Pregnancy rate in timed-mated, spontaneously cyclic rhesus monkeys, *Macaca mulatta*

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Summary. After detection of the preovulatory oestradiol-17 β peak in blood, females were placed with a male and left undisturbed during mating (N = 28, 94 cycles) or were exposed to serial diagnostic laparoscopy (1–4 occasions) to monitor ovulation (N = 87, 224 cycles). A total of 86 pregnancies resulted from timed mating in the 318 menstrual cycles (27% rate). When periovarian laparoscopy was performed, the pregnancy rate was 22.3%. In the absence of laparoscopy the pregnancy rate was 38.3% ($P < 0.003$). These pregnancy rates are similar to those of women and show an adverse effect of laparoscopy on pregnancy rate.

Introduction

Nonhuman primates are widely used as a basis for studies of human reproduction. Despite this, the baseline reproductive efficiency of nonhuman primates, as reflected by the fertilization rate, implantation rate, and rates of pre- and post-implantation pregnancy wastage, is unknown. We have therefore determined the pregnancy rate in our colony of rhesus monkeys.

Accurate documentation of the occurrence and time of ovulation is often necessary for reproductive studies. In primates, ovulation occurs spontaneously with few overt external biological signs or behavioural manifestations. Indirect criteria used to detect ovulation in nonhuman primates include rectal palpation of the ovaries (Hartman, 1932; Mahoney, 1970), sequential changes in vaginal cytology (Hutchinson, 1970; Mahoney, 1970; Mauro *et al.*, 1970) and in cervical mucus composition (David & Mastroianni, 1968; Mahoney, 1970; Ovadia *et al.*, 1971), body temperature (Hendrickx *et al.*, 1967), and sex skin colour changes (Fujiwara *et al.*, 1973). The variation in such changes, the fact that they are often obvious only in retrospect, and the variability between observers in interpreting them limit their value in accurately detecting and timing the occurrence of ovulation and render them impractical for scheduling matings.

The pre- and periovarian ovarian steroid profile, especially the preovulatory rise in oestradiol-17 β in blood, offers an objective and fairly accurate means of predicting impending ovulation. In the rhesus monkey, the interval between the occurrence of the preovulatory oestrogen peak and ovulation, a period of 30–40 h (Weick *et al.*, 1973; Pauerstein *et al.*, 1978), and the approximately 3-day duration of the gradual preovulatory rise in oestradiol-17 β that precedes the peak (Hotchkiss *et al.*, 1971) allow mating or artificial insemination to be timed to the fertile peri-ovulatory period (Parkin & Hendrickx, 1975).

If serial diagnostic laparoscopic examination of the ovaries is initiated after detection of the preovulatory oestradiol-17 β peak, it is possible to confirm ovulation visually and to time it within hours (Eddy *et al.*, 1975; Pauerstein *et al.*, 1978; Eddy, 1980). We often utilize serial diagnostic laparoscopy to time ovulation and to schedule mating, but because diagnostic laparoscopy involves anaesthesia, invasion of the peritoneal cavity, and manipulation of the reproductive tract, it may adversely affect reproductive function. To examine this possibility, we compared the pregnancy rates in animals in which mating was scheduled solely on the basis of the preovulatory oestrogen

peak with those in animals in which serial laparoscopic examinations of the ovaries were performed during the periovulatory period.

Materials and Methods

Animals

Sexually mature, regularly cyclic female rhesus monkeys (*Macaca mulatta*) were mated to 8 proven fertile male rhesus monkeys during the period August 1978 to November 1985. The 104 females were caged individually and maintained at a temperature of 24°C with 50% relative humidity. All animals were exposed to a 14 h light:10 h dark cycle (lights on at 06:00 h). Purina Monkey Chow was supplied twice daily and was supplemented by fresh fruit and sweet potatoes twice weekly. A chewable multivitamin tablet was given each day. Tap water was available *ad libitum*. All females underwent daily vaginal swabbing with a cotton-tipped wooden swab to detect menstrual bleeding. The first day of menstrual bleeding was designated Day 1 of the cycle. Menstrual calendars were maintained for all females.

Timing of mating

Beginning on Day 8, peripheral blood samples (1.0–3.0 ml) were taken daily between 08:00 and 09:00 h from the saphenous vein after tranquilizing the monkey with ketamine hydrochloride (Vetalar: Parke-Davis, Detroit, MI; 1–5 mg/kg, i.m.). The serum was assayed daily for oestradiol-17 β by radioimmunoassay following extraction of serum samples (100 μ l) with 3.0 ml hexane:ethyl acetate (9:1 v/v). Labelled oestradiol (New England Nuclear, Boston, MA), 200 pg/ml, containing bovine gamma globulin (0.1%) was added to each tube. An antiserum to oestradiol-17 β , with no significant cross-reaction with any other circulating oestrogens, was used (100 μ l) at a dilution of 1:100 of stock. Tubes were incubated for 2 h at room temperature and the bound hormone was precipitated with 200 μ l saturated ammonium sulphate. The supernatant (200 μ l) containing unbound hormone was aspirated and mixed with 2.5 ml scintillation fluid (4 g PPO/litre toluene with 2% methanol). Samples were then counted to 2% error. A 7-point standard curve was prepared by serially diluting 200 pg oestradiol-17 β (Sigma, St Louis, MO). Assay sensitivity was 3 pg per tube. Intra- and interassay coefficients of variation at 70% of maximum binding were approximately 8% and 12%, respectively (Pauerstein *et al.*, 1978).

By measuring peripheral oestradiol-17 β concentrations daily on the same day the blood sample was withdrawn, the progressive rise in this hormone was determined, together with identification of the surge that preceded ovulation. Oestradiol-17 β values in excess of 300 pg/ml signalled impending ovulation. When a preovulatory oestradiol peak was detected, the animals were divided into two groups. In one group, oestradiol measurement alone was used to schedule mating, whereas in the other serial diagnostic laparoscopies were also performed. Cycles with a low oestrogen profile in which a definitive oestrogen peak was not detected were considered anovulatory and were excluded.

Laparoscopy

In animals monitored laparoscopically, anaesthesia was induced with ketamine hydrochloride (5–15 mg/kg, i.m.). The abdomen was shaved, scrubbed with povidone iodine, and covered with sterile drapes. Using sterile techniques, a pneumoperitoneum was induced with CO₂ via a Verres needle which entered the abdominal cavity at the umbilicus and was connected to a Wolf insufflator. The Verres needle was then withdrawn and a 6 mm diameter Wolf paediatric end-view diagnostic laparoscope was inserted into the peritoneal cavity at the level of the umbilicus. A

second puncture was made in the lower right quadrant of the abdomen with the Verres needle, the blunt end of which was then used as a manipulating probe. The entire surfaces of both ovaries were examined and the ovary containing the mature follicle was identified. This ovary invariably appeared as an enlarged, spherical, opaque, fluid-filled, tense structure about 1.5–2 times the size of the contralateral non-ovulatory ovary, which had a more dense, elongated, dorso-ventrally flattened appearance (Betteridge *et al.*, 1970; Eddy *et al.*, 1975). Care was taken to avoid damage to the pelvic viscera and uterine adnexa, particularly the fimbria. After detection of the preovulatory follicle, the laparoscopy was terminated and the animal allowed to recover from the anaesthesia. Cycles in which serial laparoscopy demonstrated follicular regression or persistence of the mature follicle without ovulation were excluded.

Mating

Animals in which the presence of a preovulatory ovarian follicle was documented laparoscopically, or in which the preovulatory surge in oestradiol-17 β had been detected by RIA without laparoscopic visualization of the ovaries, were placed with a male monkey for breeding.

In animals undergoing serial diagnostic laparoscopy, the female was removed from the male's cage at various intervals and re-examined laparoscopically. If ovulation had not occurred, the female was returned to the male until ovulation had been laparoscopically documented. Animals underwent a mean of 2.33 ± 0.04 laparoscopies (range 1–4) and remained with the male 2.85 ± 0.09 days (range 1–7) for mating. In animals not undergoing laparoscopy, the male and female remained together a mean of 3.02 ± 0.09 days (range 1–5 days) and were then separated.

Pregnancy diagnosis

Pregnancy was documented by laparoscopic visualization of an enlarged uterus, by ultrasound examination, or by physical diagnosis 6–8 weeks after mating. Only animals in which pregnancy was unequivocally diagnosed were considered pregnant.

Statistical analysis

Differences in pregnancy rates between females mated with or without diagnostic laparoscopy were tested by χ^2 analysis and the Fisher exact probability test. Arithmetic means were expressed with $\pm$ s.e.m. Differences between means were tested by the paired *t* test. A value of $P < 0.05$ was considered significant.

Results

The 104 females were mated during 318 spontaneous menstrual cycles, resulting in a total of 86 pregnancies (27% pregnancy rate). Of this total, 32 pregnancies progressed to term and

Table 1. Pregnancy rate in rhesus monkeys in which ovulation was diagnosed by serial laparoscopy (Group I) or by increased oestradiol-17 β concentrations in serum (Group II)

Group/Subgroup	No. of females	No. of cycles	No. of pregnancies	Pregnancy rate (%)	Significance
Group I	87	224	50	22.3	
Group II	28	94	36	38.3	$P < 0.003$
Subgroup Ia (proven fertile)	26	52	15	28.8	
Subgroup IIa (proven fertile)	20	64	28	43.7	$P < 0.098$
Subgroup Ib (breeding pairs)	8	12	3	25.0	
Subgroup IIb (breeding pairs)	9	10	6	60.0	$P < 0.109$

spontaneous vaginal delivery; 34 underwent Caesarian section at term; 13 aborted after fetal cannulation or maternal administration of tetrahydrocannabinol, luteinizing hormone-releasing hormone analogue or prostaglandin F-2 α analogue; and 7 spontaneously aborted or delivered stillbirths.

The pregnancy results are shown in Table 1.

Discussion

This study supports the usefulness of the preovulatory oestrogen peak as an indicator of impending ovulation and a means with which to schedule timed mating during the periovulatory period in the rhesus monkey (Parkin & Hendrickx, 1975). The overall pregnancy rate of 27% (38.3% in the absence of laparoscopy) is comparable to the 30% rate calculated for women (Barrett & Marshall, 1969) and supports the usefulness of this nonhuman primate as an animal model in which to acquire baseline fertility data pertinent to the development of techniques or strategies to manipulate reproductive outcome in the human. Diagnostic laparoscopy, although allowing accurate documentation and timing of ovulation, adversely affected fertility. Although statistical significance declined as the size of the subgroups decreased, arithmetically and clinically the contrast became more apparent in progressively more fertile subgroups. The biggest difference in pregnancy rate occurred among the most fertile groups, females of documented fertility mated again with males that had previously impregnated them (25% and 60% pregnancy rates with and without laparoscopy, respectively).

Based on 12-h serial measurements of peripheral oestradiol-17 β and timed breeding periods of 30 min or less, a fertile period extending from the preovulatory oestrogen peak to 24 h later has been identified in rhesus and bonnet monkeys (*M. radiata*). Matings before or after this period do not result in pregnancy (Parkin & Hendrickx, 1975). Similar findings have been reported for the crab-eating macaque (*M. fascicularis*) (Dukelow & Brüggemann, 1979). In our study, females that underwent laparoscopy and those that did not were placed with the male at the same time relative to the preovulatory rise in oestradiol-17 β and remained with the male for similar periods (2.85 and 3.02 days, respectively) which included the alleged 24-h fertile period.

Although an adverse effect of laparoscopy is evident, the mechanism of this effect remains obscure as does the component of the procedure responsible for the effect. Laparoscopy could theoretically affect fertility adversely by altering a variety of reproductive functions from the preovulatory to the post-implantation period. Laparoscopy could adversely affect the quality of the preovulatory oocyte. We cannot rule out this possibility, but it is unlikely because laparoscopic monitoring of follicular development in response to ovarian stimulation by exogenous gonadotrophin followed by follicular aspiration does not preclude a high rate of fertilization and cleavage *in vitro*, or of pregnancy after embryo transfer (Balmaceda *et al.*, 1984). Laparoscopy can, however, delay or block ovulation. Ovulation failed to occur in 75 cycles despite the presence of a preovulatory oestradiol-17 β peak and laparoscopic observation of an enlarged stimulated ovary containing a well-defined preovulatory follicle. In this study, cycles monitored by laparoscopy were included only if ovulation was documented. Laparoscopy-induced inhibition of ovulation was therefore not a factor in the reduced pregnancy rates seen. It is possible that a proportion of those animals not laparoscoped also failed to ovulate despite the occurrence of a preovulatory oestradiol-17 β peak. Were this the case, the 'natural' pregnancy rate would be higher than calculated, thus further increasing the difference between groups.

Laparoscopy did not shorten the luteal phase. We compared luteal phase length in laparoscopically monitored cycles to those in which laparoscopy was not performed. Because laparoscopic timing of ovulation was not performed in both groups, we measured the interval from the oestradiol-17 β peak to the first day of menstruation. Among 74 cycles monitored laparoscopically,

this interval was 15.86 ± 0.37 days (range 5–23 days). In 43 cycles in which laparoscopy was not performed, the interval was 15.56 ± 0.68 days (range 5–27 days).

Because laparoscopy to document ovulation entails manipulation of the reproductive tract with displacement of the fimbriae from the surface of the ovary, ovum pick-up by the oviduct may have been adversely affected. This has been considered possibly responsible for the low ovum recovery seen in ovum-transport studies in the crab-eating macaque (Jainudeen & Hafez, 1973) and may have contributed to the low recovery rates in other studies of ovum transport in the rhesus monkey (Eddy *et al.*, 1975) and the baboon (Eddy *et al.*, 1976).

The possibility that laparoscopy may have adversely affected fertilization and gamete transport is considered unlikely, since animals are able to conceive despite repeated laparoscopies performed during the periovulatory period (Jewett & Dukelow, 1972; Rawson & Dukelow, 1973; Mahone & Dukelow, 1978). Nevertheless, this possible effect of laparoscopy cannot be ruled out.

Finally, laparoscopy may have affected implantation. Such an effect is impossible to detect because the baseline fertilization rate, implantation rate, and rate of embryo wastage are not known and were not determined in the present study. The stillbirth and abortion rates that we noted are within the range reported under normal conditions for rhesus monkeys in a stable breeding colony (Van Wagenen, 1972).

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