gestation. Transabdominal palpation was performed at regular intervals on the eighty-one pregnancies that did not go to term but these have been excluded from this report. The length of gestation was calculated on a retrospective basis from the day of parturition which was designated Day 150. Four dated conceptions resulted from caging of males and females in pairs for a 48-hr period and these were compared with the retrospective figures.

Methods

Female marmosets required for examination were caught and physically restrained by a technician. The animal was held in a vertical position with the legs fully extended caudally. It was necessary for the operator to wear thin surgical gloves to attain maximum digital sensitivity. One hand was placed on the ilio-sacral region to support the lower pubic area of the animal; by using the thumb and forefinger only of the free hand, transabdominal palpation of the uterus and ovaries was achieved.

Measurement of uterine size was performed by placing the thumb and forefinger around the periphery of the uterus and by employing these two digits as 'calipers', the distance was calculated using a millimetre scale. Measurements were always made twice and if possible by two operators. When using manual palpation, there was a tendency to close the digits slightly on removal from the abdomen and in practice it was found that the palpated size was invariably 1 to

2 mm less than the actual size. The most specific measurement of the uterus and ovaries could only be made with sterile calipers during exploratory laparotomy. Digital measurement was found to be more accurate than calipers when palpating the unanaesthetized animal, and the uncorrected figures are presented.

Successive palpations were made at approximately fortnightly intervals on all females throughout gestation. It has been advantageous to palpate animals before feeding. This minimizes the possibility of confusing faecal pellets with ovaries.

RESULTS

Uterus and ovaries in the non-pregnant female

Sexual maturity was related to definite changes in the external genitalia. In the immature animal, the vulva was small but developed to a large turgid structure with collapsing walls when maturity was reached. In this colony, puberty normally occurred at 13 to 14 months of age, although one female conceived at 11½ months and continued to term. The vulval mucosa was pale pink in immature animals, and the nipples, which were axillary in position, were small and flat.

The vulval changes which preceded maturity were accompanied by palpable changes in the uterus and ovaries. The uterus in the virgin animal was a firm club-shaped structure approximately 1.5 cm long and 4 to 5 mm wide. The ovaries were not palpable at first but could be detected just anterior to the uterus as firm ovoid structures, 3 to 4 mm in diameter, as the animal matured. In primiparous females, the uterus was frequently in a dorsal position close to the rectum but uteri of multiparae were easily detectable in the anterior pubic region of the abdomen. The cervix uteri lay well down in the pelvic region and could only be palpated with difficulty. In a small proportion of females, it was found impossible to palpate more than the anterior end of the body of the uterus.

Utero-ovarian changes during pregnancy

Pregnancy was often suspected at 15 to 20 days after conception but was rarely confirmed before 25 to 30 days. The first detectable sign was a change in shape of the uterus from a club-shaped structure to a firm spherical organ. A slight size increase was noted and, by 30 days, the uterus was normally 6 mm in diameter. An increase in size of one or both ovaries was often detected at this stage. The belief that this reflected the developing CL of pregnancy was confirmed in several animals by exploratory laparotomy. The ovaries, together with their maturing CL, often measured as much as 10 to 12 mm × 6 mm.

At about 45 days of pregnancy, the uterus was 8 mm in diameter and extremely round and firm in texture. It gradually increased in size to 13 mm at about 70 days of pregnancy. The uterine diameter did not appear to be related to the number of embryos up to this stage of pregnancy. In this series of seventy-four pregnancies, there were eleven sets of triplets, fifty-three twins and ten singletons. As the uterus increased in size beyond this point, it became soft and difficult to measure. Between 90 and 100 days, the uterus was virtually impal-

pable and could easily be confused with that of a non-pregnant animal (Table 1). Fetal masses then became palpable, although individual structures could not be distinguished. At this stage, the uterus underwent a transient period of apparent positive pressure when its outline could again be distinguished. This texture was lost when fetal heads were first palpated. These measured 8 to 10 mm in width at approximately 120 days. Fetal head measurements gradually increased to around 20 mm just before parturition. Parturition was rarely observed until a head measurement of 16 mm was reached (Table 1). The palpation records of the four dated conceptions correlated closely with the estimates based on the retrospective dating of pregnancies.

Table 1. Palpable uterine features in marmosets throughout pregnancy

<i>Uterine size (mm)</i>	<i>No. of records</i>	<i>Mean day of gestation* ± S.D. (range)</i>
6	17	30.3 ± 13.0 (10-49)
7	10	41.7 ± 9.47 (22-54)
8	16	46.1 ± 12.55 (25-69)
9	5	56.2 ± 7.14 (46-68)
10	13	60.5 ± 7.87 (46-73)
11	6	58.8 ± 7.31 (51-73)
12	7	64.4 ± 8.05 (55-75)
13	7	67.6 ± 5.1 (62-77)
14	6	74.8 ± 7.34 (61-83)
15	9	78.6 ± 6.15 (69-90)
16	3	88.3 ± 1.70 (86-90)
Uterus too soft to measure	9	97.7 ± 6.48 (90-112)
Fetal masses palpable	25	98.6 ± 8.65 (84-117)
Positive pressure present	8	109.6 ± 5.75 (101-117)
Positive pressure lost	5	121.8 ± 9.04 (108-130)
Fetal headsize (mm)		
8 to 10	8	119.6 ± 13.16 (98-137)
11 to 12	14	123.5 ± 7.03 (111-137)
13 to 14	28	131.5 ± 12.02 (110-150)
15 to 16	14	139.2 ± 8.30 (126-150)
17 to 18	10	137.8 ± 4.74 (130-150)
19 to 20	11	140.5 ± 6.15 (130-150)
20+	5	144.4 ± 1.36 (143-150)

* Counting retrospectively from day of parturition = Day 150.

Utero-ovarian changes after parturition

The uterine and ovarian changes were studied in detail in thirteen females for up to 6 weeks after parturition. Immediately after parturition, the uterus was found to be extremely soft, irregular in shape and approximately 12 mm in diameter. After 24 hr, the diameter had usually regressed to 7 to 8 mm. The uterus retained its softness for a further 4 to 5 days. The ovaries were usually firm and 3 to 4 mm in diameter. Vaginal bleeding usually continued for 2 to 3 days after parturition but was rarely observed beyond this. Regular examination of daily smears of vaginal cytology for the presence of spermatozoa has shown that the marmoset may mate as early as 3 days after parturition and

regularly throughout the postnatal period. Six females have shown a palpable increase in ovarian size, and a change in texture from firm to soft, at a variable period after parturition. Pregnancies subsequently developed in three of these animals (Table 2).

Table 2. Palpable changes in the ovaries of marmosets after parturition

Female no.	Increase in ovarian size	Increase in ovarian softness	No. of days post partum	Outcome
48	+	+	12	—
34	+	+	10	Pregnancy
51	+	+	19	—
1	+	+	8 to 10	—
3	+	—	17	Pregnancy
19	+	—	9	Pregnancy

Other features

The presentation of the fetuses could be recorded from around 120 days. In this series, 80% of fetuses were in anterior presentation and 20% in posterior (breech) presentation. There did not appear to be any fixed time at which fetuses were more likely to be in posterior presentation. Posterior presentation was observed in some individuals up to 2 days before parturition.

A number of other features were observed which might aid prediction of imminent parturition, particularly in undated pregnancies. Softening was observed in the pelvic ligaments of *C. jacchus* 3 to 4 weeks before parturition but was not considered a sufficiently accurate guide to the parturition data. Softening of the cervix was observed in the marmoset but the timing of this event varied from 4 weeks to 3 days before parturition and was not a reliable indicator. Enlargement of nipples was of little value as an indicator since many of the multiparous females were lactating for the major part of the new pregnancy. A grey secretion could be expressed from the nipples less than 1 week before parturition in a few individuals and was a good indication of impending birth. In two females, engagement of the fetal head within the pelvis was noted 24 hr before parturition. The colour of the vulval mucosa was observed to change from pink to deep purple during the last 4 weeks of gestation. Increased vulval swelling and flaccidity was also noted during this period. Neither of these features could be accurately correlated with impending parturition.

DISCUSSION

Rectal palpation of the uterus and ovaries is well recognized as a method of determining maturity, ovulation and pregnancy in macaques and baboons. These features may be detected in *C. jacchus* by means of transabdominal palpation. In the early stages of pregnancies of unknown conception date, the embryonic age may be estimated to within 15 days. The accuracy is improved to within 10 days once fetal structures become palpable.

A fetal head measurement of more than 16 mm and signs of lactation are the most reliable indicators of imminent parturition in marmosets. In the cow, the

caudal edges of the sacro-sciatic and sacro-iliac ligaments soften and become impalpable 24 to 48 hr before parturition (Arthur, 1964). Mahoney (1969) observed similar changes in the pelvic ligaments of *Macaca irus* about 6 weeks before term but concluded they were an unreliable guide to impending birth. Softening of the cervix uteri was, however, helpful in determining parturition in that species. Neither of these features appear to be of great value in *C. jacchus*. Changes in the size and texture of the ovaries shortly after parturition may be correlated with follicle formation and a post-partum oestrus in *C. jacchus*.

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