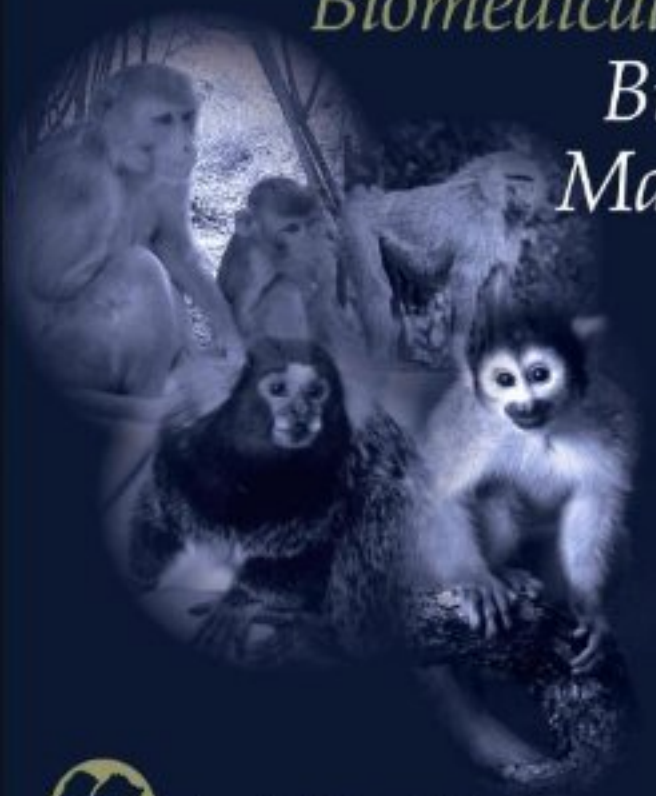
VOLUME 1

Nonhuman Primates in Biomedical Research: Biology and Management



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Nonhuman Primates in Biomedical Research

Volume 1: Biology and Management

Second Edition

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Chapter 8

Reproduction and Breeding of Nonhuman Primates

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Chapter Outline

BASIC REPRODUCTIVE BIOLOGY

Overview of Hypothalamic-pituitary-gonadal Function

In both sexes, reproductive function in nonhuman primates is ultimately regulated by gonadotropin-releasing hormone (GnRH). GnRH is synthesized in the medial basal hypothalamus and released into the hypothalamic-hypophyseal portal blood vessels in a pulsatile manner. Mechanisms responsible for generation of GnRH pulses are poorly understood but appear to involve endogenous oscillations within the GnRH neurons themselves (Terasawa, 2001; Zeleznik and Pohl, 2006). Stimulatory inputs to the GnRH pulse generator in nonhuman primates include kisspeptin (Kp), norepinephrine, glutamate, neuropeptide Y (NPY), and nitric oxide; inhibitory inputs include endogenous opiates, γ-aminobutyric acid (GABA), and corticotropin-releasing hormone (CRH) (Terasawa, 2001; Zeleznik and Pohl, 2006; Plant et al., 2009).

GnRH binds to gonadotropes in the anterior pituitary to stimulate synthesis and secretion of two glycoprotein hormones. In Old World monkeys and apes, those two gonadotropins (GTH) are luteinizing hormone (LH) and follicle-stimulating hormone (FSH). In most New World monkeys that have been extensively studied (common

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marmoset, *Callithrix jacchus*; Bolivian squirrel monkey, *Saimiri boliviensis*; Ma's owl monkey, *Aotus nancymaee*), the pituitary secretes chorionic gonadotropin (CG) instead of LH. Correspondingly, the gonads in some or all New World monkeys express a modified form of the LH receptor in which exon 10 is not expressed and which is activated selectively by CG (Gromoll et al., 2003; Müller et al., 2004a,b; Scammell et al., 2008).

The major function of LH in males is stimulation of androgen release by the Leydig cells. The testis of the rhesus monkey responds rapidly to LH; increased concentrations of serum testosterone (T) are evident within 30 minutes, with maximum concentrations achieved by 1 hour (Toivola et al., 1978; Wickings and Nieschlag, 1980a,b). Administration of human CG (hCG; a molecule with analogous functions to LH) for a 3-day duration results in a 10-fold increase in serum T concentrations in the adult rhesus macaque (Wickings et al., 1986). Treatment of adult cynomolgus (or long-tailed) macaques (*Macaca fasicularis*) with hCG for 16 days results in a 163% increase in the number of Leydig cells and a ninefold rise in plasma T concentration (Teerds, et al., 1989). Correspondingly, T is an important regulator of LH secretion, exerting negative feedback to reduce the frequency of LH and presumably GnRH pulses (reviewed by Tilbrook and Clarker, 2001). The T-mediated feedback in nonhuman primates occurs primarily through brain cell populations, although not through direct effects on GnRH neurons that appear to lack receptors for the sex steroids — androgens, estrogens, and progesterone (Saltzman et al., 2011).

In contrast to LH, the major functions of FSH in males involve development of the gonads, especially production of Leydig cells, and regulation of spermatogenesis, the latter being mediated through actions on Sertoli cells. Whereas LH release is highly sensitive to GnRH pulse frequency, FSH release is not, so that changes in GnRH pulse frequency alter the ratio of circulating FSH to LH (Zeleznik and Pohl, 2006). The major testicular hormones controlling FSH secretion are inhibin B and activins, two glycoprotein hormones produced by the Sertoli cells that inhibit and stimulate, respectively, pituitary release of FSH (McLachlan et al., 2002).

The ovarian cycle in nonhuman primates, as in other species, is governed by a complex interplay among the gonads, the gonadotropes in the anterior pituitary, and the GnRH pulse generator in the medial basal hypothalamus (reviewed by Johnson and Everitt, 2000; Messinis, 2006; Zeleznik and Pohl, 2006). During the follicular phase, LH and FSH are released in low-amplitude, circhoral pulses, reflecting negative-feedback effects of estrogens on pulse amplitude but not frequency. At midcycle, estrogens trigger positive-feedback surges in GnRH, LH, and FSH, eliciting increases in pulse frequency and/or amplitude. Fully developed gonadotropin surges in women require the presence of small amounts of progesterone (P_4); however, no such effect is seen in rhesus macaques (Zeleznik and Pohl, 2006). The luteal phase, following ovulation, is characterized by low-frequency, high-amplitude LH pulses, reflecting negative feedback primarily by P_4. In addition to ovarian steroids, inhibins secreted by granulosa and luteal cells possibly exert negative feedback specifically on FSH release; however, the precise role of inhibins in nonhuman primate ovarian cycles is not yet clear (Zeleznik and Pohl, 2006; Randolph, 2008). Interestingly, FSH concentrations are elevated during the luteal phase in squirrel monkeys, suggesting that development of antral follicles may occur during this period, possibly permitting the extremely short (~5-day) follicular phase of these species (Yeoman et al., 2000).

Estrogens generate both negative and positive feedback effects on gonadotropin release. Negative feedback effects occur at both the level of the hypothalamus and the pituitary (Mizuno and Terasawa, 2005). A variety of experimental approaches have suggested that positive feedback by estrogens at the pituitary alone is sufficient to generate preovulatory LH surges, although GnRH plays an obligate permissive role. Nonetheless, other studies have indicated that hypothalamic release of GnRH increases in response to sustained elevations of estrogens. Negative feedback effects of P_4 on GTH pulse frequency are assumed to occur at least partly within the central nervous system and are mediated at least in part by endogenous opioids. In addition, negative feedback effects of both estrogens and P_4 at the level of the brain appear to be mediated in part by kisspeptin (Kp) (Plant et al., 2009).

Puberty

Puberty is the period of development during which the individual achieves the capacity to reproduce successfully. This period is characterized by morphological, physiological, and behavioral changes driven by maturation and activation of the hypothalamic-pituitary-gonadal (HPG) axis (i.e. gonadarche) and in some species, of the hypothalamus-pituitary-adrenal (HPA) axis (i.e. adrenarche). An excellent review of puberty in nonhuman primates can be found in Plant and Witchel (2006).

In infant nonhuman primates, the pituitary and gonads secrete high levels of GTHs (i.e. LH and FSH) and steroid hormones (e.g. T, dihydrotestosterone (DHT), estrone (E_1), and (E_2)), respectively, for a period of weeks to months. This period of neonatal gonadal activity ends with the onset of the so-called juvenile or prepubertal hiatus, during which gonadotropin levels drop precipitously and the gonads enter a dormant state, especially in males (reviewed by Plant and Witchel, 2006). Gonadal "re-awakening" occurs at the time of gonadarche.

In females, the surge in gonadotropins stimulates the initiation of cyclical ovarian activity (i.e. folliculogenesis) leading to development of follicles capable of ovulation. In concert, the pituitary gonadotropes develop the capacity for positive feedback response to estrogens, leading to the first ovulation. In rhesus macaques, menarche (the first menstrual period) precedes by approximately a year the onset of regular, fertile ovulatory cycles, with the initial cycles being frequently anovulatory and irregular (Dixson, 1998; Saltzman et al., 2011). Increasing estrogen concentrations during the menarche stimulate uterine growth and development of secondary sexual characteristics such as coloration of sexual skin (Dixson 1998).

In males, body weight changes, testicular size and position, presence of an ejaculate, elevations in T, and conception are all aspects of attainment of sexual maturity. Gonadarche in male nonhuman primates is characterized by dramatic elevations in circulating concentrations of LH and to a lesser extent, FSH, reflecting primarily an increase in secretory pulse amplitude. These GTH increases, which are thought to reflect a concomitant amplification of pulsatile GnRH release from the hypothalamus, stimulate an increase in testicular volume (associated with growth of the seminiferous tubules, maturation of Sertoli cells, and proliferation of germ cells), development of Leydig cells, secretion of high levels of gonadal androgens, and initiation of spermatogenesis (Plant and Witchel, 2006). The primary androgen formed by the testis is T, which is responsible for the normal development of male structures, including secondary sexual characteristics such as facial and genital coloration (e.g. mandrill, *Mandrillus sphinx*) and specialized facial or body hair (e.g. baboon, *Papio hamadryas hamadryas*) as well as sexual behavior (Dixson, 1998). Testosterone may also be responsible for programming regions in the central nervous system (CNS) that regulate testicular function and male behavior. Normal differentiation requires the presence of T, but the prostate and external genitalia require DHT for appropriate development (Wilson et al., 1970).

The proximate trigger for gonadarche involves maturation of neural inputs to the GnRH neurons, eliciting the dramatic increase in pulsatile GnRH secretion and, consequently, increases in pituitary secretion of GTHs and stimulation of gonadal endocrine and gametogenic activity.

Studies in rhesus macaques have implicated several neurotransmitters and neuropeptides in the onset of gonadarche. These include the inhibitory neurotransmitter GABA, which plays a key role in restraining GnRH secretion during the juvenile period but exerts only modest inhibitory effects on GnRH release after the onset of puberty (Terasawa, 2005), and NPY, which has been implicated both in inhibiting GnRH release during the prepubertal hiatus and, paradoxically, in stimulating GnRH release during puberty and adulthood (Plant and Witchel, 2006).

Recent attention has focused on the role of the neuropeptide Kp and its receptor, GPR54 (also known as KiSS1R), in regulating gonadarche in humans and other primates (Plant, et al. 2009). Specifically, Kp-GPR54 signaling has been implicated in the control of hypothalamic GnRH release, pituitary GTH release, and onset of puberty in a number of mammalian species, including rhesus macaques (Roa et al., 2008).

The factors that determine the timing of these processes remain poorly understood (reviewed by Plant and Witchel, 2006) but are clearly multifactorial, involving genetic, physiological, and environmental influences. An early hypothesis – that the timing of puberty is governed by an endogenous "pubertal clock" in the CNS that initiates puberty at a specific age – is not widely accepted. Instead, the timing of puberty has long been thought to be governed by a putative "somatometer" that measures some index of somatic growth. The somatometer hypothesis is supported by compelling evidence. It has been noted that dietary restriction can result in a decline in both body weight and circulating gonadotropins when food intake is reduced in castrated males (Dubey et al., 1986). The observed decreases in FSH and LH were, however, restored by an infusion of GnRH. It has been suggested that insulin or amino acids could provide the link between nutritional status and reproductive function by influencing the synthesis of neurotransmitters critical for maintaining GnRH secretion (Steiner et al., 1983). Studies with *M. fascicularis* have shown that long-term administration of amino acids and glucose stimulates adult-like LH/FSH, presumably through the release of GnRH (Cameron et al., 1985a,b). It was concluded that blood-borne metabolic cues that specifically sustain glucose-induced elevation of insulin can stimulate the activity of GnRH-secreting cells and that these factors may be responsible for mediating maturational events within the brain (Cameron et al., 1985a,b).

The index of somatic development being monitored is not yet known. In recent years, attention has focused on a possible role of the adipocyte-produced hormone leptin, circulating concentrations of which correlate with body fat mass. Findings in humans and rhesus macaques as well as rodents suggest that leptin plays a critical, permissive role in the onset of gonadarche (Ebling, 2005; Plant and Witchel, 2006; Kaplowitz, 2008). Other indices of somatic development that have been implicated in determining the timing of puberty include insulin, growth hormone (GH), ghrelin, and metabolic fuels (Plant and Witchel, 2006; Kaplowitz, 2008; Tena-Sempere, 2008). Strenuous exercise, undernutrition, and chronic disease can all delay the onset of puberty, possibly acting through the putative somatometer (Plant and Witchel, 2006).

Finally, a number of environmental factors are known to modulate the timing of puberty in humans and nonhuman primates. Social influences can advance or delay puberty, as described below. In seasonally breeding species, aspects of pubertal maturation may be gated by seasonal cues such as photoperiod. Rhesus males show seasonal increases in sexual behavior during the second and third year prior to the rise in plasma T. This species has shown a rise in both LH and T during the third year of life, with rapid decreases in the fall months, which coincides with the breeding season (Mann et al., 1989). In the seasonal Japanese macaque (*M. fuscata*), the process of maturation occurred over a 2-year period, with full maturity achieved at $\geq$ 6.5 years of age. It was concluded that based on testis size, plasma T, and seminiferous epithelium, gonadal activity developed rapidly during a short period of time and although spermatogenesis started during the mating season at 4 years of age, full sexual maturation was attained 2 years later. Similarly, in squirrel monkeys, the onset of ovulatory cyclicity in young females and the first T surge in young males are restricted to the breeding season, presumably in response to photoperiodic cues (Coe et al., 1981). Thus, seasonality "imposes a quantum effect" on pubertal timing such that gonadarche is more closely dependent on the number of breeding seasons elapsed since an individual's birth than on age per se (Plant and Witchel, 2006). Importantly, seasonally related cues do not necessarily govern maturation of the neural processes underlying pubertal reactivation of the GnRH neurons but instead may play a permissive role in the expression of gonadarche following this reactivation (Plant and Witchel, 2006).

Female

The female nonhuman primate reproductive system functions basically as do those of other placental mammals. However, nonhuman primates are characterized by slow reproductive processes and low fecundity, and their reproductive processes, from folliculogenesis through gestation and lactation, all reflect the slow, prolonged nature of the nonhuman primate reproductive cycle. The following section provides an overview of: (1) ovarian cycles, (2) gestation, (3) lactation, and (4) reproductive senescence. The majority of information available has come from studies on a limited number of species − most notably, rhesus macaques (*Macaca mulatta*), cynomolgus macaques (*Macaca fascicularis*), baboons (*Papio anubis*), squirrel monkeys (*Saimiri* sp), and common marmosets (*Callithrix jacchus*). For detailed recent reviews on female nonhuman primate reproductive physiology, see the articles by Zeleznik and Pohl (2006) and Saltzman et al. (2011). Table 8.1 provides basic female reproductive parameters for species of importance in biomedical research.

Ovarian Cycles

As in other mammals, the development of oocytes to the point at which they undergo either ovulation or atresia proceeds from the development of primordial follicles in which the oocyte is associated with supportive layers of granulosa cells. These primordial follicles develop into early antral follicles through the growth of the oocyte, formation of a zona pellucida, proliferation of granulosa cells followed by formation of the antral cavity, and development of the thecal cell layer. This early stage of development occurs in a continuous stream largely independent of gonadotropin stimulation.

Maturation of early follicles to the preovulatory stages is under the control of LH and FSH and includes expansion of the antral cavity, secretion of follicular fluid into the antrum, expression of LH receptors by the granulosa cells, and increasing secretion of estrogens and inhibin B. Estrogen production is a result of interaction between the granulosa and thecal cells, whereby thecal cells convert C21 steroids to C19 steroids under the influence of LH and granulosa cells subsequently aromatize these androgens to estrogens under the influence of FSH. Steroidogenesis is also affected by numerous paracrine factors, including insulin-like growth factor (IGF), activin, and inhibin (Zeleznik and Pohl, 2006). The majority of preovulatory follicles will undergo atresia, a process of degeneration and resorption. Only those follicles that are at the appropriate phase of development in late follicular phase can proceed to ovulate; the appropriate phase of development includes significant increases in FSH and LH receptor content and an increasingly dense capillary network that allows the follicle to continue to develop in the face of decreasing FSH in the late follicular phase. Eventually, sustained high concentrations of estrogens generate positive feedback that causes surges in GnRH, FSH, and most notably, LH. The LH surge stimulates final preovulatory maturation including completion of the first meiotic division in the oocyte, initiation of progesterone secretion by the thecal layer, and increased expression of collagenases, prostaglandins, vascular endothelial growth factor (VEGF), and matrix metalloproteinases (MMPs) leading to the rupture of the follicle and its ejection into the oviduct.

Following ovulation, the granulosa cells remaining in the ovary respond to the LH surge through a process called luteinization, resulting in the formation of the corpus luteum (CL), made up of luteal cells that secrete steroid hormones (progesterone and estrogens) and peptide hormones (relaxin, oxytocin, and inhibin A) (Zeleznik and Pohl, 2006). In humans and macaques, the CL has an intrinsic lifespan of 14−16 days in nonconceptive cycles. As opposed to most other mammals, the regression of the CL in nonhuman primates is not the result of endogenous prostaglandins but rather appears to be the result of luteal cells undergoing

TABLE 8.1 Reproductive and Life-history Parameters for Primate Species Important to Biomedical Research

Species	Male Age at Sexual Maturity (Days)	Female Age at Sexual Maturity (Days)	Ovarian Cycle Length (Days)	Menstruation	Birth Seasonality	Age at First Parturition (Years)	Gestation Length (Days)	Interbirth Interval (Month)	Weaning Age (Days)	Placentation
Callithrix jacchus (common marmoset)	382	477	28.6	Absent/covert	Weak, bidmodal	1.44	148	6	76	Superficial, hemochorial trabecular
Saimiri sciureus (squirrel monkey)	1826	1003	9.1	Absent/covert	Strong	2.5	170	9	197	
Cebus apella (brown capuchin)		1703	20	Slight	Weak	5.64	154	22	263	
Aotus trivirgatus (owl monkey)	730	821	15.6	Absent/covert	Weak	2.4	133	9	127	
Macaca mulatta (rhesus macaque)	2007	1231	26.6	Overt	Strong	3.75	165	12	279	Interstitial, hemochorial villous
Macaca fascicularis (long-tailed macaque)	1544	1238	29.4	Overt	Weak	3.9	164	13	375	
Macaca nemstrina (pig-tailed macaque)	1095	1125		Slight	Weak	3.92	169	14	300	
Papio hamadryas (baboon)	1762	1514	30	Overt	Weak	6.1	170	24	561	
Chlorocebus aethiops (vervet monkey)	1825	1034	33	Slight	Weak	4.88	163	12	262	
Pan troglodytes (chimpanzee)	2920	3376	37.3	Overt	Weak	13.6	238	60	1691	Interstitial, hemochorial villous

Adapted from Saltzman, et al., 2011

apoptosis associated with decreases in LH responsiveness (Brannian and Stouffer, 1991; Nakano, 1997).

New World monkeys differ from Old World monkeys in that atretic follicles also undergo luteinization, forming accessory CLs or interstitial glands. These glands are steroidogenic and may contribute to the extremely high concentration of circulating progesterone characteristic of New World monkeys (Saltzman et al., 2011).

Anthropoid nonhuman primates are unusual among mammals in undergoing a menstrual cycle in which the endometrial layer of the uterus is sloughed off in a cyclical fashion associated with the fall in progesterone and estrogen at the end of the cycle's luteal phase. The possible adaptive significance of menstruation is controversial (Profet, 1993; Strassman, 1996; Finn, 1998), but there is general agreement as to the proximate causes. In the mid to late follicular phase, the endometrium, under the influence of estrogens from the ovary, undergoes edema; proliferation of stromal cells; angiogenesis; and increases in the size, number, and tortuosity of endometrial glands. During the luteal phase, progesterone, acting in concert with estrogen, causes further cell proliferation, edema, increased capillary permeability, and coiling of the spiral arterioles. At the end of the luteal phase, with declining concentrations of progesterone and estrogen, lysosomal membranes in the endometrium break down, releasing lytic enzymes; spiral arterioles constrict, causing ischemia; and vascular injury and plasminogen activators are released. As opposed to other mammals, in which the endometrium is then resorbed, in Old World monkeys the majority of the endometrial lining and blood from the ruptured arterioles are expelled through the vagina. There are some reports of menstruation occurring in New World monkeys, but the reports are not consistent.

Saltzman et al. (2011) provide a description of cyclical changes in the oviduct, cervix, and vagina resulting from cyclical changes in exposure to estrogens. In addition to these changes, some nonhuman primates displayed marked changes in external genitalia associated with the ovarian endocrine cycle. In species displaying such changes, estrogen generally stimulates swelling (tumescence) and reddening of the sexual skin. The swelling peaks during the periovulatory phase. Progesterone antagonizes these effects during the luteal phase so that detumescence occurs shortly after ovulation. Species displaying such swellings include chimpanzees, baboons, and mangabeys. Rhesus macaques display a change in coloration associated with breeding cycle phase but without notable swelling.

Pregnancy

Numerous reviews have been published on the physiology of mammalian pregnancy (Albrecht and Pepe, 1990, 1999; Ogren and Talamantes, 1994; Petraglia et al., 1996). Additional details about nonhuman primate pregnancy, parturition, and maternal physiology are found in the main section "Pregnancy management."

One feature that differs dramatically between mammalian taxa is the form of placentation. Nonhuman primates display hemochorial placentation, in which the fetal trophoblast layer (the chorion) is in direct contact with the maternal blood supply. Although it has long been proposed that hemochorial placentation evolved from an epitheliochorial form in which the chorion is not in direct contact with the maternal blood supply, recent phylogenetic analyses suggest that hemochorial placentation is likely the ancestral form in mammals (Wildman et al., 2006). Monkeys exhibit a superficial implantation in which the trophoblast adheres to the uterine wall without complete endometrial penetration whereas in apes, the entire blastocyst penetrates the endometrial epithelium and invades the uterine vasculature (Luckett, 1974; Mossman, 1987; Lee and DeMayo, 2004).

The placenta, in addition to providing the interface for transfer of nutrients from the mother to the fetus, is a complex endocrine organ that produces both steroids (e.g. estrogen, progesterone) and peptide hormones (e.g. chorionic gonadotropin, chorionic somatomammatropin, corticotrophin-releasing hormone, leptin). Saltzman et al. (2011) provide a more detailed discussion of the role of each of these hormones in nonhuman primate pregnancy. One feature of note that is unusual in nonhuman primates is the interaction of the mother, placenta, and fetus to generate placental estrogen synthesis (Albrecht and Pepe, 1999). In nonhuman primates, placental estrogen synthesis is dependent upon precursors supplied by the fetal adrenal gland. Nonhuman primate fetal adrenal glands are unique in the presence of a fetal adrenal zone that synthesizes dihydroepiandrosterone-sulfate (DHEA-S), which is then used by the syncytiotrophoblast as a substrate for estradiol synthesis. This fetal adrenal zone involutes after birth, disappearing before adulthood (McNulty et al., 1981).

Lactation

Nonhuman primates are similar to other mammals in the processes leading to milk synthesis and secretion. An excellent overview of lactational physiology is provided by Neville (2001). The continued synthesis of milk is dependent upon the presence of the hormone prolactin while contraction of myoepithelial cells (leading to the "let-down" of milk into the nipple where it can be accessed by the infant) is dependent upon the hormone oxytocin. Compared with other mammals, nonhuman primates produce milk that has low caloric density (e.g. high water content) and relatively low protein content (Oftedal, 1984; Milligan et al., 2008). These features form part of a lactation strategy that involves frequent nursing throughout the day and night combined with a relatively long period of

exclusive milk feeding of young, i.e. weaning at a relatively late age.

An unusual feature of lactation in most nonhuman primates is the occurrence of a prolonged lactation-induced anovulatory period, termed lactational amenorrhea in nonhuman primates that undergo menstrual cycles. It is well established that the suckling stimulus, rather than milk production, is the driving force behind lactation's effects upon the ovary, as the suckling stimulus results in impaired hypothalamic GnRH release that in turn causes impaired pulsatile LH release from the pituitary (Weiss et al., 1976; McNeilly, 1994). The only nonhuman primate group in which lactation-induced anovulation is not routine is the New World marmosets and tamarins. While central administration of oxytocin will inhibit LH release in rhesus macaques (Luckhaus and Ferrin, 1989), such administration increases pituitary luteotropic hormone release in the marmoset (O'Byrne et al., 1990).

Reproductive Senescence

Nonhuman primates, in common with many other mammals, display an inverted-U shaped pattern relating female fertility parameters to age (e.g. Caro et al., 1995; Smucny et al., 2004). Anovulation, insufficient luteolysis, and impairment of gestational and lactational processes are all more common at the beginning and end of reproductive life (Atsalis and Margulis, 2008a).

Reproductive senescence will be used herein to describe the process through which the hypothalamic-pituitary-gonadal axis ages, resulting ultimately in cessation of function. Walker and Herndon (2008) provides an excellent overview of what is known about reproductive senescence and menopause in nonhuman primates, with discussion of controversies stemming from differing uses of the term "menopause." Wise (2006) provides a thoughtful perspective, comparing what is known about reproductive aging in rodents with that in women. Recent findings on nonhuman primate reproductive senescence, along with commentary, are also found in Atsalis and Margulis (2008a). Female reproductive senescence differs among mammalian taxonomic groups. In nonhuman primates, the loss of the follicular pool is the primary event shaping the end of reproductive life, whereas in rodents, striking variation is seen in the size of the follicular pool remaining at the end of reproductive life as well as at maximum life span (Wise, 2006).

Within nonhuman primates, human females are unusual in experiencing follicular depletion relatively early in the maximum life span, resulting in an extended period of altered hormonal environments. These alterations stem from the declining negative feedback signals from the ovary (reduced circulating estrogens, P_4, and inhibin), resulting in elevated GTH concentrations for a time, followed by declining GTHs. These hormonal changes are believed to affect disease risks (Wise, 2006). The risk associated with bone loss due to decreasing estrogenic activity on osteoblasts is well described; however, cardiovascular effects continue to be hotly debated.

With increasing numbers of older nonhuman primates available for study, it is now clear that monkeys and apes also experience follicular depletion and associated hormonal alterations (Hodgen et al., 1977; Graham, 1979; Tardif, 1985; Tardif and Ziegler, 1992; Shideler et al., 2001; Schramm et al., 2002; Atsalis and Margulis, 2008b; Videan et al., 2008). However, the stage of life at which this occurs is generally later than that observed in humans. Atsalis and Margulis (2008a), in reviewing the data on monkeys and apes, conclude that "potentially up to 25% of a female's life can be post-reproductive," This claim is made in reference to maximal life span; in comparison, a human female reaching the maximal life span (now around 120 years) will spend around 58% of her life in a post-reproductive state. When compared with average life span (as opposed to maximal life span), most nonhuman female primates will die at or before the point at which reproductive senescence begins. These comparisons have been controversial and will continue to be refined, given the oft-made claim that human female reproductive aging is unique and may be driven by indirect fitness advantages to post-reproductive women providing resources to grandchildren; i.e. the grandmother hypothesis (Hill and Hurtado, 1991; Peccei, 2001).

Male

The male primate reproductive system, like that of other animals, functions to produce sperm capable of fertilizing an ovum and to package and deliver those sperm to the female reproductive tract. In this review, this process is broken down into: (1) spermatogenesis; (2) sperm maturation; (3) structure and function of epididymal and seminal fluid; (4) copulation and ejaculation; and (5) environmental effects. As with other areas of primate reproduction discussed in this chapter, the majority of information available has come from studies on a limited number of species – most notably rhesus macaques (*Macaca mulatta*), cynomolgus macaques (*Macaca fascicularis*), baboons (*Papio anubis*), squirrel monkeys (*Saimiri* sp.), and common marmosets (*Callithrix jacchus*). For more detailed reviews on the endocrine regulation of male reproduction, see Graham (1981), Wickings et al. (1986), McLachlan et al. (2002), and Saltzman et al. (2011).

Spermatogenesis

The process of spermatogenesis involves the multiplication and proliferation of spermatogonial stem cells,

recombination of genetic material during meiotic division of spermatocytes, and differentiation and maturation of spermatids into testicular sperm. An excellent overview of this process is provided by Sharpe (1994), and detailed discussions of recent theories about the molecular and cellular control of this process are provided by Sofikitis et al. (2008) and Cheng et al. (2010). Detailed investigations of primate spermatogenesis have been conducted in the rhesus macaque (Clermont and LeBlond, 1959; Arsenieva et al., 1961; Conaway and Sade, 1965; Fawcett et al., 1970; de Barr, 1973; Rooij et al., 1986), the cynomolgus macaque (Dang, 1970; Kluin et al., 1983; Fouquet and Dadoune, 1986), the stump-tailed macaque (*Macaca arctoides*, Clermont and Antar, 1973), the African green (or vervet) monkey (*Chlorocebus aethiops*, Clermont, 1969), *C. sabolus* (Barr, 1973), the baboon (Barr, 1973; Chowdhury and Steinberger, 1976; Chowdhury and Marshall, 1980; Afzelius et al., 1982), and the common marmoset (Weinbauer et al. 2001). Clermont and LeBlond (1959) described 12 stages in the cycle of the rhesus seminiferous epithelium. Steps were defined by changes in the nucleus and acrosomal structures, and it was noted that each stage appeared in sequence with time over a particular area in a given seminiferous tubule. Depending on the species, tubule cycle durations range from 9.5 to 14.4 days, and the total duration of spermatogenesis ranges from 36 to 48 days (Table 8.2).

A tubular cross-section may contain either a single germ cell association (single stage tubule) or different germ cell associations (multi-stage tubule). It has been suggested that variation in clonal size might lead to multi-stage organization (Zhengwei et al., 1997; Wistuba et al., 2003; Luetjens et al., 2005). Rodents and prosimian primates display a single-stage tubule structure while New World monkeys and hominoids (apes and humans) display a multi-stage tubular structure. Old World monkeys appear intermediate, with baboons and mandrills displaying a mix of single- and multi-stage tubules while macaques and vervets display predominantly single-stage tubules (Wistuba et al., 2003; Luetjens et al., 2005). Previous proposals that the multi-stage structure is associated with low spermatogenic efficiency have been disproved (Luetjens et al., 2005).

As in other mammals, the process of nonhuman primate spermatogenesis is governed by the Sertoli cells, the only somatic cells present in the seminiferous tubules (Sofikitis et al., 2008). Spermatogenesis is supported directly by FSH, with FSH receptors present on the Sertoli cells. Manipulation of FSH concentration in macaques will directly affect germ cell number and seminiferous tubule size (Wickings and Nieschlag, 1980a,b; Moudgal, 1981; Madhaw Raj et al., 1982; van Alphen et al., 1988). Studies in hypogonadotropic rhesus macaques (Marshall et al., 2005) and common marmosets (Sharpe et al., 2003) support the conclusion that mitotic growth of the A-pale spermatagonial population is gonadotropin independent, but this constitutive proliferation is amplified by exposure to LH and/or FSH. These results indicate that in nonhuman primates, FSH levels determine the number of germ cells in the testis.

LH supports spermatogenesis indirectly by controlling T production by the Leydig cells within the testis. Testosterone supports spermatogenesis directly, with androgen receptors present on the Sertoli cells. In addition, aromatized T may mediate spermatogenesis via estrogen receptors present on spermatocytes, spermatids, and Leydig and Sertoli cells (Shaha, 2008). LH stimulation of the Leydig cells within the testis is responsible for maintaining high concentrations of T, the androgen essential for spermatogenesis. The mechanism by which LH controls androgen secretion has been studied (Arslan et al., 1986), and it was noted that chronic gonadotropin exposure (hCG) resulted in the activation of the stimulatory response required for T production. It was proposed that this activation occurred via enhancement of LH/CG receptor availability on Leydig cells (Wickings et al., 1986).

Testosterone is capable of stimulating spermatogenesis in rhesus, cynomolgus, and bonnet macaques, but T stimulation alone does not appear to be sufficient to produce normal spermatogenesis (Wickings et al., 1986). Studies of hypogonadic rhesus macaques that were supplemented with T or FSH or both concluded that the differentiation of A-pale into B spermatogonia may be driven by T or FSH alone but that differentiation is amplified by the presence of both (Marshall et al., 2005).

Sperm Maturation

The primary functions of the epididymis are maturation and storage of spermatozoa. Because spermatozoa are largely synthetically inactive, this maturation process involves the interaction of sperm cells with proteins synthesized from the epididymis in a region-dependent manner. An excellent review of the role of the epididymal microenvironment in sperm maturation, concentrating on rodents models and humans, is provided in the article by Cornwall (2009).

Sperm maturation is defined by the ability to undergo capacitation and the acrosome reaction. There is a wide disparity among mammals in the role of each epididymal section in these maturation processes; therefore the acrosomal response of epidydimal sperm has been examined in both macaques and marmosets to determine the value of these animals as models of human epididymal sperm maturation processes. Both macaques and marmosets display an acrosome maturation profile similar to that of humans, with increasing in vitro acrosomal response when moving from the caput to the caudal epididymis (Moore, 1981; Moore et al., 1984; Yeung et al., 1996). In addition to

TABLE 8.2 Male Reproductive Parameters[a]

Species	Spermatogenesis (Days)	Ejaculate (Semen) Volume (ml)	Sperm			Plasma Testosterone (ng/ml)
			Concentration ($\times 10^6$/ml)	% Motility	% Normal	
S. sciureus	39	0.2–1.5	80.8–310.9 (205.9)	52 (40–80)	–	52.2 ± 11.6 [June–Sept.]
C. jacchus		0.0157–0.078	306.8–2225.1 (1154.2)	59.6 (25–82)		103.5 ± 12.8 [Dec–March]
C. aethiops		0.3–2.0 (0.9)	165.8–810.8 (439.6)	39 (15–70)		8–20
M. mulatta	36	0.4 ± 0.06	618 ± 125[b] ($n = 23$) [Jan]	57 ± 6[b]	71 ± 5	5.5 ± 0.5 nm/1 [March –May]
			758 ± 220[b] ($n = 18$) [March]	51 ± 7[b]		8.9 ± 2.0[b] [Aug.–Sept.]
			381 ± 133[b] ($n = 7$) [May]	43 ± 13[b]		23.5 ± 5.8[a] [Oct.]
			441 ± 217[b] ($n = 7$) [July]	53 ± 13[b]		
			439 ±215[b]($n = 6$) [Sept.]	57 ± 9[b]		
			348 ± 127[b]($n = 14$) [Nov.]	61 ± 6[b]		
M. fascicularis	36	0.26 ± 0.03	1638 ± 115	84 ± 1	77 ± 3	16.7 ± 1.1 [Sept.–Oct.]
						9.5 ± 0.9 [March–June]
M. arctoides	36	–	–	–	–	14.8 [June]
						6.7–7.2 [Aug.–Sept.]
M. fuscata	36	–	–	–	–	7–13
M. nemestrina	36	–	–	–	–	9–16.5
M. radiata	36	2.2 ± 0.2 [winter]	1251 ± 165	72 ± 2		21.3 ± 4.1
		1.9 ± 0.2 [summer]	1195 ± 145	74 ± 2		
P. ursinus	42	–	–	–		18.3 ± 3.2
P. troglodytes	–	0.1–2.5 (1.1)	548 (54–2750)	30 (10–60)		409 ± 45
P. pygmaeus	–	0.2–3.6	76(10–165)			1003 (628–1421)
		0.2–3.2	10–128	50	60	2367
G. gorilla	–	0.38	41	32	49	413.7 ± 219.1

[a]*From Dang and Meusy-Dessolle (1981); Graham (1981); Harrison and Lewis (1986); Wickings et al. (1986); Wiebe et al. (1988); and Kuderling et al. (2000).*
[b]*SEM.*

the ability to undergo capacitation and acrosome reactions, the epididymal sperm maturation process includes remodeling of sperm chromatin to a highly condensed form. Studies in hypogonadal cynomolgus monkeys concluded that chromatin condensation is a gonadrotropin-independent process (Golan et al., 1997).

The basic morphology of spermatozoa among nonhuman primates is similar, although species differences do exist. Generally speaking, sperm consists of four basic components: (1) the *head,* which contains chromatin and is capped by the acrosome; (2) the *neck,* which contains the basal plate, connecting pieces, and a centriole; (3) the *midpiece,* which contains the mitochondria; and (4) the *tail*.

Several comparative studies on the morphology of nonhuman primate spermatozoa utilizing scanning electron microscopy (Bedford, 1967; Matano et al., 1975; Gould and Martin, 1978) include details of the ultrastructural (and identifying) features of numerous prosimian species (Gould, 1980; Harrison and Lewis, 1986). Sperm parameters have been assessed in a number of New World species (Bush et al., 1975; Gould and Martin, 1978; Harrison and Wolf, 1985). In the platyrrhine, the midpiece often inserts eccentrically into the posterior border of the head. The posterior acrosomal margin of squirrel monkey sperm has a serrated appearance and is smaller than the capuchin sperm, which have typical paddle-shaped sperm heads (Barr, 1973).

Sperm from various Cercopithecidae species appear uniform in shape, particularly compared with sperm from the great apes (Harrison and Lewis, 1986). The sperm heads appear flat and paddle-shaped, with the midpiece long in relation to the head and the mitochondria of the midpiece small and well organized (Harrison and Lewis, 1986), whereas the sperm heads from the baboon are short, oval, paddle-shaped, and taper anteriorly (Flechon et al., 1976). The anterior segment of the acrosome in this species is surrounded by a marginal thickening and covers roughly two thirds of the head. The midpiece is characterized by a relatively long and regular helical sheath of mitochondria, with the ends of the mitochondria randomly distributed. Morphology and dimensions have been compared with those of other nonhuman primates and are very similar to those of Cercopithecidae (Flechon et al., 1976). Gould (1980) has provided an excellent description of spermatozoa for various great apes. Among these, the spermatozoa of the chimpanzee (*P. troglodytes* and *P. paniscus*) are the most uniform, with the sperm heads relatively small and thickened posteriorly and the midpiece similar to that of the gorilla.

Nonhuman primates display a wide variety of mating systems, from monogamy to promiscuity (see Chapter 5). This variety has elicited interest in the effects of sexual selection on reproductive parameters among nonhuman primates. Anderson and Dixson (2002) reported that the nonhuman primate sperm midpiece volume is associated with relatively large testis size and with multiple male mating systems, arguing that the larger midpieces may reflect increased mitochondrial loading and increased sperm motility in those species experiencing more intense sexual selection.

Epididymal and Seminal Fluids

The fluid of the epididymis contains a variety of compounds derived from rete testicular fluid, which is modified by the epididymal epithelium. Testicular fluid has low concentrations of spermatozoa and is characterized by a low glucose and high inositol content (White, 1981). Collection of fluid from the cauda epididymis indicates that the composition is similar among species, although slight variations are noted. The chief characteristics of the cauda epididymal plasma are low concentrations of inorganic ions and high levels of organic constituents such as glycerylphosphylcholine, carnitine, sialic acid, hypotaurine, glycosidases, and phosphatases. The concentrations of sodium ions are ~20 mEq/liter. The potassium ion levels are generally greater than or equal to sodium levels, although in the rhesus, the potassium concentration is twice that of sodium. It is generally accepted that pH varies along the length of the epididymis but is usually within the 6.5−7.0 range, although it may occasionally be slightly higher (White, 1981). The composition of the epididymal fluids has been reported for the rhesus monkey (Bose and Kar, 1968; Riar et al., 1973a,b; Arora et al., 1975; Jones, 1978) and to a more limited extent, for the langur (Gupta and Dixit, 1981).

Information on the biochemical parameters associated with testicular and epididymal fluids is limited, with the most data available for the rhesus. Although some species differences have been noted, these differences may be dependent on the methods used for analyses (Harrison and Lewis, 1986). Inasmuch as fluids vary in the different regions of the male reproductive tract owing to absorptive and secretive mechanisms, there are significant differences between blood and reproductive tract fluids, which in many cases are due to the presence of the blood−testis barrier (White, 1981). For example, testicular fluid in the rhesus consists of greater volumes of lactate dehydrogenase, glucose-6-phosphate dehydrogenase, lactic acid, and ascorbic acid than those present in serum, and the converse is true for glucose and total lipids.

Ackerman and Roussel (1968) reported one of the few comparative studies of semen among nonhuman primates and humans. Table 8.3 lists three biochemical parameters − lactic acid, citric acid, and fructose − found in the semen of 10 nonhuman primate species. Although our current understanding of the functional aspects of these fluids is

TABLE 8.3 Biochemical Parameters in Sperm and Plasma Fractions of Semen from Nonhuman Primates

	Lactic Acid		Citric Acid		Fructose	
Species	SF	PF	SF	PF	SF	PF
C. apella	0	4	13 ± 19	79 ± 85	0	563 ± 496
S. sciureus	42	151	3 ± 4	48 ± 14	0.4 ±1	110 ± 129
E. patas	34 ± 21	194 ± 222	31 ± 52	127 ± 62	18 ± 39	315 ± 274
C. aethiops	20 ± 12	192 ± 196	5 ± 10	122 ± 25	10 ± 16	264 ± 175
M. mulatto	32 ± 30	138 ± 115	2 ± 5	157 ± 86	14 ± 28	753 ± 900
M. fascicularis	36 ± 17	239 ± 88	0	101 ± 65	7 ± 13	299 ± 264
M. arctoides	28 ± 30	183 ± 186	6 ± 9	231 ± 121	0	262 ± 108
T. gelada	10	130	16	168	0	160
P. troglodytes	24 ± 16	160 ± 127	15 ± 24	256 ± 191	10 ± 30	497 ± 363

Sperm fraction (SF): all parameters expressed in mg/100 ml. Plasma fraction (PF): all parameters expressed in mg/100 ml.
Data adapted from Ackerman and Roussel (1968) and Harrison and Lewis (1986).

limited, state-of-the-art microanalytical and *in vitro* techniques should improve methods for analysis, thereby increasing our knowledge (Hinton, 1980; Hinton and Howards, 1982). The rhesus ejaculate has been noted to solidify immediately upon expulsion.

Copulation and Ejaculation

Reviews of male nonhuman primate sexual behavior and its endocrine control are provided in Dixson (1998) and Saltzman et al. (2011). Male nonhuman primates use a variety of behaviors to initiate sexual interactions (Dixson, 1998). Most nonhuman primates display dorso-ventral mounting postures, typical of most mammals; however, numerous variations are seen in relation to the nonhuman primates' varied locomotor styles (e.g. arboreal versus terrestrial). Nonhuman primates species vary in the number and duration of intromissions prior to ejaculation (Dixson, 1998), from single, brief intromissions (e.g. marmosets) to single but prolonged intromissions (e.g. stump-tailed macaque) to multiple, brief mounts (e.g. rhesus macaques), the most extreme case being the thick-tailed greater galago (*Otolemur crassicaudatus*), in which post-ejaculation intromissions lasting hours suggest the possibility of a genital lock. Capacitation is a phenomenon affecting the sperm that normally occurs within the female reproductive tract and, through enzymatic action, renders the sperm capable of fertilization (Dukelow and Yorozu, 1986). In most nonhuman primate species studied, this requires approximately 2 to 8 hours.

Environmental Effects

Nonhuman primates exhibit a broad array of seasonal patterning of reproduction (Table 8.1). Phylogeny appears to be a poor predictor of these traits. Prosimians are generally strongly seasonal — a number of prosimians, including the ruffed and mongoose lemurs, show a testicular volume increase as the breeding season approaches, with maximum size obtained about 1 month prior to the initiation of breeding. This volume increase has been calculated to be over 150%; a body weight increase of about 14% is also observed during this period. Apes are generally not seasonal breeders. However, the degree of seasonality in Old World and New World monkeys is highly variable and not related to phylogeny (see Saltzman et al., 2011). For example, several macaques are seasonal breeders (*M. fuscata, M. mulatta, M. sylvana*), whereas many species will breed all year round (*M. arctoides, M. fascicularis, M. nemestrina, M. radiata*).

Two nonhuman primate genera with strongly seasonal patterns in male reproductive physiology and behavior are the Central and South American squirrel monkeys (*Saimiri*) and the northernmost macaques (*Macaca*), such as rhesus macaques and Japanese macaques (*M. fuscata*). In both squirrel monkeys and rhesus macaques, males display a seasonal pattern of T production, with peaks occurring as the mating season commences. Both squirrel monkey and macaque males display T-supported characteristics that arise during the breeding season. Male squirrel monkeys undergo weight gain (the "fatted male" response (DuMond, 1968; Lindburg 1987)) of around 14%, caused largely by

retention and deposition of water along the arms, shoulders, and back (Jack, 2007), as well as increases in testicular volume of 150% (Wiebe et al., 1984) and increases in aggressive behavior related to increasing circulating androgens (Wiebe et al., 1988). During the nonbreeding season, male rhesus macaques display reduced LH pulsatility (4 to 6 pulses per day (Plant, 1980; Wickings et al., 1986)), reduced diurnal rhythms in pulsatility (Wickings et al., 1986), regression of seminiferous tubules, and few spermatocytes or spermatids. In the months leading into the mating season, LH pulsatility increases, seminiferous tubular diameter increases threefold (Conaway and Sade, 1965), and spermatogenesis commences (Zamboni et al., 1974; Wickings et al., 1986). Breeding males also display skin reddening, likely related to increasing T production.

The roles of specific environmental cues in generating these neuroendocrine changes in squirrel monkeys and rhesus macaques remain obscure; however, changing day length cues do not appear necessary, as these patterns are retained in controlled day length conditions (Wehrenberg and Dyrenfurth, 1983).

Reproductive function of adult males can be influenced by both intrasexual and intersexual stimuli in a wide variety of nonhuman primates. Typically, cues from other adult males may dampen the activity of the HPG axis whereas interactions with females will have stimulatory effects. See Saltzman et al. (2011) for a more detailed discussion of this topic.

HOUSING, HUSBANDRY, AND POPULATION MANAGEMENT FOR BREEDING

Macaques

Macaques are the nonhuman primates most commonly used in biomedical research. In 2010, the two macaque species in highest demand and, therefore, having the largest number of breeding programs were the rhesus macaque (*Macaca mulatta*) and the cynomolgus macaque (*Macaca fascicularis*). The details provided in this section regarding macaque breeding apply generally to most macaque species.

Macaques in the wild live in large multi-male, multi-female groups (see Chapter 5). Most groups comprise one male for every 2.4 females (Southwick et al., 1965). This ratio of males to females appears to promote stability within the groups. Female offspring inherit the rank of their mother and, unlike male offspring, remain closely associated with their group and hierarchy throughout their life (Lutz and Novak, 2005; see Chapter 5). In the natural environment, males will move out of their natal group at maturity and attempt to incorporate into a new group; thus, the dominance status of the male is based more upon individual factors (age, temperament, size) than on relations. In colony-reared animals, males are usually deliberately culled from the group, ensuring an increase in stability and a decrease in potential for loss of genetic heterogeneity (Pusey and Packer, 1986; Beisner et al., 2010). Maintaining stability and decreasing aggression within groups is aided by a stable dominance hierarchy within each sex. Within large social groups there will be multiple matrilines, and the possibility of one matriline being overthrown is not uncommon. This situation happens when lower ranking matrilines group together and displace the highest ranking matriline. A retrospective analysis at The California National Primate Research Center revealed that matriline overthrows were more likely to occur if the alpha female had been removed from the group (Oates-O'Brien et al., 2010). In addition, during the breeding season there appeared to be an increased risk of overthrows that was associated with increased levels of aggression between animals (Eaton et al.,1981).

Schemes for breeding macaques are numerous and include semi-free-ranging island populations, large outdoor corrals with multi-male, multi-female groups, smaller outdoor or indoor housing with single-male, multi-female groups, and individually housed females who are timed mated. The choice of grouping type is governed by the practicalities of available space, climate concerns, pathogen control concerns, and the planned use of the offspring.

Several studies have documented and compared reproductive parameters among these various breeding schemes. The parameters most often calculated and compared are: (1) conception or pregnancy rate = number of documented pregnancies/average number of females at risk for pregnancy; (2) loss rates = number of pregnancies documented but not carried to term/number of pregnancies documented; (c) production rate = number of young weaned/number of pregnancies documented.

Table 8.4 provides reproduction rates as reported in the literature for rhesus and cynomolgus macaques in a variety of breeding schemes. The highest pregnancy and reproduction rates are generally associated with provisioned, free-ranging populations. A notable example of such a population is the free-ranging colonies of rhesus macaques maintained on the island of Cayo Santiago, Puerto Rico, from the 1940s to the present (see Koford, 1965; Carpenter, 1972; Rawlins and Kessler, 1986, for history of this colony). An analysis of rhesus reproduction from 1976 to 1983 (Rawlins and Kessler, 1986) reported an average reproduction rate of 80.3%. Of the total births, 95.7% were live births. The average interbirth interval was 372 days for rhesus females who had produced a live, weaned infant in the preceding breeding season and 336 days for those females who had not.

TABLE 8.4 Reproductive Rates for Macaques Housed Under Different Captive Conditions

Species	Breeding Configuration	Pregnancy Rate[a]	Loss Rate[a]	Production Rate[a]	Reference
M. mulatta	Free-ranging, provisioned	80.3	4.3	71.3	Rawlins and Kessler, 1986
M. mulatta	Outdoor corral	73.3	13.6	NA	Hendrickx and Dukelow, 1995
M. mulatta	Gang caged harems	55	9	43	Westergaard et al., 2000
M. mulatta	Outdoor corral	80	6	69	Westergaard et al., 2000
M. fascicularis	Indoor-outdoor harems	53	22	31.8	Gardin et al., 1989
M. fascicularis	Indoor, single housing, F_0	57.9	12.8	47.4	Honjo et al., 1984
M. fascicularis	Indoor, single housing, F_1	36.8	14.6	28	Honjo et al., 1984
M. fascicularis	Free-ranging, unprovisioned	68	NA	NA	Crockett et al., 1996

[a]*Percentage, see text for definition of terms.*

Data from long-term reproduction programs have been fruitfully used to assess the variation in output from nonhuman primate populations that need to be self sustaining. Ha et al. (1999) provide a detailed analysis of reproduction in pig-tailed macaques (*Macaca nemistrina*) based upon 30 years of breeding records from the University of Washington's nonhuman primate research center. This analysis indicated that for this species, the presence of the sire and of other pregnant females in the group increased the probability of viable births while more frequent animal moves in and out of groups as well as lower parity decreased the probability of viable births. Sire presence was the single most important factor in most measures of reproductive outcome. Crockett et al. (1996) evaluated the value of computer simulations of population dynamics as a means to manage harvesting rates from a population of simian retrovirus-free cynomolgus macaques established on Tinjil Island, Indonesia. The simulation was used to determine that at intermediate birth and survival values that reflected the actual birth and survival rates, the island population of these animals would be approaching carrying capacity and that at high rates, the rate of harvesting from this animal population would need to be increased in order to not overpopulate the island, or provisioning would need to begin. This "herd management" approach can, then, be successfully employed with free-ranging breeding populations.

Various strategies may be employed during the formation of new breeding groups regardless of which type of housing conformation is used. Groups can be formed by introducing all the members at once or by incrementally adding animals in small numbers until the desired population is attained. Animals can also be introduced to each other in an environment in which there are barriers that allow sight and touch between individuals but prevent complete contact, or animals can be introduced to an environment without barriers. A study performed at Labs of Virginia (Westergaard et al., 1999) investigated the role different group formation practices had on rhesus aggression and reproductive performance. Results suggested that incremental addition of animals in small groups to the population in an enclosure with barriers that do not permit immediate contact decreased the incidence of aggression, trauma, and death. However, group formation strategy did not appear to have any effect on reproductive rates.

In addition to employing strategies to minimize aggression and trauma and to promote reproductive performance, colony managers must also be cognizant of maintaining genetic diversity. An essential component of this is maintenance of superlative parentage and medical records. Molecular techniques such as DNA fingerprinting, analysis of DNA restriction length polymorphisms (RFLPs), short tandem repeats (STR), or single nucleotide polymorphisms (SNPs) are frequently employed to confirm parentage and assess the degree of genetic diversity (von Segesser et al., 1995; Kanthaswamy and Smith, 1998, 2002; Kanthaswamy et al., 2009, 2010; Trask et al., 2011). Various management practices can be utilized to promote genetic diversity or minimize loss. These include fostering infants from one matriline onto dams in another matriline, removal of males after a designated number of breeding seasons, removal of complete matrilines, introduction of new males, or increasing the male:female ratio. Another possible method for increasing diversity is to select males for breeding who possess rare genetic alleles. However, because any procedure that alters the population dynamics within groups has the potential to result in aggression and trauma, the benefits of increasing genetic diversity must be balanced with the potential social impact.

Detailed descriptions of pregnancy, pregnancy management, and lactation are provided in the main sections "Basic reproductive biology" and "Housing, husbandry, and population management for breeding." Species of macaques vary in the seasonality of their breeding seasons. Pigtailed macaques (*Macaca nemistrina*) and cynomolgus macaques (*Macaca fasicularis*) are not known to show any seasonality and can conceive throughout the year (Ha et al., 2000a,b). However, rhesus macaques (*Macaca mulatta*) and Japanese macaques (*Macaca fuscata*) show distinct seasonality with regard to breeding. The breeding season for rhesus colonies in North America is most commonly between October and February (Eaton et al., 1981; Ehardt and Bernstein, 1986). Although rhesus macaques do not typically ovulate outside these months, they will continue to have menstrual cycles that may be more irregular than during the breeding months. As females age, they may cease to menstruate altogether during the nonbreeding months. See the section "Reproductive senescence" above for more details on reproductive aging. Throughout the breeding season, the number of ovulations varies for each individual rhesus female and has been shown to be directly related to the female's body weight, social rank, and mean luteal progesterone level (Takahata, 1980; Garcia et al. 2009, 2010; Du et al., 2010).

In captivity, female macaques generally reach puberty between 2 and 4 years of age and will deliver their first offspring on average a year later. The menstrual cycle of macaques is generally 26−30 days (Blakley et al., 1981; Walker et al., 1983). Outward indications of ovarian activity such as changes in sexual behavior, perianal swelling, and alterations in inguinal and facial sex skin coloration may aid in assessing the stage of the cycle depending on the species involved (Carpenter, 1942; Zinner et al., 2004; Engelhardt et al., 2005; Bradley and Mundy, 2008; Dubuc et al., 2009) Pubertal macaques also may have large variations in their intermenstrual cycles (Resko et al., 1982). Male macaques generally reach puberty at 3.5 years of age; however, they normally do not contribute significantly to breeding until 2 years later (Chambers et al., 1982; Honjo et al., 1984). The age at which individual animals reach sexual maturity is governed by various factors, of which the animal's nutritional status and the social ranking of its dam are thought to be most important.

The gestational period in macaques is generally 165−170 days. Most pregnancies result in the delivery of one fetus; the incidence of twinning is very uncommon (Bercovitch et al., 2002; Sugiyama et al., 2011). Normally, infants are delivered at night and the female consumes the placenta. Labor is reported to last 5−7 hours, and prior to delivery, the female may show signs such as increased grooming, restlessness, and alteration in eating and sleeping patterns (Goodlin and Sackett, 1983). In addition, there may be a decrease in the female's body temperature prior to parturition (Ruppenthal and Goodlin, 1982). Complications such as dystocia have been reported in many macaque species and have been associated with the age and parity of the dam along with the percentage of the dam's previous pregnancies that resulted in caesarean section deliveries (Stockinger et al., 2011). Unfortunately, cases of dystocia may be difficult to detect, as most animals give birth at night when staff are not usually present to observe the births. Indications of dystocia include infants with facial bruising or edema, presence of protruding limbs at the vaginal orifice, and weakness or collapse of the dam. In instances where there are clinical indications that the delivery has been unsuccessful, medical intervention must be taken immediately in order to save the life of the dam and fetus. Treatments may vary from manual extraction of the fetus and placenta if it is lodged in the vaginal canal to delivery of the fetus by caesarean section. Should ultrasound examination indicate that the fetus is no longer viable, it may be prudent to initiate fluid therapy and supportive care to stabilize the dam prior to performing surgery.

Additional complications associated with parturition in macaque colonies include neonatal abandonment by mothers. Retrospective analysis of reproductive performance in a captive colony of Japanese macaques suggested that the most important factor involved in abandonment was parity of the dam followed by her social rank. In this study, neonates were 90 times more likely to be abandoned by a primaparous than by a multiparous mother. Also, abandonment was six times more likely to occur by a low-ranking female than by a high-ranking female (Schino and Troisi, 2005). A correlation between the age of the dam and the incidence of an infant dying before 30 days of age has also been demonstrated. The incidence of death was found to be higher for offspring born to either primaparous or aged females (Gagliardi et al., 2007; Schino and Troisi, 2005). Studies have also examined the relationship between the body condition of a primaparous female at time of first conception and the time required to recover from pregnancy and lactation and also the effect on the postnatal development of the infant. Results from work carried out at the Caribbean Primate Center imply that the body condition of the female at first conception directly correlates with the growth and development of her infant and also with the time needed to recover reproductive capabilities postpartum (Mas-Rivera and Bercovitch, 2008). Females with higher body mass index (BMI) prior to conception had a more rapid recovery after parturition.

In recent years an increased emphasis has been placed on studying the role that physiological factors may play in the relationship between dams and their offspring, the reproductive success of the dams, and the temperament and success of the offspring. Studies have suggested that glucocorticoids in breast milk and biogenic amines may

have an important role in parenting behavior and subsequent development of the offspring from birth through maturity (Cleveland et al, 2004; Hinde and Capitanio, 2010; Sullivan et al., 2011).

Rejection of macaque infants necessitates either nursery rearing or fostering of the infant. Fostering is preferable over nursery rearing due to the physiological and psychological benefits that maternal rearing offers to infants (Fontenot et al., 2004; Watts and Veall, 2004). However, essential to the success of fostering is the availability of lactating dams willing to accept an infant that is not their own. Multiparous females normally are better foster mothers than are primaparous females and females that have previously demonstrated a history of neonatal abuse. Frequently, fostering may require multiple attempts before a successful outcome is attained.

If a suitable foster mother is not available or if the infant is ill and requires clinical treatment, nursery rearing of the infant may be required (Sackett et al., 2005). During the initial weeks in the nursery, infants should be kept in an environment with an ambient temperature of 30−35°C. At the New England Primate Research Center, infants are offered 5% dextrose per os on the day of birth and then offered commercially available lactose-free infant formula with iron. The volume of formula is increased weekly from 15 ml per feeding to 35 ml per feeding over the first 4 weeks of life. Each infant is fed 6 times daily between 7am and 9pm. Then, at 4 weeks formula-soaked chow (Purina Monkey Chow, 25% protein, Purina Mills, Inc.) mixed with a small amount of banana is introduced. Over the next months, the amount of chow (both soaked and hard) is increased and the infant is gradually weaned off bottle feeding. To encourage the development of normal behavioral patterns, it is important that nursery-reared infants be introduced to age-matched peers as soon as feasible. Should circumstances dictate that an infant is reared in isolation for an extended period of time, studies have shown that reversal of abnormal behavioral traits that may have developed is best accomplished by introducing the nursery-reared infant to a younger infant that has been reared by its dam (Rommeck et al., 2009). Consequences of nursery rearing of nonhuman primates can be both physiological and psychological and can last into adulthood. Important consideration should be given to the necessity of nursery rearing, the importance of socialization, and in detail the infant's environment (Sackett et al., 2002; see Chapter 7).

Specific Pathogen-free Macaques

Due to the continued increase in sophistication of molecular, genetic, and biochemical modalities used in research, investigators are also becoming much more specific in their requirements for rhesus macaques of defined genetic and pathogen-free status. Coupled with the increased awareness of the occupational health and safety risks associated with working with nonhuman primates, emphasis has been placed on developing macaque colonies that are free from common infectious agents (Holmes et al., 1995; Desrosiers, 1997).

Developing specific pathogen-free (SPF) rhesus macaque colonies is a complex process that requires an understanding of nonhuman primate biology and behavior, exemplary husbandry techniques, and knowledge of infectious disease testing, treatment, and control to be successful. The definition of SPF macaques may vary from colony to colony depending on which agents have been targeted for elimination. At the most basic level, the initial targets for elimination should be *Mycobacteria tuberculosis* and *Macacine herpes* (BV) virus (Morton et al., 2008). Although BV virus poses few problems for macaques, it does present a significant risk to personnel. Thus, from an occupational health viewpoint, most facilities prefer to utilize animals that are not infected with BV (Holmes et al., 1995; Cohen et al., 2002). In contrast, infections with *M. tuberculosis* present a serious health threat to both humans and nonhuman primates, and therefore, rigorous testing for this organism is imperative, particularly when introducing animals from other sources (Ruch 1959). In addition to BV, three other viruses have been targeted for elimination from macaque colonies designated for use in SIV/AIDS research: Simian immunodeficiency virus (SIV), Simian retrovirus D (SRV-D), and Simian T lymphotropic virus (STLV) (Mansfield, 2005; Morton et al., 2008).

Regardless of the agents identified for exclusion during formation of SPF colonies, the process is based on repeated serological testing of founder animals and immediate removal from the colony of animals that seroconvert (Mansfield, 2005). During the formation of these colonies, it is essential that there is complete separation of conventional (i.e. seropositive) animals from those identified as SPF candidates.

Before selection of the animals that will be recruited as founders for the SPF colony, it is crucial to perform pedigree analysis to ensure sufficient genetic diversity and representation of multiple family lineages within the colony. This is particularly important as established SPF colonies are frequently closed (i.e. all future breeding stock is recruited from within the colony), and thus, if the initial population is not carefully selected, the result may be narrowing of genetic diversity after successive generations of offspring are born. Pedigree and demographics programs such as the Pedigree Data Management System (PEDSYS) system developed by the Southwest National Primate Research Center (SNPRC), Southwest Foundation for Biomedical Research, are invaluable in guiding the selection of founder candidates. With the increasing demand for

animals of defined major histocompatibility complex (MHC) it may also be important to include MHC type as a criterion for selection of the founding cohort (Baskin et al., 1997). With continued refinement of the rhesus macaque model for disease research, increasing demands for animals of known genetic and disease background will be inevitable.

Target Viruses for SPF Macaque Colonies

Macacine Herpes Virus (BV)

BV is a member of the alpha herpesvirus family and is a common infection among all species of macaques in which infection is normally self-limiting (Weigler, 1992). After BV infection of a macaque, the virus becomes latent in sensory ganglia, and reactivation can occur during periods of immunosuppression or stress (Chellman et al., 1992; Weigler et al., 1995). This virus is shed in secretions such as saliva and is readily transmitted, with most animals becoming seropositive by 2 years of age (Weigler et al., 1990, 1993). Of the viruses targeted for elimination in SPF colonies, BV has proved most problematical (Ward et al., 2000; Ward and Hilliard, 2002). Various reports have documented a phenomenon of delayed seroconversion, in which an animal that has repeatedly tested BV seronegative unexpectedly develops antibodies (Ward and Hilliard, 1994). The occurrence of delayed BV seroconversion can jeopardize the integrity of SPF colonies many years after they have been founded. Although the mechanism responsible for this phenomenon is not well understood, it is hypothesized to occur when animals are infected at a very early age and then a latent viral state is established before an adequate antibody response is developed.

Simian T Lymphotropic Virus (STLV)

This group of type C retroviruses is closely related to human T lymphotropic virus types I and II (Miyoshi et al., 1983). Although STLV appears to have minimal health consequences in immunocompetent macaques, it has been linked to lymphoproliferative disorders in AIDS (Homma et al., 1984). STLV has a low zoonotic potential and therefore has limited occupational health and safety risks; however, infection of macaques has been shown to alter cytokine profiles and thus could have a confounding effect on immunological studies, particularly those involving simian AIDs (Lazo and Bailer, 1996; Carvalho et al., 2001). For this reason, STLV has been targeted for elimination from SPF programs. The incidence of STLV infection within colonies varies dramatically, with reports of 0−20% of animals being infected (Daniel et al., 1988; Lerche et al., 1994; Schillaci et al., 2005). The virus is highly cell associated with primary tropism for CD4+ lymphocytes (Gabet et al., 2003). Transmission involves transfer of infected lymphocytes from one animal to another, and it is believed that sexual or parenteral routes of infection are responsible for the spread within colonies (Ishikawa et al., 1987). The highly cell-associated nature of STLV implies that it should be relatively easy to eradicate from colonies. Commercial ELISA assays for HTLV are available and due to cross-reactivity can be used for serological screening for STLV in colonies (Meertens et al., 2001). If seropositive animals are identified, samples should be sent to a commercial laboratory for confirmational testing using Western blot assays.

Simian Retrovirus Type D (SRV-D)

This is a group of closely related retroviruses that have been isolated from most species of macaques (Daniel et al., 1984; Lerche et al., 1994; Marx et al., 1984). To date, seven SRV-D serotypes have been recognized, and it has also been shown that marked genetic variation exists among individual isolates (Marx et al., 1984; Marracci et al., 1995). SRV-D exhibits broad cellular tropism, including cells of lymphoid and nonlymphoid lineages (Lackner et al., 1988). This expansive tropism increases the potential for spread of SRV-D between animals, as the virus can be secreted in many bodily fluids (Lerche, 1992; Gardner et al., 2000). Transmission can occur both horizontally and vertically, adding to the complexity of eliminating this virus from nonhuman primate colonies (Tsai et al., 1987; Lerche et al., 1994). Another confounding problem with eradicating SRV-D is the existence of animals that are virus positive but antibody negative (Wilkinson et al., 2003). This state normally arises when infection has occurred in utero or shortly after birth. To eliminate SRV-D from a colony, it is important to incorporate serological assays to screen for antibodies with PCR and virus isolation techniques to detect the cohort of antibody-negative/virus-positive animals. Cross-reactivity of antibodies between different serotypes is directed mainly at the major capsid protein p27 and transmembrane glycoprotein gp20−22. These conserved regions are commonly used in serological assays to test for SRV-D (Kuller et al., 2005; Khan et al., 2006). Viral isolation techniques involve culturing of peripheral blood mononuclear cells (PBMCs) with permissive cell lines such as Raji cells. If the animal is SRV-D positive, syncytial cell formation will normally be seen in approximately 3 weeks. Should syncytial cell formation occur, microscopic examination of the culture or PCR analysis should be performed. When PCR results are positive, the isolate should be sequenced to confirm that it is SRV-D rather than a closely related endogenous retrovirus (Morton et al., 2008).

Simian Immunodeficiency Virus (SIV)

SIV is known to infect various African species of nonhuman primates, in which the virus causes minimal clinical disease. However, infection of Asian species of

macaques results in the induction of an immunodeficient state that frequently progresses to AIDS (Baskin et al., 1988; Lackner et al., 1988). Although since 1980, SIV has not been documented in domestic nonhuman primate centers other than in those animals experimentally infected or those accidentally housed with infected animals, SIV has been included as a target virus for elimination from SPF programs. Because SIV is a group of closely related lentiviruses, serological testing routinely involves whole-virus preparations that contain conserved regions of the genome, i.e. the core p27 region (Morton et al., 2008). A test-and-remove strategy has proved effective in eliminating this virus from colonies; provided that stringent separation is maintained between experimentally infected and noninfected animals, inadvertent transmission of SIV should not occur.

Viral Screening Tests

Various diagnostic tests, including ELISA, immunofluorescent assays, polymerase chain reaction (PCR), Western blot, and viral isolation, are used in screening animals for these four target viruses. Samples may either be tested in house or sent to a commercial testing laboratory. On-site screening is inexpensive and may decrease time between seroconversion and removal of an animal from the colony. Details for the production of ELISA plates using purified whole-virus preparations for on-site screening can be found in the literature (Daniel et al., 1988; Blewett et al., 1999; Takano et al., 2001). Regardless of which test is used, when analyzing results it is important that the sensitivity and specificity of the test is understood (Gardner et al., 2000). The test specificity reflects the percentage of animals that are negative for the disease that the test accurately reports as negative, whereas the sensitivity reflects the ability of the test to identify truly positive animals. Regardless of the test used, false-positive and false-negative results will occur on occasion. Increased numbers of false-negative results may be seen during the early periods after formation of an SPF colony, whereas more false positives may be reported after the colony is established (Mansfield, 2005). Confirmational testing should always be performed at a reference laboratory when positive or questionable results are reported. To increase throughput, many commercial laboratories are now employing multiplex microbead immunoassay technology (Luminex Corp. Austin TX), which facilitates the concurrent detection of numerous pathogens in one sample (Khan et al., 2006).

Formation of Specific Pathogen-free Colonies

The methods employed in the formation of SPF colonies will vary depending on the infrastructure of the parent facility. Ideally, candidate animals 8–12 months of age should be selected. Selection criteria include virological status, genetic background, and potential health and behavioral concerns. Once identified, founder animals are placed into small social groups (3–4 individuals) and blood samples collected for virological testing. The size of the peer groups should be such that it encourages normal behavior but limits the risk of virus transmission should an animal seroconvert. Candidate SPF founders should undergo quarterly viral screening for at least 2 years before being placed into breeding groups (Mansfield, 2005). Owing to the potential of delayed seroconversion, testing for BV should continue on a quarterly basis throughout the animal's life. The initial breeding groups formed are often referred to as Level 1 SPF animals. Progeny from the SPF Level 1 cohorts are frequently referred to as Level 2 SPF. Should seroconversion occur in a Level 1 group, the affected animal should be removed. The remainder of the cohort should be isolated from the SPF colony and undergo rigorous serological testing to confirm continued seronegative status.

Housing strategies play a major role in the development and success of SPF colonies. Depending on their geographical location and size, facilities may house their breeding groups in harems of 8–10 animals or in large outdoor corrals containing up to 150 individuals. In the formation of SPF colonies, harem breeding may be more advantageous, particularly in the initial period. In this configuration, seroconversion of one animal poses a threat of virus transmission to a maximum of 10 contact individuals. In contrast, seroconversion within corral housing can result in potential exposure of 100+ animals. Additionally, the testing and removal strategy for viral screening may be more efficiently accomplished in smaller group settings. Normally breeding harems are housed in indoor pens or outdoors in "corn cribs." Indoor housing may be beneficial if there are requirements for eradication of additional pathogens such as bacteria, parasites, and protozoa. At some facilities, breeding strategies such as timed-mating or assisted reproductive technologies are employed as an adjunct to harem or coral breeding. Both of these are useful in pathogen elimination and in the production of timed pregnancies for research protocols. However, these approaches are expensive to implement and frequently result in a reduction in colony reproductive performance. Table 8.5 lists advantages and disadvantages of various reproductive strategies used in breeding macaques.

The success of SPF colonies depends not only on continued screening for the target viruses but also on a rigorous surveillance program for other potentially detrimental pathogens. Ideally, preventative health examinations should be performed on a quarterly basis. Procedures normally performed at this time include clinical examination, collection of blood samples for virological testing, DNA banking and MHC typing, intradermal

TABLE 8.5 Relative Advantages and Disadvantages of Breeding Schemes Relative to SPF Production

Corral Breeding		Harem Breeding	
Advantages	**Disadvantages**	**Advantages**	**Disadvantages**
Lower infrastructure costs	Break in SPF status of one animal puts many at risk	Easier to maintain SPF status	Increased infrastructure costs
Increased genetic diversity	"Test and remove" more difficult to implement	"Test and remove" strategy easier to implement	Decreased genetic diversity
Improved reproductive performance	Increased risk of trauma	Easier to maintain accurate pedigree records	Possible decrease in reproductive performance
	Pathogen containment more difficult	Pathogen containment easier to accomplish	
	Additional immunizations required	Monitoring of disease and illness easier in smaller groups	
	Only feasible in areas with temperate climates		

tuberculin testing, and vaccination based on colony risk assessment. Additional tests such as the Primagam and lateral flow assays may be useful in diagnosing *M. tuberculosis*, particularly in the initial stages of colony formation (Garcia et al., 2004; Lyashchenko et al., 2007). Should clinical problems be noted, further diagnostic tests must be performed to identify the causative problem. If an animal dies or is euthanized, a full necropsy and histological examination of tissue must be performed. Clinical and necropsy findings should be entered into each animal's medical record to enable tracking of diseases and to aid in implementing prevention or treatment programs.

Maintenance of the SPF status of a colony requires exemplary husbandry and strict separation of SPF animals from conventional populations. As fomites pose a potential route of transmission of agents from animal to animal, it is essential that dedicated equipment is used in each colony. All personnel working in the area should be aware of the potential routes of spread of agents between animals and of the importance of changing personnel protective equipment between areas. Staff that need to work with both conventional and SPF animals should always perform procedures with the SPF colony before performing procedures with the conventional colony.

Ideally, once SPF colonies have been established, all future breeding stock should be recruited from within the colony. It is therefore vital to incorporate a wide range of genetic lineages when selecting founder animals. Introduction of animals from outside sources may prove a significant risk to the integrity of the SPF status. The primary reason for introducing animals from other facilities is to increase genetic diversity within the colony. If this is necessary, addition of new males will provide the most benefit. Prior to their introduction into the colony, the animals from outside sources should undergo extended virus testing and quarantine for at least 1 year.

In addition to the four target viruses discussed above, demand has also increased for animals that are known to be free of various other pathogens, including bacteria, protozoa, and other viruses. Elimination of these agents may prove beneficial to research protocols and may also decrease the occupational health and safety risks associated with working with nonhuman primates. At some nonhuman primate centers, an "Expanded Specific Pathogen-Free Program" has been implemented. The primary pathogens targeted in these programs include simian foamy virus, rhesus lymphocryptovirus, rhesus cytomegalovirus, rhesus rhadinovirus and simian virus 40.

In summary, it is anticipated that over the next years not only will the demand for SPF macaques for use in biomedical research increase dramatically but also investigators will request animals with more specifically defined genetic composition. These requirements will result in an onus on nonhuman primate breeding facilities to produce animals of ever-increasing quality. The ease at which animals of defined pathogenic and genetic background can be produced will vary from facility to facility, with housing strategies and infrastructure having a major impact. Indoor housing of macaques in small cohorts should facilitate the elimination of bacterial, protozoal, and parasitic pathogens if required. Harem housing also enables accurate identification of dams and sires of progeny and improves the ease

of making clinical assessments. However, in contrast to corral breeding, production of macaques in harem groups can result in decreased genetic diversity of offspring produced and lower production rates. A strong veterinary and animal care program is central to the production of SPF macaques, and its importance should not be underestimated. Training programs for all personnel who have contact with the animals should include discussion of the routes of pathogen transmission, zoonotic potential of pathogens, and use of personal protective equipment. In addition, the importance of prompt reporting of clinical problems is essential to prevent disease outbreaks in the colony. Computerized medical, pathology, and demographic databases should be considered essential in colony management to aid in the identification of disease trends and housing, breeding, and pedigree data.

Baboons

Baboons breed continuously throughout the year, which is a major advantage when research protocols depend on a regular, consistent supply of pregnancies or newborn infants. The prominent perineal skin of the female baboon enables reliable and inexpensive daily visual assessment of ovarian function status and of pregnancy, which is valuable for reproductive research and breeding colony management.

Female baboons in captivity generally reach puberty between 3 and 4 years of age (as determined by observation of the menstrual cycle). Females have a regular menstrual cycle that is physically visible by the size and appearance of skin in the perineal area, commonly called the "sex skin" in nonhuman primates. The sex skin swells and shrinks according to reproductive hormone levels. In an unpublished study of 32 juvenile females, cycles were read starting at 3 years of age, using the scoring system of Hendrickx and Kraemer (1969). The average age of cycle commencement was 3.6 years (K.S. Rice, unpublished observations).

The average menstrual cycle length in baboons is 33 days, with follicular and luteal phases, just as in humans. The correlation between sex skin turgescence and ovulation has been well documented (see the section "Detection of ovarian cycle phase" below) so that determining the onset of the menstrual cycle in puberty, producing timed pregnancies in group-caged baboons, and identifying cycle irregularities in the perimenopausal period are both feasible and economical.

Endometriosis develops spontaneously in baboons, as in humans. Although endometriosis is undesirable in a breeding colony because it affects fecundity, the existence of this condition in baboons demonstrates their physiological similarity to humans and is thus a useful model for testing agents meant to inhibit endometrial growths (Hendrickx, 1967; Hendrickx and Kraemer, 1969; Pauerstein et al., 1978; Stevens, 1997; Chen et al., 1998).

Cycle reading has been used to produce timed pregnancies in baboons for years at the facility with the world's largest captive baboon breeding program, the Southwest National Primate Research Center (SNPRC), Southwest Foundation for Biomedical Research. Reading the baboon cycle three times per week (usually Monday, Wednesday, and Friday) produced accurate predictions of conception within 2 days. Detection of pregnancy is best confirmed indirectly by lack of sex skin swelling. Therefore, it is possible to predict a pregnancy as early as 15 days (if the cycle length is known and regular). The pregnancy can be confirmed with ultrasound, which requires sedation but does not require manual palpation of the uterus, which might predispose to pregnancy loss. Ultrasound confirmation of pregnancy is also appealing since the result is instantly visible whereas chemical confirmation from a blood or urine sample further delays the answer.

The baboon gestation period is about 6 months (Sunderland et al., 2008) and most baboons deliver at around 185 days' gestation. Pregnancy loss is most likely in the first 90 days. Viable offspring that do not need supportive care have been born as early as 155 days' gestation. Pregnancies may extend 2 weeks past the due date with no adverse events. Breech presentations are occasionally observed but successful deliveries have been accomplished with manual turning of fetus.

Baboons have a single discoid placenta similar to that of humans. This anatomical similarity to humans is important when measuring maternal-infant placental transfer. Shearer et al. (1995) have demonstrated that baboons, like humans and unlike macaques, have four IgG subclasses (IgG 1, 2, 3, and 4). Maternal immunity is transferred to the fetus through IgG subclasses so this trait is important in an animal model used to test the efficacy of human vaccine regimens designed to enhance placental transfer of maternal antibodies to the fetus (Ha et al., 2000a,b).

Most baboon babies are born at night (Sunderland et al., 2008), regardless of whether they are group or singly housed. In most cases, the placenta is consumed immediately after delivery. Baboons generally continue to lactate as long as the infant nurses. Success with surrogate mothers has been limited (K.S. Rice, personal observation).

Baboons continue to cycle regularly for at least 15 years and usually well into their mid twenties. Documentation of a female baboon reaching menopause (6 months acyclic with no vaginal bleeding) before the late twenties or early thirties is rare (Chen et al., 1998; Honore and Tardif, 2009).

Male baboons attain puberty, as determined by testicular enlargement, between 5 and 6 years of age (Beehner et al., 2009). Generally, males are not selected as breeders until they are at least 6 or preferably 8 years old because to

be good breeders, males must exhibit authority to maintain social harmony.

Baboon breeding arrangements have been described by Else et al. (1986) and Ha et al. (2000a,b). Baboons breed best in harems, though they may also be maintained in very large multi-male, multi-female groups with sufficient space. Optimal productivity has been found with breeding groups of a single male and 10−15 females (K.S. Rice, personal observation). Stable breeding groups with little movement in or out maintain social stability and help minimize the chance of miscarriage. A single male breeder also tends to maintain social harmony among his group members such that the best success is achieved by introducing females in small groups instead of one by one. Good integration is experienced by introducing a small group of new females to the male and allowing them to socialize for several hours, then returning the main group of female breeders to the group cage. Although establishing social rank may necessitate some physical altercations, the male is more apt to promote integration because of the bonds established by introducing new females in this manner.

Baboons are predictable in their behavior, generally calm, and easy to handle in captivity. Since baboons tolerate weather extremes well, they can be housed in outdoor facilities in most environments. The types of outdoor large group housing used for the SNPRC colony afford easy access to the animals and allow moderately large social groups (up to 20 animals) that closely approximate a natural setting.

When a new breeding group is started, the group is allowed the first 3 months to acclimate, after which a pregnancy rate of about 80% is expected. Females who do not become reproductive can be moved into another group with success. Sometimes it helps to move low-ranking or more submissive females to groups with younger females.

Other factors to monitor are pregnancy retention, live births, and successful mothering. A relatively common phenomenon in harem groups is for a more dominant female to "steal" another female's infant, in which case it is difficult, if not impossible, for the mother to retrieve her infant. If a female steals another mother's infant, the practice has been to retrieve the infant and put the baby back on the mother. If it happens again and the baby stealer is lactating, she is allowed to keep the infant. Females are kept in breeding, and about three pregnancy losses or three infant deaths are allowed before that baboon is removed from breeding. SNPRC keep infants with their mothers for a minimum of 9 months. From practice, this seems to promote the best environment for producing offspring that will have normal behavior.

The best guide to population management in baboons may be medium-term supply and demand. Evaluation of the demand for animals over a 5- to 10-year span will help to determine the numbers of animals needed at specific ages. Based on this scenario and on knowledge of mortality (life-table analysis) and reproduction (e.g. animal age at first pregnancy, prime reproductive years, stable breeding group design), an optimal breeding colony size can be identified. Other factors to consider are recovery periods for surgical interventions (e.g. catheter implant for tether studies, fectectomy or caesarean section), sufficient reserve male breeders, and facility renovation plans that may affect breeding space.

Squirrel Monkeys

Squirrel monkeys have a distinct breeding season that spans approximately 3 months followed by a birth season approximately 5 months later (Williams et al., 2002). Seasonal breeding has been related to annual rainfall cycles and seasonal food availability (Baldwin and Baldwin, 1981; Boinski, 1987), changes in light cycle (Rosenblum and Cooper, 1968), and relative humidity (DuMond, 1968). Boinski (1987) found a strong tendency toward birth synchrony in *Saimiri oerstedii* in Costa Rica and suggested this tendency might be an anti-predator adaptation.

During prebreeding and breeding season, male squirrel monkeys undergo marked physical changes including increases in body weight, spermatogenesis, and circulating levels of androgens (Wiebe et al., 1984, 1989). The increase in body mass is distributed primarily over the upper torso (DuMond and Hutchinson, 1967; Williams et al., 1986; Boinski, 1987) and is related to the increased levels of androgens (Nadler and Rosenblum, 1972; Coe and Rosenblum, 1978). These annual weight gains begin during the third year of life; however, most laboratories do not consider a male squirrel monkey to be sexually mature until he is 4 years old.

Along with the physiological changes in adult male squirrel monkeys there are behavioral changes (Williams et al., 1986). Sexual interactions between males and females are not seen during the nonbreeding season. The breeding season is characterized by increased levels of sexually related responses such as genital displays, anogenital inspection, and copulation. The breeding season is characterized by a reduction in aggressive responses compared with prebreeding season levels.

Female squirrel monkeys have an unusually short ovarian cycle that is characterized by exceptionally high circulating steroid hormones (Ghosh et al., 1982; Aksel et al., 1991). Females are seasonally polyestrous, with each cycle spanning 10−12 days (Diamond et al., 1984; Aksel et al., 1985). Although there are no obvious, external signs of estrous, the female's cycle can be monitored by the cytology of vaginal epithelial cells (Travis and Holmes, 1974), by ultrasound monitoring of follicular development (Schuler et al., 2007), or by monitoring circulating levels of serum progesterone

(Aksel et al., 1985). Serum progesterone concentrations greater than 100 ng/ml can be used to identify cycling squirrel monkeys. Yeoman et al. (2000) found a mid-cycle follicle stimulating hormone (FSH) rise coincident with the LH surge as well as elevations in circulating FSH during the luteal phase, suggesting that considerable follicular development occurred prior to luteolysis. Average gestation length in squirrel monkeys is 150−155 days.

These hormonal changes, consistent with estrous cycles, were associated with behavioral changes (Williams et al., 1986). The female squirrel monkeys with cyclic hormonal levels received more genital investigations and more sexual invitations than did noncycling females. Cycling females also showed changes in their behavior, tending to follow the male more than noncycling females did and to sniff the male more frequently. Copulations occurred on the day of and day following ovulation.

Squirrel monkeys adapt to many different social group configurations. For breeding purposes, squirrel monkeys do well in single-male/multi-female groups with ratios as high as 1 male to 10−12 females. Infants begin to eat solid food at around 1 month of age and will stop nursing at 4−5 months. Squirrel monkey females show a high rate of allomaternal care, with unrelated females carrying and nursing infants as old as 6 months (Williams et al., 1994). Since squirrel monkey infants are large (up to 18% of the dam's nonpregnant body weight), allomaternal care may be an important strategy to increase infant survivability.

Squirrel monkey females reach their maximum reproductive potential between the ages of 6 and 13 years. In females younger than age 4, fetal and neonatal mortality is high. Although females can produce healthy infants later into their teens, their probability of becoming pregnant decreases with age. The generation time for squirrel monkey females is approximately 9 years, with females producing 5−10 surviving infants during their lifetime.

Owl Monkeys

Owl monkeys are usually reported as highly monogamous nonhuman primates, with groups most often composed of an adult male and female and up to three infants and juveniles (Wright, 1981). The sire provides most of the care for the infant and only gives the infant to the dam to nurse (Jantschke et al., 1998). If the father dies when the infant is still young, other siblings, but not the mother, will assume the caregiver role. Owl monkeys are unique in that the female will actively refuse to carry the young if the male is unavailable, going so far as to violently pull the infant off its back.

Owl monkeys are highly territorial and have extremely small home ranges given the size of this nonhuman primate (Wright, 1989). Male−male aggression is common and is a factor in keeping groups apart (Moynihan, 1964). Agonistic encounters involve back arching, stiff-legged jumping, pilo-erection, urination and defecation, and giving clicking/grunting alarm calls. Identical agonistic displays occur between conspecifics and other species (Wright, 1989). They have loud contact calls (Moynihan, 1976). Olfaction is an important component of communication, and Aotus marks substrates by rubbing a gland at the base of its tail and exuding a brown, oily substance (Wolovich and Evans, 2007).

Owl monkeys are typically described as nonseasonal breeders in captivity, although some field data suggest that these animals can show seasonality in habitats with extreme environmental fluctuations (Fernandez-Duque and Huntington, 2002). Two birth peaks per year have been noted in the field − one at the end of the dry season and the other in the middle of the wet season (Wright, 1985). Females cycle approximately every 16 days (Bonney et al., 1979). The mean age of first birth in Aotus females is 3 years (Gozalo and Montoya, 1990). Gestation is 133−141 days (Gozalo and Montoya, 1990). Owl monkeys have a relatively short interbirth interval of about 1 year, which is possible owing to the high levels of paternal care that alleviate much of the energy burden to the mother (Garber and Leigh, 1997).

Although there are no data on female puberty in owl monkeys, male puberty begins around 13−15 months of age (Dixson et al., 1980). In field studies, young males tend to disperse at the age of 2−3 years (Fernandez-Duque and Huntington, 2002) and may pass into a nomadic, "vagabond" stage before pair-bonding with a female. Once animals reach 18−24 months of age, they can be paired to form new breeding groups. Male−female pairings are successful in approximately 75−80% of attempts. Isosexual pairs can be established but are more difficult; up to 65% of female−female pairs and 44% of male−male pairs are successful.

Marmosets

Marmosets and tamarins are small, South American primates of the subfamily Callitrichidae. The only callitrichine species commonly used in biomedical research is the common marmoset (*Callithrix jacchus*). Recent reviews of standard husbandry and management practices for breeding marmosets include those of Tardif et al. (2003), Layne and Power (2003), and Rensing and Oerke (2005).

Sexual maturity − as defined by occurrence of ovulation in females and ejaculation of motile sperm in males − occurs at 11−13 months of age. However, ovulation may be suppressed for periods of years following puberty under certain conditions (Abbott, 1987; Tardif et al., 1994). A standard recommendation is that animals not be paired for breeding until around 18 months of age, as successful

reproduction is limited in pubescent animals and there is potential value in additional social experience for breeders with their natal group, as described below.

Female marmosets display no overt signs of ovulation. Copulations may occur at any point during the cycle and during pregnancy, although there is an increase in sexual activity during the female's ovulatory period. Average gestation length is 143−144 days (Hearn, 1986; Jaquish et al., 1995). Lactation lasts for 65−90 days and infants begin to take solid food at around 30 days of age and are completely weaned from milk at around 80−100 days of age. As opposed to most nonhuman primates, callitrichids do not display any meaningful delays in ovulation associated with lactation. Marmoset females typically ovulate 9−11 days following parturition.

Marmosets are typically housed for breeding as mated pairs. Aggression between males and females is relatively uncommon, and establishing breeding pairs is straightforward. Marmosets are cooperative breeders − group members including the breeding male and older offspring in the group participate in the care of dependent offspring, including transport and provisioning the infants with solid food once weaning begins. Therefore, it is preferable for the breeding female to remain not only with her mate but also with her older offspring. There is some suggestion that callitrichids − particularly tamarins − display more adept parenting skills if they were previously exposed to and participated in the care of younger siblings (Tardif et al., 1984; Tardif, 1997). Generally, older offspring will not reproduce as long as the original mated pair remains intact. Daughters are often reproductively suppressed and do not ovulate (French, 1997; Saltzman et al., 1997a,b), and mothers and daughters generally will not accept copulation attempts from sons/brothers.

Therefore, "nuclear" marmoset families are a stable housing condition as long as the original breeding pair remains intact, i.e. there is generally no need to remove older offspring from the group unless cage size requires it. Once the reproductive male or female dies or is removed from the group, the group may become unstable as new breeders are placed in the cage. Marmoset groups will sometimes include two breeding females if an unrelated male is present, but this configuration often leads to high infant mortality, especially due to infanticide (Rothe and Koenig, 1991; Saltzman et al., 2004, 2008).

Marmosets and tamarins are the only anthropoid nonhuman primates that routinely produce litters. In the wild, marmosets typically produce litters of 2, but in captivity they may produce litters up to 5, and triplets are often the most common litter size. Ovulation number and litter size are related to maternal condition, with a higher average maternal weight for larger litters than for smaller litters (Tardif and Jaquish, 1997). Females will not routinely rear more than two litters at a time, and it is thus reasonable to have plans in place for the handling of supernumerary offspring. Infants may be hand-reared for short periods (around 3 weeks) and successfully reintroduced into either their original group or into an experienced group that has had visual and olfactory exposure to the infant while it was being hand-reared. Supernumerary infants may also be hand-reared by rotating a different infant out of the group each day (Ziegler et al., 1981). Infants may also be cross-fostered to families with only one infant of a similar age.

The reproductive potential of marmosets is often cited as 4.0−4.5 young/year or up to 80 offspring in a lifetime. However, because marmosets have relatively high infant mortality and relatively short life spans, these figures likely represent maxima rather than averages. A recent examination of reproductive output in a population of around 400 dams found an average yearly production of weaned offspring of around 2.3 and a lifetime production of 7.75. So, while the pace of marmoset reproduction is clearly faster than in larger-bodied Old World monkeys (e.g. 2.3 versus 0.44 young/year for provisioned macaques) (Fedigan et al., 1986), it is considerably less than the oft reported maximum.

PREGNANCY MANAGEMENT

Detection of Ovarian Cycle Phase

Ovulation in most nonhuman primates occurs with few, if any, behavioral or externally perceptible cues, yet the ability to predict ovulation is essential to many reproductive and developmental studies. This section provides information on the methods commonly used to assess cyclical ovarian change in nonhuman primates, including ovulation and menses.

Monitoring female reproduction by cyclical changes in vaginal cytology (e.g. changes in vaginal epithelial cornification), such as is done in rodents, is not commonly used in assessing monkeys but has been evaluated for those nonhuman primates with no or limited menstrual flow, primarily prosimians and New World monkeys (Travis and Holmes, 1974; Hendrickx and Newman, 1978; Stolzenberg et al., 1979; Dukelow, 1983; Nagle and Denari, 1983; Izard and Rasmussen, 1985; Gluckman et al., 2004).

In contrast to prosimians and New World species, ovarian cyclicity is easily determined in the higher nonhuman primates due to an overt menses. In almost all breeding colonies, monitoring menses is done in one of two ways: (1) visual examination of the external genitalia for fresh blood, or (2) use of vaginal swabs to detect menstrual blood. Vaginal swabs or smears are taken by placing a cotton-tipped swab into the vaginal canal, preferably in the anterior fornix, and then visually examining the swab for the presence of blood. In some instances it may be

beneficial, especially in young pubertal or oligomenorrheic animals, to smear the swab on a glass microscope slide and examine it for red blood cells.

In approximately 16% of the menstrual cycles of baboons, overt menstruation does not occur in successive cycles, making it difficult to utilize gross observation as an end point without collecting vaginal smears (Hendrickx, 1971). Vaginal smears make it possible to detect menstruation in approximately 95% of the cycles but is less convenient to measure than are the changes that occur in the perineum (sex skin).

The sex skin (perineal swelling) is a very reliable way of accurately dating the pregnancy in several Old World monkeys (baboon, pig-tailed macaques, and others) and the chimpanzee. The visible cyclic changes in the sex skin or perineum that correlate with the menstrual cycle have been well described in the baboon (Hendrickx, 1971). The turgescent phase encompasses an initial turgescent stage (average 4 days) when the perineal area starts to swell with a decrease in wrinkling of the skin, which changes color from dull pink to a pinkish red. During the subsequent maximum turgescent stage (average 13 days), the skin of the perineum is fully distended with no wrinkles and attains its deepest and most intense bright red color (Figure 8.1a). The deturgescent phase is similarly divided into two stages. Initial deturgescence (average 5 days) begins with a loss of color, a decrease in size of the swelling, and a corresponding increase in wrinkles (Figure 8.1b). During the following quiescent stage (average 12 days), the perineum is of minimal size and the labia and clitoris have many wrinkles with an overall pinkish red color. The dull epithelial surface of the perineum, which begins to slough during the deturgescent stage, is usually completely shed by the end of the quiescent phase.

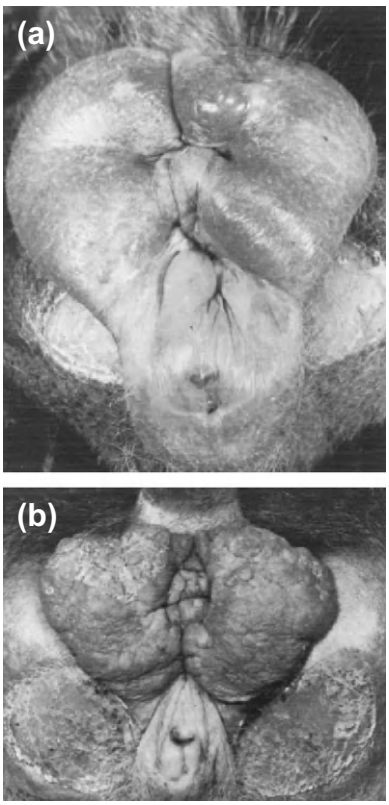

FIGURE 8.1 Perineum of an adult baboon at two different stages of the menstrual cycle. (a) Maximum turgescent stage characterized by full distention of the perineal skin which has a smooth, shiny appearance and is a deep, intense red color. (b) Late turgescent stage identified by a loss of turgidity and color and an increase in perineal wrinkles.

Timed matings in baboons have established that Day −3 deturgescence (the third day before the onset of deturgescence) is the optimal day for mating (Hendrickx, 1971). Endocrinological data indicate that ovulation occurs most often on Day −1 or −2 deturgescence (Wildt et al., 1977; Shaikh et al., 1982), therefore Day −1 or −2 should be designated as Day 0 of pregnancy. Bielert et al. (1976) demonstrated a positive correlation among sex skin color, circulating levels of estradiol, and increased sexual activity as indicated by ejaculations in periovulatory rhesus monkeys.

Another common method for detecting ovulation is endocrinological evaluation of urine or serum. During the normal estrous cycle, an estrogen peak occurs 15−24 hours prior to a peak in luteinizing hormone (LH). The latter triggers ovulation and the formation of the corpus luteum with a subsequent rise in progestins. Analysis of these hormones (i.e. the estrogens, LH, or progesterone) provides presumptive evidence that ovulation has occurred; the pattern of change in circulating estradiol, progesterone, and LH has been described for most nonhuman primates commonly used in studies.

A considerable body of evidence has been gathered about ovulation and its relationship to ovarian and pituitary endocrine events in *M. mulatta* (Hotchkiss et al., 1971; Weick et al., 1973; Parkin and Hendrickx, 1975; Monfort et al., 1987), *M. radiata* (Lasley et al., 1974; Parkin and Hendrickx, 1975), *M. fascicularis* (Monfort et al., 1987; Behboodi et al., 1991), *Papio* spp. (Wildt et al., 1977; Shaikh et al., 1982), *Saimiri* (Aksel et al., 1985), *Callithrix* (Harlow et al., 1984), and *P. troglodytes* (Gould and Faulkner, 1981).

Hotchkiss et al. (1971) reported that estradiol will rise over a 3-day period prior to ovulation in *M. mulatta*, indicating that this parameter may be useful for predicting ovulation. Weick et al. (1973) showed that plasma estradiol levels peak 9−15 hours prior to the preovulatory LH surge and approximately 30−40 hours before ovulation in the same species. Of particular relevance to the use of either ovarian hormone as a marker for ovulation are the observations by Bielert et al. (1976), who noted that sexual interaction increased between heterosexual pairs in daily time-limited matings that coincided with the preovulatory estradiol peak.

Additional information on the temporal relationship between the preovulatory estradiol peak and ovulation has been provided in *M. radiata* (Lasley et al., 1974). Estradiol levels begin to rise 1−3 days prior to the peak and return to baseline within 2 days; additionally, estradiol peak occurred between cycle days 7 and 12 in 14 of 15 cycles. Ovulation was confirmed by measuring progestins, which rose significantly the day following the estradiol peak. Observations at laparotomy confirmed that ovulation occurred 24−48 hours after the peak. The favorable temporal relationship between the estradiol peak and ovulation as well as the approximate 3-day duration of the preovulatory estradiol rise (Hotchkiss et al., 1971) has made measurement of this hormone a useful parameter for predicting ovulation.

Circumstances frequently arise in which detection of urinary or fecal metabolites of ovarian hormones provide a more practical means of determining cycle phase. Radioimmunoassays (RIAs) and enzyme-linked immunosorbant assays (ELISAs) have been developed and applied to the detection of urinary estrone conjugates (E_1C), progesterone metabolites, and LH. These results may be used for detection of ovulation and for monitoring reproductive function in rhesus and cynomolgus macaques (Monfort et al., 1986, 1987), marmosets and tamarins (Eastman et al., 1984; Harlow et al., 1984; Heger and Neubert, 1987), capuchin monkeys (Nagle et al., 1980), and squirrel monkeys (Travis and Holmes, 1974). In *M. mulatta*, E_1C measurements in both nonconceptive and conceptive ovarian cycles demonstrate profiles that are both qualitatively and quantitatively similar to measurements of circulating serum estradiol.

Thus, measurement of E_1C provides a practical and noninvasive approach in prospective and retrospective longitudinal studies of individual animals provided that adequate facilities for collection of urine are available. A 1.5- to 2.0-fold E_1C increase above the mean early follicular baseline has been observed 2 to 3 days before the E_1C peak, which occurs in the majority of *M. fascicularis* between Days 8 and 15 of the menstrual cycle (Behboodi et al., 1991). These results are similar to those reported for women (Munro et al., 1991). A single 2-hour mating before or the day of the E_1C peak resulted in a conception rate of 38.6%, which is comparable to a 40% conception rate in contemporary controls mated every other day over a 5-day period during midcycle (three times). In contrast, breeding 2 days prior to or 2 days after the peak significantly reduced the conception rate (Behboodi et al., 1991).

In marmosets, excreted estrone conjugate concentrations largely reflect the metabolism of progesterone and, therefore, follow a different pattern in relation to ovulation, rising at or immediately after the ovulatory LH surge (Eastman et al., 1984). Comparisons among closely related marmoset and tamarin species reveal significant variation among these species in the metabolism of ovarian steroids, and results from any individual species should be compared specifically with standards from that species (Hodges and Eastman, 1984; Ziegler et al., 1987).

As interest in determining endocrine parameters of nonhuman primates in the wild has developed, methods to store, process, and analyze fecal samples have become increasingly sophisticated (Ziegler and Wittwer, 2005). Fecal samples have also been shown to be of value in assessing ovarian steroid concentrations in nonhuman primates of interest to biomedical research, including macaques (e.g. O'Neill et al., 2004), squirrel monkeys (Moorman et al., 2002), and marmosets and tamarins (Ziegler et al., 1996). These methods may prove to have particular importance in the future for monitoring breeding animals maintained in large enclosures or free-ranging conditions.

If ovulation does not occur, several techniques have been utilized to induce this process − namely, treatment with exogenous gonadotropins (Dukelow, 1970, 1979; Kuehl and Dukelow, 1975; Kholkute and Nandekar, 1983) or gonadotropin-releasing hormone (GnRH) (Hodges et al., 1988; Yeoman et al., 1988). In reference to marmosets, of particular note is the fact that prostaglandins will induce luteal regression, therefore allowing for relatively precise timing or synchronization of ovulation (Summers et al., 1985).

Laparoscopy involves anesthetizing the animal and inserting a laparoscopic telescope into the abdominal cavity with subsequent insufflation with an inert gas, which allows visualization of abdominal structures including the ovary. With this technique, it is possible to evaluate developing follicles, sites of ovulation, or the presence of a corpus luteum. Laparoscopic evaluation of folliculogenesis and ovulation have been conducted in prosimians and in a number of the New and Old World species (Dukelow et al., 1973; Dukelow, 1975; Dukelow and Ariga, 1976; Tardif et al., 1993). The dynamic changes in follicular structure and vascular patterns noted at laparoscopy have been used to identify the occurrence of ovulation within a short interval (approximately 6 hours) in *Papio* spp. (Wildt et al., 1977). Moreover, the sequential changes in ovarian follicular development have been correlated with perineal swelling, changes in vaginal cytology, and serum ovarian hormone levels (progesterone and estrogens). In most breeding colonies, the use of these procedures is normally restricted to situations in which confirmation of ovulation is needed or determination of a pathological condition is required. It should be noted that potential detrimental effects of general anesthesia and carbon dioxide pneumoperitoneum on oocyte quality have been proposed (Lavy et al., 1988).

Ultrasonography has been used as a means of imaging ovarian development in the rhesus (Morgan et al., 1987),

the long-tailed macaque (VandeVoort and Tarantal, 1991), and the marmoset (Oerke et al., 1996). Because of its reliability for documenting the response to ovarian stimulation and aspiration of follicles, ultrasonography can be used to recover oocytes for in vitro fertilization in macaques.

Detection and Monitoring of Pregnancy

Pregnancy is diagnosed at the earliest point by using assays that measure the presence of chorionic gonadotropin (CG). CG is produced by the trophoblast and functions to prevent luteolysis; detection of CG is the basis for over-the-counter pregnancy tests in women. In the 1970s and 1980s, hemagglutination inhibition tests for urinary CG were developed for use in Old World monkeys. These tests include the Subhuman Primate Pregnancy Test Kit (SHPT) and the Nonhuman Primate Pregnancy Test (NHPPT) developed by Hodgen and Ross (1974) using an antiserum to the β subunit of ovine LH, which is common to the CG of humans, gorillas, orangutans, chimpanzees, baboons, and macaques. This antiserum is, however, dissimilar to FSH and LH of baboons and macaques. Only 0.2 ml of neat urine (or an equivalent amount of urine extract) is required for testing, and results are obtained within 3 hours after the samples are collected. This method is reliable for confirming pregnancy on gestation day (GD) 16 (fivefold concentration of urine required) or by GD 18 (neat urine tested). Collecting aliquots of freshly voided urine gives the most satisfactory result because it minimizes the time and collection of samples in addition to urine debris. This test became the method of choice for routine breeding management situations and has been used successfully in rhesus (Hodgen and Ross, 1974) and long-tailed macaques (Boot and Huisin't Veld, 1981), baboons (Hodgen and Niemann, 1975), and chimpanzees (Hodgen et al., 1976). Both false-positive and false-negative tests are reported, due in part to the variability in urinary macaque CG excretion from animal to animal.

ELISA, which may now be used for accurate measurement of CG in serum or urine in macaques (Munro et al., 1991), is a particularly valuable method for studies in which changes in CG concentrations may be relevant. In 2001, Shimizu et al. (2001) reported on a noninstrumented ELISA for pregnancy detection in macaques that used a color change visible to the naked eye, similar to a human pregnancy test. The false-positive rate for this test was zero, but the false-negative rate was relatively high, such that these investigators reported an accuracy of 70%. Recently, Lohstroh et al. (2007) reported on the validation of a chemiluminescent immunoassay measurement of CG in macaque urine, adapted to the platform of the Bayer ACS-180 autoanalyzer. Using this method, CG was on average first detectable at GD 12−13.

Determination of CG concentration has also become a standard means for diagnosing pregnancy in many New World species. In the squirrel monkey, the concentrations of hormone gradually increases during early pregnancy and reaches maximum values at midgestation (Diamond et al., 1987). Analysis of CG is most accurate between 40 and 60 days of pregnancy; however, single determinations have an inherent 10% risk of false-negative responses due to low CG levels (Hodgen et al., 1978). CG in the marmoset is excreted throughout pregnancy, and maximum levels can be detected between the 8th and 9th week of gestation (Hearn et al., 1988; Hobson et al., 1977). Gestational levels of CG are first noted about 20 days after the LH peak in tamarins and continue to be elevated for another 80 days (Kleiman et al., 1978; Heistermann et al., 1987; Ziegler et al., 1987). In the owl monkey, CG can be detected about 16 weeks prepartum until birth.

Shaikh et al. (1976) found that pregnancy confirmation in baboons is more reliably detected by a plasma CG RIA than by the urine hemagglutination inhibition assay (Hodgen and Ross, 1974). Although plasma CG RIA is more time-consuming (results obtained 18 hours after sample collection), pregnancy is identified on GD 16 with 96.6% reliability. Plasma estradiol and progesterone RIA determinations, which can be obtained more quickly, have the same level of accuracy (96.6%) on GD 16 when evaluated according to a computer-derived formula.

A quantitative radioreceptor assay (RRA) was employed for early diagnosis of pregnancy in *M. fascicularis* by determining serum CG levels 3−4 weeks after conception (Yoshida et al., 1987). Serum CG levels increased to 50 μg/ml in the majority of animals evaluated. Three weeks after conception, 86% of all pregnant animals showed a positive response, and by 4 weeks after conception, a 95% positive response was reported. Five percent of the tested animals yielded false-negative responses at 4 weeks due to low CG levels; no false-positive responses were reported.

Monitoring steroid hormone metabolites in urine has also been used as a means of detecting pregnancy in some New World species. Measurement of hydroxypregnenolone excretion has been used for this purpose in marmosets (Hodges et al., 1983; Heger and Neubert, 1987), tamarins, and owl monkeys (Kleiman et al., 1978; Heistermann et al., 1987; Ziegler et al., 1987). In a study by Czekala et al. (1981), pregnancy was monitored via small urine volumes and measurements of immunoreactive E_t and LH/CG bioactivity in four diverse species: the orangutan, pygmy chimpanzee, Douc langur, and capuchin (Czekala et al., 1981). Measurement of E_t alone was sufficient to detect and monitor pregnancy in most species. However, in some species it may be necessary to assess individual estrogens if a more precise evaluation is necessary. Measurement of LH/CG bioactivity usually allows for earlier detection of

pregnancy than does E_t alone and provides additional information on implantation and placental function.

Before the early 1960s, the only means of diagnosing pregnancy in nonhuman primates was by palpation of the uterus. Hartman (1932) was the first investigator to describe in detail the method of bimanual rectal palpation for accurately determining the stage of pregnancy by the size of the uterus and size of the fetal head and for following involution of the uterus postpartum. This palpation procedure is still useful for diagnosing pregnancy in both indoor and outdoor breeding colonies as long as it is performed by well-trained and experienced individuals. The examination is best done using ketamine hydrochloride (Ketaset; 10 mg/kg) or similar anesthesia with the animal lying on its side or placed in the supine position (Figure 8.2). In larger species (i.e. *M. mulatta*, *M. nemestrina*, *Papio* spp.), the examiner inserts the middle finger of the right hand into the rectum as far as possible, pressing toward the abdominal wall while placing the left hand ventrally for counterpressure. In smaller species (i.e. *M. fascicularis*), use of the little finger may be more appropriate. For most Old World monkeys, with the exception of large baboons, the entire length of the uterus may be appreciated if the animal is not pregnant or in the early stages of pregnancy; the ovaries may be identified on either side of the uterine body. The cervix in most macaques is readily distinguished by the sharp ridge marking its cranial border. The vagina is difficult to identify since it is collapsed against the symphysis pubis. If the ovaries are palpated, their size can be described by subjective terms such as "tiny (infantile), small, medium, large, or very large." Wilson et al. (1970) devised a set of gauges covering the range of ovarian and uterine sizes; however, he found that palpation of the ovaries did not yield reliable information about reproductive events.

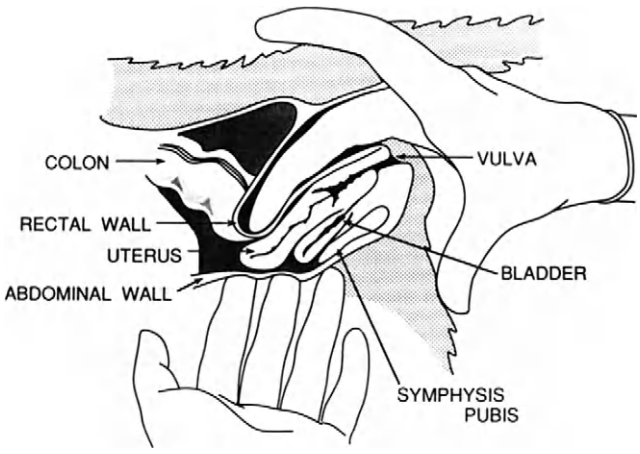

FIGURE 8.2 Diagrammatic representation of bimanual palpation for pregnancy detection. *Adapted from Wilson et al. (1970). (From Teratology, 3. Copyright © 1970, John Wiley & Sons, Inc. Reprinted with permission of Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.)*

Hartman (1932) and Mahoney (1970, 1972, 1975) appear to be among the few who developed the skills required to obtain useful information by ovarian palpation of *M. mulatta* and *M. fascicularis.*

Diagnosing pregnancy by bimanual palpation is easier from approximately GD 16 onward in macaques and baboons because of the relatively rapid changes that occur anatomically. Pregnancy is confirmed on the basis of size and consistency of the uterus until the 11th or 12th week (in macaques), at which time the head of the fetus can be palpated directly. Uterine palpation can be used to accurately diagnose pregnancy by GD 25 in *M. mulatta* and by GD 20−21 in the baboon provided that the day(s) of mating is known. Abdominal palpation can also be used to diagnose pregnancy in New World species. In the squirrel monkey, this method is reliable by the 6th week of gestation, when a 4- to 5-mm mass is detectable initially in the lower abdominal region cranial to the pelvis. The mass is larger and spongier by about 10−12 weeks (Kaplan, 1977). In the marmoset, estimates of uterine size based on external palpation have allowed formulation of an equation that permits prediction of parturition time (Gengozian et al., 1974; Phillips and Grist, 1975).

One of the more recent techniques employed to detect and monitor pregnancy in monkeys has been diagnostic ultrasound. This method provides a reliable means for evaluating and maintaining reproductive colonies in addition to its application for experimental purposes. For macaques, animals may be hand held by experienced animal handlers, placed in restraint chairs (if previously trained), or immobilized with ketamine hydrochloride (10 mg/kg) for examinations. To detect pregnancy, the uterus is scanned transabdominally in both serial sagittal and transverse planes (Tarantal and Hendrickx, 1988a,d). Because of the characteristic thin abdominal wall in these species (*M. mulatta* and *M. fascicularis*), the high-frequency transducers (i.e. 7.5 or 10 MHz) provide optimal resolution and image quality. The uterus will usually be found midline, although anatomical variations and abdominal/pelvic adhesions can result in alternate locations. In many cases, the uterine body may be highly mobile and can be found flexed to the right, left, ventral (anteflexed), or dorsal (retroflexed) in relation to the cervix. This is frequently the case for females with an elongated lower uterine segment. In roughly 90% of all cases evaluated, a central linear echo is noted within the normal nongravid uterus. This echo represents the uterine "cavity"/interface between apposing layers of endometrium. The uterine or endometrial cavity echo (ECE) is a useful landmark for (1) identifying the uterus, (2) detecting early pregnancy, and (3) assessing uterine pathology.

Pregnancy can be identified in both the rhesus and long-tailed macaques as early as GD 14−16 (Tarantal and Hendrickx, 1988a). During this period of development, the

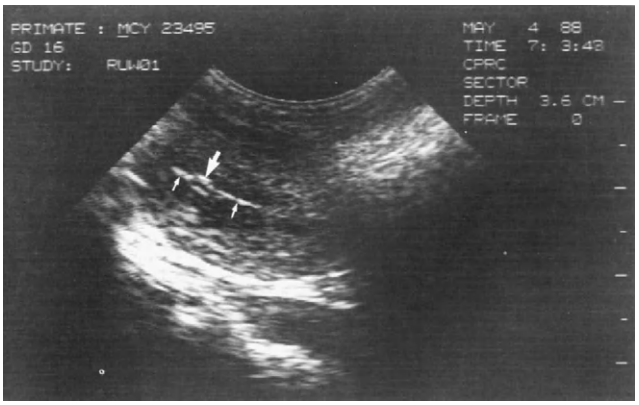

FIGURE 8.3 Sonogram (sagittal section) of macaque uterus on gestational day (GD) 16. Note endometrial (uterine) cavity echo (small arrows) and developing gestational sac (large arrow).

ECE shows a slight irregularity, thickening, or split in the upper third of the uterine body (Figure 8.3). This represents the developing gestational sac (GS), which by GD 18 appears as an ovoid fluid-filled structure approximately 5.5 ± 1.4 mm in mean diameter. Early pregnancy (GD 14 or 16–30) can be accurately detected and assessed using the sonographic developmental guidelines previously established (Table 8.6). These guidelines are useful for assessing early pregnancies at risk provided that normal developmental variation and methods for mating are kept in mind. In addition, no false positives or negatives result when incorporating these techniques (Tarantal and Hendrickx, 1988d). Once pregnancy is identified, a variety of measurements [mean GS size, yolk sac diameter, greatest length (GL) of the embryo, and heart rate] are important for monitoring normal development (Tarantal and Hendrickx, 1988a,b,c) (Table 8.7).

Of interest is the sonographic appearance of "implantation bleeding." Hypoechoic areas may appear to surround the developing GS or appear within discrete regions such as cranial and caudal to the GS. This can be distinguished

TABLE 8.6 Sonographic Developmental Guidelines[a]

Feature	GD
Thickening and/or split in ECE	14–15
Gestational sac	16–18
Yolk sac	18–20
Embryo	21–25
Cardiac motion	21–25

[a]*Gestational days (GD) 14–25. ECE, endometrial cavity echo.*
From Hendrickx and Dukelow (1995).

TABLE 8.7 Mean Gestational Sac Size (GS), Yolk Sac Diameter (YS), Greatest Length (GL), and Embryonic Heart Rates (EHR) for Both Rhesus and Long-tailed Macaque[a]

GD	Mean GS (mm)	YS (mm; range)	GL (mm)	EHR (bpm)
14	1.9 ± 0.2	–	–	–
15	2.6 ± 0.5	–	–	–
16	2.3 ± 0.4	–	–	–
17	3.7 ± 1.0	–	–	–
18	5.5 ± 1.4	1–2	–	–
19	5.9 ± 2.4	1–2	–	–
20	7.0 ± 2.6	2–3	–	–
21	8.9 ± 2.8	2–3	2.0 ± 0.0	60–80
22	9.3 ± 2.9	2–3	2.9 ± 0.6	72–104
23	10.7 ± 2.3	3	3.4 ± 0.8	80–120
24	12.4 ± 3.3	2–3	4.7 ± 0.9	80–144
25	13.0 ± 3.4	2–4	4.9 ± 0.9	92–144
26	13.5 ± 3.3	2–5	5.4 ± 1.9	100–140
27	14.9 ± 3.4	3–4	6.5 ± 1.5	100–144
28	16.0 ± 3.2	3–4	6.5 ± 1.8	108–148
29	18.1 ± 3.9	2–4	7.9 ± 1.5	120–152
30	18.3 ± 3.6	2–4	8.9 ± 1.7	120–156
31	20.7 ± 3.9	3–4	8.8 ± 1.9	128–160
32	19.9 ± 4.0	3–4	10.5 ± 2.3	132–160
33	23.0 ± 3.6	3–5	12.0 ± 2.1	128–160
34	24.2 ± 4.7	3–5	12.9 ± 2.6	128–160
35	24.2 ± 3.5	3–5	14.0 ± 2.7	124–168
36	28.1 ± 3.9	4–5	15.6 ± 2.0	124–156
37	27.0 ± 3.5	4–5	16.3 ± 1.9	144–176
38	29.2 ± 3.6	4–5	17.2 ± 1.9	140–184
39	28.1 ± 5.1	4–5	18.1 ± 2.1	132–184
40	31.9 ± 3.8	4–5	18.7 ± 2.0	132–168
41	32.2 ± 3.4	4–6	20.4 ± 1.9	140–180
42	32.6 ± 3.3	4–5	21.4 ± 2.1	140–172
43	33.2 ± 3.7	4–7	24.2 ± 2.9	144–172
44	34.4 ± 3.4	4–9	24.4 ± 2.2	144–176
45	33.4 ± 3.8	4–9	26.6 ± 3.7	144–180
46	37.2 ± 3.4	6–9	27.3 ± 3.1	148–184
47	38.4 ± 2.6	5–9	28.7 ± 4.7	144–180

[a]Macaca mulatta, Macaca fascicularis. *Gestational days (GD) 14–47. Both species similar in size during organogenesis (see Tarantal and Hendrickx, 1988a,b).*
From Hendrickx and Dukelow (1995).

from early signs of threatened or impending abortion where intrauterine hemorrhage may be associated with hematoma formation. Both can result in extensive vaginal hemorrhage. Early resorptions can also be identified by an inappropriate GS size for the expected GD, poor development of the chorionic villi/placenta, or a diminishing GS when examined consecutively. A small volume of intrauterine fluid within an enlarged uterus is also suggestive of early abortion/resorption, although small volumes of intrauterine fluid may be observed in some nongravid animals during the follicular phase of the menstrual cycle.

The incidence of live-born twins in Old World monkeys is extremely rare (Tarantal and Hendrickx, 1988d). Compilation of the published data for the rhesus indicates a twinning rate of 0.2% (10/4991). However, the appearance of twin GS − one with a viable embryo and one anembryonic − has been noted sonographically, which suggests that the incidence of multiple gestations is probably greater than reported. These data are similar to the "vanishing twin" phenomena as noted in the human, although the outcome in the macaque appears less favorable.

For those nonhuman primate females determined to be nonpregnant, recording baseline data of uterine size and appearance has been suggested (Tarantal and Hendrickx, 1988d). These data include measurements of total uterine length (uterine body and cervix, normal range for *M. mulatta* (Mm) is 52.0 ± 7.1 mm; *M. fascicularis* (Mf) is 43.6 ± 9.2 mm); uterine body length (Mm 28.2 ± 5.0 mm; Mf 23.7 ± 4.3 mm), width (Mm 17.8 ± 3.4 mm; Mf 16.8 ± 2.8 mm), and height (Mm 17.5 ± 3.4 mm; Mf 16.3 ± 2.7 mm); uterine shape, contour, and homogeneity; appearance of the ECE; and endometrial thickness. This information is particularly useful for documenting early absorptions/resorptions and for evaluating nonreproductive females. Alterations in uterine size, contour, and/or appearance (i.e. changes in echogenicity) may suggest a pathological process such as leiomyoma, carcinoma, adenomyosis, endometritis, or endometrial hyperplasia. Foreign bodies and seminal plugs/coagulum can be readily identified within the vaginal canal, fornices, or cervix/endocervical canal by an increase in echogenicity (hyperechoic) and in some cases by acoustic shadowing.

Of particular importance is the detection of endometriosis, a relatively frequent finding in laboratory-housed macaques with a history of repeat hysterotomies. Endometriomas may occur in single or multiple sites attached to the uterus and/or adnexal structures, either homogeneous or septated, and will usually appear cystic with well-defined borders and some internal echoes. Diagnosis can be confirmed by ultrasound-guided aspiration of "chocolate fluid," which is a characteristic feature. In some cases, endometriomas may be complex in appearance (cystic and solid components) or predominantly solid if of longstanding duration. Other types of cystic structures can be imaged in the mesentery (common; 1−10 mm in length; benign), ovary (may be follicles or theca lutein cysts), uterus (hydro-, pyo-, or hematometra), or cervix (nabothian cysts) and can be aspirated, if deemed appropriate. Other pelvic and/or uterine solid neoplasms can be detected and biopsied to confirm the diagnosis.

The use of ultrasound-guided interventional procedures in these species has been described and its application in both the pregnant (Tarantal, 1990) and nonpregnant female (Tarantal et al., 1990) established. The primary advantage of these techniques is that they obviate extensive surgical procedures with all the associated risks, costs, and potential trauma.

Among the New World monkeys species, ultrasound has also been used to diagnose pregnancy and monitor fetal growth in squirrel monkeys (Narita et al., 1988). Pregnancy can be identified on GD 25 with detection of the GS; cardiac activity can be confirmed approximately 2 weeks later. Pregnancy in marmosets can be diagnosed by ultrasound at around GD 30 by the presence of a hypoechoic area between the hyperechoic uterine walls. Gestational sacs are clearly evident by around day 30, and heart beats are reliably detected by around day 50.

Prenatal Growth and Development

Hendrickx and Dukelow (1995) reported routine observations for evaluating prenatal growth sonographically for *M. mulatta* and *M. fascicularis* at the California Primate Research Center. These routine observations included mean GS dimension (GD 14−50), GL (~GD 23−60), head measurements (biparietal and occipitofrontal diameters, head area, and circumference), abdominal area and circumference, and humerus and femur lengths (see Tarantal and Hendrickx, 1988b,c for methods). These measurements are compared with normative growth data or predicted values (Tables 8.8−8.10) for each species and are used in combination to obtain greater accuracy in either predicting or confirming gestational age (Tarantal and Hendrickx, 1988b). In addition, embryonic/fetal heart rates can be obtained during the examination period and compared with the expected normal rates.

Documentation of normal and abnormal growth and development of the conceptus is particularly pertinent, both from a colony maintenance standpoint and for research-oriented purposes. The standard sonographic evaluation performed during the second trimester (~GD 75−90) is incorporated in order to assess anatomical configuration, determine gender, monitor condition, and evaluate placental development. This is the optimal time for making judgments about conformation of the fetus since the volume of amniotic fluid (i.e. the ratio of fetus to the fluid volume) provides an excellent sonographic "window."

TABLE 8.8 Predicted Values for Gestational Sac (GS) and Greatest Length (GL) for the Rhesus and Long-tailed Macaque (*M. mulatta* and *M. fascicularis*)

GD	GS (mm)	GL (mm)	GD	GS (mm)	GL (mm)
15	1.4	–	38	28.7	16.9
16	2.7	–	39	29.6	18.2
17	3.9	–	40	30.7	19.5
18	5.1	–	41	31.7	20.9
19	6.3	–	42	32.8	22.4
20	7.5	–	43	33.9	23.9
21	8.7	2.9	44	34.9	25.5
22	10.0	3.3	45	36.0	27.1
23	11.2	3.8	46	37.0	28.8
24	12.3	4.3	47	38.0	30.5
25	13.5	4.9	48	39.0	32.3
26	14.7	5.5	49	40.1	34.2
27	15.9	6.2	50	41.1	36.1
28	17.1	6.9	51	–	38.1
29	18.3	7.7	52	–	40.1
30	19.4	8.5	53	–	42.2
31	20.6	9.4	54	–	44.4
32	21.7	10.3	55	–	46.7
33	22.9	11.3	56	–	49.0
34	24.0	12.3	57	–	51.3
35	25.1	13.4	58	–	53.8
36	26.2	14.5	59	–	56.3
37	27.4	15.7	60	–	58.8

Note: *GS and GL are measured as described in Table 8.5. During early gestation (GD 14–25), GS used for predicted gestational age (pGA); for GD > 25–60, GL used for pGA (see Tarantal and Hendrickx, 1988b).*
From Hendrickx and Dukelow (1995).

TABLE 8.9 Predicted Values for Biparietal Diameter (BPD) for the Rhesus (MM) and Long-Tailed (MF) Macaque[a]

GD	MM	MF	GD	MM	MF	GD	MM	MF
47	10.90	10.91	87	30.14	29.42	127	43.35	41.39
48	11.44	11.44	88	30.55	29.81	128	43.58	41.59
49	11.97	11.96	89	30.96	30.20	129	43.81	41.78
50	12.50	12.48	90	31.36	30.58	130	44.03	41.96
51	13.03	13.00	91	31.76	30.95	131	44.25	42.14
52	13.56	13.51	92	32.16	31.32	132	44.47	42.32
53	14.09	14.02	93	32.55	31.69	133	44.68	42.49
54	14.61	14.53	94	32.94	32.05	134	44.88	42.65
55	15.13	15.03	95	33.33	32.41	135	45.08	42.81
56	15.64	15.54	96	33.71	32.76	136	45.27	42.96
57	16.16	16.03	97	34.09	33.11	137	45.46	43.10
58	16.67	16.53	98	34.46	33.46	138	45.64	43.24
59	17.18	17.02	99	34.83	33.80	139	45.82	43.38
60	17.68	17.51	100	35.19	34.14	140	45.99	43.51
61	18.19	18.00	101	35.55	34.47	141	46.15	43.63
62	18.69	18.48	102	35.91	34.80	142	46.31	43.75
63	19.18	18.96	103	36.26	35.12	143	46.47	43.86
64	19.68	19.44	104	36.61	35.44	144	46.62	43.96
65	20.17	19.91	105	36.95	35.75	145	46.76	44.06
66	20.66	20.38	106	37.29	36.06	146	46.90	44.15
67	21.13	20.85	107	37.63	36.36	147	47.03	44.24
68	21.62	21.31	108	37.96	36.66	148	47.16	44.32
69	22.10	21.77	109	38.28	36.96	149	47.28	44.39
70	22.58	22.23	110	38.60	37.25	150	47.39	44.46
71	23.05	22.68	111	38.92	37.53	151	47.50	44.52
72	23.52	23.13	112	39.23	37.81	152	47.60	44.57
73	23.98	23.58	113	39.54	38.08	153	47.70	44.62
74	24.44	24.02	114	39.84	38.35	154	47.79	44.67
75	24.90	24.46	115	40.14	38.62	155	47.87	44.70
76	25.36	24.89	116	40.44	38.88	156	47.95	–
77	25.81	25.32	117	40.72	39.13	157	48.03	–
78	26.26	25.75	118	41.01	39.38	158	48.09	–
79	26.70	26.17	119	41.29	39.63	159	48.15	–
80	27.15	26.59	120	41.56	39.87	160	48.21	–
81	27.58	27.01	121	41.83	40.10	161	48.26	–

(*Continued*)

Development of the brain (i.e. lateral, third, and fourth ventricles; thalamus; midbrain; cerebellum; cerebral hemispheres; choroid plexus; cranial base), face (eyes, nose, mouth), heart, abdominal viscera, and axial and appendicular skeleton can all be assessed with accuracy. In addition, placental location (mono versus bidiscoid) and development (aging changes) are particularly important, especially when monitoring for placenta previa or placental abruptions. Those animals documented with either

TABLE 8.9 Predicted Values for Biparietal Diameter (BPD) for the Rhesus (MM) and Long-Tailed (MF) Macaque[a]—cont'd

GD	MM	MF	GD	MM	MF	GD	MM	MF
82	28.02	27.42	122	42.10	40.33	162	48.30	–
83	28.45	27.83	123	42.36	40.55	163	48.33	–
84	28.88	28.23	124	42.61	40.77	164	48.36	–
85	29.30	28.64	125	42.86	40.98	165	48.39	–
86	29.72	29.03	126	43.11	41.19			–

Note: *For GD 50–60, use GL and BPD for pGA; for GD >60, use BPD and FL for pGA. See Tarantal and Hendrickx (1988b) for a description of methods for obtaining measurements, techniques for use, and accuracy/reliability.*
[a]*Gestational days (GD) 47–165. Used in combination with femur length (FL; see Table 8.8) to confirm/predict gestational age (pGA).*
From Hendrickx and Dukelow (1995).

TABLE 8.10 Predicted Values for Femur Length (FL) for the Rhesus (MM) and Long-tailed (MF) Macaque[a]

GD	MM	MF	GD	MM	MF	GD	MM	MF
50	2.61	2.84	89	19.05	18.84	128	34.48	32.08
51	3.00	3.27	90	19.48	19.22	129	34.81	32.37
52	3.40	3.70	91	19.91	19.60	130	35.15	32.65
53	3.79	4.13	92	20.33	19.97	131	35.48	32.94
54	4.19	4.56	93	20.76	20.35	132	35.80	33.22
55	4.60	4.99	94	21.19	20.72	133	36.13	33.49
56	5.00	5.42	95	21.61	21.09	134	36.44	33.77
57	5.41	5.84	96	22.04	21.46	135	36.76	34.04
58	5.82	6.27	97	22.46	21.83	136	37.06	34.31
59	6.23	6.69	98	22.88	22.20	137	37.37	34.57
60	6.64	7.12	99	23.30	22.56	138	37.66	34.83
61	7.06	7.54	100	23.72	22.92	139	37.96	35.09
62	7.47	7.96	101	24.13	23.28	140	38.25	35.35
63	7.89	8.38	102	24.55	23.64	141	38.53	35.60
64	8.31	8.80	103	24.96	23.99	142	38.81	35.85
65	8.73	9.22	104	25.37	24.34	143	39.08	36.10
66	9.15	9.63	105	25.78	24.70	144	39.35	36.34
67	9.58	10.05	106	26.19	25.04	145	39.62	36.58
68	10.00	10.46	107	26.60	25.39	146	39.87	36.81
69	10.43	10.88	108	27.00	25.73	147	40.13	37.05
70	10.85	11.29	109	27.40	26.07	148	40.37	37.27

TABLE 8.10 Predicted Values for Femur Length (FL) for the Rhesus (MM) and Long-tailed (MF) Macaque[a]—cont'd

GD	MM	MF	GD	MM	MF	GD	MM	MF
71	11.28	11.70	110	27.80	26.41	149	40.62	37.50
72	11.71	12.11	111	28.20	26.75	150	40.85	37.72
73	12.14	12.52	112	28.59	27.08	151	41.08	37.94
74	12.57	12.92	113	28.98	27.42	152	41.30	38.16
75	13.00	13.33	114	29.37	27.74	153	41.52	38.37
76	13.43	13.73	115	29.75	28.07	154	41.73	38.58
77	13.87	14.13	116	30.14	28.40	155	41.94	38.78
78	14.30	14.53	117	30.52	28.72	156	42.14	–
79	14.73	14.93	118	30.89	29.04	157	42.33	–
80	15.16	15.33	119	31.27	29.35	158	42.52	–
81	15.59	15.73	120	31.64	29.67	159	42.70	–
82	16.03	16.12	121	32.00	29.98	160	42.87	–
83	16.46	16.52	122	32.37	30.29	161	43.04	–
84	16.89	16.91	123	32.73	30.59	162	43.20	–
85	17.32	17.30	124	33.09	30.90	163	43.36	–
86	17.76	17.68	125	33.44	31.19	164	43.50	–
87	18.19	18.07	126	33.79	31.49	165	43.64	–
88	18.62	18.45	127	34.13	31.79			–

Note: *For GD >60, use BPD and FL for pGA. See Tarantal and Hendrickx (1988b) for a description of methods for obtaining measurements, techniques for use, and accuracy/reliability.*
[a]*Gestational days (GD) 50–165. Used in combination with biparietal diameter (BPD; see Table 8.7) to confirm/predict gestational age (pGA).*
From Hendrickx and Dukelow (1995).

a marginal or a complete previa during the second trimester can be reevaluated later in gestation and scheduled for caesarean section, as required. It is important to rescan during the latter stages of development, as the placenta may "migrate" (i.e. as the uterus grows, the discs are displaced craniad). Diagnosis of this condition requires accurate localization of the placenta in relation to the cervix on longitudinal scans. It should be noted that a distended urinary bladder may alter the relationship of the placenta to the cervix, which can lead to misinterpretation. Animals that display retroplacental or subchorionic hemorrhage are also closely monitored; if continued and considerable hemorrhage is noted, emergency surgery is performed. Of the nine concealed abruptions detected during three breeding seasons (GD 51–125; 9/873 or 1%), no maternal deaths occurred. One interesting feature that has been frequently associated with abruptions is the proliferation of

decidua, particularly near the lower uterine segment (Tarantal and Hendrickx, 1988d). Continued surveillance within the colony for repeat incidence has resulted in the removal of females at risk and a resultant decrease in abruptions.

The use of a modified biophysical profile (BPP) as performed in the human fetus has been incorporated with a variety of observations related to fetal activity in utero. Similar to the human, the nonhuman primate fetus is very active in utero, particularly during the early fetal period (GD 50–70) (Tarantal and Hendrickx, 1988d). By GD 80–100, vigorous whole-body movements are less frequently observed, and more selective activities such as darting eye movements, oral activities, and extension and flexion of the limbs and head may be noted. The BPP has proven useful for evaluating fetal status and well-being during the third trimester in unanesthetized dams (chair restrained; A. F. Tarantal and M. S. Golub, unpublished observations), primarily for experimental purposes. An observation period of 20 minutes on GD 115, 125, 135, and 145 includes documentation of changes in fetal heart rate and quantitation of respiratory and motor activity, muscle tone, and whole-body startle reflex.

A comprehensive overview of the many studies and methods for analyzing growth of the nonhuman primate fetus, including body and organ weights and dimensional and proportional growth, has been provided by Brizzee and Dunlap (1986) and will not be repeated herein.

Jaquish et al. (1995) provided growth curves for crown–rump length and biparietal diameter in marmosets from around day 30 to term (Figures 8.4, 8.5). Tardif et al. (1998) demonstrated that these curves could be used to reliably predict delivery dates to within ± 5 days.

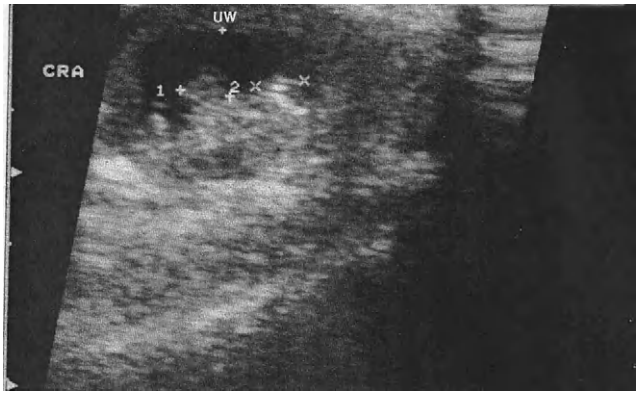

FIGURE 8.4 Method of measuring crown–rump length (CRL) in the common marmoset (*Callithrix jacchus*). There are two embryos measured in this image. 1 (designated by +) and 2 (designated by x) (day 67 gestation). Embryo 1 measures 3.7 mm and embryo 2, 3.6 mm. UW, uterine wall. Arrowheads on the left margin = 1 cm. *(From Jaquish et al., 1995,* American Journal of Primatology, *36:259–275.)*

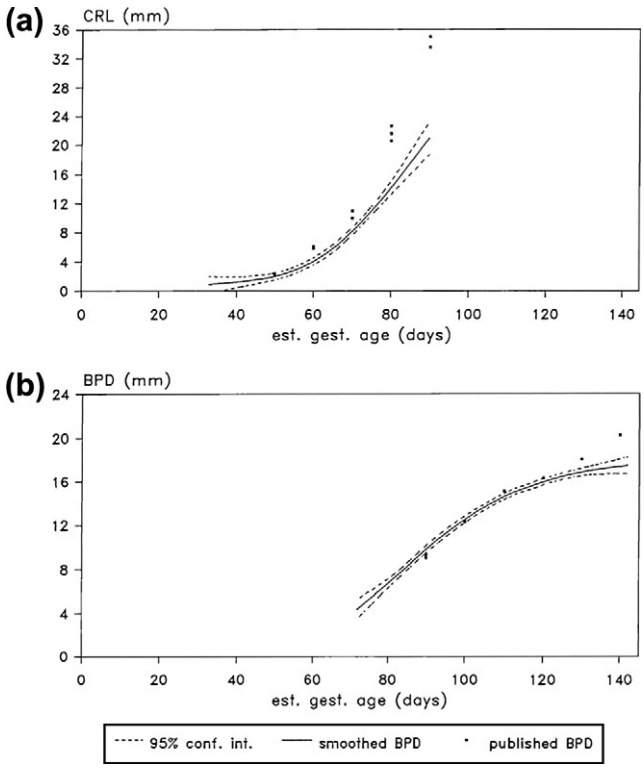

FIGURE 8.5 Ultrasound measurements of crown–rump length (CRL) and biparietal diameter (BPD) in common marmosets (*Callithrix jacchus*). (a) Comparison of fitted curve for ultrasound measured crown–rump length (CRL) to published CRL measures for the common marmoset (*Callithrix jacchus*) (Chambers and Hearn, 1985). The ultrasound measures agreed with those from gross specimens until approximately day 70 of gestation; (b) Comparison of fitted curve for ultrasound measured biparietal diameter (BPD) to published BPD measures (Chambers and Hearn, 1985). The ultrasound measures are in close agreement with those taken from gross specimens. *(From Jaquish et al., 1995,* American Journal of Primatology, *36:259–275.)*

Crown–rump length measures taken between GD 50 and 80 (at 3- to 14-mm) provided the most reliable estimator of delivery date.

Maternal Changes with Pregnancy

In nonpregnant *S. sciureus*, daily water consumption ranges from 20 to 160 ml (mean, 110 ml) and increases to 346 ml per day during the fifth month of pregnancy. A significant decrease to nonpregnant levels has been noted 2–16 days prior to delivery (Clewe, 1969). Travis and Holmes (1974) reported a linear increase in water consumption from the day of conception through GD 138, with a correlative significant increase in mean daily urine output. Evaluation of four species of nonhuman primates (*M. mulatta*, *M. speciosa*, *E. patas*, and *P. troglodytes*) showed that significant upper ureteral dilatation occurs during pregnancy, similar to findings in humans (Roberts and Wolf, 1971).

An increase in plasma volume compared with red cell mass occurs in nonhuman primates throughout gestation. This hydremia of pregnancy is important in maintaining the health of the fetus by ensuring adequate uterine perfusion. An approximate 30% increase in blood volume has been reported for *M. mulatta* (Allen and Ahlgren, 1968), with increases in total volumes of red and white blood cells (WBCs), hemoglobin, total plasma protein, and albumin; all values decrease substantially at parturition. Other studies have indicated a shift in the albumin:globulin ratio and an increase in sedimentation rate and plasma fibrinogen (Allen and Siegfried, 1966; Knapp et al., 1974). Neutrophilia has also been observed beginning ~GD 50 (Allen and Siegfried, 1966). Studies by Spicer and Oxnard (1967) also showed a decrease in hemoglobin late in pregnancy that was well correlated with reductions in mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and iron and with an increase in mean corpuscular volume. A further demonstration of changes both pre- and postpartum was provided by Switzer et al. (1970); packed cell volumes decreased during the first trimester (attributed to implantation bleeding) and again 72−96 hours postdelivery. Sedimentation rates also increased during the third trimester, with a peak 72 hours postpartum. Interestingly, WBCs decreased mid gestation, with a reversed ratio of lymphocytes: neutrophils during the second trimester; eosinophils also decreased during the third trimester, with lowest values detected at parturition.

Reference values for hematological parameters and clinical chemistry screens from the CPRC rhesus (GD 45, 90, 135, 165; $n = 13$) and long-tailed (GD 25, 50, 75, 100; $n = 10$) macaque colonies are presented in Tables 8.11−8.14. For both species, samples were collected from unanesthetized females in their cages (from an arm or leg extended out of a partially opened door). In contrast to finding in the previously cited studies, the samples indicated only marginal changes in all parameters. These differences may be attributed to the methods used for collection and to the frequency of sampling. For clinical chemistry screens, rhesus showed a minor increase in blood urea nitrogen (BUN), glucose, and alkaline phosphatase (ALP) and a decrease in albumin and total proteins at term. Marginal decreases were also observed for carbon dioxide, potassium, and γ-glutamyltransferase. On GD 100, the long-tailed macaques showed reductions in BUN, total proteins, and alkaline phosphatase (ALP).

Of interest are studies performed in *M. nemestrina* that specifically addressed the effects of pregnancy on high-density lipoprotein (HDL) concentrations. Although HDL decreased (Schiller et al., 1983), increases in low density lipoproteins (LDL) were noted in late pregnancy (Rudel et al., 1981). In addition, HDL levels were predictive of pregnancy outcome since no changes in HDL were observed in pregnancies that resulted in spontaneous abortion (Schiller et al., 1983). It was hypothesized that this lack of a decrease may be attributed to fetal−placental dysfunction (i.e. decreased HDL-cholesterol utilization for steroid biosynthesis).

The average total weight gain during pregnancy for *M. nemestrina* has been reported to be 19% above the preconception weight (Goodlin and Sackett, 1983). It was also observed that individual animals lost from 5 to 10% of their mean preconception weight during the first 45 days of pregnancy. By GD 60, a body weight gain was initiated that peaked at roughly GD 160. Maternal body weight changes during pregnancy from the CPRC rhesus and long-tailed macaque colonies are shown in Figure 8.5. Rhesus data were collected periodically during gestation (GD 60, 90, 120, 150, and postpartum) from females participating in chair-restrained blood pressure monitoring beginning on GD 90. Long-tailed macaque body weights were collected from sham controls (GD 20 to > 150) hand-caught for oral gavage during GD 20−50. Similar to observations in *M. nemestrina*, a decline in body weight was observed prior to GD 50 in the longtailed macaques. Lunn (1983) reported on body weight changes throughout pregnancy in common marmosets. Generally, females did not display reliable gains over their pre-pregnant weights until approximately GD 80.

Prenatal Mortality

Information on pregnancy loss in nonhuman primates is largely provided by studies in the more commonly used Old World species, particularly macaques and baboons. Prenatal mortality occurs throughout gestation in these species, but the level is particularly high during the very early embryonic stages, when pregnancy confirmation may be uncertain and/or unreliable. Assessment of the magnitude of early embryonic mortality is additionally complicated by the occurrence of "placental sign" (implantation bleeding), which is normally seen in macaques during early pregnancy. Morphological examinations of normal and abnormal embryos in the rhesus monkey (Heuser and Streeter, 1941) and baboon (Hendrickx and Binkerd, 1980) as well as the chimpanzee (Heuser, 1940) have provided information on embryonic death during the peri-implantation period. These studies indicate preimplantation losses of 26.3 and 25.0% for rhesus monkeys and baboons, respectively. Corresponding values during the post-implantation period (approximately GD 10−22) have been estimated at 14.3% in baboons, 28% in rhesus monkeys, and 50% in chimpanzees.

During the subsequent period of organogenesis (approximately days 20−50), the incidence of embryonic loss for several nonhuman primates is significantly lower than in the peri-implantation period. Microscopic examination of embryos of five Old World species (green

TABLE 8.11 Hematology Reference Values for *M. mulatta*[a]

Parameter	GD 45	(Range)	GD 90	(Range)	GD 135	(Range)	GD 165	(Range)
RBC ($\times 10^6$/μl)	5.6 ± 0.5	(4.7–6.4)	5.6 ± 0.5	(4.9–6.2)	5.4 ± 0.5	(4.5–6.3)	4.7 ± 0.7	(3.9–6.3)
HgB (g/dl)	21.7 ± 1.0	(10.2–14.1)	12.9 ± 0.7	(11.7–13.9)	12.7 ± 0.9	(11.4–14.4)	11.2 ± 1.6	(9.5–14.5)
HCT (%)	38.9 ± 3.2	(31.2–44.7)	39.9 ± 2.4	(36.8–43.0)	38.4 ± 3.0	(33.8–43.9)	33.3 ± 5.0	(27.4–43.7)
MCV (fl)	69.9 ± 3.3	(65–73)	71.8 ± 2.9	(67–77)	71.5 ± 2.4	(68–76)	71.2 ± 3.0	(67–76)
MCH (pg)	22.9 ± 1.1	(21.0–25.0)	23.3 ± 1.1	(21.5–25.6)	23.6 ± 1.1	(21.5–25.2)	24.0 ± 1.3	(22.1–26.3)
MCHC (pg/fl)	32.7 ± 0.9	(31.5–35.1)	32.5 ± 0.8	(31.4–33.4)	32.9 + 0.7	(31.5–34.2)	33.7 ± 0.8	(32.6–34.8)
PP (g/dl)	7.2 ± 0.3	(6.7–7.8)	6.8 ± 0.4	(6.3–7.8)	6.9 ± 0.3	(6.2–7.4)	6.6 ± 0.4	(5.8–7.2)
Fibrin. (mg/dl)	192 ± 64	(<100–300)	192 ± 86	(<100–400)	154 ± 52	(100–200)	277 ± 101	(100–500)
WBC ($\times 10^3$/μl)	8.0 ± 2.4	(4.7–13.4)	8.8 ± 2.8	(5.1–14.7)	7.9 ± 2.1	(4.8–11.0)	7.9 ± 2.0	(4.8–12.6)
Seg. Neutr.								
%	52.8 ± 11.2	(33–68)	58.9 ± 11.9	(41–77)	61.8 ± 10.6	(45–85)	70.2 ± 8.4	(58–82)
/μl	4331 ± 1,819	(1974–8040)	5384 ± 2634	(2142–1,319)	4987 ± 1861	(2208–8670)	5566 ± 1589	(3264–8820)
Lymph								
%	42.5 ± 11.5	(28–65)	35.5 ± 12.7	(18–55)	31.2 ± 9.1	(13–45)	25.5 ± 7.3	(14–39)
/μl	3288 ± 966	(2175–4947)	2891 ± 778	(1406–4550)	2410 ± 822	(1326–3630)	1972 ± 574	(1050–2772)
Mon.								
%	3.3 ± 2.1	(1–7)	4.0 ± 2.0	(1–8)	5.2 ± 2.6	(1–10)	3.8 ± 2.5	(1–8)
/μl	262 ± 218	(0–670)	321 ± 214	(0–630)	374 ± 141	(75–546)	312 ± 275	(57–1008)
Eos.								
%	2.9 ± 1.2	(1–4)	2.4 ± 1.0	(1–4)	2.3 ± 0.9	(0–4)	2.0 ± 0.0	(0–2)
/μl	137 ± 179	(0–536)	158 ± 167	(0–504)	137 ± 105	(0–312)	30 ± 58	(0–148)
Baso.								
%	1.0 ± 0.0	(0–1)	1.0 ± 0.0	(0–1)	1.0 ± 0.0	(0–1)	1.0 ± 0.0	(0–1)
/μl	13 ± 32	(0–103)	15 ± 42	(0–147)	7.3 ± 26.4	(0–95)	6 ± 20	(0–72)

RBC, red blood cells; HgB, hemoglobin; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; PP, plasma protein; Fibrin, fibrinogen; WBC, white blood cells; Seg. Neutr., segmented neutrophils; Lymph., lymphocytes; Mono., monocytes; Eos., eosinophils; Baso., basophils.

[a]*Gestational days (GD) 45–165 (n = 13; mean ± SD).*

From Hendrickx and Dukelow (1995).

TABLE 8.12 Hematology Reference Values for *M. fascicularis*[a]

Parameter	GD 25	(Range)	GD 50	(Range)	GD 75	(Range)	GD 100	(Range)
RBC ($\times 10^6$/μl)	5.7 ± 0.6	(4.8–7.0)	6.0 ± 0.5	(5.2–6.4)	6.4 ± 0.5	(5.7–7.2)	6.4 ± 0.5	(5.6–7.0)
HgB (g/dl)	10.8 ± 0.8	(9.5–12.1)	11.7 ± 0.6	(10.5–12.3)	12.4 ± 0.9	(11.5–14.2)	12.2 ± 0.8	(11.4–13.4)
HCT (%)	36.3 ± 2.8	(31.4–41.8)	37.8 ± 2.7	(32.4–40.4)	40.4 ± 2.8	(37.2–45.4)	40.2 ± 2.1	(37.3–43.1)
MCV (fl)	64 ± 2	(59–66)	64 ± 2	(59–67)	63 ± 2	(60–67)	63 ± 3	(58–67)
MCH (pg)	19.0 ± 1.1	(17.2–20.4)	19.7 ± 0.8	(18.5–20.2)	19.3 ± 0.9	(18.1–20.7)	19.1 ± 0.9	(17.6–20.2)
MCHC (pg/fl)	29.9 ± 0.9	(28.7–31.5)	31.0 ± 0.9	(29.7–32.4)	30.6 ± 1.2	(28.7–32.2)	30.4 ± 0.9	(28.6–31.7)
WBC ($\times 10^3$/ μl)	8.1 ± 1.6	(5.9–10.2)	8.0 ± 1.5	(6.4–11.1)	7.6 ± 1.8	(4.3–11.0)	7.9 ± 1.6	(6.3–10.7)
Seg. Neutr.								
%	53 ± 10	(40–71)	58 ± 6	(47–68)	58 ± 6	(50–66)	65 ± 11	(52–83)
/μl	4243 ± 872	(2542–5429)	4597 ± 797	(3584–6380)	4400 ± 1058	(2709–6283)	5138 ± 1512	(3816–8881)
Lymph								
%	40 ± 8	(22–48)	36 ± 7	(29–50)	35 ± 6	(29–46)	30 ± 9	(16–43)
/μl	3280 ± 1083	(1298–4896)	2876 ± 775	(1856–4150)	2719 ± 806	(1419–3818)	2402 ± 947	(1188–4429)
Mono.								
%	5 ± 3	(2–12)	4 ± 3	(1–10)	6 ± 3	(2–12)	4 ± 3	(0–10)
/μl	445 ± 314	(142–1224)	360 ± 290	(83–790)	441 ± 281	(150–1080)	312 ± 241	(0–760)
Eos.								
%	2 ± 2	(0–6)	1 ± 2	(0–7)	1 ± 1	(0–2)	1 ± 1	(0–4)
/μl	132 ± 139	(0–378)	123 ± 192	(0–588)	51 ± 52	(0–130)	80 ± 101	(0–272)
Baso.								
%	0 ± 0	(0–1)	0 ± 0	(0)	0 ± 0	(0–1)	0 ± 0	(0–1)
/μl	19 ± 41	(0–99)	0 ± 0	(0)	21 ± 43	(0–103)	0 ± 0	(0)

RBC, red blood cells; HgB, hemoglobin; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; WBC, white blood cells; Seg. Neutr., segmented neutrophils; Lymph., lymphocytes; Mono., monocytes; Eos., eosinophils; Baso., basophils.

[a]*Gestational days (GD) 25–100 (*n *= 10; mean ± SD).*

From Hendrickx and Dukelow (1995).

TABLE 8.13 Clinical Chemistry Reference Values for *M. mulatta*[a]

Parameter	GD 45	(Range)	GD 90	(Range)	GD 135	(Range)	GD 165	(Range)
Cl (mM/l)	107.8 ± 2.1	(104–111)	108.8 ± 2.2	(106–114)	109.8 ± 1.7	(107–112)	111.3 ± 1.7	(109–116)
TCO_2 (mM/l)	23.6 ± 5.0	(16–37)	25.0 ± 4.0	(19–36)	21.6 ± 2.3	(17–25)	22.7 ± 2.5	(18–26
K (mM/l)	4.6 ± 0.5	(4.0–5.8)	4.8 ± 0.3	(4.1–5.3)	4.8 ± 0.4	(4.3–5.6)	4.0 ± 0.3	(3.6–4.7)
Na (mM/l)	144.1 ± 1.7	(142–149)	143.5 ± 1.9	(140–146)	144.2 ± 1.8	(141–148)	147.0 ± 2.7	(142–153)
AG (mM/l)	17.3 ± 4.5	(7–26)	14.5 ± 4.2	(4–21)	17.8 ± 3.7	(12–26)	17.1 ± 2.8	(13–22)
Alb (g/dl)	3.9 ± 0.4	(3.1–4.8)	3.4 ± 0.5	(2.9–4.6)	3.2 ± 0.3	(2.6–3.5)	2.6 ± 0.3	(2.1–3.1)
BUN (mg/dl)	13.2 ± 1.6	(11–16)	12.1 ± 1.8	(9–15)	12.0 ± 1.7	(8–14)	23.5 ± 2.9	(20–29)
Glucose (mg/dl)	50.4 ± 5.7	(44–64)	47.2 ± 8.5	(38–70)	52.4 ± 11.6	(22–67)	58.7 ± 23.4	(39–95)
TP (g/dl)	7.4 ± 0.6	(6.7–8.8)	6.9 ± 0.5	(6.0–7.8)	7.0 ± 0.3	(6.5–7.5)	5.9 ± 1.1	(2.7–7.1)
ALT(U/l)	32.5 ± 13.3	(17–69)	34.5 ± 12.3	(15–58)	30.7 ± 16.9	(12–66)	30.8 ± 17.6	(11–74)
ALP(U/l)	111.2 ± 43.1	(26–196)	96.5 ± 53.2	(18–232)	135.1 ± 62.1	(35–268)	138.4 ± 68.6	(40–281)
Ca (mg/dl)	9.8 ± 0.5	(9.1–10.6)	9.4 ± 0.3	(8.8–9.9)	9.3 ± 0.3	(8.7–9.9)	9.3 ± 0.4	(8.7–9.8)
Cr (mg/dl)	0.9 ± 0.1	(0.7–1.1)	0.8 ± 0.1	(0.7–0.9)	0.8 ± 0.1	(0.6–1.1)	0.9 ± 0.1	(0.7–1.1)
P (mg/dl)	3.9 ± 0.5	(3.4–4.8)	3.8 ± 0.7	(2.9–5.3)	4.0 ± 0.5	(3.5–5.6)	3.8 ± 0.6	(2.6–4.6)
γGT (U/l)	41.2 ± 7.0	(31–58)	50.9 ± 13.0	(30–71)	49.7 ± 18.6	(25–104)	39.2 ± 8.8	(25–53)

Note: *C1, chloride; TCO_2, total carbon dioxide; K, potassium; Na, sodium; AG, anion gap; Alb, albumin; BUN, blood urea nitrogen; TP, total protein; ALT, alanine aminotransferase; ALP, alkaline phosphatase; Ca, calcium; Cr, creatinine; P, phosphorus; γGT, γ-glutamyltransferase.*

[a]*Gestational days (GD) 45–165 (n = 13; mean ± SD).*

From Hendrickx and Dukelow (1995).

monkey, long-tailed, rhesus, and bonnet macaques, and baboons) indicate embryonic mortality rates of 2.4–18.2% during organogenesis (Hendrickx and Binkerd, 1980).

Jaquish et al. (1996) reported on patterns of prenatal survival and loss in the marmoset, based upon repeated ultrasound examinations. In that study of 50 pregnancies, rates of prenatal loss – both total pregnancy loss and litter size reduction – were high relative to rates for other nonhuman primates, a fact possibly related to production of litters. Of the 50 pregnancies, 22% spontaneously aborted, with 7 of the 11 aborted pregnancies occurring after completion of organogenesis and in three cases, with evidence of placental abruption (as a hypoechoic area between the uterine wall and the placenta). In addition, five pregnancies that resulted in singleton births started with two or three embryos identified at the first ultrasound. Similarly, high rates of loss were also reported by Heger et al. (1988) in a study tracking excreted steroid concentrations in a large population of breeding females. In that case, 28% of females aborted prior to day 40, with an additional 11.5% of pregnancies lost in days 50–70 and 4.4% lost thereafter.

Reported figures for the incidence of stillbirths among 11 species of indoor-housed nonhuman primate colonies, including the green monkey, mangabey, baboon, langur, and five species of macaques, ranged from 5.9 to 20% (Hendrickx and Binkerd, 1980). These rates are markedly higher than the 2.7% figure reported for free-ranging rhesus monkeys (Koford, 1965), and the increase may be attributable to the artificial housing conditions or to increased likelihood of discerning stillbirths in indoor-housed colonies. In one large breeding population of common marmosets housed at the Southwest National Primate Research Center, the stillbirth rate was 16.6% (S. Tardif, unpublished observation).

The primary factors that have been implicated in prenatal mortality in nonhuman primates are adverse maternal factors (diet, health, infections, and stress) and various uterine conditions that compromise fetal viability (Small, 1982). Studies carried out in rhesus monkeys indicate that normal prenatal growth is maintained on a protein-deficient diet due to the ability of the gravid nonhuman primate uterus to adapt to such nutritional restrictions (Riopelle, 1985). However, severe protein

TABLE 8.14 Clinical Chemistry Reference Values for *M. fascicularis*[a]

Parameter	GD 25	(Range)	GD 50	(Range)	GD 75	(Range)	GD 100	(Range)
Cl (mM/l)	110 ± 2	(108–113)	109 ± 2	(105–112)	109 ± 3	(106–114)	109 ± 3	(107–114)
TCO_2 (mM/l)	24 ± 2	(20–27)	24 ± 5	(16–30)	22 ± 5	(12–28)	20 ± 3	(16–24)
K (mM/l)	4.8 ± 0.4	(4.1–5.4)	5.0 ± 0.6	(4.3–5.9)	4.7 ± 0.5	(4.1–5.7)	4.9 ± 0.5	(4.2–5.9)
Na (mM/l)	146 ± 2	(144–148)	146 ± 3	(143–153)	146 ± 3	(143–151)	144 ± 1	(142–147)
Alb (g/dl)	4.0 ± 0.3	(3.7–4.5)	3.5 ± 0.3	(2.8–3.8)	3.1 ± 0.4	(2.4–4.0)	3.2 ± 0.3	(2.7–3.8)
BUN (mg/dl)	21 ± 6	(11–29)	21 ± 6	(15–31)	21 ± 5	(16–32)	18 ± 3	(14–22)
Glucose (mg/dl)	62 ± 9	(46–76)	48 ± 11	(30–64)	46 ± 13	(31–70)	49 ± 6	(40–59)
TP (g/dl)	7.8 ± 0.4	(7.1–8.2)	7.3 ± 0.5	(6.5–7.9)	6.9 ± 0.6	(6.3–8.2)	6.9 ± 0.4	(6.4–7.4)
ALT (U/l)	47 ± 16	(22–76)	54 ± 34	(15–132)	51 ± 30	(21–112)	50 ± 31	(18–117)
ALP (U/l)	175 ± 49	(114–290)	166 ± 51	(119–275)	123 ± 41	(86–212)	145 ± 31	(104–199)
Ca (mg/dl)	10.0 ± 0.4	(9.5–10.8)	10.0 ± 0.6	(9.2–11.1)	9.7 ± 0.5	(9.0–10.3)	9.2 ± 0.4	(8.8–9.9)
Cr (mg/dl)	0.9 ± 0.1	(0.8–1.0)	0.9 ± 0.1	(0.8–1.1)	0.8 ± 0.1	(0.6–1.0)	0.8 ± 0.1	(0.7–0.9)
P (mg/dl)	3.9 ± 1.1	(1.7–4.7)	3.3 ± 0.8	(1.7–4.5)	2.7 ± 0.8	(1.4–4.3)	3.6 ± 0.6	(2.4–4.3)

Note: *Cl, chloride; TCO_2, total carbon dioxide; K, potassium; Na, sodium; Alb, albumin; BUN, blood urea nitrogen; TP, total protein; ALT, alanine aminotransferase; ALP, alkaline phosphatase; Ca, calcium; Cr, creatinine; P, phosphorus.*

[a]*Gestational days (GD) 25–100 (*n = *10; mean ± SD).*

From Hendrickx and Dukelow (1995).

deprivation during pregnancy results in maternal as well as fetal mortality in this species (Kohrs et al., 1976). The effects of experimentally induced diabetes mellitus on fetal metabolism and well-being have also been examined in rhesus monkeys (Mintz et al., 1972). Administration of the pancreatic β cell cytotoxin streptozotocin before conception and during the first trimester of pregnancy was associated with a 27% mortality rate during the second and third trimesters. The drug effects included fetal hyperinsulinemia, enhanced pancreatic islet cell responsiveness, enlarged placentas, and polyhydramnios.

Both spontaneous and experimentally induced maternal infections are important factors in reproductive failure in a variety of nonhuman primate species (Hendrickx and Binkerd, 1980). The following infections have been implicated as causative agents in abortions or stillbirths: Chagas' disease (*Trypanosoma cruzi*-like) in marmosets; T-strain mycoplasmas in talapoins and patas monkeys; measles virus in rhesus monkeys (Renne et al., 1973); rubella virus in long-tailed monkeys and baboons; and mumps virus in rhesus monkeys.

Observations in rhesus monkey colonies indicate that maternal psychological factors may be as important for fetal viability as maternal physiological conditions during pregnancy. The high level of abortion (50–70%) among pregnant animals captured in a native environment and shipped to the USA for experimental purposes may be partially attributable to the high degree of stress associated with handling techniques (Myers, 1972). Experiments carried out in rhesus monkeys to study the effect of maternal stress on pregnancy indicate that excitability and discomfort associated with labor and delivery may have deleterious effects on the fetus. Brief episodes of experimentally induced stress in near-term rhesus monkeys cause fetal deterioration in the form of fetal bradycardia and decreased arterial oxygenation (Morishima et al., 1978) or fetal asphyxia and concomitant disturbances in the acid–base balance as a result of impaired uteroplacental circulation. Boot et al. (1985) have also demonstrated that housing conditions (i.e. cage size and density) can adversely affect the pregnancy outcome and may be related to stress.

Changes in the mother's environment during pregnancy (e.g. social change, location change) have been noted to influence pregnancy outcome in several nonhuman primate species. A variety of reproductive deficiencies were noted ranging from poor infant survival to a complete inhibition of ovulation and infertility. Specific hypothalamic factors (i.e. suppression of GnRH or LH/FSH) have been suggested as the mechanism responsible for these changes

(e.g. Abbott et al., 1988). Although stress appeared to be contributory in New World species under these conditions and in Old World species as described earlier, other environmental effects such as airline travel and various forms of restraint and immobilization do not appear to result in an altered pregnancy outcome in captive macaques. It has been noted that for pigtailed, long-tailed, and rhesus macaques in addition to baboons, jet transport during various periods of gestation does not alter the rate of viable offspring (Sackett, 1981) or increase the rate of spontaneous abortion (A. G. Hendrickx, unpublished observations). Of the 154 pregnant rhesus females (GD 30−150) shipped via air flight to other institutions for experimental purposes from 1982 to 1990, only 0.7% (1/154) produced a nonviable fetus upon arrival; no abortions either during or within 2 weeks of shipment were observed. Other studies have shown that daily capture of gravid pig-tailed macaques during GD 30−130 does not alter gestational length or survival of the offspring (Newell-Morris et al., 1989). Experience at the CPRC indicates that frequent chair restraint and/or hand-catching by experienced animal handlers during all stages of pregnancy in both rhesus and long-tailed macaques do not affect pregnancy maintenance or outcome (Tarantal and Hendrickx, 1988b, 1989).

Various types of placental insufficiency have also been associated with high rates of fetal mortality, including infections of the placenta (placentitis) (Kaplan, 1979), impaired placental circulation (infarctions and abruptio placentae), and abnormal placental location (placenta previa). The role of ultrasound in the detection of placental abruptions, either subchorionic or retroplacental, is significant (Tarantal and Hendrickx, 1988b). Use of this technique, particularly for concealed hemorrhage in conjunction with emergency hysterotomy, can result in major improvements in maternal mortality in addition to retrieval of viable fetal tissues for experimental purposes.

In a histological study of stillborn fetuses from a variety of nonhuman primates species, a necropsy examination confirmed placentitis in seven of eight cases as the primary cause of fetal demise. The most commonly isolated organisms responsible for ascending genital infections leading to placentitis and subsequent fetal anoxia were Group D streptococci and β-hemolytic, coagulase-positive *Staphylococcus aureus.* Infection of the placenta was accompanied by infiltration of inflammatory cells, edema, necrosis, and hemorrhage which interfered with fetal oxygenation (Andrews, 1974). Gram-positive cocci, especially α-hemolytic *Streptococcus viridans*, were implicated in 11 of 17 cases of abortions and stillbirths in rhesus monkeys (Swindle et al., 1982). Acute placentitis and fetal bronchopneumonia were the most consistent histopathological findings in these cases.

Although it is difficult to document dystocia in nonhuman primates because of the high incidence of night births, contributing causes may include cephalopelvic disproportion, positional abnormalities, uterine malformation and inertia, uncontrolled hemorrhage, and toxemia (Hendrickx and Giles-Nelson, 1971). Adverse pregnancy outcomes (i.e. stillbirths) have been associated with breech rather than cephalic presentation in term pregnancies of long-tailed (Cho et al., 1985) and pig-tailed (Goodlin and Sackett, 1983) macaques. Parturition in marmosets can lead to complications, primarily in primiparous births (Hill, 1969). No evidence has linked litter size with parturition difficulties (Poole and Evans, 1982).

NONHUMAN PRIMATES FROM FOREIGN BREEDING PROGRAMS

Introduction

Recent trends in utilization of nonhuman primates for research within the USA indicate that foreign breeding operations will continue to be a valued source of animals in order to meet demands within the biomedical community. This is especially true for *Macaca fascicularis*, the cynomolgus macaque. In addressing issues related to foreign sourcing of research nonhuman primates, the ultimate goal is to provide the appropriate research model to the investigator, which requires production of a well-defined research nonhuman primate relative to genetic origin and SPF status. Furthermore, reliable animal availability requires a secure means of transportation into the USA, with successful completion of Centers for Disease Control (CDC) import quarantine. Investigators and colony managers should understand some of the unique issues surrounding acquisition of research nonhuman primates from foreign sources. Suppliers, importers, and end users of these animals should continually work to improve processes to meet quality and supply expectations.

Historical Perspective

The invaluable role of nonhuman primates in biomedical research has always required attention to securing adequate numbers of research quality nonhuman primates. Research model specifications minimally address a defined species, health status, gender, and maturity. Since the early 1960s in the USA, the National Institutes of Health began planning and implementing programs to generate nonhuman primate research models for use in federally funded research. (Whitehair, 1999). For decades after inception, the National Primate Research Centers (NPRCs) relied on feral or bred nonhuman primates from foreign suppliers to replace and expand breeding stock. Similarly, commercial and academic institutions conducting research with nonhuman

primates relied primarily on animals imported from their native countries of origin. Today, the NPRCs are more self-sustaining. Additionally, there are several established commercial breeders of research nonhuman primates in the USA. However, to meet the demand for research quality nonhuman primates, in particular cynomolgus macaques; there is still a critical need to import animals from breeding programs outside the USA and Canada. In this discussion, it should be noted that although a large portion of research involving nonhuman primates remains in North America, the countries of Japan, Europe, and China also conduct significant nonhuman primate research. Furthermore, other Asian countries are working in this field. The geographical distribution of nonhuman primate research is not static. The term "foreign breeding source" in this chapter is meant to define sites apart from the USA and Canada. A review of US production of research nonhuman primates from government or commercial sources is beyond the scope of this discussion.

Foreign breeders of research nonhuman primates may be located in countries where the particular species is free ranging (either native or introduced and established) or alternatively, in countries that rely on importation of the nonhuman primate species from abroad specifically for breeding purposes. The location of the foreign breeding operation may affect the availability of breed stock, veterinary care, housing, and biosecurity practices. Important aspects in this regard include characterization of the research model with respect to phenotype and genotype of the breeding animals and their offspring; management of endemic diseases and vectors; species conservation and Convention on International Trade in Endangered Species (CITES) implications; safe and manageable in-transit handling and biosecurity during animal export to the USA; proximity to necessary diagnostic resources for SPF screening, and also the impetus or need to conduct F1+ breeding. Regardless of their respective locations, a collaborative relationship should exist between the research scientist and the supplier so that the research facility can adequately communicate expectations of quality and be able to audit the breeding program's procedures and facilities. On this latter point, recognized accreditation programs such as Association for Assessment and Accrediation of Laboratory Animal Care (AAALAC) may be a useful indicator of a program's level of competence.

Breeding research monkeys outside the USA is a well established practice. An early program to breed nonhuman primates in the country of origin includes the Peruvian Primatology Project, which in conjunction with the Pan American Health Organization began in 1975 to promote investigation into conservation, management, and reproduction of certain New World nonhuman primates (Montoya, 2003). This program continues to provide animals for scientific research. The Washington NPRC also supported the inception of a free-range breeding colony of cynomolgus macaques for research and conservation in Indonesia on Tinjil Island in 1988−1990 (Kyes 1993). Several "in country" commercial breeders of cynomolgus macaques also began in the mid-1980s, including captive breeding programs in the Philippines (1983) and in Mauritius (1985) (Hobbs et al., 1987; Stanely 2003). China began developing captive breeding of imported cynomolgus monkeys to supply biomedical research in 1985. It is interesting to note that cynomolgus monkeys are not native to China and that Chinese cynomolgus breeding centers have imported animals from several origin countries. China had programs for captive breeding of domestic rhesus monkeys in 1978 for both conservation and research purposes. (Hsu and Jia 2003). The drive to breed monkeys for research in source countries reflects the situation during the 1980s when the majority of cynomolgus monkeys imported to the USA were feral source and shipped from Indonesia and the Philippines. By 1994, Indonesia had enacted guidance to cease export of feral nonhuman primates (Pamungkas and Sajuthi, 2003), and the Philippines followed suit shortly thereafter.

It is worth noting that several free-ranging populations of nonhuman primates, introduced into new habitats and now established, have also been managed as a resource for behavioral and scientific research. These animals include rhesus macaques on Cayo Santiago (Whitehair, 1999), African green monkeys in the Caribbean (Ervin and Palmour, 2003), and cynomolgus monkeys on the island of Mauritius (Stanely, 2003).

Current Considerations

Breeding research nonhuman primates outside the USA remains attractive from a cost perspective. The goals of foreign breeding centers should continue to focus on meeting international standards of accreditation as well as on implementing an industry standard for the level of professional diversity and training of staff involved in the program.

Current information on the importation of research nonhuman primates in the USA is reflected in summary data from the National Center for Infectious Disease, CDC Division of Quarantine and Migration. During fiscal year 2009, this report documented that a total of 24 414 nonhuman primates were imported into the USA (Mullan, 2009). This figure is down slightly from the previous 4 years, which had posted numbers just exceeding 25 000 animals. Of the total imports, 92.3% were cynomolgus macaques, 5.0% rhesus macaques, and 1.5% African green monkeys. New World species, represented by squirrel monkeys and marmosets, combined for 0.7%. The relatively small remainder of the imports included 10 other

nonhuman primate species. By far the majority of nonhuman primates imported into the USA for research were macaques, primarily cynomolgus monkeys. Originating countries (and percent of total imports) included China (59.8%), Mauritius (17.4%), Vietnam (11.3%), Cambodia (5.9%), Indonesia (1.2%), and the Philippines (1.2%).

When acquiring nonhuman primates from foreign breeding programs, it is useful to be able to review the facilities' management practices, disease surveillance records, preventative medicine program, welfare and enrichment plan, clinical records of animals, and breeding history of the colony. Breeding history of the colony should include species and sub-species information on the parents and offspring. Another important point of review is whether the colony is closed or receiving expansion/replacement breeders from an outside source. Programs that employ even some F_0 breeding should be scrutinized for relative practices of health quarantine and source and genetic background evaluation of animals to be introduced into the colony. This is necessary to limit the risk of contagious disease and to avoid admixture of differing sub-species into the breeding colony.

The genetic composition of the parents and offspring may a have critical role in meeting production goals and in providing research models suitable to meet particular research expectations. An understanding of the ancestry of the parents and some level of genetic information on the offspring from foreign breeding centers should be achieved prior to the acquisition of animals for a particular study.

It is well established that species of nonhuman primates commonly used in research often include subspecies and, further, that subspecies' differences can affect responses to a particular study. Variation in test and reference data between subspecies of nonhuman primates has been reported. Such differences may include a subspecies' response in infectious disease research, immunological expression, physiological and biochemical parameters, breeding performance, and even behavior. Such findings have been noted in squirrel monkeys, baboons, rhesus macaques, and cynomolgus macaques (Abee, 2000; Williams-Blangero et al., 2002; Williams-Blangero and VandeBerg, 2003; Leuchte et al., 2004; Kawamoto et al., 2007; Stevison and Kohn 2008; Van Andel et al., 2008). It should be noted that morphological inspection alone may be inadequate for distinguishing subspecies or hybrid animals. Ideally, to be able to compare data from various research studies and, further, to manage consistency within control and test animals, it is preferable to use a single sub-species that is genetically and physiologically well defined. Genetic admixture and hybrid animals can impart differences in response to test articles or procedures, which may confound results. (Williams-Blangero and VandeBerg, 2003)

Over 19 000 cynomolgus macaques (*Macaca fascicularis*) were imported into the USA for use in biomedical research in fiscal year 2009. These animals were imported from China, Mauritius, Vietnam, Cambodia, Indonesia, and the Philippines (Mullan, 2009). The genetic character of all these animals is not consistent across all sources but varies as a reflection of the wild cynomolgus (*Macaca fascicularis*) population from which the parents were derived. Furthermore, variability may arise from human influence if animals of different genetic backgrounds are intermixed in breeding. In general, the captive breeding colonies of *Macaca fascicularis* are derived from one of three populations of free-ranging cynomolgus monkeys or an admixture of these three populations. These three sources include cynomolgus derived from Indochina, Insular Asia (Indonesia, Philippines), and a Mauritian cynomolgus group (Stevison and Kohn, 2008). The last is a species introduced to the island of Mauritius between 400 and 500 years ago, likely from a relatively very small founder population originating in Java and/or Sumatra (Stanely, 2003; Tosi and Coke, 2007; Blancher et al., 2008; Stevison and Kohn, 2008). The insular cynomolgus macaques split from the continental Indochinese cynomolgus approximately 1 million years ago (Blancher et al., 2008). Cynomolgus macaques of the Philippines appear to have originated 40 000 to 110 000 years ago, by natural or human-assisted colonization of an Indonesian founder population in the Philippines (Smith et al., 2007; Blancher et al., 2008). Genetic evidence indicates that Indochinese cynomolgus macaques were influenced by genetic inflow from rhesus macaques, both in the past and possibly to current times. This event occurs to varying degrees in geographically distinct intergrade zones of cohabitation on the mainland (Leuchte et al., 2004; Kanthaswamy et al., 2008; Stevison and Kohn, 2008, 2009). Indochinese cynomolgus breeding animals originating from widely different locations may display varying proportions of hybridization, and this may have important implications on their study use, depending upon the type of research to be conducted using the offspring. It might be preferable to keep local populations of Indochinese animals segregated in breeding systems in order to produce a consistent model (Stevison and Kohn, 2008).

Particular research goals and methods employing cynomolgus monkeys may dictate a preference relative to the animal's origin of derivation. In addition to variations referenced earlier, there are important differences in the distribution of major histocompatibility complex (MHC) class I and II alleles. Mauritian cynomolgus monkeys display a great deal of homogeneity relative to MHC I and II along with a relatively low genetic variability compared with Indonesian or Indochinese cynomolgus macaques (Krebs et al., 2005; Blancher et al., 2008; Kanthaswamy et al., 2008). This characteristic of low variability in MHC

class I alleles found in Mauritian monkeys may make them good candidates for studies requiring identical or well-defined test cohorts relative to MHC I and II. Research where this characteristic may be beneficial includes immunological studies such as HIV vaccine trials and other infectious disease or organ/tissue transplant studies (Leuchte et al., 2004; Krebs et al., 2005; Van Andel et al., 2008; Stevison and Kohn 2008). Indonesian cynomolgus reportedly are a very good model for studying human pneumonic plague and possibly other human infectious diseases (Van Andel et al., 2008). Toxicology and other specific research may have goals and concerns in using cynomolgus monkeys with low genetic variability. These populations may have "lost" specific genes over generations, which could confer a susceptibility to a certain disease (Smith et al., 2007).

Rhesus macaques (*Macaca mulatta*), from captive breeding in China, were the second most frequently imported research nonhuman primate in the USA in fiscal year 2009 (Mullan, 2009). It should be pointed out that similar to genetic, behavioral, and research differences reported for various origins of *Macaca fascicularis*, genetic, behavioral, and research differences also exist between both Chinese wild and captive-bred rhesus macaques and Indian-derived rhesus macaques (Satkoski et al., 2008). Indian-derived rhesus monkeys have been bred domestically by the NPRCs following the embargo on the export of rhesus from India in 1978. Indian rhesus are highly sought for use in HIV vaccine research, transplant studies, and other immunology research due to their relatively well defined and unique MHC I presentation. This feature is of value in evaluating immune responses between various test groups in a study. Interestingly, the mtDNA of Chinese rhesus more closely matches that of the Taiwanese (*Macaca cyclopis*) and the Japanese macaque (*Macaca fuscata*) than it does the Indian rhesus monkey. Furthermore, free-ranging Chinese rhesus populations show significant differences in genetic character based upon geographical division across an East−West mapping. Rhesus monkeys in captive breeding in China may in some cases represent an admixture of breeders from different wild Chinese populations (Satkoski et al., 2008).

Challenges to Foreign Breeding

Foreign-sourced research nonhuman primates are expected to be defined with respect to specific pathogen-free (SPF) status. Depending upon the location of the breeder, certain obstacles may need to be addressed relative to managing SPF production. Implementation of site-specific protocols addressing species-specific disease surveillance, preventative medicine, occupational health, and zoonoses training of employees are necessary. When breeding units are exposed to the outdoors, there may be concerns related to endemic parasitic diseases with specific intermediate hosts or vectors (*Plasmodium* sp., microfilaria, trematodes, cestodes) that are uncommon or not found within the USA. In this regard, foreign suppliers may have instituted both preventative programs and prophylactic treatment to block transmission of these parasites. Continued surveillance for these parasites upon arrival of these animals in the USA should still be practiced. The risk of animals' exposure to certain zoonotic disease may be related to a high prevalence of the disease within the colony's employees or their familial contacts. Such disease examples may include rubeola, *Shigella* sp., and *Mycobacterium tuberculosis*. All nonhuman primate breeding programs must address occupational health and use of primary or personal protective equipment relative to human / nonhuman primate interactions. Macaque breeding colonies often have a goal of producing SPF animals (free of SRV, STLV-1, and B virus), but this goal may pose a diagnostic challenge due to the lack of local or in-house test systems with adequate sensitivity and specificity. Furthermore, when PCR capability is needed, there may be obstacles in sending samples to a distant laboratory. This is a critical point when trying to maintain an SPF macaque colony or introduce new animals for expansion or as replacement breeders. Similarly, when investigating suspicious disease outbreaks, it may be difficult to find local laboratories capable of performing preferred diagnostics (e.g. direct PCR or culture for Mycobacterium). Retrieving permits to ship samples out of the country may hinder expedient delivery of samples to a qualified laboratory.

Securing a consistent and proper nutritionally balanced diet for the breeding colony is also an area of consternation in some foreign breeding facilities. Importation of nonhuman primate chow is often prohibitively expensive. Availability issues for nonhuman primate diets can be overcome by working with certified local feed producers. On occasion there may still be difficulty in obtaining fixed-formula ingredients. The goal is to provide a stable, contaminant-free formulated chow that meets NRC guidelines for the given species. Local feed producers should have a qualified nutritionist on staff and be equipped with an adequate means of analyzing the diet being produced for the breeding colony. Animal drinking water must also be regularly evaluated as to source, safety, and sanitation.

Management of foreign breeding operations should be evaluated relative to the level of training and support of the staff directly involved in veterinary care, husbandry activities, and the behavioral/welfare program. Availability of trained staff with the opportunity to participate in laboratory animal science training and continuing education varies from program to program. In some cases there may be disparity in the level of formal training when comparing numerous breeding operations. In such cases, a practical

approach is warranted. Furthermore, although there may be local governance of breeding and transporting nonhuman primates, AAALAC accreditation is useful for assessing the animal care and welfare standards. The level of both responsibility and program input by the veterinary staff is another important factor. Attention to the response of management to the veterinary staff's input may reflect on the program's overall quality. The relationship among all working toward a goal can be strengthened by the presence of an Institutional Animal Care and Use Committee (IACUC) or a similar body.

Acquisition of research nonhuman primates by importation also relies on safe and humane transport. Transportation challenges may include maintaining the biosecurity of the animals; safety of animals and human contacts; impact of public perception of research nonhuman primates as cargo; meeting the vital husbandry needs of the species during transit, and compliance with numerous regulatory agencies (Elmore, 2008). Over the past 20 years, numerous air carriers transiting through Europe have abandoned nonhuman primate cargo, in part due to animal rights campaigns as well as public perception of risk following disease outbreaks that publicized a link to nonhuman primates. Consider as examples the popular press handling of issues related to Ebola/Reston, severe acute respiratory syndrome (SARS), and simian acquired immune deficiency syndrome (SAIDS). In the past, difficulties arranging importation of nonhuman primate cargo through Europe led to chartered aircraft importation of nonhuman primates, but that imposed a significant business expense on the importation process. It should be noted that these difficulties in air transportation have not occurred with imports from China using Chinese air carriers.

Imported nonhuman primates must undergo government-regulated quarantine upon arrival in the USA. Import quarantine is a period when the animals are not available for research use. Quarantine protocols incur additional expenses to comply with regulatory guidelines. During an imposed import quarantine period, animals are segregated in dedicated animal rooms with restricted access. There are elevated requirements for personal protective equipment (PPE) and for disposition of potentially contaminated crates, equipment, and waste from quarantine rooms. Further costs associated with routine disease surveillance as well as specified testing may be imposed in response to any disease outbreak or mortality during quarantine. Failure or delays in completing the quarantine period on schedule may have research, occupational health, and added financial consequences.

Recent trends in utilization of nonhuman primates for research within the USA indicate that foreign breeding operations will continue to be a valued source of animals to meet the demands of the biomedical community. This is especially true for *Macaca fascicularis*, the cynomolgus macaques. Investigators and colony managers should understand some of the unique issues surrounding acquisition of research nonhuman primates from foreign sources. Suppliers, importers, and end users of these animals should continually work to improve processes to meet expectations of quality and supply.

REFERENCES

Abee, C. R. (2000). Squirrel monkey (*Saimiri* spp.) research and resources. *ILAR J., 41*, 2–9.

Abbott, D. H. (1987). Behaviourally mediated suppression of reproduction in female primates. *J. Zoo., 213*, 455–470.

Abbott, D. H., Hodges, J. K., & George, L. M. (1988). Social status controls LH secretion and ovulation in female marmoset monkeys (*Callithrix jacchus*). *J. Endocrinol., 17*, 329–339.

Ackerman, D. R., & Roussel, J. D. (1968). Fructose, lactic acid and citric acid content of the semen of eleven sub-human primate species and of man. *J. Reprod. Fertil., 17*, 563–566.

Afzelius, B. A., Johnsonbaugh, R. E., Kim, J.-W., Plüen, L., & Ritzen, E. M. (1982). Spermiogenesis and testicular spermatozoa of the olive baboon (*Papio anubis*). *J. Submicrosc. Cytol., 14*, 627–639.

Aksel, S., Diamond, E. J., Hazelton, J., Wiebe, R. H., & Abee, C. R. (1985). Progesterone as a predictor of cyclicity in Bolivian squirrel monkeys during the breeding season. *Lab. Anim. Sci., 35*, 54–57.

Aksel, S., Hazelton, J., Jennings, R. A., & Abee, C. R. (1991). Age related hormonal changes in estrous cycles of squirrel monkeys (*Saimiri boliviensis boliviensis*). *Am. J. Primatol., 24*, 86.

Albrecht, E. D., & Pepe, G. J. (1990). Placental steroid hormone biosynthesis in primate pregnancy. *Endocrinol. Rev., 11*, 124–150.

Albrecht, E. D., & Pepe, G. J. (1999). Central integrative role of oestrogen in modulating the communication between the placenta and fetus that results in primate fetal-placenta development. *Placenta., 20*, 129–139.

Allen, J. R., & Ahlgren, S. A. (1968). A comparative study of the hematologic changes in pregnancy in the *Macaca mulatta* monkey and the human female. *Am. J. Obstet. Gynecol., 100*, 894–903.

Allen, J. R., & Siegfried, L. M. (1966). Hematologic alterations in pregnant rhesus monkeys. *Lab. Anim. Care, 16*, 465–471.

Anderson, M. J., & Dixson, A. F. (2002). Motility and the midpiece in primates. *Nature, 416*, 496.

Andrews, E. J. (1974). Pulmonary pathology in stillborn nonhuman primates. *J. Am. Vet. Med. Assoc., 164*, 715–718.

Arora, R., Dinakar, N., & Prasad, M. R. N. (1975). Biochemical changes in the spermatozoa and luminal contents of different regions of the epididymis of the rhesus monkey, *Macaca mulatta. Contraception, 11*, 689–700.

Arsenieva, M., Dubinin, N. P., Orlova, N. N., & Bakulina, E. D. (1961). Radiation analysis duration of meiotic phases in spermatogenesis of monkey (*Macaca mulatta*). *Dokl. Biol. Sci. (Engl. Transl.)., 141*, 984–986.

Arslan, M., Mahmood, S., Khurshid, S., Naqvi, S. M. S., Afzal, M. A., & Baig, S. M. (1986). Changes in circulating levels of immunoreactive follicle stimulating hormone, luteinizing hormone, and testosterone during sexual development in the rhesus monkey, *Macaca mulatta. J. Med. Primatol., 15*, 351–359.

Atsalis, S., & Margulis, S. W. (2008a). Primate reproductive aging: from lemurs to humans. In S. Atsalis, S. W. Margulis & P. R. Hof (Eds.), *Primate Reproductive Aging: cross-taxon perspectives on reproduction* (pp. 186−194). Basel: Karger.

Atsalis, S., & Margulis, S. W. (2008b). Perimenopause and menopause: documenting life changes in aging female gorillas. In S. Atsalis, S. W. Margulis & P. R. Hof (Eds.), *Primate Reproductive Aging: cross-taxon perspectives on reproduction* (pp. 119−146). Basel: Karger.

Baldwin, J. D., & Baldwin, J. I. (1981). *The Squirrel Monkeys, Genus Saimiri. Ecology and Behavior of Neotropical Primates*. Vol. 1 (pp. 277−330). Rio de Janeiro: Academia Brasileira de Ciencias.

Barr, A. B. (1973). Timing of spermatogenesis in four nonhuman primate species. *Fertil. Steril., 24*, 381−389.

Baskin, G. B., Murphey-Corb, M., Watson, E. A., & Martin, L. N. (1988). Necropsy findings in rhesus monkeys experimentally infected with cultured Simian Immunodeficiency Virus (SIV)/Delta. *Vet. Pathol., 25*, 456−467.

Baskin, G. B., Bontrop, R. E., Niphius, H., Noort, R., Rice, J., & Heeney, J. L. (1997). Correlation of major histocompatibility complex with opportunistic infections in simian immunodeficiency virus-infected rhesus monkeys. *Lab. Invest., 77*, 305−309.

Bedford, J. M. (1967). Observations on the fine structure of spermatozoa of the bush baby (*Galago senegalensis*), the African green monkey (*Cercopithecus aethiops*) and man. *Am. J. Anat., 121*, 443−460.

Beehner, J. C., Gesquiere, L., Seyfarth, R. M., Cheney, D. L., Alberts, S. C., & Altmann, J. (2009). Testosterone related to age and life-history stages in male baboons and geladas. *Horm. Behav., 56*, 472−480.

Behboodi, E., Katz, D. F., Samuels, S. J., Tell, L., Hendrickx, A. G., & Lasley, B. L. (1991). The use of a urinary estrone conjugates assay for detection of optimal mating time in the cynomolgus macaque (*Macaca fascicularis*). *J. Med. Primatol., 20*, 229−234.

Bercovitch, F. B., Widdig, A., Berard, J. D., Nuernberg, P., Kessler, M. J., Schmidtke, J., et al. (2002). Multiple sirehood in free-ranging twin rhesus macaques (*Macaca mulatta*). *Am. J. Primatol., 57*, 31−34.

Beisner, B. A., Jackson, M. E., Cameron, A., & McCowan, B. (2010). Effects of natal male alliances on aggression and power dynamics in rhesus macaques. *Am. J. Primatol*. Nov 30. [Epub ahead of print].

Bielert, C., Czaka, J. A., Eisele, S., Scheffler, G., Robinson, J. A., & Goy, R. W. (1976). Mating in the rhesus monkey (*Macaca mulatta*) after conception and its relationship to oestradiol and progesterone levels throughout pregnancy. *J. Reprod. Fertil., 46*, 179−187.

Blakley, G. B., Beamer, T. W., & Dukelow, W. R. (1981). Characteristics of the menstrual cycle in nonhuman primates. IV. Timed mating in *Macaca nemestrina*. *Lab. Anim., 15*, 351−353.

Blancher, A., Bonhomme, M., Crouau-Roy, B., Terao, K., Kitano, T., & Saitou, N. (2008). Mitochondrial DNA sequence phylogeny of 4 populations of the widely distributed cynomolgus macaque (*Macaca fascicularis*). *J. Hered., 11*, 1−11.

Blewett, E. L., Saliki, J. T., et al. (1999). Development of a competitive ELISA for detection of primates infected with monkey B virus (*Herpesvirus simiae*). *J. Virol Methods., 77*, 59−67.

Boinski, S. (1987). Mating patterns in squirrel monkeys *(Saimiri oerstedi)*: implications for seasonal sexual dimorphism. *Behav. Ecol. Sociobiol., 21*, 13−21.

Bonney, R. C., Dixson, A. F., et al. (1979). Cyclic changes in the circulating and urinary levels of ovarian steroids in the adult female owl monkey (*Aotus trivirgatus*). *J. Reprod. Fertil., 56*, 271−280.

Boot, R., & Huis in't Veld, L. G. (1981). Pregnancy diagnosis in *Macaca fascicularis*: hemagglutination inhibition test for urinary chorionic gonadotropin and implantation bleeding. *J. Med. Primatol., 10*, 141−148.

Boot, R., Leussink, A. B., & Vlug, R. F. (1985). Influence of housing conditions on pregnancy outcome in cynomolgus monkeys (*Macaca fascicularis*). *Lab Anim., 19*, 42−47.

Bose, A. R., & Kar, A. B. (1968). Distribution of sialic acid in the genital organs of male rhesus monkeys: effects of castration and replacement therapy. *Curr. Sci., 37*, 168−169.

Bradley, B. J., & Mundy, N. I. (2008). The primate palette: the evolution of primate coloration. *Evol. Anthropol., 17*, 97−111.

Brannian, J. D., & Stouffer, R. L. (1991). Progesterone production by monkey luteal cell subpopulations at different stages of the menstrual cycle: changes in agonist responsiveness. *Biol. Reprod., 44*, 141−149.

Brizzee, K. R., & Dunlap, W. P. (1986). Growth. In W. R. Dukelow & J. Erwin (Eds.), *Comparative Primate Biology*, Vol. 3 (pp. 363−413). New York: Alan R. Liss.

Bush, D. E., Russell, L. H., Flowers, A. I., & Sorenson, A. M. (1975). Semen evaluation in capuchin monkeys (*Cebus apella*). *Lab. Anim. Sci., 25*, 588−593.

Cameron, J. L., Hansen, P. D., McNeill, T. H., Koerker, D. J., Clifton, D. K., Rogers, K. V., et al. (1985a). Metabolic cues for the onset of puberty in primate species. In S. Venturoli, C. Flamigni & J. R. Givens (Eds.), *Adolescence in Females* (pp. 59−77). Chicago: Year Book Medical Publishers.

Cameron, J. L., Koerker, D. J., & Steiner, R. A. (1985b). Metabolic changes during maturation of male monkeys: possible signals for onset of puberty. *Am. J. Physiol., 249*, E385−E391.

Carpenter, C. R. (1942). Sexual behavior of free ranging rhesus monkeys (*Macaca mulatta*). I. Specimens, procedures and behavioral characteristics of estrus. *J. Comp. Psychol., 33*, 113−142.

Carpenter, C. R. (1972). Breeding colonies of macaques and gibbons on Santiago Island, Puerto Rico. In W. I. B. Beveridge (Ed.), *Breeding Primates* (pp. 76−87). Basel: Karger.

Caro, T. M., Sellen, D., Parish, A., Frank, R., Brown, D. M., & Voland, E. (1995). Termination of reproduction in nonhuman and human female primates. *Int. J. Primatol., 16*, 205−220.

Carvalho, E. M., Bacellar, O., et al. (2001). Cytokine profile and immunomodulation in asymptomatic human T-lymphotropic virus type 1-infected blood donors. *J. Acquired Immunodef. Synd., 27*, 1−6.

Chambers, P. L., & Hearn, J. P. (1985). Embryonic, foetal, and placental development in the common marmoset monkey (*Callithrix jacchus*). *J. Zool., 207*, 545−561.

Chambers, K. C., Resko, J. A., & Phoenix, C. H. (1982). Correlation of diurnal changes in hormones with sexual behavior and age in male rhesus macaques. *Neurobiol. Aging., 3*, 37−42.

Chellman, G. J., Lukas, V. S., Eugui, E. M., et al. (1992). Activation of B virus (*Herpesvirus simiae*) in chronically immunosuppressed cynomolgus monkeys. *Lab. Anim. Sci., 42*, 146−151.

Chen, L. D., Kushwaha, R. S., McGill, H., Rice, K. S., & Carey, K. D. (1998). Effect of naturally reduced ovarian function of plasma lipoprotein and 27-hydroxycholesterol levels in baboons (*Papio* sp.). *Atherosclerosis, 136*, 89−98.

Cheng, C. Y., Wong, E. W. P., Yan, HH. N., & Mruk, D. D. (2010). Regulation of spermatogenesis in the microenvironment of the

seminiferous epithelium: new insights and advances. *Mol. Cell. Endocrinol., 315*, 49–56.

Cho, F., Hanari, K., Suzuki, M., & Honjo, S. (1985). Relationship between fetal position and stillbirth in the cynomolgus monkeys (*Macaca fascicularis*): Retrospective analysis. *J. Med. Primatol., 14*, 169–174.

Chowdhury, A. K., & Marshall, G. R. (1980). Irregular pattern of spermatogenesis in the baboon (*Papio anubis*) and its possible mechanism. In A. Steinberger & E. Steinberger (Eds.), *Testicular Development, Structure and Function* (pp. 129–137). New York: Raven Press.

Chowdhury, A. K., & Steinberger, E. (1976). A study of germ cell morphology and duration of spermatogenic cycle in the baboon, *Papio anubis*. *Anat. Ret., 185*, 155–170.

Clermont, Y. (1969). Two classes of spermatogonial stem cells in the monkey, *Cercopithecus aethiops*. *Am. J. Anat., 126*, 57–72.

Clermont, Y., & Antar, M. (1973). Duration of the cycle of the seminiferous epithelium and the spermatogonial renewal. *Physiol. Rev., 52*, 198–235.

Clermont, Y., & LeBlond, C. P. (1959). Differentiation and renewal of spermatogonia in the monkey, *Macaca rhesus*. *Am. J. Anat., 104*, 237–273.

Cleveland, A., Westergaard, G. C., Trenkle, M. K., & Higley, J. D. (2004). Physiological predictors of reproductive outcome and mother-infant behaviors in captive rhesus macaque females (*Macaca mulatta*). *Neuropsychopharmacol., 29*, 901–910.

Clewe, T. H. (1969). Observations on reproduction of squirrel monkeys in captivity. *J. Reprod. Fertil. Suppl., 6*, 151–156.

Coe, C. L., & Rosenblum, L. A. (1978). Annual reproductive strategy of the squirrel monkey (*Saimiri sciureus*). *Folia Primatol., 29*, 19–42.

Coe, C. L., Chen, J., Lowe, E. L., Davidson, J. M., & Levine, S. (1981). Hormonal and behavioral changes at puberty in the squirrel monkey. *Horm. Behav., 15*, 36–53.

Cohen, J. L., Davenport, D. S., Stewart, J. A., Deitchman, S., Hilliard, J. K., & Chapman, L. E. (2002). Recommendations for prevention of and therapy for exposure to B virus (*Cercopithecine herpesvirus 1*). *Clin. Infect. Dis., 35*, 1191–1203.

Conaway, C. H., & Sade, D. S. (1965). The seasonal spermatogenic cycle in free-ranging rhesus monkeys. *Folia Primatol., 3*, 1–12.

Cornwall, G. A. (2009). New insights into epidydimal biology and function. *Hum. Reprod. Update, 15*, 213–227.

Crockett, C. M., Kyes, R. C., & Sajuthi, D. (1996). Modeling managed monkey populations: sustainable harvest of longtailed macaques on a natural habitat island. *Am. J. Primatol., 40*, 343–360.

Czekala, N. M., Hodges, J. K., & Lasley, B. L. (1981). Pregnancy monitoring in diverse primate species by estrogen and bioactive luteinizing hormone determinations in small volumes of urine. *J. Med. Primatol., 10*, 1–15.

Dang, D. C. (1970). *Le cycle de I'epithelium seminifere du singe crabier (*Macaca fascicularis*). PhD Dissertation*. Paris: Faculté des Sciences.

Dang, D. C., & Meusy-Dessolle, N. (1981). Annual plasma testosterone cycle and ejaculatory ability in the laboratory housed crab-eating macaque (*Macaca fascicularis*). *Reprod. Nutr. Dev., 21*, 59–68.

Daniel, M. D., King, N. W., Letvin, N. L., Hunt, R. D., & Seghal, P. K. (1984). A new type D retrovirus isolated from macaques with an immunodeficiency syndrome. *Science, 223*, 602–605.

Daniel, M. D., Letvin, N. L., Seghal, P. K., Schmidt, D. K., Silva, D. P., et al. (1988). Prevalence of antibodies to 3 retroviruses in a captive colony of macaque monkeys. *Int. J. Cancer, 41*, 601–608.

de Rooij, D. G., van Alphen, M. M. A., & van de Kant, H. J. G. (1986). Duration of the cycle of the seminiferous epithelium and its stages in the rhesus monkey (*Macaca mulatta*). *Biol. Reprod., 35*, 587–591.

Desrosiers, R. C. (1997). The value of specific pathogen-free rhesus monkey breeding colonies for AIDS research. *Aids Res. Hum. Retroviruses, 13*, 5–6.

Diamond, E. J., Aksel, S., Hazelton, J. M., Jennings, R. A., & Abee, C. R. (1984). Seasonal changes of serum concentrations of estradiol and progesterone in Bolivian squirrel monkeys (*Saimiri sciureus*). *Am. J. Primatol., 6*, 103–113.

Diamond, E. J., Aksel, S., Hazelton, J. M., Wiebe, R. H., & Abee, C. R. (1987). Serum oestradiol, progesterone, chorionic gonadotrophin and prolactin concentrations during pregnancy in the Bolivian squirrel monkey (*Saimiri sciureus*). *J. Reprod. Fertil., 80*, 373–381.

Dixson, A. F. (1998). *Primate Sexuality: comparative studies of the prosimians, monkeys, apes and human beings*. Oxford, UK: Oxford University Press.

Dixson, A., Gardner, J., & Bonney, R. C. (1980). Puberty in the male owl monkey (*Aotus trivirgatus griseimembra*): a study of physical and hormonal development. *Int. J. Primatol., 1*, 129–139.

Du, Y., Fan, T. Y., Tan, Y., Xiong, Z., & Wang, Z. (2010). Seasonal changes in the reproductive physiology of female rhesus macaques (*Macaca mulatta*). *J. Am. Assoc. Lab. Anim. Sci.,, 49*, 289–293.

Dubey, A. K., Cameron, J. L., Steiner, R. A., & Plant, T. M. (1986). Inhibition of gonadotropin secretion in castrated male rhesus monkeys (*Macaca mulatta*) induced by dietary restriction: analogy with the prepubertal hiatus of gonadotropin release. *Endocrinology, 118*, 518–525.

Dubuc, C., Brent, L. J. N., Accamando, A. K., Gerald, M. S., MacLarnon, A., Semple, S., et al. (2009). Sexual skin color contains information about the timing of the fertile phase in free-ranging rhesus macaques. *Int. J. Primatol., 30*, 777–789.

Dukelow, W. R. (1970). Induction and timing of single and multiple ovulations in the squirrel monkey (*Saimiri sciureus*). *J. Reprod. Fertil., 22*, 303–309.

Dukelow, W. R. (1975). The morphology of follicular development and ovulation in nonhuman primates. *J. Reprod. Fertil. Suppl., 22*, 23–51.

Dukelow, W. R. (1979). Human chorionic gonadotropin: induction of ovulation in the squirrel monkey. *Science, 206*, 234–235.

Dukelow, W. R. (1983). The squirrel monkey. In J. P. Hearn (Ed.), *Reproduction in New World Primates* (pp. 151–179). Hingham, MA: MTP Press.

Dukelow, W. R., & Ariga, S. (1976). Laparoscopic techniques for biomedical research. *J. Med. Primatol., 5*, 82–99.

Dukelow, W. R., & Yorozu, Y. (1986). Sperm capacitation and fertilization. In W. R. Dukelow & J. Erwin (Eds.), *Comparative Primate Biology*, Vol. 3 (pp. 277–289). New York: Alan R. Liss.

Dukelow, W. R., Jewett, D. A., & Rawson, J. M. R. (1973). Follicular development and ovulation in *Macaca fascicularis, Saimiri sciureus* and *Galago sengalensis*. *Am. J. Phys. Anthropol., 38*, 207–210.

DuMond, F. V. (1968). The squirrel monkey in a seminatural environment. In L. A. Rosenblum & R. W. Cooper (Eds.), *The Squirrel Monkey* (pp. 87–145). New York: Academic Press.

DuMond, F. V., & Hutchinson, T. C. (1967). Squirrel monkey reproduction: the "fatted" male phenomenon and seasonal spermatogenesis. *Science, 158*, 1067–1070.

Eaton, G. G., Modahl, K. B., & Johgnson, D. F. (1981). Aggressive behavior in a confined troop of Japenese Macaques: effects of density, season and gender. *Aggress. Behav., 7*, 145−164.

Eastman, S.-A. K., Makawiti, D. W., Collins, W. P., & Hodges, J. K. (1984). Pattern of excretion of urinary steroid metabolites during the ovarian cycle and pregnancy in the marmoset. *J. Endocrinol., 102*, 19−26.

Ebling, F. J. (2005). The neuroendocrine timing of puberty. *Reproduction, 129*, 675−683.

Ehardt, C. L., & Bernstein, I. S. (1986). Matrilineal overthrows in rhesus monkey groups. *Int. J. Primatol., 7*, 157−181.

Elmore, D. B. (2008). Quality management for the international transportation of nonhuman primates. *Vet. Ital., 44*, 141−147.

Else, J. G., Tarara, R., Suleman, M. A., & Eley, R. M. (1986). Enclosure design and reproductive success of baboons used for reproductive research in Kenya. *Lab. Anim. Sci., 36*, 168−172.

Engelhardt, A., Hodges, J. K., Niemitz, C., & Heistermann, M. (2005). Female sexual behaviour, but not sex skin swelling, reliably indicates the timing of the fertile phase in wild long-tailed macaques (*Macaca fascicularis*). *Horm. Behav., 47*, 195−204.

Ervin, F., & Palmour, R. (2003). Primates for 21st century biomedicine: the St. Kitts Vervet (*Chlorocebus aethips*, *SK*). In *International Perspectives: The Future of Nonhuman Primate Resources, Proceedings of the Workshop Held April 17−19, 2002* (pp. 49−53). National Research Council.

Fawcett, D. W., Eddy, E. M., & Phillips, D. M. (1970). Observations on the fine structure and relationships of the chromatid body in mammalian spermatogenesis. *Biol. Reprod., 2*, 129−153.

Fedigan, L. M., Fedigan, L., Gouzoules, H., & Koyama, N. (1986). Lifetime reproductive success in female Japanese macaques. *Folia Primatol., 47*, 143−157.

Fernandez-Duque, E., & Huntington, C. (2002). Disappearances of individuals from social groups have implications for understanding natal dispersal in monogamous owl monkeys (*Aotus azarai*). *Am. J. Primatol., 57*, 219−225.

Finn, C. A. (1998). Menstruation: a nonadaptive consequence of uterine evolution. *Q. Rev. Biol., 73*, 163−173.

Flechon, J.-E., Kraemer, D. C., & Hafez, E. S. E. (1976). Scanning electron microscopy of baboon spermatozoa. *Folia Primatol., 26*, 24−35.

Fontenot, M. B., Galentine, D. M., & Cummings, L. J. (2004). Fostering procedures for orphaned infants in a breeding colony of African green monkeys. *Contemp. Top. Lab. Anim. Sci., 43*, 41−43.

Fouquet, J. P., & Dadoune, J. P. (1986). Renewal of spermatogonia in the monkey (*Macaca fascicularis*). *Biol. Reprod., 35*, 199−207.

French, J. A. (1997). Proximate regulation of singular breeding in callitrichid primates. In N. G. Solomon & J. A. French (Eds.), *Cooperative Breeding in Mammals* (pp. 34−75). New York, NY: Cambridge University Press.

Gabet, A. S., Gessalin, A., & Wattel, E. (2003). High simian T-cell leukemia virus type 1 proviral loads combined with genetic stability as a result of cell-associated provirus replication in naturally infected, asymptomatic monkeys. *Int. J. Cancer, 107*, 74−83.

Gagliardi, C., Liukkonen, J. R., Phillippi-Falkenstein, K. M., Harrison, R. M., & Kubisch, H. M. (2007). Age as a determinant of reproductive success among captive female rhesus macaques (*Macaca mulatta*). *Reproduction, 133*, 819−826.

Garber, P. A., & Leigh, S. R. (1997). Ontogenetic variation in small-bodied New World primates: implications for patterns of reproduction and infant care. *Folia Primatol., 68*, 1−22.

Garcia, M. A., Yee, J., Bouley, D. M., Moorhead, R., & Lerche, N. W. (2004). Diagnosis of tuberculosis in macaques, using whole-blood in vitro interferon-gamma (PRIMAGAM) testing. *Comp. Med., 54*, 86−92.

Garcia, C., Shimizu, K., & Huffman, M. (2009). Relationship between sexual interactions and the timing of the fertile phase in captive female Japanese macaques (*Macaca fuscata*). *Am. J. Primatol., 71*, 868−879.

Garcia, C., Huffman, M., & Shimizu, K. (2010). Seasonal and reproductive variation in body condition in captive female Japanese macaques (*Macaca fuscata*). *Am. J. Primatol., 72*, 277−286.

Gardner, I. A., Stryhn, H., Lind, P., & Collins, M. T. (2000). Conditional dependence between tests affects the diagnosis and surveillance of animal diseases. *Prev. Vet. Med., 45*, 107−122.

Gengozian, N., Smith, T. A., & Gosslee, D. G. (1974). External uterine palpation to identify stages of pregnancy in the marmoset. *Saguinus fuscicollis. J. Med. Primatol., 3*, 236−243.

Ghosh, M., Hutz, R. J., & Dukelow, W. R. (1982). Serum estradiol-17β, progesterone and relative LH levels in *Saimiri sciureus*: cyclic variations and the effect of laparoscopy and follicular aspiration. *J. Med. Primatol., 11*, 312−318.

Gluckman, T. L., Walz, S. E., Schultz-Darken, N., & Bolton, I. D. (2004). Cytologic assessment of the vaginal epithelium in the common marmoset (*Callithrix jacchus*): a preliminary new approach to reproductive screening. *Contemp. Topics Lab. Anim. Sci., 43*, 28−31.

Golan, R., Cooper, T. G., Oschry, Y., Weinbauer, G. F., Shochat, L., & Lewin, L. M. (1997). Epidydimal maturation of chromatin in spermatozoa from control monkeys (*Macaca fasciularis*) and those treated with Cetrorelix, a gonadotropin-releasing hormone antagonist. *Biol. Reprod., 57*, 354−359.

Goodlin, B. L., & Sackett, G. P. (1983). Parturition in *Macaca nemestrina*. *Am. J. Primatol., 4*, 283−308.

Gould, K. G. (1980). Scanning electron microscopy of the primate sperm. *Int. Rev. Cytol., 63*, 323−355.

Gould, K. G., & Faulkner, J. R. (1981). Development, validation, and application of a rapid method for detection of ovulation in great apes and in women. *Fertil. Steril., 35*, 676−682.

Gould, K. G., & Martin, D. E. (1978). Comparative morphology of primate spermatozoa using scanning electron microscopy. II. Families *Cercopithecidae, Lorisidae, Lemuridae*. *J. Hum. Evol., 7*, 637−642.

Gozalo, A., & Montoya, E. (1990). Reproduction of the owl monkey (*Aotus nancymai*) (Primates: Cebidae) in captivity. *Am. J. Primatol., 21*, 61−68.

Graham, C. E. (1979). Reproductive function in aged female chimpanzee. *Am. J. Phys. Anthropol., 50*, 291−300.

Graham, C. E. (1981). Endocrine control of spermatogenesis in primates. *Am. J. Primatol., 1*, 157−165.

Gromoll, J., Wistuba, J., Terwort, N., Godmann, M., Müller, T., & Simoni, M. (2003). A new subclass of the luteinizing hormone/chorionic gonadotropin receptor lacking exon 10 messenger RNA in the New World monkey (Platyrrhini) lineage. *Biol. Reprod., 69*, 75−80.

Gupta, C. L., & Dixit, V. P. (1981). Effects of danazol on the epididymal function in male langurs (*Presbytis entellus entellus* Dufresne). *Andrologia, 13*, 314−321.

Ha, J. C., Robinette, R. L., & Sackett, G. P. (1999). Social housing and pregnancy outcome in captive pigtailed macaques. *Am. J. Primatol., 47*, 153–163.

Ha, J. C., Robinette, R. L., & Davis, A. (2000a). Survival and reproduction in the first two years following a large-scale primate colony move and social reorganization. *Am. J. Primatol., 50*, 131–138.

Ha, J. C., Robinette, R. L., & Sackett, G. P. (2000b). Demographic analysis of the Washington Regional Primate Research Center pigtailed macaque colony, 1967–1996. *Am. J. Primatol., 52*, 187–198.

Harlow, C. R., Hearn, J. P., & Hodges, J. K. (1984). Ovulation in the marmoset monkey: endocrinology, prediction and detection. *J. Endocrinol., 103*, 17–24.

Harrison, R. M., & Lewis, R. W. (1986). The male reproductive tract and its fluids. In W. R. Dukelow & J. Erwin (Eds.), *Comparative Primate Biology*, Vol. 3 (pp. 101–148). New York: Alan R. Liss.

Harrison, R. M., & Wolf, R. H. (1985). Sperm parameters and testicular volumes in *Saguinus mystax*. *J. Med. Primatol., 14*, 281–285.

Hartman, C. G. (1932). Studies in the reproduction of the monkey *Macacus (pithecus) rhesus*, with special reference to menstruation and pregnancy. *Contrib. Embryo Carnegie Inst., 23*, 1–1612.

Hearn, J. P. (1986). The embryo-maternal dialogue during early pregnancy in primates. *J. Reprod. Fertil., 76*, 809–819.

Hearn, J. P., Hodges, J. K., & Gems, S. (1988). Early secretion of chorionic gonadotrophin by marmoset embryos in vivo and in vitro. *J. Endocrinol., 119*, 249–255.

Heger, W., & Neubert, D. (1987). Determination of ovulation and pregnancy in the marmoset (*Callithrix jacchus*) by monitoring of urinary hydroxypregnanalone excretion. *J. Med. Primatol., 16*, 151–164.

Heger, W., Merker, H.-J., & Neubert, D. (1988). Frequency of prenatal loss in marmoset monkeys (*Callithrix jacchus*). In D. Neubert, H.-J. Merker & A. G. Hendrickx (Eds.), *Non-human Primates: Developmental Biology and Toxicology* (pp. 129–140). Berlin: Ueberreuter Wissenschaft.

Heistermann, M., Prove, E., Wolters, H.-J., & Mika, G. (1987). Urinary oestrogen and progesterone excretion before and during pregnancy in a pied bare-faced tamarin (*Saguinus bicolor bicolor*). *J. Reprod. Fertil., 80*, 635–640.

Hendrickx, A. G. (1967). The menstrual cycle of the baboon as determined by the vaginal smear, vaginal biopsy, and perineal swelling. In H. Vagtborg (Ed.), *The Baboon in Biomedical Research*, Vol. II (pp. 437–459). Austin, TX: The University of Texas Press.

Hendrickx, A. G. (1971). *Embryology of the Baboon*. Chicago: University of Chicago Press.

Hendrickx, A. G., & Binkerd, P. E. (1980). Fetal deaths in nonhuman primates. In I. H. Porter & E. B. Hook (Eds.), *Embryonic and Fetal Death* (pp. 45–69). New York: Academic Press.

Hendrickx, A. G., & Dukelow, W. R. (1995). Breeding. In B. T. Bennett, C. R. Abee & R. Henrickson (Eds.), *Nonhuman Primates in Biomedical Research: biology and management* (pp. 335–376). New York, NY: Academic Press.

Hendrickx, A. G., & Giles-Nelson, V. (1971). Reproductive failure. In E. S. E. Hafez (Ed.), *Comparative Reproduction of Nonhuman Primates* (pp. 403–425). Springfield, IL: Thomas.

Hendrickx, A. G., & Kraemer, D. C. (1969). Observations on the menstrual cycle, optimal mating time and pre-implantation embryos of the baboon, *Papio anubis* and *Papio cynocephalus*. *J Reprod. Fertil. Suppl., 6*, 119–128.

Hendrickx, A. G., & Newman, L. M. (1978). Reproduction of the greater bushbaby (*Galago crassicaudatus panganiensis*) under laboratory conditions. *J. Med. Primatol., 7*, 26–43.

Heuser, C. H. (1940). Chimpanzee ovum in early stages of implantation (about 10½ days). *J. Morphol., 66*, 155–173.

Heuser, C. H., & Streeter, G. L. (1941). Development of the macaque embryo. *Contrib. Embryol. Carnegie Inst., 29*, 15–56.

Hill, K., & Hurtado, A. M. (1991). The evolution of premature reproductive senescence and menopause in human females: an evaluation of the "grandmother hypothesis." *Hum. Nat., 2*, 313–350.

Hill, W. C. O. (1969). Obstetric mishaps in marmosets. *Acta Zool. Pathol. Antverp., 48*, 149–154.

Hinde, K., & Capitanio, J. P. (2010). Lactational programming? Mother's milk energy predicts infant behavior and temperament in rhesus macaques (*Macaca mulatta*). *Am. J. Primatol., 72*, 522–529.

Hinton, B. T. (1980). The epididymal microenvironment. A site of attack for a male contraceptive? *Invest. Urol., 18*, 1–10.

Hinton, B. T., & Howards, S. S. (1982). Micropuncture and microperfusion techniques for the study of testicular physiology. *Ann. NY Acad. Sci., 383*, 29–43.

Hobbs, K. R., Welschman, M. D., Nazareno, J. B., & Resuello, R. G. (1987). Conditioning and breeding facilities for the cynomolgus monkey (*Macaca fascicularis*) in the Philippines: a progress report on the SICONBREC project. *Lab. Anim., 21*, 131–137.

Hobson, B. M., Hearn, J. P., Lunn, S. F., & Flockhart, J. H. (1977). Urinary excretion of biologically active chorionic gonadotropin by the pregnant marmoset (*Callithrix jacchus jacchus*). *Folia Primatol., 28*, 251–258.

Hodgen, G. D., & Niemann, W. H. (1975). Application of the subhuman primate pregnancy test kit to pregnancy diagnosis in baboons. *Lab. Anim. Sci., 25*, 757–759.

Hodgen, G. D., & Ross, G. T. (1974). Pregnancy diagnosis by a hemagglutination inhibition test for urinary macaque chorionic gonadotropin (mCG). *J. Clin. Endocrinol. Metab., 38*, 927–930.

Hodgen, G. D., Niemann, W. H., Turner, C. K., & Chen, H.-C. (1976). Diagnosis of pregnancy in chimpanzees using the nonhuman primate pregnancy test kit. *J. Med. Primatol., 5*, 247–252.

Hodgen, G. D., Goodman, A. L., O'Connor, A., & Johnson, D. K. (1977). Menopause in rhesus monkeys: model for study of disorders in the human climanteric. *Am. J. Obstet. Gynecol., 127*, 581–584.

Hodgen, G. D., Stolzenberg, S. J., Jones, D. C. L., Hildebrand, D. F., & Turner, C. K. (1978). Pregnancy diagnosis in squirrel monkeys: hemagglutination test, radioimmunoassay and bioassay of chorionic gonadotropin. *J. Med. Primatol., 7*, 59–64.

Hodges, J. K., & Eastman, S. A. K. (1984). Monitoring ovarian function in marmosets and tamarins by the measurement of urinary estrogen metabolites. *Am. J. Primatol., 6*, 187–197.

Hodges, J. K., Henderson, C., & Hearn, J. P. (1983). Relationship between ovarian and placental steroid production during early pregnancy in the marmoset monkey (*Callithrix jacchus*). *J. Reprod. Fertil., 69*, 613–621.

Hodges, J. K., Green, D. I., Cottingham, P. G., Saver, M. J., Edwards, C., & Lightman, S. L. (1988). Induction of luteal regression in the marmoset monkey (*Callithrix jacchus*) by a gonadotrophin-releasing hormone antagonist and the effects on subsequent follicular development. *J. Reprod. Fertil., 82*, 743–752.

Holmes, G. P., Chapman, L. E., Stewart, J. A., Strauss, S. E., Hilliard, J. K., & Davenport, D. S. (1995). Guidelines for the

prevention and treatment of B-virus infections in exposed persons. The B virus Working Group. *Clin. Infect. Dis., 20*, 421−439.

Homma, T., Kanki, P. I., King, N. W., et al. (1984). Lymphoma in macaques: association with virus of human T lymphotrophic family. *Science, 225*, 716−718.

Honjo, S., Cho, F., & Terao, K. (1984). Establishing the cynomolgus monkey as a laboratory animal. *Adv. Vet. Sci. Comp. Med., 28*, 51−80.

Honore, E. K., & Tardif, S. D. (2009). Reproductive biology of baboons. In J. L. VandeBerg, S. Williams-Blangero & S. D. Tardif (Eds.), *The Baboon in Biomedical Research* (pp. 89−110). New York: Springer.

Hotchkiss, J., Atkinson, L. E., & Knobil, E. (1971). Time course of serum estrogen and luteinizing hormone (LH) concentrations during the menstrual cycle of the rhesus monkey. *Endocrinology, 89*, 177−182.

Hsu, C. K., & Jia, R. (2003). Chinese Macaques − East Meets West. In *International Perspectives: The Future of Nonhuman Primate Resources, Proceedings of the Workshop Held April 17−19, 2002* (pp. 197−199). National Research Council.

Ishikawa, K., Fukasaw, M., Tsujimoto, H., et al. (1987). High simian T-cell leukemia virus type 1 proviral loads combined with genetic stability as a result of cell-associated provirus replication in naturally infected, asymptomatic monkeys. *Int. J. Cancer, 40*, 233−239.

Izard, M. K., & Rasmussen, D. T. (1985). Reproduction in the slender loris (*Loris tardigradus malabaricus*). *Am. J. Primatol., 8*, 153−165.

Jack, K. M. (2007). The Cebines: toward an explanation of variable social structure. In C. J. Campbell, A. F. Fuentes, K. C. Mackinnon, M. Panger & S. Beader (Eds.), *Primates in Perspective* (pp. 107−123). New York: Oxford University Press.

Jantschke, B., Welker, C., & Klaiber-Schuh, C. (1998). Rearing without paternal help in the bolivian owl monkey *Aotus azarae boliviensis*: a case study. *Folia Primatol., 69*, 115−120.

Jaquish, C. E., Toal, R. L., Tardif, S. D., & Carson, R. L. (1995). Use of ultrasound to monitor prenatal growth and development in the common marmoset (*Callithrix jacchus*). *Am. J. Primatol., 36*, 259−275.

Jaquish, C. E., Tardif, S. D., Toal, R. L., & Carson, R. L. (1996). Patterns of prenatal survival in the common marmoset (*Callithrix jacchus*). *J. Med. Primatol., 25*, 57−63.

Johnson, M. H., & Everitt, B. J. (2000). *Essential Reproduction*. Oxford: Blackwell Science.

Jones, R. (1978). Comparative biochemistry of mammalian epididymal plasma. *Comp. Biochem. Physiol. B, 61B*, 365−370.

Kanthaswamy, S., & Smith, D. G. (1998). Use of microsatellite polymorphisms for paternity exclusion in rhesus macaque (*Macaca mulatta*). *Primates 39*, 135−39, 145.

Kanthaswamy, S., & Smith, D. G. (2002). Assessment of genetic management at three specific-pathogen-free rhesus macaque (*Macaca mulatta*) colonies. *Comp. Med., 52*, 414−423.

Kanthaswamy, S., Satkoski, J., George, D., Kou, A., Erickson, B. J.-A., & Smith, D. G. (2008). Hybridization and stratification of nuclear genetic variation in *Macaca mulatta* and *M. fascicularis*. *Int. J. Primatol., 29*, 1295−1311.

Kanthaswamy, S., Capitanio, J. P., Dubay, C. J., Ferguson, B., Folks, T., Ha, J. C., et al. (2009). Resources for genetic management and genomics research on nonhuman primates at the National Primate Research Centers (NPRCs). *J. Med. Primatol., 38*(Suppl. 1), 17−23.

Kanthaswamy, S., Kou, A., & Smith, D. G. (2010). Population genetic statistics from rhesus macaques (*Macaca mulatta*) in three different housing configurations at the California National Primate Research Center. *J. Am. Assoc. Lab. Anim. Sci., 49*, 598−609.

Kaplan, J. N. (1977). Breeding and rearing squirrel monkeys (*Saimiri sciureus*) in captivity. *Lab. Anim. Sci., 27*, 557−567.

Kaplan, C. G. (1979). Intrauterine infections in nonhuman primates. *J. Med. Primatol., 8*, 233−243.

Kaplowitz, P. B. (2008). Link between body fat and the timing of puberty. *Pediatrics, 121*, S208−S217.

Kawamoto, Y., Kawamoto, S., Matsubayashi, K., Nozawa, K., Watanabe, T., Stanley, M. A., et al. (2007). Genetic diversity of longtailed macaques (*Macaca fascicularis*) on the island of Mauritius: an assessment of nuclear and mitochondrial DNA polymorphism. *J. Med. Primatol., 37*, 45−54.

Khan, I. H., Mendoza, S., Yee, J., Deane, M., Ventkateswarren, K., Zhou, S. S., et al. (2006). Simultaneous detection of antibodies to six nonhuman primate viruses by multiplex microbead immunoassay. *Clin. Vaccine Immunol., 13*, 45−52.

Kholkute, S. D., & Nandekar, T. D. (1983). Follicular growth and induction of ovulation by gonadotrophins in the common marmoset (*Callithrix jacchus*). *Ind. J. Exp. Biol., 21*, 536−538.

Kleiman, D. G., Gracey, D. W., & Hodgen, G. D. (1978). Urinary chorionic gonadotropin levels in pregnant golden lion tamarins. *J. Med. Primatol., 7*, 333−338.

Kluin, P. M., Kramer, M. F., & de Rooij, D. G. (1983). Testicular development in *Macaca irus* after birth. *Int. J. Androl., 6*, 25−43.

Knapp, K. K., Hunter, R. L., Hendrickson, R. V., & Hendrickx, A. G. (1974). Plasma proteins of the pregnant and normal rhesus monkey (*Macaca mulatta*). *J. Med. Primatol., 3*, 351−358.

Koford, C. B. (1965). Population dynamics of rhesus monkeys on Cayo Santiago. In I. Devore (Ed.), *Primate Behavior* (pp. 160−174). New York: Holt, Rinehart and Winston.

Kohrs, M. B., Harper, A. E., & Kerr, G. R. (1976). Effects of a low-protein diet during pregnancy of the rhesus monkey. I. Reproductive efficiency. *Am. J. Clin. Nutr., 29*, 136−145.

Krebs, K. C., Jin, Z., Rudersdorf, R., Hughes, A. L., & O'Connor, D. H. (2005). Unusually high frequency MHC Class I alleles in Mauritian origin macaques. *J. Immunol., 175*, 5230−5239.

Kuderling, I., Schneiders, A., Sonksen, J., Nayudu, P. L., & Hodges, J. K. (2000). Non-invasive collection of ejaculates from the common marmoset (*Callithrix jacchus*) using penile vibrostimulation. *Am. J. Primatol., 52*, 149−154.

Kuehl, T. J., & Dukelow, W. R. (1975). Ovulation induction during the anovulatory season in *Saimiri sciureus*. *J. Med. Primatol., 4*, 23−31.

Kuller, L., Watanabe, R., Anderson, D., & Grant, R. (2005). Development of a whole-virus multiplex flow cytometric assay for antibody screening of a specific pathogen-free primate colony. *Diag. Microbiol. Infect. Dis., 53*, 185−193.

Kyes, R. C. (1993). Survey of the long-tailed macaques introduced onto Tinjil Island, Indonesia. *Am. J. Primatol., 31*, 77−83.

Lackner, A. A., Rodriguez, M. H., Bush, C. E., Munn, R. J., Kwang, D., et al. (1988). Distribution of a macaque immunosuppressive type D retrovirus in neural, lymphoid, and salivary tissues. *J. Virol., 62*, 2134−2142.

Lasley, B., Hendrickx, A. G., & Stabenfeldt, G. H. (1974). Estradiol levels near the time of ovulation in the bonnet monkey (*Macaca radiata*). *Biol. Reprod., 11*, 237−244.

Lavy, G., Restrepo-Candelo, H., Diamond, M., Shapiro, B., Grunfeld, L., & DeCherney, A. H. (1988). Laparoscopic and

transvaginal ova recovery: the effect on ova quality. *Fertil. Steril., 49*, 1002–1006.

Layne, D. G., & Power, R. A. (2003). Husbandry, handling, and nutrition for marmosets. *Comp. Med., 53*, 351–359.

Lazo, A., & Bailer, R. T. (1996). Constitutive cytokine release by simian T-cell lymphotropic virus type I (STLV-1) and human T-lymphotropic virus Types I/II(HTLV-I / II) transformed cell lines. *J. Med. Primatol., 25*, 257–266.

Lee, K. Y., & DeMayo, F. J. (2004). Animal models of implantation. *Reproduction, 128*, 679–695.

Lerche, N. W. (1992). Epidemiology and control of type D retrovirus infection in captive macaques. In H. Ishida & M. Goodman (Eds.), *Topics in Primatology*, Vol. 3 (pp. 439–447). University of Toyko Press.

Lerche, N. W., Yee, J. L., & Jennings, M. B. (1994). Establishing specific retrovirus-free breeding colonies of macaques: an approach to primary screening and surveillance. *Lab. Anim. Sci., 44*, 217–221.

Leuchte, N., Berry, N., Köhler, B., Almond, N., LeGrand, R., Thorstensson, R., et al. (2004). MhcDRB-sequences from cynomolgus macaques (*Macaca fascicularis*) of different origin. *Tissue Antigens, 63*, 529–537.

Lindburg, D. G. (1987). Seasonality of reproduction in primates. In G. Mitchell & J. Erwin (Eds.), *Comparative Primate Biology*, Vol. 2B (pp. 167–218). New York: Alan R. Liss.

Lohstroh, P., Laughlin, L., Gee, N., & Lasley, B. (2007). Development, validation and application of a chemiluminescent immunoassay for the measurement of circulating chorionic gonadotropin levels in the laboratory macaque. *J. Med. Primatol., 36*, 164–169.

Luckett, W. P. (1974). Comparative development and evolution of the placenta in primates. In W. P. Luckett (Ed.), *Contributions to Primatology: The Reproductive Biology of the Primates* (pp. 142–234). Basel: Karger.

Luckhaus, J., & Ferrin, M. (1989). Effects of intraventricular oxytocin infusion on LH and cortisol secretion in ovariectomized rhesus monkeys. *Acta Endocrinol., 120*(Suppl.), 59.

Luetjens, C. M., Weinbauer, G. F., & Wistuba, J. (2005). Primate spermatogenesis: new insights into comparative testicular organisation, spermatogenic efficiency and endocrine control. *Biol. Rev., 80*, 475–488.

Lunn, S. F. (1983). Body weight changes throughout pregnancy in the common marmoset *Callithrix jacchus*. *Folia Primatol., 41*, 204–217.

Lutz, C. K., & Novak, M. A. (2005). Primate natural history and social behavior: implications for laboratory housing. In S. Wolfe-Coote (Ed.), *The Laboratory Primate* (pp. 134–135). London: Elsevier Academic Press.

Lyashchenko, K. P., Greenwald, R., Esfandiardi, J., et al. (2007). Prima-TB STAT-PAK Assay, a novel, rapid lateral-flow test for the tuberculosis in nonhuman primates. *Clin. Vaccine Immunol., 14*, 1158–1164.

McLachlan, R. I., O'Donnell, L., Meachem, S. J., Stanton, P. G., De Kretser, D. M., et al. (2002). Identification of specific sites of hormonal regulation in spermatogenesis in rats, monkeys, and man. *Rec. Prog. Horm. Res., 57*, 149–179.

Madhaw Raj, H. G., Murty, G. S. R. C., Sairam, M. R., & Talbert, L. M. (1982). Control of spermatogenesis in primates: effects of active immunization against FSH in the monkey. *Int. J. Androl. Suppl., 5*, 27–33.

Mahoney, C. J. (1970). A study of the menstrual cycle in *Macaca irus* with special reference to the detection of ovulation. *J. Reprod. Fertil., 21*, 153–163.

Mahoney, C. J. (1972). The breeding of primates. In *The UFAW Handbook on the Care and Management of Laboratory Animals* (4th ed.). (pp. 398–414). Edinburgh: Churchill-Livingstone.

Mahoney, C. J. (1975). The accuracy of bimanual rectal palpation for determining the time of ovulation and conception in the rhesus monkey (*Macaca mulatta*). *Lab. Anim. Hand., 6*, 127–138.

Mann, D. R., Gould, K. G., Collins, D. C., & Wallen, K. (1989). Blockade of neonatal activation of the pituitary-testicular axis: effect on peripubertal luteinizing hormone and testosterone secretion and on testicular development in male monkeys. *J. Clin. Endocrinol. Metab., 68*, 600–607.

Mansfield, K. G. (2005). Development of specific pathogen free nonhuman primate colonies. In S. Wolfe-Coote (Ed.), *The Laboratory Primate* (pp. 229–230). New York: Elsevier.

Marracci, G. H., Kelley, R. D., Pilcher, K. Y., et al. (1995). Simian AIDS type D serogroup 2 retrovirus: isolation of an infectious molecular clone and sequence analyses of its envelope glycoprotein gene and 3′ long terminal repeat. *J. Virol., 69*, 2621–2628.

Marshall, G. R., Ramaswamy, S., & Plant, T. M. (2005). Gonadotropin-independent proliferation of the pale type A spermatogonia in the adult rhesus monkey (*Macaca mulatta*). *Biol. Reprod., 73*, 222–229.

Marx, P. A., Maul, D. H., Osborn, K. G., Lerche, N. W., Moody, P., Lowensteine, L. J., et al. (1984). Simian AIDS: isolation of a type D retrovirus and transmission of the disease. *Science, 223*, 1083–1086.

Mas-Rivera, A., & Bercovitch, F. B. (2008). Postpartum recuperation in primiparous rhesus macaques and development of their infants. *Am. J. Primatol., 70*, 1047–1054.

Matano, Y., Matsubayashi, K., & Omichi, A. (1975). Scanning electron microscopy of primate spermatozoa. In S. Kondo, M. Kawai & A. Ehara (Eds.), *Contemporary Primatology* (pp. 121–124). Basel: Karger.

McNeilly, A. S. (1994). Suckling and the control of gonadotropin secretion. In E. Knobil & J. D. Neill (Eds.), *The Physiology of Reproduction* (2nd ed). (pp. 1179–1212). New York: Raven Press.

McNulty, W. P., Novy, M. J., & Walsh, S. W. (1981). Fetal and postnatal development of the adrenal glands in *Macaca mulatta*. *Biol. Reprod., 25*, 1079–1089.

Meertens, L., Rigoulet, J., Mauclere, P., VanBeveren, M., Chen, G. M., Diop, O., et al. (2001). Molecular and phylogenetic analysis of 16 novel simian T cell leukemia virus Type 1 from Africa: close relationship of STLV-1 from *Allenopithecus nigrovirdis* to HTLV-1 subtype B strains. *Virology, 287*, 275–285.

Messinis, I. E. (2006). Ovarian feedback, mechanism of action and possible clinical implications. *Hum. Reprod. Update, 12*, 557–571.

Milligan, L. A., Gibson, S. V., Williams, L. E., & Power, M. L. (2008). The composition of milk from Bolivian squirrel monkeys (*Saimiri boliviensis boliviensis*). *Am. J. Primatol., 70*, 35–43.

Mintz, D. H., Chez, R. A., & Hutchinson, D. L. (1972). Subhuman primate pregnancy complicated by streptozotocin-induced diabetes mellitus. *J. Clin. Invest., 51*, 837–847.

Miyoshi, I., Fujishita, M., Taguchi, H., et al. (1983). Natural infection in nonhuman primates with adult T-cell leukemia virus or a closely related agent. *Int. J. Cancer, 32*, 333–336.

Mizuno, M., & Terasawa, E. (2005). Search for neural substrates mediating inhibitory effects of oestrogen on pulsatile luteinising hormone-releasing hormone release in vivo in ovariectomized female rhesus monkeys (*Macaca mulatta*). *J. Neuroendocrinol., 17*, 238–245.

Monfort, S. L., Jayaraman, S., Shideler, S. E., Lasley, B. L., & Hendrickx, A. G. (1986). Monitoring ovulation and implantation in the cynomolgus macaque (*Macaca fascicularis*) through evaluations of urinary estrone conjugates and progesterone metabolites: a technique for the routine evaluation of reproductive parameters. *J. Med. Primatol., 15*, 17−26.

Monfort, S. L., Hess, D. L., Shideler, S. E., Samuels, S. J., Hendrickx, A. G., & Lasley, B. L. (1987). Comparison of serum estradiol to urinary estrone conjugates in the rhesus macaque (*Macaca mulatta*). *Biol. Reprod., 37*, 832−837.

Montoya, E. (2003). Center for the Breeding and Conservation of Primates of the Peruvian Primatology Project. In: *International Perspectives: The Future of Nonhuman Primate Resources, Proceedings of the Workshop Held April 17−19, 2002* (pp. 81−90). National Research Council.

Moynihan, M. (1964). *Some Behavior Patterns of Platyrrhine Monkeys. I. The night monkey (*Aotus trivirgatus*)*. Washington: Smithsonian Institution.

Moynihan, M. (1976). *The New World Primates: adaptive radiation and the evolution of social behavior, languages, and intelligence*. Princeton, NJ: Princeton University Press.

Moore, H. D. M. (1981). An assessment of the fertilizing ability of spermatozoa in the epididymis of the marmoset monkey (*Callithrix jacchus*). *Int. J. Androl., 4*, 321−330.

Moore, H. D. M., Hartman, T. D., & Holt, W. V. (1984). The structure and epididymal maturation of the spermatozoon of the common marmoset (*Callithrix jacchus*). *J. Anat., 138*, 227−235.

Moorman, E. A., Mendoza, S. P., Shideler, S. E., & Lasley, B. L. (2002). Excretion and measurement of estradiol and progesterone metabolites in the feces and urine of female squirrel monkeys (*Saimiri sciureus*). *Am. J. Primatol., 57*, 79−90.

Morgan, P. M., Hutz, R. J., Kraus, E. M., Cormie, J. A., Dierschke, D. J., & Bavister, B. D. (1987). Evaluation of ultrasonography for monitoring follicular growth in rhesus monkeys. *Theriogenology, 27*, 769−780.

Morishima, H. O., Pederson, H., & Finster, M. (1978). The influence of maternal psychological stress on the fetus. *Am. J. Obstet. Gynecol., 131*, 286−290.

Morton, W. R., Agy, M. B., Capurenio, S. V., & Grant, R. F. (2008). Specific pathogen-free macaques: definition, history, and current production. *ILAR J., 49*, 137−144.

Mossman, H. W. (1987). *Vertebrate Fetal Membranes*. New Brunswick: Rutgers University Press.

Moudgal, N. R. (1981). A need for FSH in maintaining fertility in adult male subhuman primates. *Arch. Androl., 7*, 117−125.

Mullan R.J. (2009). Nonhuman Primate Importation and Quarantine: United States Fiscal Year 2009. Presented at Association of Primate Veterinarians 37th Annual Workshop, November 4−7, 2009, Broomfield, Co. NCID, CDC Quarantine and Migration Health.

Müller, T., Gromoll, J., Simula, A. P., Norman, R., Sandhowe-Klaverkamp, R., & Simoni, M. (2004a). The carboxyterminal peptide of chorionic gonadotropin facilitates activation of the marmoset LH receptor. *Exp. Clin. Endocrinol. Diabetes, 112*, 574−579.

Müller, T., Simoni, M., Pekel, E., Luetjens, C. M., Chandolia, R., Amato, F., et al. (2004b). Chorionic gonadotrophin beta subunit mRNA but not luteinising hormone beta subunit mRNA is expressed in the pituitary of the common marmoset (*Callithrix jacchus*). *J. Mol. Endocrinol., 32*, 115−128.

Munro, C. J., Stebenfeldt, G. H., Cragun, J. R., Addiego, L. A., Overstreet, J. W., & Lasley, B. L. (1991). Relationship of serum estradiol and progesterone concentrations to the excretion profiles of their major urinary metabolites as measured by enzyme-immunoassay and radioimmunoassay. *Clin. Chem., 37*, 838−844.

Myers, R. E. (1972). The pathology of the rhesus monkey placenta. *Acta Endocrinol., 71*(Suppl.), 221−257.

Nadler, R. D., & Rosenblum, L. A. (1972). Hormonal regulation of the 'fatted' phenomenon in squirrel monkeys. *Anat. Rec., 173*, 181−187.

Nagle, C. A., & Denari, J. H. (1983). The cebus monkey (*Cebus apella*). In J. P. Hearn, (Ed.), *Reproduction in New World Primates* (pp. 39−67). Hingham, MA: MTP Press.

Nagle, C. A., Riarte, A., Quiroga, S., Azorero, R. M., Carril, M., Denari, J. H., et al. (1980). Temporal relationship between follicular development, ovulation and ovarian hormonal profile in the capuchin monkey (*Cebus apella*). *Biol. Reprod., 23*, 629−635.

Nakano, R. (1997). Control of the luteal function in humans. *Semin. Reprod. Endocrinol., 15*, 335−344.

Narita, H., Hamano, M., & Cho, F. (1988). Pregnancy diagnosis by the ultrasonographical device and observation of fetal growth in the squirrel monkey (*Saimiri sciureus*). *Exp. Anim., 37*, 393−397.

Neville, M. C. (2001). Anatomy and physiology of lactation. *Pediatr. Clin. North Am., 48*, 13−34.

Newell-Morris, L. L., Fahrenbruch, C. E., & Sackett, G. P. (1989). Prenatal psychological stress, dermatoglyphic asymmetry and. pregnancy outcome in the pigtailed macaque (*Macaca nemestrina*). *Biol. Neonate., 56*, 61−75.

Oates-O'Brien, R. S., Farver, T. B., Anderson-Vicino, K. C., McCowan, B., & Lerche, N. W. (2010). Predictors of matrilineal overthrows in large captive breeding groups of rhesus macaques (*Macaca mulatta*). *J. Am. Assoc. Lab. Anim. Sci., 49*, 196−201.

O'Byrne, K. T., Lunn, S. F., & Coen, C. W. (1990). Central oxytocin stimulates luteinizing hormone release in the marmoset, a primate which fails to show lactationally-induced infertility. *J. Neuroendocrinol., 2*, 419−421.

Oerke, A. K., Eispanier, A., & Hodges, J. K. (1996). Noninvasive monitoring of follicle development, ovulation and corpus luteum formation in the marmoset monkey (*Callithrix jacchus*) by ultrasonography. *Am. J. Primatol., 39*, 99−113.

Oftedal, O. T. (1984). Milk composition, milk yield, and energy output at peak lactation: a comparative review. *Symp. Zool. Soc. Lond., 51*, 33−85.

Ogren, L., & Talamantes, F. (1994). The placenta as an endocrine organ: polypeptides. In E. Knobil & J. D. Neill (Eds.), *The Physiology of Reproduction* (2nd ed). (pp. 875−945). New York: Raven Press.

O'Neill, A. C., Fedigan, L. M., & Ziegler, T. E. (2004). Ovarian cycle phase and same-sex mating behavior in Japanese macaque females. *Am. J. Primatol., 63*, 25−31.

Pamungkas, J., & Sajuthi, D. (2003). Sustainable primate resources through SPF breeding programs in Indonesia. In: *International Perspectives: The Future of Nonhuman Primate Resources, Proceedings of the Workshop Held April 17−19, 2002* (p. 20). National Research Council.

Parkin, R. F., & Hendrickx, A. G. (1975). The temporal relationship between the preovulatory estrogen peak and the optimal mating period in rhesus and bonnet monkeys. *Biol. Reprod., 13*, 610−616.

Pauerstein, C. J., Eddy, C. A., Croxatto, H. D., Hess, R., Siler-Khodr, T. M., & Croxatto, H. B. (1978). Temporal relationships of

estrogen, progesterone, and luteinizing hormone levels to ovulation in women and infrahuman primates. *Am. J. Obstet. Gynecol., 130*, 876–886.

Peccei, J. S. (2001). Menopause: adaptation or epiphenomenon? *Evol. Anthropol., 10*, 43–57.

Petraglia, F., Florio, P., Nappi, C., & Genazzani, R. A. (1996). Peptide signaling in human placenta and membranes: autocrine, paracrine, and endocrine mechanisms. *Endocr. Rev., 17*, 156–186.

Phillips, I. R., & Grist, S. M. (1975). The use of transabdominal palpation to determine the course of pregnancy in the marmoset (*Callithrix jacchus*). *J. Reprod. Fertil., 43*, 103–108.

Plant, T. M. (1980). The neuroendocrine basis of the diurnal variation of testicular testosterone secretion in the adult rhesus monkey (*Macaca mulatta*). In A. Steinberger & E. Steinberger (Eds.), *Testicular Development, Structure and Function* (pp. 419–423). New York: Academic Press.

Plant, T. M., & Witchel, S. F. (2006). Puberty in nonhuman primates and humans. In J. D. Neill (Ed.) (3rd ed). *Knobil and Neill's Physiology of Reproduction*, Vol. 2 (pp. 2177–2230). St. Louis, MO: Elsevier.

Plant, T. M., Ramaswamy, S., & Dipietro, M. J. (2009). Repetitive activation of hypothalamic G protein-coupled receptor 54 with intravenous pulses of kisspeptin in the juvenile monkey (*Macaca mulatta*) elicits a sustained train of gonadotropin-releasing hormone discharges. *Endocrinology, 147*, 1007–1013.

Poole, T. B., & Evans, R. G. (1982). Reproduction, infant survival and productivity of a colony of common marmosets (*Callithrix jacchus*). *Lab. Anim., 16*, 88–97.

Profet, M. (1993). Menstruation as a defense against pathogens transported by sperm. *Q. Rev. Biol., 68*, 335–386.

Pusey, A. E., & Packer, C. (1986). Dispersal and philoparty. In B. Smuts, D. L. Cheney, R. M. Seyfarth, R. W. Wrangham & T. T. Struhsaker (Eds.), *Primate Societies* (pp. 250–266). Chicago: The University of Chicago Press.

Randolph, J. F., Jr. (2008). The endocrinology of the reproductive years. *J. Sex. Med., 5*, 2274–2281.

Rawlins, R. G., & Kessler, M. J. (1986). *The Cayo Santiago Rhesus Macaque: History, Behavior, and Biology.* Albany: State University of New York Press.

Renne, R. A., McLaughlin, R., & Jenson, A. B. (1973). Measles virus-associated endometritis, cervicitis, and abortion in a rhesus monkey. *J. Am. Vet. Med. Assoc., 163*, 639–641.

Rensing, S., & Oerke, A. K. (2005). Husbandry and management of New World species: marmosets and tamarins. In S. Wolfe-Coote (Ed.), *The Laboratory Primate* (pp. 145–162). New York, NY: Elsevier.

Resko, J. A., Goy, R. W., Robinson, J. A., & Norman, R. L. (1982). The pubescent rhesus monkey: some characteristics of the menstrual cycle. *Biol. Reprod., 27*, 354–361.

Riar, S. S., Setty, B. S., & Kar, A. B. (1973a). Studies on the physiology and biochemistry of mammalian epididymis: biochemical compositon of epididymis. a comparative study. *Fertil. Steril., 24*, 355–363.

Riar, S. S., Setty, B. S., & Kar, A. B. (1973b). Studies on the physiology and biochemistry of mammalian epididymis: histology, enzyme and electrolyte composition of epididymis. A comparative study. *Indian J. Exp. Biol., 11*, 365–372.

Riopelle, A. J. (1985). Prenatal protein deprivation. In E. S. Watts (Ed.), *Nonhuman Primate Models for Human Growth and Development* (pp. 171–185). New York: Alan R. Liss.

Roa, J., Aguilar, E., Dieguez, C., Pinilla, L., & Tena-Sempere, M. (2008). New frontiers in kisspeptin/GPR54 physiology as fundamental gatekeepers of reproductive function. *Front. Neuroendocrinol., 29*, 48–69.

Roberts, J. A., & Wolf, R. H. (1971). Hydronephrosis of pregnancy: a naturally occurring disorder in nonhuman primates closely resembling that in man. *Folia Primatol., 15*, 143–147.

Rommeck, I., Gottlieb, D. H., Strand, S. C., & McCowan, B. (2009). The effects of four nursery rearing strategies on infant behavioral development in rhesus macaques (*Macaca mulatta*). *J. Am. Assoc. Lab. Anim. Sci., 48*, 395–401.

Rosenblum, L. A., & Cooper, R. W. E. (1968). *The Squirrel Monkey.* New York: Academic Press.

Rothe, H., & Koenig, A. (1991). Variability of social organization in captive common marmosets (*Callithrix jacchus*). *Folia Primatol., 57*, 28–33.

Ruch, T. C. (1959). *Disease of Laboratory Primates*. Philadelphia: W.B. Saunders.

Rudel, L. L., McMahan, M. R., & Shah, R. N. (1981). Pregnancy effects on nonhuman primate high density lipoproteins. *J. Med. Primatol., 10*, 16–25.

Ruppenthal, G. C., & Goodlin, B. L. (1982). Monitoring temperature of pigtailed macaques (*Macaca nemestrina*) during pregnancy and parturition. *Am. J. Obstet. Gynecol., 143*, 971–973.

Sackett, G. P. (1981). Pregnancy outcome following jet transport stress in nonhuman primates. *J. Med. Primatol., 10*, 149–154.

Sackett, G. P., Ruppenthal, G. C., & Davis, A. E. (2002). Survival, growth, health and reproduction following nursery rearing compared with mother rearing in pigtailed monkeys (*Macaca nemistrina*). *Am. J. Primatol., 56*, 165–183.

Sackett, G. P., Ruppenthal, G., & Elias, K. (2005). *Nursery Rearing of Nonhuman Primates in the 21st Century (Developments in Primatology: progress and prospects)*. Berlin: Springer.

Saltzman, W., Schultz-Darken, N. J., & Abbott, D. H. (1997a). Familial influences on ovulatory function in common marmosets (*Callithrix jacchus*). *Am. J. Primatol., 41*, 159–177.

Saltzman, W., Severin, J. M., Schultz-Darken, N. J., & Abbott, D. H. (1997b). Behavioral and social correlates of escape from suppression of ovulation in female common marmosets housed with the natal family. *Am. J. Primatol., 41*, 1–21.

Saltzman, W., Pick, R. R., Salper, O. J., Liedl, K. J., & Abbott, D. H. (2004). Onset of plural cooperative breeding in common marmoset families following replacement of the breeding male. *Anim. Behav., 68*, 59–73.

Saltzman, W., Liedl, K. J., Salper, O. J., Pick, R. R., & Abbott, D. H. (2008). Post-conception reproductive competition in cooperatively breeding common marmosets. *Horm. Behav., 53*, 274–286.

Saltzman, W., Tardif, S. D., & Rutherford, J. N. (2011). Hormones and reproductive cycles in primates. In D. O. Norris & K. H. Lopez (Eds.), *Hormones and Reproduction of Vertebrates*, Vol. 5 (pp. 291–328). London: Elsevier.

Satkoski, J., George, D., Smith, D. G., & Kanthaswamy, S. (2008). Genetic characterization of wild and captive rhesus macaques in China. *J. Med. Primatol., 37*, 67–80.

Scammell, J. G., Funkhouser, J. D., Moyer, F. S., Gibson, S. V., & Willis, D. L. (2008). Molecular cloning of pituitary glycoprotein a-subunit and folicle stimulating hormone and chorionic gonadotropin b-subnits from New World squirrel monkey and owl monkey. *Gen. Comp. Endocrinol., 155*, 534–541.

Schillaci, M. A., Jones-Engel, L., Engel, G. A., Paramastri, Y., Iskandar, E., Wilson, B., Allan, J. S., et al. (2005). Prevalence of enzootic simian viruses among urban performance monkeys in Indonesia. *Trop. Med. Int. Health., 10*, 1305−1314.

Schiller, H. S., Sackett, G. P., Frederickson, T., & Risler, L. J. (1983). Maintenance of high-density lipoprotein blood levels prior to spontaneous abortion in pig-tailed macaques (*Macaca nemestrina*). *Am. J. Primatol., 4*, 127−133.

Schino, G., & Troisi, A. (2005). Neonatal abandonment in Japanese macaques. *Am. J. Phys. Anthropol, 126*, 447−452.

Schramm, R. D., Paprocki, A. M., & Bavister, B. D. (2002). Features associated with reproductive ageing in female rhesus monkeys. *Hum. Reprod., 17*, 1597−1603.

Schuler, A. M., Westberry, J. M., Parks, V. J., Kuehl, C. J., & Abee, C. R. (2007). Ultrasound-guided follicular aspiration in squirrel monkeys. *J. Med. Primatol., 36*, 113−117.

Shaha, C. (2008). Estrogens and spermatogenesis. *Adv. Exp. Med. Biol., 636*, 42−64.

Shaikh, A. A., Allen-Rowlands, C., Dozier, T., & Kraemer, D. C. (1976). Diagnosis of early pregnancy in the baboon. *Conception, 14*, 391−402.

Shaikh, A. A., Celaya, C. L., Gomez, I., & Shaikh, S. A. (1982). Temporal relationship of hormonal peaks to ovulation and sex skin deturgescence in the baboon. *Primates, 23*, 444−452.

Sharpe, R. M. (1994). Regulation of spermatogenesis. In E. Knobil & J. R. Neill (Eds.), *The Physiology of Reproduction* (pp. 1363−1394). New York: Raven Press.

Sharpe, R. M., Fraser, H. M., Brougham, M. F., McKinnell, C., Morris, K. D., Kelnar, C. J., et al. (2003). Role of the neonatal period of pituitary-testicular activity in germ cell proliferation and differentiation in the primate testis. *Hum. Reprod., 18*, 2110−2117.

Shearer, M. H., Stevens, F. J., Westholm, F. A., Jensen, H. B., Chanh, T. C., Carey, K. D., et al. (1995). Serologic cross-reactions among primate immunoglobulins. *Dev. Comp. Immunol., 19*, 547−557.

Shideler, S. E., Gee, N. A., Chen, J., & Lasley, B. L. (2001). Estrogen and progestogen metabolites and follicle-stimulating hormone in the aged macaque female. *Biol. Reprod., 65*, 1718−1725.

Shimizu, K., Lohstroh, P. N., Laughlin, L. S., Gee, N. A., Todd, H., Shideler, S. E., et al. (2001). Noninstrumented enzyme-linked immunosorbant assay for detection of early pregnancy in macaques. *Am. J. Primatol., 54*, 57−62.

Small, M. F. (1982). Reproductive failure in macaques. *Am. J. Primatol., 2*, 137−147.

Smith, D. G., McDonough, J. W., & George, D. A. (2007). Mitochondrial DNA variation within and among regional populations of longtailed macaques (*Macaca fascicularis*) in relation to other species of the *Fascicularis* group of macaques. *Am. J. Primatol., 69*, 182−198.

Smucny, D. A., Abbott, D. H., Mansfield, K. G., Schultz-Darken, N. J., Yamamoto, M. E., & Alencar, A. I. (2004). Reproductive output, maternal age and survivorship in captive common marmoset females (*Callithrix jacchus*). *Am. J. Primatol., 64*, 107−121.

Sofikitis, N., Giotitas, N., Tsouinapi, P., Baltogiannis, D., Giannakis, D., & Pardalidis, N. (2008). Hormonal regulation of spermatogenesis and spermiogenesis. *J. Steroid Biochem. Mol. Biol., 109*, 323−330.

Southwick, C. H., Beg, M. A., & Siddiqi, M. R. (1965). Rhesus Monkeys in North India. In I. DeVore (Ed.), *Primate Behaviour: field studies of monkeys and apes* (pp. 111−159). New York: Academic Press.

Spicer, E. J. F., & Oxnard, C. E. (1967). Some haematological changes during pregnancy in the rhesus monkey (*Macaca mulatta*). *Folia Primatol., 6*, 236−242.

Stanely, M. A. (2003). The breeding of naturally occurring B virus-free cynomolgus monkeys (*Macaca fascicularis*) on the Island of Mauritius. In: *International Perspectives: The Future of Nonhuman Primate Resources, Proceedings of the Workshop Held April 17−19, 2002* (pp. 46−48). National Research Council.

Steiner, R. A., Cameron, J. L., McNeill, T. H., Clifton, D. K., & Bremner, W. J. (1983). Metabolic signals for the onset of puberty. In R. L. Norman (Ed.), *Neuroendocrine Aspects of Reproduction* (pp. 149−182). New York: Academic Press, ORPRC Symp. Primate Reprod. Biol.

Stevens, V. C. (1997). Some reproductive studies in the baboon. *Hum. Reprod. Update., 3*, 533−540.

Stevison, L. S., & Kohn, M. H. (2008). Determining genetic background in captive stocks of cynomolgus macaques (*Macaca fascicularis*). *J. Med. Primatol., 37*, 311−317.

Stevison, L. S., & Kohn, M. H. (2009). Divergence population genetic analysis of hybridization between rhesus and cynomolgus macaques. *Molec. Ecol., 18*, 2457−2475.

Stockinger, D. E., Torrence, A. E., Hukkanen, R. R., Vogel, K. W., Hotchkiss, C. E., & Ha, J. C. (2011). Risk factors for dystocia in pigtailed macaques (*Macaca nemestrina*). *Comp. Med., 61*, 170−175.

Stolzenberg, S. J., Jones, D. C., Kaplan, J. N., Barth, R. A., Hodgen, G. D., & Madan, S. M. (1979). Studies with timed-pregnant squirrel monkeys (*Saimiri sciureus*). *J. Med. Primatol., 8*, 29−38.

Strassman, B. I. (1996). The evolution of endometrial cycles and menstruation. *Q. Rev. Biol., 71*, 181−220.

Sugiyama, Y., Kurita, H., Matsui, T., & Shimomura, T. (2011). Twinning frequency of Japanese macaques (*Macaca fuscata*) at Takasakiyama. *Primates, 52*, 19−23.

Sullivan, E. C., Hinde, K., Mendoza, S. P., & Capitanio, J. P. (2011). Cortisol concentrations in the milk of rhesus monkey mothers are associated with confident temperament in sons, but not daughters. *Dev. Psychobiol., 53*, 96−104.

Summers, P. M., Wennink, C. J., & Hodges, J. K. (1985). Cloprostenol-induced luteolysis in the marmoset monkey (*Callithrix jacchus*). *J. Reprod. Fertil., 73*, 133−138.

Sunderland, N., Heffernan, S., Thomson, S., & Hennessy, A. (2008). Maternal parity affects neonatal survival rate in a colony of captive bred baboons (*Papio hamadryas*). *J. Med. Primatol., 37*, 223−228.

Swindle, M. M., Craft, C. F., Marriott, B. M., Strandberg, J. D., & Luzarragu, M. (1982). Ascending intrauterine infections in rhesus monkeys. *J. Am. Vet. Med. Assoc., 181*, 1367−1370.

Switzer, J. W., Valerio, D. A., Martin, D. P., Valerio, M. G., & Rininger, B. F. (1970). Hematologic changes associated with pregnancy and parturition in *Macaca mulatta. Lab. Anim. Care, 20*, 930−939.

Takahata, Y. (1980). The reproductive biology of a free-ranging troop of Japanese monkeys. *Primates, 21*, 303−329.

Takano, J., Narita, T., Fujimoto, K., Mukai, R., & Yamada, A. (2001). Detection of B virus infection in cynomolgus monkeys by ELISA using simian agent 8 as alternative antigen. *Exp. Anim., 50*, 345−347.

Tarantal, A. F. (1990). Interventional ultrasound in pregnant macaques: embryonic/fetal applications. *J. Med. Primatol., 19*, 47−58.

Tarantal, A. F., & Hendrickx, A. G. (1988a). The use of ultrasound for early pregnancy detection in the rhesus and cynomolgus macaque. *J. Med. Primatol., 17*, 105–112.

Tarantal, A. F., & Hendrickx, A. G. (1988b). Prenatal growth in the cynomolgus and rhesus macaque (*Macaca fascicularis* and *Macaca mulatta*) – a comparison by ultrasonography. *Am. J. Primatol., 15*, 309–323.

Tarantal, A. F., & Hendrickx, A. G. (1988c). Characterization of prenatal growth and development in the crab-eating macaque (*Macaca fascicularis*) by ultrasound. *Anat. Rec., 222*, 177–184.

Tarantal, A. F., & Hendrickx, A. G. (1988d). The use of ultrasonography for evaluating pregnancy in macaques. In D. Neubert, H.-J. Merker & A. G. Hendrickx (Eds.), *Non-human Primates: developmental biology and toxicology* (pp. 91–99). Berlin: Ueberreuter Wissenschaft.

Tarantal, A. F., & Hendrickx, A. G. (1989). Evaluation of the bioeffects of prenatal ultrasound exposure in the cynomolgus macaque (*Macaca fascicularis*): I. Neonatal/infant observations. *Teratology, 39*, 137–147.

Tarantal, A. F., Vandevoort, C. A., & Overstreet, J. W. (1990). Intrauterine insemination with ultrasound guidance in the long-tailed macaque (*Macaca fascicularis*). *J. Med. Primatol., 19*, 447–453.

Tardif, S. D. (1985). Histologic evidence for age-related differences in ovarian function in tamarins (*Saguinus* sp.; primates). *Biol. Reprod., 33*, 993–1000.

Tardif, S. D. (1997). The bioenergetics of parental behavior and the evolution of alloparental care in marmosets and tamarins. In N. Solomon & J. French (Eds.), *Cooperative Breeding in Mammals* (pp. 11–33). Cambridge: Cambridge Univ. Press.

Tardif, S. D., & Jaquish, C. E. (1997). Number of ovulations in the marmoset monkey (*Callithrix jacchus*): relation to body weight, age and repeatability. *Am. J. Primatol., 42*, 323–329.

Tardif, S. D., & Ziegler, T. E. (1992). Features of reproductive senescence in tamarins (*Saguinus* spp.), a New World primate. *J. Reprod. Fertil., 94*, 411–421.

Tardif, S. D., Richter, C. B., & Carson, R. L. (1984). Effects of sibling rearing experience on future reproductive success in two species of Callitrichidae. *Am. J. Primatol., 6*, 377–380.

Tardif, S. D., Lacker, H. M., & Feuer, M. (1993). Follicular development and ovulation in the marmoset monkey as determined by repeated laparoscopic examination. *Biol. Reprod., 48*, 1113–1119.

Tardif, S. D., Hyde, K., & Digby, L. (1994). Evidence for suppression of ovulation in singly-housed female common marmosets (*Callithrix jacchus*). *Lab. Primate Newsl., 33*, 1–4.

Tardif, S. D., Jaquish, C. E., Toal, R. L., Layne, D. G., & Power, R. A. (1998). Estimation of gestational ages in the common marmoset (*Callithrix jacchus*) from published prenatal growth curves. *J. Med. Primatol., 27*, 28–32.

Tardif, S. D., Smucny, D. A., Abbott, D. H., Mansfield, K., Schultz-Darken, N., et al. (2003). Reproduction in captive common marmosets (*Callithrix jacchus*). *Comp. Med., 53*, 364–368.

Teerds, K. J., Rommerts, F. F. G., van der Kant, H. J. G., & de Rooij, D. G. (1989). Leydig cell number and function in the adult cynomolgus monkey (*Macaca fascicularis*) is increased by daily hCG treatment but not by daily FSH treatment. *J. Reprod. Fertil., 87*, 141–146.

Tena-Sempere, M. (2008). Ghrelin as a pleotropic modulator of gonadal function and reproduction. *Nat. Clin. Pract. Endocrinol. Metab., 4*, 666–674.

Terasawa, E. (2001). Luteinizing hormone-releasing hormone (LHRH) neurons: mechanisms of pulsatile LHRH release. *Vitam. Horm., 63*, 91–129.

Terasawa, E. (2005). Role of GABA in the mechanism of the onset of puberty in nonhuman primates. *Int. Rev. Neurobiol., 71*, 113–129.

Tilbrook, A. J., & Clarker, I. J. (2001). Negative feedback regulation of the secretion and actions of gonadotropin-releasing hormone in males. *Biol. Reprod., 64*, 735–742.

Toivola, P. T. K., Bridson, W. E., & Robinson, J. A. (1978). Effects of LH-RH on the secretion of LH, FSH and testosterone in adult male rhesus monkeys. *Endocrinol., 102*, 1815–1821.

Tosi, A. J., & Coke, C. S. (2007). Comparative phylogenetics offer new insights into the biogeographic history of *Macaca fascicularis* and origin of the Mauritian macaques. *Molec. Phylogenet. Evol., 42*, 498–504.

Trask, J. A., Malhi, R. S., Kanthaswamy, S., Johnson, J., Garnica, W. T., Malladi, V. S., et al. (2011). The effect of SNP discovery method and sample size on estimation of population genetic data for Chinese and Indian rhesus macaques (*Macaca mulatta*). *Primates, 52*, 129–138.

Travis, J. C., & Holmes, W. N. (1974). Some physiological and behavioral changes associated with oestrus and pregnancy in the squirrel monkey (*Saimiri sciureus*). *J. Zool., 174*, 41–66.

Tsai, C. C., Follis, K. E., et al. (1987). Vertical transmission of simian retroviruses. *Lab. Invest., 56*(1A).

Van Alphen, M. M. A., van de Kante, H. J. G., & de Rooij, D. G. (1988). Follicle-stimulating hormone stimulates spermatogenesis in the adult monkey. *Endocrinology, 123*, 1449–1455.

Van Andel, R., Sherwood, R., Gennings, C., Lyons, C. R., Hutt, J., Gigliotti, A., et al. (2008). Clinical and pathological features of cynomolgus macaques (*Macaca fascicularis*) infected with aerosolized *Yersinia pestis. Comp. Med., 58*, 68–75.

VandeVoort, C. A., & Tarantal, A. F. (1991). The macaque model for in vitro fertilization: superovulation techniques and ultrasound-guided follicular aspiration. *J. Med. Primatol., 20*, 110–116.

Videan, E. N., Fritz, J., Heward, C. B., & Murphy, J. (2008). Reproductive aging in female chimpanzees (*Pan troglodytes*). In S. Atsalis, S. W. Margulis & P. R. Hof (Eds.), *Primate Reproductive Aging* (pp. 103–118). Basel: Karger.

von Segesser, F., Scheffrahn, W., & Martin, R. D. (1995). Parentage analysis within a semi-free-ranging group of Barbary macaques *Macaca sylvanus*. *Mol. Ecol., 4*, 115–120.

Walker, M. L., Gordon, T. P., & Wilson, M. E. (1983). Menstrual cycle characteristics of seasonally breeding rhesus monkeys. *Biol. Reprod., 29*, 841–848.

Walker, M. L., & Herndon, J. G. (2008). Menopause in nonhuman primates? *Biol. Reprod., 79*, 398–406.

Ward, J. A., & Hilliard, J. K. (1994). B virus-specific pathogen-free (SPF) breeding colonies of macaques: issues, surveillance, and results in 1992. *Lab. Anim. Sci., 44*, 222–228.

Ward, J. A., & Hilliard, J. K. (2002). Herpes B virus-specific pathogen-free breeding colonies of macaques: serologic test results and the B-virus status of the macaque. *Cont. Topics Lab. Anim. Med., 41*, 36–41.

Ward, J. A., Hilliard, J. K., & Pearson, S. (2000). Herpes B-virus specific-pathogen-free breeding colonies of macaques (*Macaca mulatta*): diagnostic testing before and after elimination of the infection. *Comp. Med., 50*, 317–322.

Watts, S. A., & Veall, D. (2004). Fostering an infant rhesus monkey on to a nonlactating female in a group-housed breeding colony. *Contemp. Top. Lab. Anim. Sci., 43*, 32−34.

Wehrenberg, W. B., & Dyrenfurth, I. (1983). Photoperiod and ovulatory menstrual cycles in female macaque monkeys. *J. Reprod. Fertil., 68*, 119−122.

Weick, P. F., Dierschke, D. J., Karsch, F. J., Butler, W. R., Hotchkiss, J., & Knobil, E. (1973). Periovulatory time courses of circulating gonadotropic and ovarian hormones in the rhesus monkey. *Endocrinology, 93*, 1140−1147.

Weigler, B. J. (1992). Biology of B virus in macaque and human hosts: a review. *Clin. Infect. Dis., 14*, 555−567.

Weigler, B. J., Roberts, J. A., Hird, D. W., Lerche, N. W., & Hilliard, J. K. (1990). A cross sectional survey for B virus antibody in a colony of group housed rhesus macaques. *Lab. Anim. Sci., 40*, 257−261.

Weigler, B. J., Hird, D. W., Hilliard, J. K., et al. (1993). Recommendations for prevention of and therapy for exposure to B virus (Cercopithecine herpesvirus 1). *Clin. Infect. Dis., 167*, 257−263.

Weigler, B. J., Scinicariello, F., & Hilliard, J. K. (1995). Risk of venereal B virus (cercopithecine herpesvirus 1) transmission in rhesus monkeys using molecular epidemiology. *J. Infect. Dis., 171*, 1139−1143.

Weinbauer, G. F., Aslam, H., Krishnamurty, H., Brinkworth, M. H., Einspanier, A., & Hodges, J. K. (2001). Quantitative analysis of spermatogenesis and apoptosis in the common marmoset (*Callithrix jacchus*) reveals high rates of spermatogonial turnover and high spermatogenic efficiency. *Biol. Reprod., 64*, 120−126.

Weiss, G., Butler, W. R., Dierschke, D. J., & Knobil, E. (1976). Influence of suckling on gonadotropin secretion in the postpartum rhesus monkey. *Proc. Soc. Exp. Biol. Med., 153*, 330−331.

Westergaard, G. C., Izard, M. K., Drake, J. H., Suomi, S. J., & Higley, J. D. (1999). Rhesus macaque (*Macaca mulatta*) group formation and housing: wounding and reproduction in a specific pathogen free (SPF) colony. *Am. J. Primatol., 49*, 339−347.

Westergaard, G. C., Izard, M. K., & Drake, J. H. (2000). Reproductive performance of rhesus macaques (*Macaca mulatta*) in two outdoor housing conditions. *Am. J. Primatol., 50*, 87−93.

Wistuba, J., Schrod, A., Greve, B., Hodges, J. K., Aslam, H., Weinbauer, G. F., et al. (2003). Organisation of the seminiferous epithelium in primates: relationship to spermatogenic efficiency, phylogeny and mating system. *Biol. Reprod., 69*, 582−591.

White, I. G. (1981). Epididymal compounds and their influence on the metabolism and survival of spermatozoa. *Am. J. Primatol., 1*, 143−155.

Whitehair L.A. (1999). Development and major contributions of the extramural comparative medicine area, NIH (1962−1999). In C.W. McPherson, S.F. Mattingly, (Eds.), *50 Years of Laboratory Animal Science*, pp. 50−73.

Wickings, E. J., & Nieschlag, E. (1980a). Pituitary response to LHRH and TRH stimulation and peripheral steroid hormones in conscious and anesthetized adult male rhesus monkeys (*Macaca mulatta*). *Acta Endocrinol., 93*, 287−293.

Wickings, E. J., & Nieschlag, E. (1980b). Suppression of spermatogenesis over two years in rhesus monkeys actively immunized with follicle stimulating hormone. *Fertil. Steril., 34*, 269−274.

Wickings, E. J., Marshall, G. R., & Nieschlag, E. (1986). Endocrine regulation of male reproduction. In W. R. Dukelow & J. Erwin (Eds.), *Comparative Primate Biology*, Vol. 3 (pp. 149−170). New York: Alan R. Liss.

Wiebe, R. H., Diamond, E., Akesel, S., Liu, P., Williams, L. E., & Abee, C. R. (1984). Diurnal variations of androgens in sexually mature male Bolivian squirrel monkeys (*Saimiri sciureus*) during the breeding season. *Am. J. Primatol., 7*, 291−297.

Wiebe, R. H., Williams, L. E., Abee, C. R., Yeoman, R. R., & Diamond, E. J. (1988). Seasonal changes in serum dehydroepiandrosterone, androstenedione and testosterone levels in the squirrel monkey (*Saimiri boliviensis boliviensis*). *Am. J. Primatol., 14*, 285−291.

Wiebe, R. H., Abee, C., Yeoman, R., & Williams, L. (1989). Origin of androgens in the male squirrel monkey during the breeding season. *Am. J. Primatol., 18*, 171.

Wildman, D. E., Chen, C., Erez, O., Grossman, L. I., Goodman, M., & Romero, R. (2006). Evolution of the mammalian placenta revealed by phylogenetic analysis. *Proc. Nat. Acad. Sci. USA, 103*, 3203−3208.

Wildt, D. E., Doyle, L. L., Stone, S. C., & Harrison, R. M. (1977). Correlation of perineal swelling with serum ovarian hormone levels, vaginal cytology, and ovarian follicular development during the baboon reproductive cycle. *Primates, 18*, 261−270.

Wilkinson, R. C., Murrell, C. K., Guy, R., Davis, G. M., Hall, J. M., North, D. C., et al. (2003). Persistence and dissemination of simian retrovirus type 2 DNA in relation to viremia, seroresponse, and experimental transmissibility in *Macaca fascicularis*. *J. Virol., 77*, 10751−10759.

Williams, L., Vitulli, W., McElliney, T., Wiebe, R. H., & Abee, C. R. (1986). Male behavior through the breeding season in *Saimiri boliviensis boliviensis*. *Am. J. Primatol., 11*, 27−35.

Williams, L., Gibson, S., McDaniel, M., Bazzel, J., Barnes, S., & Abee, C. (1994). Allomaternal interactions in the Bolivian squirrel monkey (*Saimiri boliviensis boliviensis*). *Am. J. Primatol., 34*, 145−156.

Williams, L. E., Brady, A. G., Gibson, S. V., & Abee, C. R. (2002). The squirrel monkey breeding and research resource: a review of *Saimiri* reproductive biology and behavior and breeding performance. *Primatologie, 5*, 303−334.

Williams-Blangero, S., & VandeBerg, J. L. (2003). Genetic considerations in the management of captive nonhuman primates. In: *International Perspectives: The Future of Nonhuman Primate Resources, Proceedings of the Workshop Held April 17−19, 2002* (pp. 114−119). National Research Council.

Williams-Blangero, S., VandeBerg, J. L., & Dyke, B. (2002). Genetic management of nonhuman primates. *J. Med. Primatol., 31*, 1−7.

Wilson, J. G., Fradkin, R., & Hardman, A. (1970). Breeding and pregnancy in rhesus monkeys used for teratological testing. *Teratology, 3*, 59−72.

Wise, P. M. (2006). Aging of the female reproductive system. In E. J. Masoro & S. N. Austad (Eds.), *Handbook of the Biology of Aging* (pp. 570−590). London: Elsevier.

Wolovich, C., & Evans, S. (2007). Sociosexual behavior and chemical communication of *Aotus nancymaae*. *Int. J. Primatol., 28*, 1299−1313.

Wright, P. C. (1981). The night monkeys, genus *Aotus*. In A. Coimbra-Filho & R. Mittermeier (Eds.), *Ecology and Behavior of Neotropical Primates*, Vol. 1 (pp. 211−240). Rio de Janeiro, Academia Brasileira de Ciencias.

Wright, P. C. (1985). *The Costs and Benefits of Nocturnality of* Aotus trivirgatus *(the Night Monkey)*. PhD thesis. New York: City University of New York.

Wright, P. C. (1989). The nocturnal primate niche in the New World. *J. Hum. Evol., 18*, 635–658.

Yeoman, R. R., Aksel, S., Hazelton, J. M., Williams, L. E., & Abee, C. R. (1988). In vitro bioactive luteinizing hormone assay shows cyclical, seasonal hormonal changes and response to luteinizing-hormone releasing hormone in the squirrel monkey (*Saimiri boliviensis boliviensis*). *Am. J. Primatol., 14*, 167–175.

Yeoman, R. R., Wegner, F. H., Gibson, S. V., Williams, L. E., Abbott, D. H., & Abee, C. R. (2000). Midcycle and luteal elevations of follicle stimulating hormone in squirrel monkeys (*Saimiri boliviensis*) during the estrous cycle. *Am. J. Primatol., 52*, 207–211.

Yeung, C.-H., Cooper, T. G., & Weinbauer, G. F. (1996). Maturation of monkey spermatozoa in the epididymis with respect to their ability to undergo the acrosome reaction. *J. Androl., 17*, 427–432.

Yoshida, T., Suzuki, K., Cho, F., & Honjo, S. (1987). Serum chorionic gonadotropin levels determined by radioreceptor assay and early diagnosis of pregnancy in the cynomolgus monkey (*Macaca fascicularis*). *Am. J. Primatol., 12*, 101–106.

Zamboni, L., Conaway, C. H., & Van Pelt, L. (1974). Seasonal changes in production of semen in free-ranging rhesus monkeys. *Biol. Reprod., 11*, 251–267.

Zeleznik, A. J., & Pohl, C. R. (2006). Control of follicular development, corpus luteum function, the maternal recognition of pregnancy, and the neuroendocrine regulation of the menstrual cycle in higher primates. In J. D. Neill (Ed.) (3rd ed). *Knobil and Neill's Physiology of Reproduction*, Vol. 2 (pp. 2449–2510). St. Louis, MO: Elsevier.

Zhengwei, Y., McLachlan, R. I., Bremner, W. J., & Wreford, N. G. (1997). Quantitative (stereological) study of the normal spermatogenesis in the adult monkey (*Macaca fascicularis*). *J. Androl., 18*, 681–687.

Ziegler, T. E., & Wittwer, D. J. (2005). Fecal steroid research in the field and laboratory: improved methods for storage, transport, processing and analysis. *Am. J. Primatol., 67*, 159–174.

Ziegler, T. E., Stein, F. J., Sis, R. F., Coleman, M. S., & Green, J. H. (1981). Supplemental feeding of marmoset (*Callithrix jacchus*) triplets. *Lab. Anim. Sci., 31*, 194–195.

Ziegler, T. E., Bridson, W. E., Snowden, C. T., & Eman, S. (1987). Urinary gonadotropin and estrogen excretion during the postpartum estrus, conception and pregnancy in the cotton-top tamarin (*Saguinus oedipus oedipus*). *Am. J. Primatol., 12*, 127–140.

Ziegler, T. E., Scheffler, G., Wittwer, D. J., Schultz-Darken, N., Snowdon, C. T., & Abbott, D. H. (1996). Metabolism of reproductive steroids during the ovarian cycle in two species of callitrichids, *Saguinus oedipus* and *Callithrix jacchus*, and estimation of the ovulatory period from fecal steroids. *Biol. Reprod., 54*, 91–99.

Zinner, D. P., van Schaik, C. P., Nunn, C. L., & Kappeler, P. M. (2004). Sexual selection and exaggerated sexual swellings of female primates. In M. Kappeler & C. P. van Schaik (Eds.), *Sexual Selection in Primates: New and Comparative Perspectives* (pp. 71–89). Cambridge: Cambridge University Press.