Morphological and Hormonal Parameters in Two Species of Macaques: Impact of Seasonal Breeding

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ABSTRACT   To compare physiological and developmental differences between two cogeneric species that differ by seasonal vs. aseasonal breeding, values for morphological measurements, testicular volume, serum testosterone, estradiol, and dehydroepiandrosterone-sulfate levels were obtained from 53 rhesus during the early breeding season, as well as 41 pig-tailed macaque males maintained at the Tulane Primate Center. The two species exhibited similar body size, testosterone, and estradiol levels, but differed substantially in testicular volume (3.00 ± 1.7 vs. 1.72 ± 1.3 cc), abdominal skinfold measures (15.7 ± 9.2 vs. 9.0 ± 7.7 mm), and DHEA-S levels (18.0 ± 11.7 vs. 7.6 ± 5.4 μg/dl). Significant interaction effects for species by age group were found for weight, tricep circumference, length, and estradiol level. In addition, length was more closely related to testicular volume among rhesus compared to pig-tailed macaques, suggesting different developmental patterns between the species. Predictors of hormonal levels differed between the two species. In the rhesus, estradiol levels were related to testicular volume and testosterone levels while there were no anthropometric predictors of testosterone or DHEA-S. For the pig-tailed macaques, testicular volume was related to tricep circumference, testosterone to triceps skinfold and testicular volume, and estradiol to weight. It is argued that rhesus have larger testes for body size and more abdominal fat deposits during the early breeding season relative to pig-tailed macaques reflecting the increased demands of sperm competition in a seasonally breeding species. Hormonal differences associated with the difference in breeding system appear to be primarily related to adrenal rather than testicular activity. Am J Phys Anthropol 117:218–227, 2002.
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The role of sperm competition among primates, including humans, has received much recent attention (Short, 1981; Harcourt et al., 1981; Baker and Bellis, 1995). The existence of such "sperm competition" implies that by increasing the volume of spermatozoa and ejaculate, males may outcompete each other for successful fertilization. Species with multimale groups have larger testes relative to body size than those with single-male polygamous mating systems (Harvey and Harcourt, 1984), which the authors conclude is the result of sperm competition. Additional evidence of sperm competition comes from the fact that increased testicular size to body size among rhesus macaques and baboons is related to an increase in the ratio of tubules to interstitial tissue (Amann and Howards, 1980; Harcourt et al., 1981). This suggests that differences in testicular size are primarily related to differences in Sertoli-cell number without changes in Leydig-cell number and the production of steroid hormones, including testosterone, which has important impacts on growth and body composition (Cassorla et al., 1984; Zemel and Katz, 1986; Preece et al., 1994). Coe et al. (1992) demonstrated differences in testosterone levels across primate species, with cebids and callitrichids showing particularly elevated levels, although it is unclear if these differences represent phylogenetic differences in testicular function or are more directly related to simple differences in body size.

Compared to the impact of single-male vs. multimale mating systems, much less is known about the consequences of seasonal breeding on testicular size among primates. Bercovitch and Nurnberg (1996) and Bercovitch (1997) suggested that seasonal breeding primates should have increased testicular volume due to the male's inability to maintain exclusive access to females during the breeding sea-

son. However, Harcourt et al. (1995) failed to find an effect of breeding seasonality on average testicular volumes across species of primates.

Comparison of closely-related species that differ by breeding seasonality (seasonal vs. aseasonal) provides one opportunity to investigate the impact of breeding seasonality on testicular size in primates, and to investigate the physiological and development mechanisms that may be involved. The more closely related the species, the more differences in hormonal and morphometric measures are likely to reflect the effects of ecological rather than phylogenetic differences. Consequently, we compared testicular volume and gonadal hormones, as well as various measures of body size, among two species of macaques, *Macaca mulatta* and *Macaca nemestrina*, which differ by the presence/absence of seasonal breeding.

The rhesus macaque (*Macaca mulatta*) is the most abundant species of macaque and is, therefore, readily available to study for both behavior and morphometric projects. Rhesus macaques are multi-male, multi-female breeders. Although in the laboratory females copulate during any day of their cycle in any month of the year, most females in natural settings exhibit a limited period of receptivity during each cycle (Bernstein, 1993). Numerous studies clearly demonstrated rhesus macaques as being highly seasonal in breeding and birthing in both captive and natural settings (Conaway and Koford, 1964; Drickamer, 1974; Wilson et al., 1978). Gestation lasts approximately 165 ± 5 days, and males reach sexual maturity in their fourth or fifth year of life.

In contrast, pig-tailed macaques (*Macaca nemestrina*) demonstrate a polygamous, multi-male non-seasonal breeding system where females in estrous are present year round in the group (Caldecott, 1986; Oi, 1996). Pig-tailed gestation is approximately 170 ± 8.5 days, and males reach sexual maturity within 3–4.5 years of life (Sirianni and Swindler, 1985). Pig-tailed macaques are much more difficult to study both in the wild and in captivity than rhesus macaques because of their relatively small numbers.

Rhesus macaques exhibit variation in amount of adipose tissue over the breeding season, changes which have been related to fluctuating estradiol levels (Bercovitch, 1992). Since estradiol can be produced by the aromatization of testosterone in adipose tissues (Longcope et al., 1978), this raises the possibility of differences in estradiol levels between rhesus and pig-tailed macaques. In addition to testosterone and estradiol, dehydroepiandrosterone-sulfate (DHEA-S) also has been implicated in regulating growth and development of muscle, bone, and other somatic and reproductive structures in both human and non-human primates (Prader, 1984; Zemel and Katz, 1986; Weirman et al., 1986; Crawford and Handelsman, 1996; Gordon et al., 1999).

Based on the premise that sperm competition and adipose reserves will be more important during the breeding season for rhesus compared to the non-seasonally breeding pig-tailed macaques, but that increases in testicular size are primarily related to spermatogenic potential and not changes in hormone-producing cells of the testes, we predict that:

1. Rhesus will have relatively larger testes, but similar testosterone levels when compared to pig-tailed macaques.
2. Rhesus will have greater fat reserves than pig-tailed macaques, and this difference will be related to differences in estradiol.
3. Differences in somatic growth between the two species may be related to DHEA-S, or estradiol, but not testosterone levels.

We emphasize that these predictions refer to differences in male rhesus macaques during the early breeding season and average year-round levels in the pig-tailed macaque sample. By sampling pubertal as well as adult animals, we are able to investigate not only species differences in these parameters, but gain insight into the developmental patterns by which they are established.

MATERIALS AND METHODS

This study utilized 53 male rhesus macaques of Indian origin between 3 and 14 years of age and 41 male pig-tailed macaques between 3 and 12 years of age, maintained at the Tulane Regional Primate Research Center (Covington, LA). Animals were housed in one-half and one-quarter acre corrals as well as corncribs and individual restraining cages. Of the 41 pig-tailed macaques used, 33 were housed in outdoor corrals (0.0003–0.0006 animals per square meter), 7 in individual cages (0.022 animals/ m^2), and one in a corncrib (0.006 animals/m^2). Of the 53 rhesus, 38 were housed in outdoor corrals (0.00007–0.0008 animals/m^2), 10 in individual cages (0.022 animals/m^2), and 5 in corncribs (0.004–0.008 animals/m^2). These values are reported for accurate representation of data. There was no effect of cage size on any of the variables of interest, and so these values were not included in further analyses. In general, the corrals demonstrate a consistent and seminatural ecological context (Sade, 1964). All animals were provisioned with Purina Monkey Chow (Ralston Purina Co., St. Louis, MO) daily. Diet was supplemented with fresh fruit weekly, and water was available ad libitum. Matrilineal data and precise chronological ages were maintained in the Center's computer system.

All measurements took place between October 1998 and January 1999, the beginning to middle of the rhesus breeding season. Sampling during this time should maximize differences between the seasonal and aseasonal breeding species. However, given morphological and hormonal changes during the breeding season (Bercovitch, 1992), changes

within the rhesus may have confounded differences between the two species. In addition, older and larger rhesus macaques were sampled first, which may have potentially biased results from the rhesus sample. Statistical methods using date of data collection were used to lessen these biases, as discussed below.

All animals were anesthetized with ketamine hydrochloride (10 mg/kg), a widely accepted dissociative anesthetic used in studies requiring transient animal tranquilization (Chen et al., 1981; Bercovitch and Nurnberg, 1996), and which has demonstrated no significant effects on serum androgen levels or production rates (Zaidi et al., 1982). Each animal was examined once, between 0800–1030 hr, to eliminate any circadian effect. All animals were held in a standardized position with their bodies lying on the left side and arms and legs extended perpendicular to the vertebral axis. The head and muzzle were aligned in the Frankfort plane relative to the torso (Coelho, 1985). Body weight was determined to the nearest 0.1 kg. A Dean fiberglass tape measure was placed parallel to the vertebrate column and measured to the nearest 0.01 cm from the occipital node to the ischial callosity. The upper arm circumference, a good predictor of lean body mass (Schartz and Kemnitz, 1992), was measured to the nearest 0.01 cm by wrapping the tape measure around the halfway point between the olecranon and the tip of the scromion. Tricep skinfold was measured to the nearest millimeter using nonflexible, sliding calipers; periumbilicular (abdominal) skinfold was measured 1 cm below the umbilicus; and the subscapular skinfold was measured 1 cm below the inferior angle of the scapula (Coelho, 1985). Past studies indicated that abdominal skinfold in male rhesus macaques is significantly correlated with amount of total body fat (Kemnitz and Franken, 1986). Comparison with other studies (Schwartz and Kemnitz, 1992) suggests that the rhesus in this study were comparable in terms of morphometric measures to other populations. In order to minimize interobservational error, morphometric measurements were made by one investigator (M.P.M.) when possible. However, a small number (<5%) were made by another investigator.

Both left and right testes were digitally restrained within the scrotum, and length and breadth of each testis were measured to the nearest millimeter using the nonflexible, sliding calipers. The epididymis was excluded in all measurements, and scrotal skin thickness was not accounted for. Testicular volumes were calculated using the formula for determining the volume of a prolate spheroid (testicular volume = $\pi LW^2/6$) (Harrison et al., 1977). Left testicular volume (to the nearest 0.01 cm^3) was reported because of its high correlation (0.97) to total testicular volume, and to maintain comparable consistency to past reports (Bercovitch and Rodriguez, 1993).

TABLE 1. Morphometric and hormonal measures for rhesus and pig-tailed macaques

Variable	Rhesus	Pig-tailed	P-value[1]
Age (years)	7.65 ± 3.24	6.60 ± 2.75	0.002
Weight (kg)	10.54 ± 3.10	10.67 ± 3.77	0.59
Testicular volume (cc)	3.00 ± 1.77	1.72 ± 1.26	0.00
Tricep circumference (cm)	18.3 ± 2.64	18.21 ± 3.07	0.81
Triceps skinfold (mm)	4.82 ± 1.46	4.33 ± 1.71	0.12
Subscapular skinfold (mm)	6.98 ± 2.15	6.39 ± 2.21	0.20
Abdominal skinfold (mm)	15.72 ± 9.14	9.02 ± 7.73	0.000
Length (cm)	47.15 ± 5.05	45.18 ± 4.21	0.002
Testosterone (ng/ml)	4.59 ± 3.19	3.53 ± 3.21	0.10
Estradiol (pg/ml)	5.80 ± 4.52	5.06 ± 6.22	0.72
DHEA-S (pg/ml)[2]	17.98 ± 11.65	7.57 ± 5.44	0.000

[1] P-value is difference between species, controlled for age using ANOVA.
[2] DHEA-S, dehydroepiandrosterone-sulfate.

For each animal, a blood sample was collected from the femoral vein, using a 4-ml SST Vacutainer Collection Tube with serum separator (Beckton-Dickson) and a 21-gauge needle. All blood samples were collected within 5 min of ketamine administration immediately following capture in order to minimize capture stress from significantly influencing gonadal and adrenal hormone concentrations (Sapolsky, 1986). The sera from the blood collected were aliquoted into two containers and frozen at −40°C until assayed for estradiol, testosterone, and dehydroepiandrosterone-sulfate (DHEA-S) using solid-phase RIA procedures (Coat-A-Count, Diagnostic Products Corp, LA).

Individuals were grouped into age categories corresponding to the major phases of the macaque life cycle:

Age group 1: ages 4–5 (adolescents); 10 rhesus, 22 pig-tailed
Age group 2: ages 6–9 (subadults); 29 rhesus, 10 pig-tailed
Age group 3: ages 10–14 (prime adults); 14 rhesus, 9 pig-tailed

Differences between species were assessed using *t*-tests for independent samples. Differences between species, age groups, and the interaction of these two variables were assessed using ANOVA procedures, with species and age category included as fixed variables. Multivariate modeling procedures were employed to determine which morphometric and hormonal variables were significant predictors of one another. For all statistical tests, alpha was set at $P < 0.05$.

RESULTS

Mean species differences

Table 1 shows the mean morphometric measures and hormonal values for both the rhesus and pig-

tailed samples. The two species were remarkably similar in terms of weight, left tricep circumference, left tricep skinfold, and subscapular skinfold. However, the rhesus showed substantially larger left testicular volumes and abdominal fat. In addition, the rhesus were slightly, but significantly longer than the pig-tailed macaques.

Despite the larger testes of the rhesus males, serum levels of testosterone and estradiol did not differ substantially between the two species. Testosterone was slightly higher among the rhesus, but not significantly so. Estradiol showed no significant difference. On the other hand, levels of DHEA-S were substantially and significantly higher among the rhesus. The sample of rhesus was slightly older; however, controlling for this age difference between the two samples (using ANOVA with species as a fixed variable and age as a covariate) did not impact the significant morphological or hormonal differences between the two species.

Age patterns of morphology and hormones

Figure 1 shows the age-related patterns of morphometric characteristics for both species. All morphometric measures increased with age. Testicular volume reached maximal level at age group 2 (6–9 years). Patterns of abdominal fat appeared to differ, with pig-tailed showing maximal levels in age group 3 (10–14 years), while rhesus reached maximal levels by age group 2. However, ANOVA procedures showed significant effects of age group (f = 22.47; $P < 0.001$) and species (f = 16.88; $P < 0.0001$), but no significant age group by species interaction for testicular volume (f = 0.25; $P = 0.78$). For abdominal fat there was also a significant effect of age group (f = 8.94; $P < 0.0001$) and species (f = 8.22; $P < 0.01$), but no age group by species interaction (f = 1.77; $P = 0.18$).

The two species did demonstrate different age patterns for weight, tricep circumference, and length. The rhesus seemed to put on more weight at an earlier age, but peaked at age category 3 (10–14 years). The pig-tailed sample also peaked in weight at age category 3 (10–14 years). Length showed a different pattern, with rhesus males showing an increase in length throughout the age groups, while the pig-tailed started out longer but peaked at age group 2. In terms of tricep circumference, it is the rhesus that peaked in age group 2, while the pig-tailed showed an increase throughout the age groups.

ANOVA confirmed the different age-related patterns between the two groups. Weight exhibited significant effects of species (f = 3.97; $P < 0.05$) and age group (f = 33.32; $P < 0.001$), as well as an interaction for species by age group (f = 9.14; $P < 0.0001$). Triceps circumference showed significant effects of age group (f = 24.96; $P < 0.0001$), as well as an age group by species interaction (f = 5.48; $P = 0.006$). For length there were significant effects of species (f = 8.36; $P < 0.01$) and age group (f = 31.21; $P <$ 0.0001), as well a significant interaction between the two (f = 5.18; $P = 0.007$).

Figure 2 shows the age pattern of the hormones testosterone, estradiol, and dehydroepiandrosterone-sulfate (DHEA-S) for both species. Testosterone increased with age in both species, with an earlier rise among rhesus, but little difference in the fully adult age groups. ANOVA showed no significant effect of age group (f = 1.89; $P = 0.17$), species difference (f = 2.24; $P = 0.11$), or age group by species interaction (f = 0.85; $P = 0.43$). For estradiol, clear differences in the pattern of hormones by age were evident. Estradiol levels not only increased in age group 2 among the rhesus compared to pig-tailed, they also showed a clear decline in the oldest adult group compared to pig-tailed, for whom estradiol levels continued to increase. These differences were supported by ANOVA, which showed not only a significant difference between age groups (f = 18.81; $P = 0.001$), but an age group by species interaction as well (f = 6.65; $P = 0.002$).

DHEA-S showed a very different age pattern compared to estradiol and testosterone, with declining levels from the youngest age group for both species. ANOVA showed a significant difference among age groups (f = 3.04; $P = 0.05$) but no species by age group interaction, indicating a similar pattern of decline in both groups (f = 0.37; $P = 0.49$).

Impact of seasonality

As discussed above, measurements were collected over the course of the beginning to the middle of the rhesus breeding season. Changes in testicular size related to the breeding season could have confounded differences in testicular volume between the two species. Additionally, this might have distorted the relationship between these variables within the two species. Table 2 shows bivariate correlations between selected measures and time of data collection (dates collapsed into week intervals: weeks 1–8 for the rhesus, and 9–12 for the pig-tailed) among the two species. Among the rhesus there was a significant and negative correlation of week with testicular volume, triceps, and subscapular skinfolds, and testosterone and DHEA-S. We attribute the negative association of these measures with week of sampling to two facts: 1) larger males were sampled first, thereby potentially biasing the findings of larger testicular size in the early weeks of data collection; and 2) variation in testicular volume over the breeding season. In contrast, among the pig-tailed macaques there was a significant and positive correlation of week with testicular volume, weight, length, triceps circumference, triceps skinfold, and estradiol. We attribute the positive association of these measures with week of sampling to the fact that smaller pig-tailed males were sampled first. In order to control for the confounding effects of sampling, week of data collection was included as a control in subsequent analyses of the relationship between morphometric and hormonal measures.
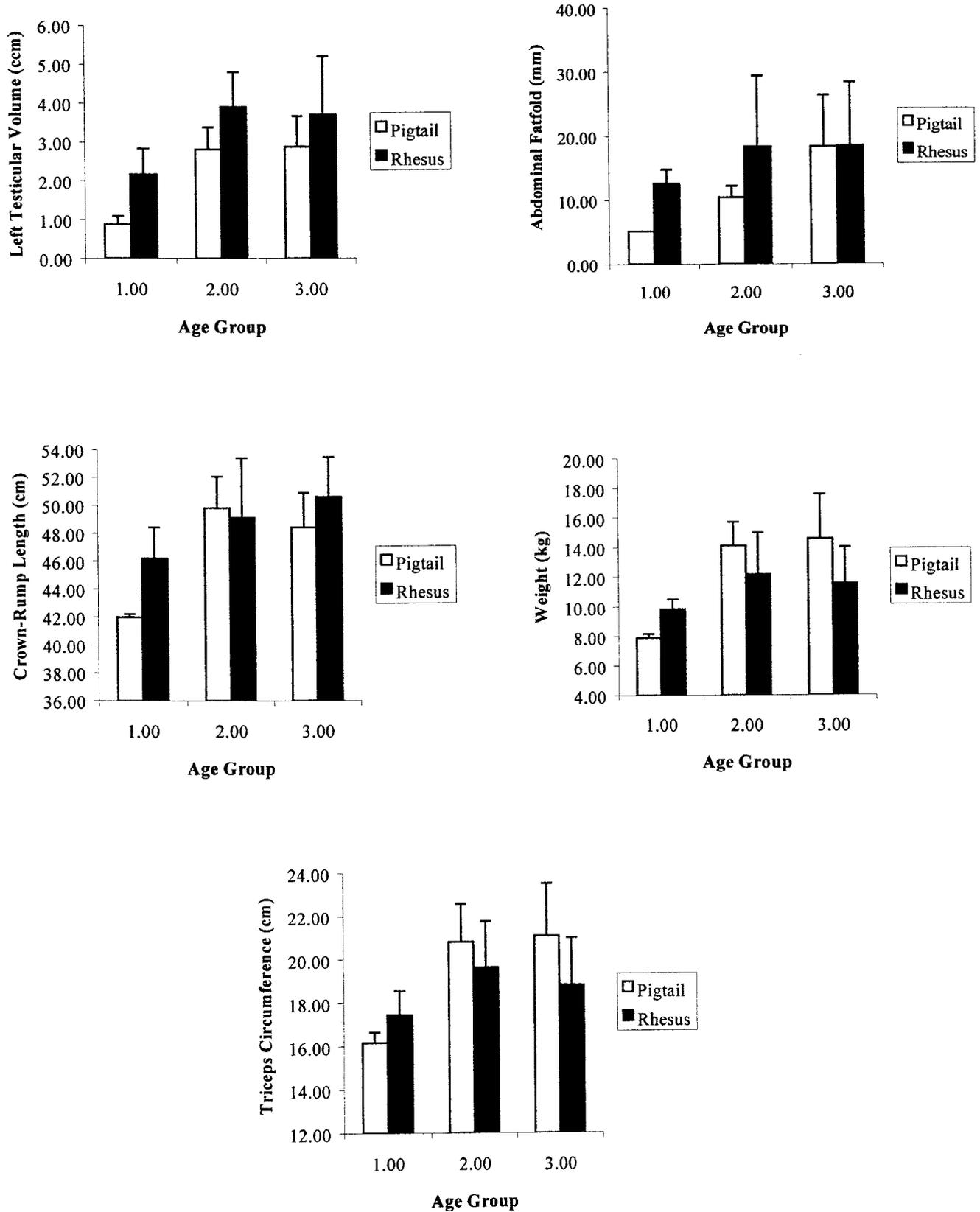
Fig. 1. Age-related patterns of morphometric traits. Despite differences in left testicular volume and abdominal skinfold thickness between the two species, age related patterns did not show significant differences between the two groups (see text for explanation). Age related patterns of weight, length, and triceps circumference differed between the two species.
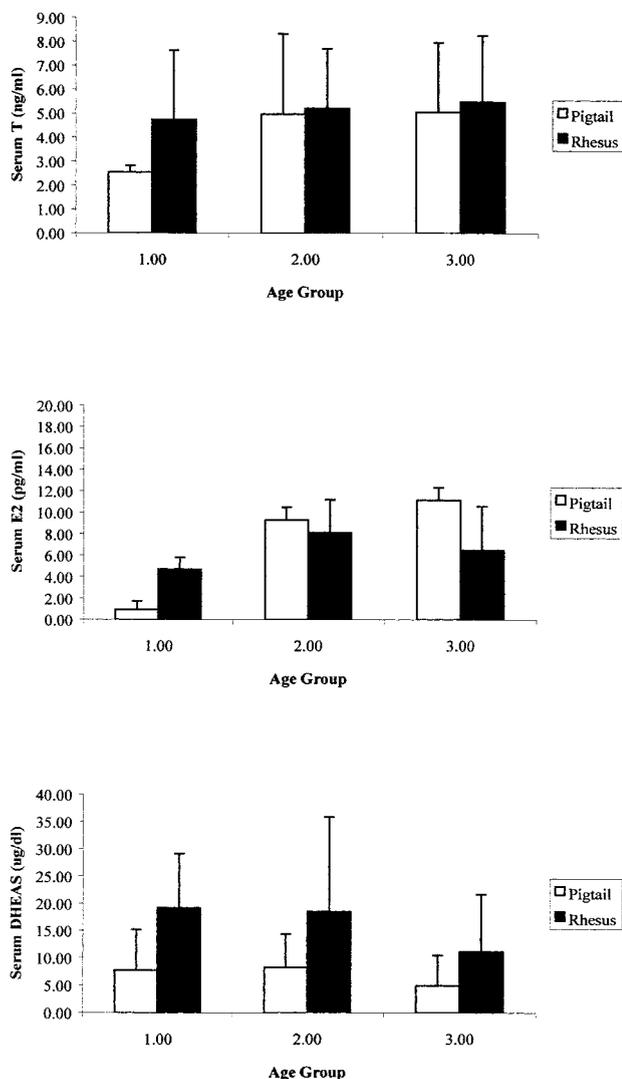
Fig. 2. Age-related patterns of hormone levels. **Testosterone.** Levels for both species increased with age. Species differences were evident in the pubertal group, with no difference between the two adult groups. However, multivariate ANOVA confirmed the increase with age, but showed no significant age group by species interaction. **Estradiol.** Species differences in age-related patterns of estradiol were evident. Not only did the rhesus show a more substantial increase in the pubertal group compared to the pig-tailed, but they also exhibited a decline in the mature adult group. Multivariate ANOVA confirmed significant differences between age groups, and also showed a significant species by age group interaction. **DHEA-S.** In contrast to testosterone and estradiol, DHEA-S levels declined with age from the youngest age group for both species. DHEA-S, dehydroepiandrosterone-sulfate; E2, estradiol; T, testosterone.

Thus the relationships between morphological and hormonal measures shown in Table 3 represent partial correlations controlling for age and week of data collection. As expected, most morphometric measures were significantly related for both species, though the lack of a significant relationship of triceps skinfold to any morphometric measure other than subscapular skinfold among the pig-tailed was notable. Testicular volume itself was strongly related to all other morphometric measures among the

TABLE 2. *Correlation of selected morphometric and hormonal measures with week of sampling[1]*

Variable	Rhesus macaques	Pig-tailed macaques
Testicular volume	−0.553***	0.541***
Length	−0.131	0.616***
Weight	−0.176	0.553***
Tricep circumference	−0.244	0.526***
Subscapular skinfold	−0.330*	−0.274
Triceps skinfold	−0.528**	0.622***
Periumbilical skinfold	−0.193*	0.201
Testosterone	−0.443**	0.064
Estradiol	−0.015	0.537**
DHEA-S	−0.318*	0.371

[1] Bivariate correlations between selected measures and time of data collection (dates collapsed into week intervals). DHEA-S, dehydroepiandrosterone-sulfate.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

rhesus and all but tricep and subscapular skinfolds among the pig-tailed.

Of the hormones, testosterone and estradiol were also closely related to the morphometric measures among the pig-tailed macaques. Among the rhesus macaques, testosterone was significantly correlated to testicular volume and triceps skinfold, while estradiol was significantly correlated with weight and length in addition to testicular volume and triceps skinfold. DHEA-S, on the other hand, showed fewer strong relationships with morphometric measures. Among the rhesus, DHEA-S showed a significant relationship with triceps skinfold. Among the pig-tailed, DHEA-S was significantly related to subscapular and triceps skinfolds. Within the hormones themselves, testosterone and estradiol levels showed a significant correlation, but neither was related to DHEA-S.

Given the interdependent nature of the morphometric measures and the potential confounding of time of data collection and age, multivariate ANOVA models were used to determine which, if any, of the morphometric measures were independent predictors of testicular volume for each species. Three different morphometric variables were included in each model: length as a long-term measure of overall growth, triceps circumference as a measure of muscle mass, and abdominal skinfold as a measure of energetic status. Age category and week of data collection were included as controls.

The results shown in Table 4 indicate that for the rhesus, length and abdominal skinfold were significant predictors of testicular volume. Week was also a significant predictor of testicular volume, indicating that even when controlling for age-related sampling biases, testicular volume does vary over the breeding season, consistent with a seasonal breeding pattern. In contrast, among the pig-tailed macaques, triceps circumference was the only morphometric predictor of testicular volume. In addition, when controlled for age-related sampling biases, week was not a significant predictor, indicating lack

TABLE 3. *Correlations of morphometric and hormonal measures*

	Weight	Testicular volume	Triceps circumference	Abdominal skinfold	Triceps skinfold	Subscapular skinfold	Length	Testosterone	Estradiol	DHEA-S[1]
A. Rhesus (n = 53)										
Age	0.164	0.194	0.138	0.231	0.064	0.197	0.445**	0.096	0.041	−0.326**
Weight		0.529**	0.757**	0.753**	0.478**	0.672**	0.639**	0.216	0.357*	−0.098
Testicular volume			0.516**	0.264*	0.311*	0.378*	0.633**	0.339*	0.491**	0.135
Triceps circumference				0.497**	0.295*	0.607**	0.538**	0.299*	0.453**	0.073
Abdominal skinfold					0.377**	0.549**	0.440**	0.138	0.217	−0.127
Triceps skinfold						0.351*	0.465**	−0.071	0.088	0.296*
Subscapular skinfold							0.464**	0.136	0.255	−0.170
Length								0.127	0.406**	−0.072
Testosterone									0.463**	−0.101
Estradiol										0.118
B. Pig-tailed (n = 41)										
Age	0.740**	0.780**	0.721**	0.691**	0.420**	0.565**	0.692**	0.456**	0.665**	−0.487**
Weight		0.777**	0.859**	0.526**	0.204	0.348*	0.730**	0.449**	0.488**	−0.220
Testicular volume			0.654**	0.498**	0.231	0.289	0.620**	0.441**	0.688**	−0.212
Triceps circumference				0.200	0.209	0.214	0.595**	0.393*	0.393*	−0.225
Abdominal skinfold					0.070	0.391*	−0.548**	0.438**	0.314*	−0.027
Triceps skinfold						0.307*	−0.013	0.516**	0.187	−0.367*
Subscapular skinfold							0.206	0.354*	0.479**	−0.284*
Length								0.376*	0.596**	−0.156
Testosterone									0.402*	−0.019
Estradiol										0.243

[1] DHEA-S, dehydroepiandrosterone-sulfate.
* $P < 0.05$.
** $P < 0.01$.

TABLE 4. *ANOVA model of testicular volume*[1]

| | Rhesus (N = 53) 0.67 | | Pig-tailed (N = 41) 0.74 | |
| Adjusted R^2 | | | | |
Variable	F-statistic	P	F-statistic	P
Age category	3.28	0.041	0.28	0.76
Length	22.50	0.001	2.57	0.12
Triceps circumference	3.72	0.06	9.54	0.004
Abdominal skinfold	4.55	0.038	0.20	0.66
Week	29.26	0.001	0.06	0.82

[1] ANOVA with age category as a fixed variable and anthropometric measures as covariates.

of change in testicular volume consistent with lack of seasonal breeding.

In order to understand the relationship of testicular volume, morphometric measures, and hormone levels more closely, we ran a separate ANOVA multivariate model for each of the three hormones. For each of the hormones, we included all of the variables significant at the zero order in Table 2 and deleted them until only significant predictors remained. Age category and week of data collection were included in all models as controls.

The results are shown in Table 5. Testicular volume and tricep skinfold were significant predictors of testosterone for the pig-tailed macaques, whereas no morphometric variables were able to significantly predict testosterone levels in the rhesus. For estradiol, testosterone and testicular volumes were significant predictors among the rhesus. In contrast, weight was the only significant predictor of estradiol for the pig-tailed macaques. In terms of DHEA-S, neither the rhesus nor the pig-tailed macaques

TABLE 5. *Predictors of hormone levels in rhesus and pig-tailed macaques*[1]

| a) Testosterone, | Rhesus (n = 53) R^2 = 0.124 | | Pig-tailed (n = 41) R^2 = 0.497 | |
Variable	F	P	F	P
Age category	0.068	0.93	0.63	0.54
Testicular volume	0.82	0.37	3.84	0.06
Triceps skinfold			9.30	0.004
Week	5.32	0.03		

| b) Estradiol, | Rhesus (n = 53) R^2 = 0.173 | | Pig-tailed (n = 41) R^2 = 0.611 | |
Variable	F	P	F	P
Age category	1.45	0.245	0.957	0.393
Testicular volume	3.91	0.017		
Testosterone	14.07	0.000		
Weight			9.02	0.005
Week	10.74	0.002	0.94	0.339

| c) DHEA-S, | Rhesus (n = 53) R^2 = 0.148 | | Pig-tailed (n = 41) R^2 = 0.297 | |
Variable	F	P	F	P
Age category	3.01	0.06	6.03	0.005
Week	7.09	0.01	16.80	0.001

[1] Best-fit ANOVA models with age category as a fixed variable and anthropometrics, hormones, and date of collection as covariates. DHEA-S, dehydroepiandrosterone-sulfate.

showed any significant predictors, with the exceptions of age and time of data collection.

DISCUSSION

The results obtained here demonstrate that, despite very similar overall body size, rhesus macaques have substantially larger testes, greater abdominal fat deposits, and higher DHEA-S levels, but

similar testosterone and estradiol levels, when compared to pig-tailed macaques. In addition, the two species differ in the age-related patterns of some morphometric measures and exhibit different relationships of testosterone, estradiol, and DHEA-S to measures of body composition. Taken as a whole, these findings: 1) provide additional support for the importance of sperm competition among seasonally breeding macaques; 2) suggest the importance of adipose tissue for males among seasonally breeding primates; 3) raise questions about the basis for species differences in DHEA-S levels; and 4) suggest differences in the relationship of testicular and somatic development in the two species that may be mediated by estradiol.

Species differences and seasonality

Using comparative data, Harcourt et al. (1995) suggested no significant relationship between seasonality of breeding and testes to body size across 58 primate species. Rather, they confirmed significant differences in testes to body size ratio between primates of multi-male and single-male mating systems, independent of breeding seasonality. Their work further suggested that any average differences between two closely related multi-male breeding species should not be confounded by differences in breeding seasonality, although it is not clear when during the breeding season their measurements were made.

Our finding of larger testes among rhesus as compared to pig-tailed macaques extends previous findings of the effects of multi-male vs. single-male mating systems on testes to body size ratio (Harvey and Harcourt, 1984) to include differences in testes size associated with seasonal breeding. Not only do we utilize sample sizes of significantly larger magnitude than that of Harcourt et al. (1995), but we attempt to control for changes associated with timing during the breeding season in all ANOVA models for predicting testicular volume and hormone levels *within* the two species (Tables 4 and 5). Significant differences in average testicular volume, while controlling for age, as well as differences in predictors of testicular volume between the two species, led us to conclude that these differences are associated with seasonal breeding of the rhesus macaques. However, when comparing the two species, we were unable to completely control for changes in testicular volume that may be experienced by rhesus across the early to middle breeding season. Thus, our results speak to differences in testicular volume during the breeding season and cannot necessarily be extended to species differences year round. It is possible that these differences may disappear outside of the rhesus breeding season.

The lack of a difference in testosterone levels between the two species, despite the larger testes of the rhesus, suggests that the difference in testicular size is associated with greater sperm and not hormone-producing capacity. This is consistent with earlier observations that both macaques and baboons have high ratios of tubules to interstitial tissue, and therefore greater testicular volume is associated with greater sperm-producing capabilities (Amann and Howards, 1980; Harcourt et al., 1981).

In addition, the fact that periumbilical, but not other, skinfolds were thicker among the rhesus males suggests the impact of seasonal breeding on energetic demands of mating. The periumbilical skinfold represents abdominal fat which is thought to be easily mobilized, in contrast to the shoulder fat depot represented by subscapular and triceps skinfolds (Pond, 1998), and thus the periumbilical skinfold can act as short-term energy storage for males that experience negative energy balance and weight loss during a restricted breeding season.

The lack of a species difference in estradiol levels, despite clear differences in thickness of the periumbilical skinfolds, is contrary to our original prediction and suggests that peripheral conversion of testosterone by aromatization in adipose tissue may not be important to circulating estradiol levels for either of these two species. In addition, while we did find higher DHEA-S levels among rhesus macaques, there is no evidence that it is directly related to greater abdominal fat in that species. However, the substantially higher levels of DHEA-S among the rhesus suggest some difference in adrenal androgen metabolism between the two species. Whether this difference is related to the relative size of the adrenal gland, its responsiveness to stimulation, the relative production of cortisol and DHEA-S, or peripheral conversion of estradiol and testosterone (Deslypere et al., 1985), or some combination of these factors in the two species, remains unclear.

Age-related changes

The restricted age range and cross-sectional nature of our research design may obscure relationships between testicular and somatic growth evident only on a much finer time scale. Thus the interpretation of age-related changes in morphometric and hormonal levels as representative of growth processes must be treated with caution. However, despite substantial species differences in testicular volume, abdominal fat, and DHEA-S levels, these variables show little difference in age-related patterns between the two species, nor is there a significant difference in the age-related pattern of testosterone. Instead, the two species differ in age-related patterns of estradiol and other morphometric dimensions, including weight, triceps circumference, and length, suggesting that estradiol may play an important role in species differences in maturation.

Estradiol is a potent regulator of bone growth (Klein et al., 1996; Culter, 1997), and the fact that estradiol is significantly related to testicular volume, even when controlling for testosterone, age, and date of sample collection among the rhesus, suggests that estradiol may play an important role

in skeletal maturation and hence final adult length. Follicle-stimulating hormone (FSH) has been shown to induce Sertoli-cell proliferation in juvenile rhesus (Arslan et al., 1993; Schlatt et al., 1995). Sertoli cells from pubertal animals are particularly sensitive to the effects of FSH (Lee et al., 1983). Thus, increased testicular size in rhesus compared to pig-tailed macaques suggests greater FSH stimulation of the testes during pubertal maturation, most likely a result of greater testicular sensitivity. FSH also stimulates aromatase activity (Lee et al., 1983) and, as a consequence, testicular production of estrogen.

In addition, the fact that testicular volume is more closely related to length among the rhesus than the pig-tailed macaques suggests that differences in the impact of estradiol may result in a tighter association between skeletal and testicular growth during pubertal maturation. Among rhesus, initiation of pubertal maturation depends on photoperiod cues that also govern the seasonality of mating (Mann et al., 2000). A greater increase in testicular size during that period may lead to a more sharply defined increase in estradiol, leading to a more sharply defined period of skeletal growth and pubertal maturation.

CONCLUSIONS

Our findings of substantial differences in testicular volume, abdominal skinfolds, and circulating DHEA-S levels between rhesus and pig-tailed macaques provide comparative evidence for the importance of sperm competition and its relationship to the metabolism of adipose tissue in seasonally breeding primates, previously described for squirrel monkeys (DuMond and Hutchison, 1967; Mendoza et al., 1978) and rhesus on Cayo Santiago (Bercovitch, 1992). In addition, our results suggest species differences in age-related patterns of morphological but not testicular development. Thus our findings are consistent with the relative independence of the spermatogenic and hormonal functions of the testes, with species differences in testicular size related to sperm production and those in somatic structures related to the peripheral metabolism of testicular and adrenal hormones.

ACKNOWLEDGMENTS

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