The role of gonadal steroids in feedback regulation of gonadotropin secretion at different stages of primate development

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Abstract. The serum gonadotropin response to castration was assessed in 8 foetal, 2 neonatal, 30 juvenile, and 2 adult rhesus monkeys (M. mulatta). In the 30 castrated juvenile monkeys and 8 sham-operated controls, concentrations of oestrone, oestradiol, androstenedione, dihydrotestosterone, testosterone and 17OHP-progesterone were measured in 10 ml serum pools before, one month after, and one year after the surgical procedure. Castration during foetal life (83–137 days gestation) was followed within 48–72 h by a significant rise in serum FSH levels in males, but had no effect on the already high levels in females. Similarly, castration of males during the first post-natal month raised serum FSH and LH into the adult castrate range; however, after 3 months of age serum gonadotropin levels again declined to the normal juvenile range in spite of the open feedback loop. Orchiectomy of prepubertal juvenile monkeys (age 3 months–23/12 years) had no immediate effect on serum gonadotropins, but was followed by a delayed rise in FSH (at age 23/12–43/12 years) and LH (at age 27/12–44/12 years) to adult castrate levels. Orchiectomy of older prepubertal (by serum testosterone) or adult males resulted within a few days in a progressive and sustained rise in serum FSH and a more gradual rise in LH. Prepubertal gonadotropin regulation appeared to be sexually dimorphic, since ovariectomy in juvenile females (age 3 months–15/12 years) was followed by generally elevated, if somewhat erratic, serum FSH values, with a secondary rise in both FSH and LH levels at 2–23/12 years. In both sexes, prepubertal castration caused a significant and sustained decline in serum concentrations of oestradiol; castrated males also showed a decline in serum testosterone levels. Although prepubertal castration also caused in both sexes a slight decline in serum oestrone, and ovariectomy a decline in serum androstenedione and dihydrotestosterone, these effects were not sustained one year later, and values were not significantly different from sham-operated controls. Taken together, these data lend support to a model of primate sexual maturation in which the primary regulator of gonadotropin secretion in both sexes during the prolonged juvenile phase is central inhibition of the hypothalamic GnRH regulator. However, during foetal and neonatal life, and again following the onset of puberty, the major modulator of gonadotropin secretion becomes sex steroid-mediated feedback inhibition.

During primate sexual maturation, a characteristic triphasic and sexually dimorphic temporal pattern in circulating gonadotropin concentrations occurs, which is imposed by the interaction of two regulatory mechanisms: central inhibition of hypothalamic gonadotropin releasing hormone (GnRH) neurosecretion, and the feedback effects of sex steroids in the circulation (Winter et al. 1981; Winter 1983). Thus, at mid-gestation, gonadotropin secretion appears to be unrestrained in the female foetus, but is partially inhibited in the male. By term, gonadotropins are suppressed in both sexes, presumably by the action of placental sex steroids, since parturition is normally
followed by a rise in FSH and LH levels in the neonate. This transient neonatal surge is succeeded in primates by a juvenile period of varying length during which gonadotropin secretion is suppressed, apparently by a central inhibitory mechanism, since at this time it can only be restored by selective lesions to the brain or by pulsatile infusion of GnRH. However, even during this time gonadal steroids appear to play some role in gonadotropin regulation, since moderate elevations of serum FSH and less frequently LH may be seen in agonadal children (Winter & Faiman 1972). Finally at puberty, there is a third rise in gonadotropin secretion as this central inhibition wanes; the impact of sex steroid negative feedback becomes more visible, so that abnormal elevations of circulating gonadotropins become apparent in individuals with even partial deficiencies of gonadal endocrine function (Salbenblatt et al. 1985).

The purpose of the present study was to analyze the significance of gonadal sex steroids upon gonadotropin secretion at different stages of development through an examination of the responses to castration of foetal, neonatal, juvenile and adult monkeys (M. mulatta).

Materials and Methods

Prenatal study

Intra-uterine castration was carried out via hysterotomy under ketamine and halothane anaesthesia in 4 male and 4 female rhesus monkeys at a mean gestational age of 110 days (range 83–137 days; term is 165 days). Following surgery and replacement of the foetus within the uterus, a silastic catheter was placed in the interplacental vein running between the two placental discs, and exteriorized in order to obtain 0.5 ml foetal blood specimens before and 48 or 72 h after castration for analysis of FSH.

Neonatal study

Two male rhesus monkeys were castrated at 1 month of age. Brachial venous blood samples (1 ml) were obtained weekly under manual restraint on two occasions before castration and weekly for 23 weeks after surgery for analysis of serum FSH and LH.

Juvenile study

Castration was carried out in 14 male rhesus monkeys (aged 3/12–29/12 years) and 16 female monkeys (aged 4/12–29/12 years). In addition, sham-castration, during which the gonads were exposed and then replaced, was carried out in 4 male (age 13/12–19/12 years) and 4 female (age 1–2 years) monkeys. In each animal, a sample (15–20 ml) for steroid assyas was obtained by pooling serum from four weekly venipunctures before surgery; a second pool was obtained in similar fashion during the month following surgery, and a third pool was obtained one year later. Blood samples for FSH and LH determination (2 ml) were drawn at 09.00 h by brachial venipuncture under light ketamine sedation; these specimens were obtained weekly for 2 months before and 3 months after surgery, and then every 2 weeks for 2–3 years, or until stable serum FSH values in the adult castrate range were observed. All sera were stored at −20°C. After weaning, animals were maintained in individual cages. All received iron supplements, and none became anaemic. Weight gain and health were considered to be satisfactory. Histological examination of the removed gonads showed features appropriate for the stage of development. Leydig cells were visible in the testes of the younger animals (3–6 months), but absent in the rest. All prepubertal ovaries contained primordial, primary and vesicular follicles, but no corpora lutea.

Adult study

Two adult male rhesus monkeys (age 5–6 years) were castrated. Blood samples (2 ml) for determination of FSH and 1.1H were obtained for 2 days prior to surgery and on days 1, 2, 4, 6, 8 and 39 following surgery.

Gonadotropin assays

FSH and LH serum concentrations were analyzed by heterologous RIA methods (Faiman et al. 1975). A rat FSH trace and guinea pig anti-hFSH antiserum were used in the FSH assay, while ovine LH trace and a guinea pig anti-hCG antiserum were used in the LH assay; in both, the standard was a crude monkey pituitary gonadotropin preparation LER1909-2, kindly provided by Dr L. E. Reichert, Albany Medical College. This standard contains the bioassay equivalents of 0.05 NIH-FSH-S1 units and 0.003 NIH-LH-S1 units per mg. The LH assay used in this study does not cross-react with the non-pituitary LH-like substance found in monkey serum (Peckham et al. 1977). The minimum detectable levels (0.2 ml sample) of the RIA were 0.68 mg/l for FSH and 0.8 mg/l for LH. Normal prepubertal ranges for serum FSH and LH were obtained by combining the results from the eight sham castrate animals with unpublished data from a longitudinal study of hormone levels in 15 male and 15 female monkeys from age 4 days to 5 years.

Steroid assays

Concentrations of oestrone, oestradiol, androstenedione, 5α-dihydrotestosterone, testosterone and 17-hydroxyprogesterone were measured in 10 ml pooled
serum samples to which a mixture containing 2000 cpm of each corresponding [3H]steroid was added for recovery estimation. The serum was extracted with 60 ml methylene chloride, which was dried. The residue was partitioned between 10 ml 1.0 N NaOH (oestrogens) and 20 ml 1:1 benzene/petroleum ether (other steroids).

The aqueous phase was neutralized and extracted with 70 ml ethyl ether; the extract was washed, dried, and transferred to a column containing 3 g Sephadex LH-20 (Pharmacia, Dorval, PQ), which was eluted with benzene/methanol 85:15. The fractions containing estrone (7—14 ml) and oestradiol (14—22 ml) were collected; an aliquot of each fraction was counted for [3H]-recovery estimation and the remainder dried under nitrogen prior to RIA (Winter et al. 1976).

The organic phase from the partition step was washed with 2% glacial acetic and with water, and then dried. The residue was transferred to a column containing 3 g Lipidex 5000 (Packard International, Downer’s Grove, IL) which was eluted with 95:5 hexane/chloroform. The fractions containing androstenedione (10—18 ml), dihydrotestosterone (25—45 ml) testosterone (45—60 ml) and 17OH-progesterone (60—90 ml) were collected for estimation of [3H]-recovery and RIA (Winter et al. 1976).

Results were corrected for recovery (mean ± sd) of [3H]-labelled oestrone (86.6 ± 8.3%), oestradiol (95.2 ± 7.5%), androstenedione (64.8 ± 3.8%), dihydrotestosterone (40.5 ± 4.8%), testosterone (60.8 ± 4.6%) and 17OH-progesterone (44.3 ± 3.2%). The minimum detectable limits of the RIA methods ranged from 10 to 20 pg per sample. Intra-assay coefficients of variance approximated ± 10%, while inter-assay variance ranged from ± 12.3% for oestrone to ± 21.6% for androstenedione. Differences between groups were assessed by analysis of variance. The effects of castration were evaluated using Student’s t-test for paired samples.

Results

**Foetal castration**

The responses to foetal castration are shown in Fig. 1. As expected (Clements et al. 1976), the mean (SE) basal serum FSH concentrations in the
females (11.5 ± 2.1 mg/l) were significantly (P < 0.01) higher than those in the males (3.1 ± 0.7 mg/l). Following orchectomy, serum FSH levels rose significantly (P<0.01) within 48–72 h to reach 4.9 ± 0.5 mg/l. After ovariectomy, mean serum FSH levels were 12.7 ± 2.1 mg/l, a value not significantly different from the pre-castration level.

**Neonatal castration**

In two one month-old male monkeys (Fig. 2), orchectomy elicited a similar brisk rise in serum FSH. One showed a concomitant LH response, while in the other there was no change in the

![Fig. 2](image-url)  
**Fig. 2.**  
Serum concentrations of FSH and LH (in mg LER 1909-2/l) in two neonatal male rhesus monkeys following orchectomy. The shaded area represents the normal range in intact male monkeys (Fuller et al. 1982). The vertical arrow shows the time of castration.

already somewhat high values. This gonadotropin response was not, however, sustained. After three months of age, serum FSH and LH levels rapidly declined, approaching by 4–6 months the range of age-matched intact controls.

**Juvenile castration**

In the two youngest juvenile males, whose serum testosterone concentrations were 1.6 and 2.7 nmol/l at 3 months of age, orchectomy had no discernible effect over the next 2 years on serum FSH and LH concentrations, which remained essentially within the range seen in intact or sham-operated animals (example of one in Fig. 3). In 11 older male juveniles aged 7/12–28/12 years, with serum testosterone values of 0.1–0.6 nmol/l, orchectomy had no immediate effect but later, as
they approached the age at which male puberty normally occurs in this species (Plant 1985), there was a brisk rise in gonadotropin levels to the adult castrate range (example of one in Fig. 4). The median age for this delayed FSH response was $2^{4/12}$ years (range $2^{3/12} - 4^{4/12}$ years). There did not appear to be any consistent seasonal timing to the peripubertal gonadotropin rise. In a single older juvenile male, aged $2^{9/12}$ years, the FSH response to orchiectomy occurred immediately, although a rise in serum LH to adult castrate values did not occur until 6 months later.

In juvenile females, the response to castration was quite different from that in males. Ovariectomy of two 4-month old monkeys (example of one in Fig. 5) was followed by a pattern of highly variable serum FSH values, with many well above the normal juvenile range. After age 2 years, both animals showed a further rise in serum FSH into the adult castrate range. In both females, serum LH remained normal until two years of age, and thereafter increased slightly. Of ten juvenile females ovariectomized between $6^{1/2}$ and $1^{10/12}$ years, seven showed a rise in serum FSH within 2 weeks, with a somewhat delayed LH response (example in Fig. 6). Each of these later showed a secondary gonadotropin rise to adult castrate values. The timing of the LH rise (median $2^{6/12}$ years; range $3^{3/12} - 3^{5/12}$ years) and of the secondary FSH rise (median $2^{3/12}$ years; range $1^{11/12} - 2^{10/12}$ years) was significantly earlier ($P < 0.02$) than the corresponding phenomena in the castrated males. In a single juvenile female aged 1 year the initial FSH rise was delayed for 3 months, but thereafter the pattern was similar. In two others, both aged $1^{10/12}$ years, no initial gonadotropin response was seen; rather, serum FSH and LH remained normal for 6 months, following which there was a brisk rise to adult castrate levels in a manner similar to that seen in juvenile males. The four oldest juvenile females, aged $2^{3/12} - 2^{9/12}$ years, all showed within a few weeks a brisk and sustained serum FSH response, with a somewhat delayed and variable LH response (example in Fig. 7).

None of the eight sham-operated juvenile controls (age 1–2 years) showed any significant change in mean (± SD) serum concentrations of

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**Fig. 4.**

The effect of orchiectomy on serum concentrations of FSH, LH, testosterone (T) and oestradiol (E2) in a $1^{11/12}$ year old male rhesus monkey. Details are as in the legend to Fig. 3.
Serum concentrations of FSH and LH (in mg LER 1909-2/l) in a 4 month old female rhesus monkey following ovariectomy at the time shown by the vertical arrow. The shaded areas show the normal ranges in female monkeys. The shaded bar and connected arrow to the right show the mean follicular phase and mid-cycle values in adult monkeys. Serum concentrations of testosterone (T) and oestradiol (E2) in ng/dl before and after castration and one year later are shown at the top; these may be converted to SI units by multiplying by 0.03 nmol/l and 36.71 pmol/l, respectively.

FSH (males 1.4 ± 0.6 mg/l; females 1.5 ± 0.7 mg/l) or LH (males 2.0 ± 0.9 mg/l; females 2.0 ± 0.8 mg/l) during two subsequent years of observation.

Adult castration
Both adult males showed a significant rise in serum FSH within four days after orchiectomy. As in the younger animals, the LH response was more delayed and less striking in magnitude (example in Fig. 8).

Sex steroid changes after castration
In Fig. 9, mean serum concentrations of six steroids are compared before, 1–4 weeks after, and one year after surgery in the 30 castrated and 8 sham-operated juvenile monkeys. There were no significant differences in pre-surgery values between the castrated and sham-operated groups. Although, because of the inclusion of several young (less than 6 month) animals in the male castrate group, their mean (± sE) pre-surgery serum testosterone level (0.8 ± 3 nmol/l) was higher than that of the sham-operated males (0.3 ± 0.06 nmol/l), this difference was not statistically significant. The only sex difference (P = 0.05) in steroid levels before surgery was a higher grand mean (± sE) level of oestradiol in females (25.6 ± 3.6 pmol/l) than in males (14.6 ± 3.6 pmol/l). Sham-operation had no significant effect on sex steroid concentrations one month or one year after surgery.

Orchiectomy was followed by a significant (P < 0.01) decline within a month in mean serum concentrations of oestrone, oestradiol and testosterone. One year later, mean serum oestradiol and testosterone levels were still significantly lower than before castration. However, it must be noted that there was no significant difference one month or one year after surgery between ca-
Fig. 6.
The effect of ovariectomy on serum concentrations of FSH, LH, testosterone (T) and oestradiol (E₂) in a 1½ year old female rhesus monkey. Details are as in legend to Fig. 5.

Fig. 7.
The effects of ovariectomy on serum concentrations of FSH, LH, testosterone (T) and oestradiol (E₂) in a 2½ year old female rhesus monkey. Details are as in legend to Fig. 5.
estrated and sham-operated males in the levels of any of the six steroids measured.

Ovariectomy was followed one month later by a fall in serum concentrations of oestradiol (P < 0.001), androstenedione (P < 0.01) and dihydrotestosterone (P < 0.05). However, one year later only serum oestradiol levels remained significantly lower (P < 0.001) than pre-castration values. One month after surgery, serum oestradiol concentrations (mean ± SE) in the castrate females (7.3 ± 0.7 pmol/l) were significantly lower (P < 0.01) than those in the sham-operated females (22.0 ± 5.8 pmol/l); however, one year later this difference had disappeared. For all the other steroids, there was no significant difference following surgery between ovariectomized and sham-operated juvenile females.

Discussion

During primate sexual maturation, circulating concentrations of pituitary gonadotropins vary with age and also show striking sexual dimorphism. Presumably these age- and sex-specific patterns (Winter et al. 1981; Winter 1983) reflect developmental changes in the relative contributions of two interacting mechanisms for gonadotropin regulation, central inhibition of hypothalamic GnRH release and feedback inhibition by sex steroids acting upon the hypothalamus and/or pituitary. Similar maturational phenomena undoubtedly also occur in rodents and domestic animals (Foster 1980), although some stages, such as the juvenile phase between infancy and puberty, may be so shortened as to become insignificant.

In both human (Clements et al. 1976) and monkey (Ellinwood, & Resko 1980) foetuses at mid-gestation, serum gonadotropin concentrations are unequivocally higher in females than in males. This sex difference is thought to reflect the operation of feedback inhibition of gonadotropin secretion by steroids such as testosterone that are secreted in large amounts by the foetal testis at this time. The present data support this notion of feedback inhibition by some testicular secretion product, since orchietomy elicited an immediate gonadotropin response in males, but ovariectomy had no effect on the already high serum FSH values of females. A similar sex difference in the serum LH response to foetal castration has been reported by Ellinwood et al. (1982). These workers have recently demonstrated (Resko & Ellinwood 1985) that implantation of crystalline testosterone or dihydrotestosterone was sufficient to prevent the post-castration gonadotropin rise in foetal male monkeys.

Shortly after parturition, separation from the inhibitory effects of placental sex steroids initiates in both sexes a neonatal surge in gonadotropin secretion (Winter et al. 1981). In both monkey (Fuller et al. 1982) and human (Winter et al. 1975) neonates, the magnitude and duration of the rise in serum FSH is much greater in females, presumably also the result of feedback inhibition in males at this time by testicular steroids. The present data confirm previous reports (Plant 1980) that neonatal orchietomy elicits an immediate rise in gonadotropin secretion in a manner similar to that seen in sexually mature animals. However, these
Mean serum concentrations (± SE) of oestrone, oestradiol, androstenedione, dihydrotestosterone, testosterone and 17OH-progesterone in sham-operated and castrated juvenile rhesus monkeys before, immediately after and one year after surgery. The asterisk indicates a significant difference (P < 0.01) from pre-surgical levels. Values of oestrone and oestradiol may be converted to SI units by multiplying by 36.71 pmol/l; the multiplier for the other steroids is 0.03 nmol/l.

Fig. 9.

high gonadotropin values are not sustained in the castrate neonate, but decline to the normal juvenile range by about 6 months of age, a process which presumably reflects post-natal maturation of central mechanisms for inhibition of the hypothalamic GnRH pulse generator. It is of interest that the peak values and patterns of serum FSH in these castrate male infants are similar to those we have observed in intact female infants (Fuller et al. 1982).

We have termed the period between infancy and puberty the juvenile phase. The effectiveness of central inhibition of GnRH release in the male juvenile monkey is demonstrated by the lack of any immediate gonadotropin response to orchietomy. The fall in serum testosterone levels after castration in this group reflects the inclusion of several younger animals (under 6 months of age) whose serum testosterone values were distinctly higher (Robinson & Bridson 1978) than those observed after 6 months of age. Indeed, through most of the juvenile period, serum testosterone
and oestradiol concentrations were indistinguishable in castrate and intact male monkeys, a further testimony to the efficacy of central inhibition of gonadotropin release. During this period male monkeys become refractory to a single bolus of GnRH (Monroe et al. 1983), although responsiveness could presumably be restored by prolonged pulsatile GnRH infusion. In this regard, the male rhesus monkey appears to be at least quantitatively different from man, since prepubertal children remain responsive to GnRH administration, at least in terms of FSH release, and one can usually observe values of serum FSH above the normal prepubertal range in children with congenital or traumatic anorchia (Winter & Faiman 1972). Furthermore, in boys there is evidence for continued low-level secretion of sex steroids throughout the juvenile period (Forti et al. 1981). Therefore, it seems likely, in the human male, that central inhibition of gonadotropin release before puberty may be supplemented by some degree of steroid-mediated feedback inhibition. In the male monkey, the latter mechanism appears to be relatively non-operative through the juvenile period or at least not apparent because of the efficacy of central inhibition.

In this regard, there appears to be more precise concordance between monkey and human juvenile females, since in both ovariectomy is followed by elevated, if somewhat variable, FSH levels. At the present time we have no obvious explanation why this initial response was delayed or absent in three animals. Subsequently, at around the time when the peripubertal nocturnal rise in LH secretion normally occurs (Terasawa et al. 1984), there was a secondary rise of both FSH and LH to adult castrate values. This biphasic pattern of gonadotropin secretion is also seen in agonadal girls (Winter & Faiman 1972). Presumably the initial gonadotropin rise after ovariectomy reflects removal of steroid-mediated feedback inhibition, while the secondary rise at puberty reflects waning of the central inhibitory mechanism. It seems likely that the important steroidal mediator of feedback inhibition is oestradiol, since similar numbers of ovarian vesicular follicles were observed throughout the juvenile phase, and ovariectomy caused a significant and sustained decline in serum oestradiol concentrations. Rapisarda et al. (1983) have demonstrated how the influence of oestradiol-mediated feedback inhibition becomes more apparent at puberty, as the effect of the central inhibitory mechanism wanes. Although steroid-mediated feedback inhibition may be demonstrated during the juvenile period, this observation does not in any way lend credence to the so-called gonadostat hypothesis for the onset of puberty. Rather it would appear that central mechanisms primarily mediate the continued suppression of pituitary gonadotropin secretion throughout the juvenile period, since complete sexual maturation can be induced by prolonged pulsatile GnRH administration (Wildt et al. 1980).

The end of the juvenile period, and thus the onset of puberty, as signalled by the rise in serum FSH to adult castrate levels in the ovariectomized or orchiectomized monkeys, occurs earlier in females than males. One unanswered question is the extent to which this sex difference in the duration of central inhibition or any apparent sexual dimorphism in feedback inhibition of gonadotropin secretion is conditioned by pre-natal sex differences in exposure to testosterone. Thus Steiner et al. (1976) have reported that ovariectomized females who had been exposed to virilizing amounts of testosterone during foetal life are less sensitive to the feedback inhibitory effects of oestradiol than are castrate females who had not been so exposed.

In both sexes the post-castration rise in FSH consistently preceded that of LH. This sequence, which also occurs in normal puberty (Faiman & Winter 1974), may reflect a progressive increase in the frequency of the hypothalamic GnRH pulse generator with a resulting shift in pituitary secretion of FSH and LH. Thus, in monkeys bearing hypothalamic lesions, an increase in GnRH pulse frequency from 3-hourly to hourly causes a relative enhancement of LH secretion (Wildt et al. 1981; Pohl et al. 1983).

In summary, the present data support a model of primate sexual maturation in which steroid-mediated feedback inhibition of gonadotropin secretion becomes established by mid-gestation in the foetus and remains operative, to some degree, throughout life. The neonatal gonadotropin surge reflects removal of placental steroid effects. Complete sexual maturation shortly after birth is averted in primates by the maturation during infancy of a central mechanism that continues to suppress pulsatile GnRH throughout a juvenile period that varies in duration from around 2–3 years in monkeys to a decade or so in man. In various species the onset of puberty has been
defined in terms of phenomena as disparate as the growth of pubic hair, vaginal opening or first ovulation. For primates of either sex, a practical operative definition for the neuroendocrine onset of puberty is that time at which the influence of the central inhibitory mechanism wanes, as reflected by an immediate rise in serum gonadotropin levels after castration to adult castrate levels. In intact primates this period appears to coincide with the initiation of nocturnal LH release (Terasawa et al. 1984) and enhanced responsiveness to a bolus of GnRH (Monroe et al. 1983). It appears to antedate the pubertal rise in daytime sex steroid concentrations (Plant 1985) and the resulting adolescent growth spurt (Watts & Gavan 1982) by about 6 months.

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References


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