—Mini Review—

Reproductive Hormones and the Ovarian Cycle in Macaques

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Abstract: *Nonhuman primates, in particular macaques, demonstrate marked similarities to humans in almost all aspects of their anatomy, endocrinology, and physiology. These similarities underlie the value of these primates for studies across a broad range of disciplines. In reproductive biology, nonhuman primates have been used as models to study the mechanisms and processes associated with fertility, infertility, pregnancy, and parturition. This review provides an overview of reproductive studies for which commonly used female macaque species such as rhesus (Macaca mulatta), long-tailed (M. fascicularis), and Japanese macaques (M. fuscata) are appropriate subjects, and a summary of the advantages and problems of using nonhuman primates in such research are described.*

Key words: *Estradiol-17β, Progesterone, LH, FSH, CG, Macaque*

Introduction

Because of their close phylogenetic relation to humans, higher primates have attracted considerable interest in a wide range of research areas. They demonstrate marked similarities to humans in almost all aspects of their anatomy, endocrinology, and physiology. These similarities in the biological mechanisms of humans and nonhuman primates underlie the value of these animals for research across a broad range of disciplines. Especially studies on the reproductive biology of nonhuman primates have been increasing in the last several decades.

This review provides an overview of the reproductive hormones profile and ovarian cycle information available for the more commonly used female macaque species, the Japanese macaque, the rhesus macaque, and the long-tailed macaque, which represent large-, medium-, and small-sized macaques, respectively. In addition, the advantages and problems of using nonhuman primates in biomedical research are described.

Received: August 1, 2008
Accepted: September 4, 2008
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Comparison of Steroid Hormones Levels Across Species

During female reproductive cycle

1. Menstrual cycles

Many of the macaque species show menstrual bleeding and have a menstrual cycle. The cycle lengths are regularized at about one-month intervals, which approximate the length seen in women. Based on intermenstrual intervals, the cycle lengths have been reported as 28.7 to 29.4 days in women [1]. The luteal phase is usually consistent and approximately 15 days in length [2]. The follicular phase is generally more variable. The cycle lengths of the three species of macaques are similar to those of the humans, with ovulation occurring during days 12 to 15 of the menstrual cycle. Average cycle lengths for the rhesus macaque range from 25.5 to 29.5 days [3, 4], 28 to 32 days for the long-tailed macaque [5] and 26 to 31 days for the Japanese macaque [6, 7].

It should be noted that the Japanese macaque is unique among higher primates in that it shows a distinct seasonal pattern of breeding activity both in outdoor and indoor environments [7, 8]. Autumn to winter is its breeding season and infertility persists during summer. In the non-breeding season, ovarian folliculogenesis is impaired and anovulatory cycles persist throughout the season, which was characterized by low levels of estrogen and progesterone. The incidence of menstrual bleeding is largely different between the indoor and outdoor groups. Menstruation in outdoor groups tends to cease completely throughout the non-breeding season. On the other hand, menstruation in the indoor

group is frequently observed even in the non-breeding season in Japanese macaques. The rhesus macaque also shows seasonal breeding, however, the long-tailed macaque shows reproduction which is unaffected by season. After menarche, normal ovulatory menstrual cycles persist throughout the year in long-tailed macaques. The gonadotropins and sex steroids showed typical patterns [9] and menstrual bleeding is observed at about one-month intervals.

2. Profiles of steroid hormones

The two most important steroid hormones of females are estradiol and progesterone and concentrations of these hormones have been measured throughout the cycle for a variety of macaques.

During the early follicular phase, estradiol concentrations of 50–100 pg/ml increase to 150–200 pg/ml. Then, they peak at concentrations of approximately 350 pg/ml. Based on one-a-day sampling, it was reported that estradiol and LH reach peak concentrations in the rhesus macaque on the same day [10], unlike in the human in which estradiol peaks on the day before the LH peak. However, the peak in circulating estradiol levels in the rhesus macaque is actually reached 9–15 hours before the LH peak and at least 36 hours prior to ovulation [11]. Also, unlike in the human, a secondary rise in estradiol during the luteal phase is not routinely observed in the rhesus female. Luteal phase estradiol concentrations of <60 pg/ml have been reported, but, other than the secondary increase during the luteal phase, estradiol concentrations in the rhesus macaque appear to be secreted throughout the menstrual cycle in a manner very similar to that in the human.

The other macaque species appear to secrete estradiol in a very similar fashion to the rhesus macaque. The long-tailed macaque has follicular phase estradiol concentrations of 50–150 pg/ml, which rapidly increase to a midcycle peak [12]. The estradiol peak in the long-tailed macaque occurs one day before the LH peak, which is similar to the cycle of the rhesus macaque [12]. The cyclical hormonal changes throughout the menstrual cycle in Japanese macaques are shown in Fig. 1. In the Japanese macaque, the secretion patterns of estradiol throughout the menstrual cycle suggest that daily concentrations are similar to the cycle of rhesus macaques. Concentrations of estradiol are low until day -4, and then rise to a preovulatory peak on day -1. After the peak, they decline rapidly to a nadir on day 3. The secondary increase during the luteal phase is not observed in the Japanese macaque.

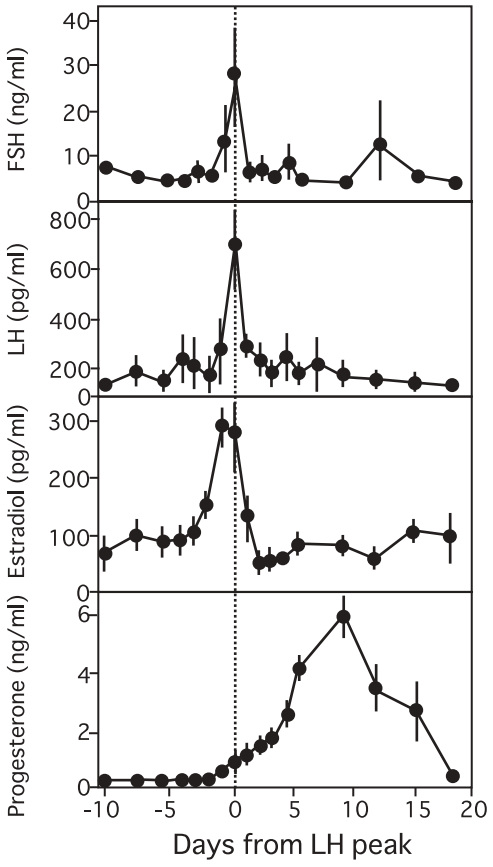

Fig. 1. Concentrations of serum follicle stimulating hormone (FSH), luteinizing hormone (LH), estradiol-17β, and progesterone in female Japanese macaques during the menstrual cycle (mean ± SEM).
Vertical dotted line is the day of the LH peak. The data are normalized to the day of the LH peak (day 0).

The duration of the luteal phase in the rhesus macaque is similar to that of women and is very consistent in length, 14–17 days [14]. Progesterone is the major gonadal steroid secreted throughout the luteal phase. During the follicular phase, minimal progesterone levels (<0.5 ng/ml) are found in the peripheral circulation. About 1 or 2 days before ovulation occurs, progesterone concentrations begin to increase. Maximum concentrations of 4–6 ng/ml are reached on about the 15th day of the cycle and remain relatively constant for approximately 7 days before gradually declining to follicular phase levels before menstruation. The secretion pattern for progesterone during the menstrual cycle of the rhesus macaque is nearly identical to that of the human, but quantitatively

the circulating concentrations in the human are 3–4 times higher [15, 16].

In the long-tailed macaque, slightly higher progesterone peaks are attained during the luteal phase [17]. Peak values of 7–10 ng/ml are reported to be attained about the middle of the 15- to 16-day luteal phase. These peaks are reached approximately one week after the estradiol and LH peaks. Progesterone concentrations are also low (<1.0 ng/ml) during the follicular phase of the Japanese macaque. As in other species, the initial rise in progesterone follows the rise in LH and reaches peak concentrations of about 5.0 ng/ml in 9–10 days [6].

3. Profiles of Gonadotropins

In the rhesus macaque, menstrual cycle patterns of gonadotropins closely resemble those observed in the human [18]. Briefly, during the follicular phase, blood levels of FSH and LH are approximately equivalent. Then at days 12–14 LH rapidly elevates to a very large peak or surge on the day of ovulation; blood levels of FSH also increase at ovulation. Throughout the luteal phase the levels of FSH and LH are low. The long-tailed macaque exhibits a 1-day elevation in serum LH between days 10 and 13 of the menstrual cycle [19]. Patterns of FSH are comparable to those of the rhesus macaque and the human [20]. Like other macaques, menstrual cycle patterns of FSH and LH in the Japanese macaque are very similar to the human. During anovulatory cycles, LH secretion is absent.

During pregnancy

1. Implantation bleeding

Implantation bleeding has been observed in the rhesus, long-tailed [18], and Japanese macaques [Shimizu, unpublished]. The occurrence of vaginal hemorrhage at approximately the same time that normal menstruation would appear negates its usefulness as a reliable indication of pregnancy. Seventy-six percent of rhesus macaques showed implantation bleeding in contrast to 93% of long-tailed macaques [18]. The onset of vaginal hemorrhage occurs on gestational day (GD, days after estimated day of conception) 19.2 ± 0.8 in long-tailed, and GD 20.2 ± 0.2 in rhesus macaques. The duration of the bleeding is considerably shorter in long-tailed macaques [18]. Implantation bleeding also occurs in the Japanese macaque and the duration is slightly longer than that of long-tailed macaques [Shimizu, unpublished].

2. Profiles of steroid hormones

In the rhesus macaque, progesterone fluctuates between 3 and 4 ng/ml during the second and third trimesters, which is similar to the concentrations detected during the luteal phase of the menstrual cycle [21]. During human pregnancy, large quantities of estriol are produced by the placenta, which is due to the presence of the 16α hydroxylase enzyme in the fetus. This is in direct contrast to macaques, in which little or no estriol is produced and estradiol is the major circulating estrogen [22]. Increasing concentrations of serum estrone and estradiol occur in the rhesus macaque with advancing gestational age [3]. Studies of the long-tailed macaque indicate that serum levels of chorionic gonadotropin (CG) and estradiol are similar to the rhesus macaque [23]. In contrast to other authors who found a progesterone peak approximately coinciding with the CG peak in the rhesus macaque [24] and the long-tailed macaque [23], Hein *et al.* reported a progesterone peak preceding the CG peak [25].

Hormone profiles of pregnant Japanese macaques are also similar to other macaques. Briefly, serum estradiol-17β increases during the late luteal phase of a fertile cycle, concomitantly with serum CG. A subsequent decline to a nadir in serum estradiol-17β is followed by a progressive increase until term. Serum progesterone is maintained at about 2.5 ng/ml during the period from GD 60-135, followed by a progressive rise, with a peak at 9.4 ± 2.3 days before delivery. After parturition, serum estrogen and progesterone decreased to a nadir.

3. Profiles of Gonadotropins

The predominant circulating gonadotropin of primate pregnancy, CG, originates in the placenta. Human chorionic gonadotropin (hCG) is secreted by the synctiotrophoblastic layer of the placenta [26] and is detectable within 9 days of the midcycle peak of LH [27]. Whereas hCG shows a steady increase to reach levels (80–100 IU/ml) by GD 90, and remains elevated at somewhat reduced levels for the remainder of gestation, in nonhuman primates CG increases early in pregnancy and then declines to undetectable levels [28]. Because of the high levels of hCG during pregnancy and its chemical similarities to LH, circulating levels of LH are difficult to distinguish during this period. However, FSH and LH levels fall to undetectable values during human pregnancy and LH is not an essential luteotropin of pregnancy in rhesus monkeys [29].

Chorionic gonadotropin is first detected in the peripheral blood of primates almost simultaneously with the beginning of implantation [30]. Although CG

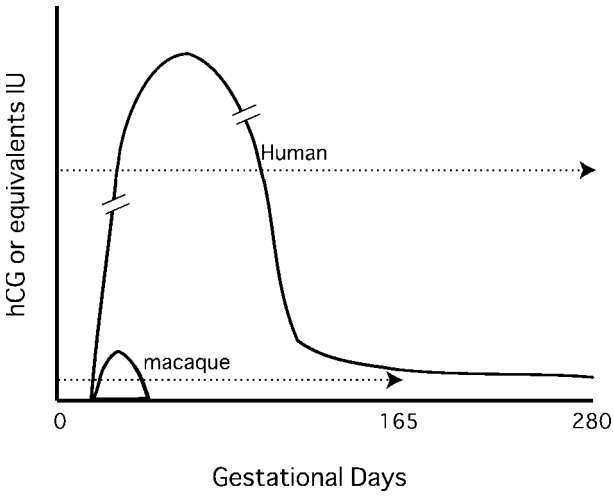

Fig. 2. Schematic patterns of chorionic gonadotropin excreted during gestation in the human and macaques.
The dotted lines show duration of pregnancy. The IU equivalents are standardized to international standard human chorionic gonadotropin [29].

secretion is a uniform characteristic of pregnancy among primates, the pattern of CG secretion among these primates is highly variable [31]. The peak concentrations of CG in macaques are about 100 to 1,000 times less than in women. Three basic patterns of CG secretion are known. Although, in all primates, CG is first detected at the time of, or soon after, implantation, among humans it is present at maximal levels in the first trimester and is present continuously throughout gestation. However, in macaques, CG levels decline to near or below the limits of detection during mid- and late- pregnancy, with a rapid decline before parturition [31] (Fig. 2).

Similar to other macaques, in Japanese macaques, serum FSH decreases during the luteal phase of a fertile cycle and falls to a nadir. After day 40 of pregnancy, serum FSH is maintained at low levels throughout pregnancy. A significant rise in serum CG is seen during the period from day 14.7 ± 1.4 to day 31.8 ± 1.0 of pregnancy. Serum estradiol-17β increases during the late luteal phase of a fertile cycle, concomitantly with serum CG. It subsequently declines to a nadir, followed by a progressive increase until term.

4. Pregnancy detection

The initial detection of CG peripherally has been noted ~12 days postovulation in the rhesus macaque. In early pregnancy, transient levels of CG observed, with a peak in production on GD22-24. Urinary CG levels in the macaque remain elevated for a very short period of time. It cannot be detected in blood or urine after GD 40. This is similar to other macaques such as the long-tailed and Japanese macaques. Recently a practical, noninstrumented enzyme-linked immunosorbent assay (NELISA) for the measurement of urinary CG has been developed for the detection of early pregnancy in macaques [32]. It has been reported that the macaque CG NELISA detected pregnancy as early as GD 14, with an average earliest detection of GD 16.5 ± 1.4 in long-tailed macaques.

5. Duration of gestation period

The duration of gestation is similar in most old world monkeys. Rhesus macaques usually deliver during GD 165–170 [33]. Long-tailed macaque deliveries are reported to occur during GD 155–165 [18, 33]. For the Japanese macaque, deliveries are reported to occur during GD 160–174 [33] in indoor individual cages.

Conclusion

The increased use of nonhuman primates in biomedical research during the last several decades, particularly within the various disciplines of reproductive biology, is a reflection of our increasing awareness of the occurrence of several fundamental differences between the reproductive processes of the human and common non-primate laboratory mammals. There is a considerable lack of information on many of the basic reproductive parameters exhibited by some of the primate species that are frequently used in biomedical researches. Moreover, the differences that may occur within primate species or families are often ignored.

This review provides basic background information relating to the physiology of reproduction in nonhuman primates. It will be useful to researchers who are selecting primate species for biomedical research in reproductive biology.

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