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The endocrinology of pregnancy and fetal loss in wild baboons

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Abstract

An impressive body of research has focused on the mechanisms by which the steroid estrogens (E), progestins (P), and glucocorticoids (GC) ensure successful pregnancy. With the advance of non-invasive techniques to measure steroids in urine and feces, steroid hormones are routinely monitored to detect pregnancy in wild mammalian species, but hormone data on fetal loss have been sparse. Here, we examine fecal steroid hormones from five groups of wild yellow baboons (*Papio cynocephalus*) in the Amboseli basin of Kenya to compare the hormones of successful pregnancies to those ending in fetal loss or stillbirth. Using a combination of longitudinal and cross-sectional data, we analyzed three steroid hormones (E, P, GC) and related metabolites from 5 years of fecal samples across 188 pregnancies. Our results document the course of steroid hormone concentrations across successful baboon pregnancy in the wild and demonstrate that fecal estrogens predicted impending fetal loss starting 2 months before the externally observed loss. By also considering an additional 450 pregnancies for which we did not have hormonal data, we determined that the probability for fetal loss for Amboseli baboons was 13.9%, and that fetal mortality occurred throughout gestation (91 losses occurred in 656 pregnancies; rates were the same for pregnancies with and without hormonal data). These results demonstrate that our longstanding method for early detection of pregnancies based on observation of external indicators closely matches hormonal identification of pregnancy in wild baboons.

Keywords: Fetal loss; Miscarriage; Fecal steroids; Estrogens; Progestins; Glucocorticoids; Baboon; *Papio*; Pregnancy

Introduction

In human and non-human primates, steroid hormones are critically important for regulating key physiological events essential to the maintenance of pregnancy and development of the fetus. Consequently, considerable research has focused on the mechanisms by which the steroids estrogen, progesterone, and glucocorticoids ensure successful pregnancy (reviewed in Pepe and Albrecht, 1995). In primate pregnancy, progesterone is essential for many homeostatic mechanisms necessary for gestation, including suppressing maternal immunity to prevent the rejection of the developing fetus and promoting quiescence to ensure that pregnancy is maintained until term (Albrecht and Pepe, 1985, 1990). Estrogen has a central integrative role in the developmental regulation of several key aspects critical to pregnancy maintenance and fetal maturation, including enhancing uteroplacental blood flow, stimulating the biosynthesis of progesterone (Albrecht et al., 1980, 1991; Albrecht and Pepe, 1984, 1990), and activating the hypothalamic–pituitary–adrenocortical (HPA) axis in the fetus and thereby the initial production of fetal adrenal glucocorticoids (Pepe and Albrecht, 1995). Glucocorticoids, in turn, are necessary for maintaining an environment to support fetal growth and development, and glucocorticoid excess or insufficiency can result in disturbances in fetal homeostasis (Keller-Wood and Wood, 2001). Moreover, elevated glucocorticoids are related to the initiation of labor in humans, a

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mechanism that may also be present in other primates (Smith et al., 1993).

In addition to sufficient steroid hormone concentrations, the maintenance of pregnancy and fetal development in primates also depends on the timely orchestration of maternal, placental, and fetal physiology. Early pregnancy relies on a temporary period of steroid secretion by the corpus luteum, to be replaced entirely by placental steroid production by mid-gestation (Bosu and Johansson, 1975; Castracane and Goldzieher, 1986; Csapo and Pulkkinen, 1978; Hodges et al., 1984; Oakey, 1970). With advancing pregnancy, the placenta is joined by the fetal adrenal in producing large amounts of steroid hormones or indispensable C_{19} steroid precursors (Pepe and Albrecht, 1995), and proper fetal development is not possible without a fully functioning fetoplacental unit. Consequently, any failure in the close communication between the developing placenta and fetus in the biosynthesis and actions of steroid hormones can result in pregnancy failure. In other words, pregnancy failure (i.e., embryonic or fetal loss) can be a direct consequence of disruptions in the functional interaction between the placenta and fetal adrenal with respect to steroid biosynthesis. As such, changes in steroid hormone profiles will carry the first signs of pregnancy failure.

Alternatively, even if the underlying mechanisms of pregnancy failure are unknown, steroid hormones can still reflect early symptoms of impending fetal loss. Measurements of hormones are used routinely for detecting pregnancy in both captive and wild mammalian species, and with the advance of non-invasive techniques to measure steroid hormones in urine and feces, more studies have come to rely on hormone profiles to detect fetal loss as well (Campbell et al., 2001; Exner et al., 2003; Fortman et al., 1993; Guo et al., 1999; Kuehl et al., 1992; Lasley et al., 1995; Schwarzenberger et al., 1996; Tardif et al., 2005). However, despite the impact that fetal loss has on individual reproductive success, the reproductive endocrinology of fetal loss in wild populations remains largely undescribed (but see Curtis et al., 2000; Hackländer and Arnold, 1999; Schwarzenberger et al., 1996). Documenting fetal loss as well as generating an adequate sample size for detailed analyses is both labor-intensive and requires years of reproductive data, particularly for species with long gestation periods. Because over 5 years of fecal hormone data in addition to over 30 years of background reproductive and life-history data have been collected on the yellow baboons (*Papio cynocephalus*) of the Amboseli basin in Kenya, this dataset is ideal for comparing the steroid hormones of successful pregnancies with those of pregnancy failures.

Thus, the objectives of the present study were (1) to describe the normative patterns of fecal steroid hormones (estrogens, progestins, and glucocorticoids) across pregnancy in wild baboons, (2) to use fecal hormones to validate our longstanding visual method of pregnancy detection, (3) to investigate whether fecal steroids can be used to detect fetal loss, and if so (4) to determine at which stage of pregnancy failure can be detected, and finally (5) to document the rate and timing of fetal loss across pregnancy in wild baboons. To meet these objectives, we examined fecal steroid hormones from 188 pregnancies across 5 years from five groups of Amboseli baboons.

Methods

Study site and study population

The data for this study come from five groups of yellow baboons in the Amboseli basin of Kenya. Individual life-history data for members of these study groups cover more than three decades (e.g., Alberts and Altmann, 1995; Alberts et al., 1996, 2003; Altmann and Alberts, 2003b, see http://www.princeton.edu/~baboon for a complete bibliography and the Baboon Project Monitoring Guide, which outlines data collection protocols; Altmann et al., 1988; Pereira, 1988; Shopland, 1987). Subjects included all females with at least one pregnancy between Dec 1999 and Dec 2004. For this population, the mean age for first pregnancy is 5.5 years, and the ages of all subjects ranged from 4.8 to 23.2 years.

Hormone data

The fecal sample collection, storage, and extraction were as described previously (Khan et al., 2002; Lynch et al., 2003). In brief, samples were mixed thoroughly, placed in 95% ethanol and stored in a charcoal refrigerator (~20–25°C) until shipped to the University of Nairobi (once every 2 weeks), where the ethanol was evaporated, and samples were freeze dried. Following freeze drying, samples were stored at −20°C until shipped to the US. After transport to Princeton University, each fecal sample was sifted to remove vegetative matter, and 0.2 g fecal powder was extracted into 2 ml 90% methanol and then run through a prepped Oasis cartridge for solid phase extraction. Prior to assay, all samples were stored at −20°C.

As part of the continuing Amboseli baboon research, repeated fecal samples are collected opportunistically from all group members (starting December 1999). In particular, we attempted to collect every fecal sample that we observed being deposited over the course of the day. All data collection procedures adhered to the Institutional Animal Care and Use Committee guidelines of Princeton University. For this study, we analyzed fecal samples for all pregnant females from December 1999 to June 2004.

A total of 1388 fecal samples from 188 pregnancies (75 different females) were assayed for estrogen (E), progestin (P), and glucocorticoid (GC) metabolites using modified protocols of commercially available radioimmunoassay kits (see protocols in Altmann et al., 2004; Khan et al., 2002; Lynch et al., 2003). The primary antibody in the Total Estrogens Kit (ICN Diagnostics, Costa Mesa, CA) cross-reacts 100% with estradiol-17β and estrone, 9.0% with estriol, 7.0% with estradiol-17α, and 2.5% with equilin (ICN Diagnostics). Inter-assay coefficients of variation for the estrogen assays ($n = 10$) were 13% and 7% for a low and high control and 14% and 11% for a low and high fecal pool, respectively. The primary antibody in the ^{125}I Direct Progesterone Kit (Pantex, Santa Monica, CA) cross-reacts 100% with progesterone, 0.5% with 17a-hydroxyprogesterone, and 0.1% with androstenedione (Pantex). For progesterone assays ($n = 10$), the inter-assay coefficients of variation were 12% for a control standard, and 12% and 9% for a low and high fecal pool. The primary antibody in the Corticosterone Kit for Rats and Mice (ICN Diagnostics, Costa Mesa, CA) has high cross-reactivities with the major cortisol metabolites present in baboon feces (Wasser et al., 2000). Inter-assay coefficients of variation for the corticosterone assays ($n = 10$) were 12% and 10% for a low and high control standard and 8% and 12% for a low and a high fecal pool. Intra-assay coefficients of variation for all assays were below 15% (any above 15% were re-assayed). All hormones are expressed as nanograms per gram dry feces.

Determination of pregnancy

Demographic and reproductive data were extracted from the long-term database, BABASE. All pregnancies had been previously scored by one of us (J. A.) based on the same criteria for the past 30 years of the project. These criteria include primarily the cessation of sexual cycling (>40 days) without evidence of menstruation (i.e., vaginal bleeding followed by sexual swelling within a week).

The first day of pregnancy was counted as the day a female's sexual swelling began to deturgesce (d-date).

Determination of fetal loss

In most cases, assigned pregnancies that did not result in a live birth were considered to be fetal losses. In a few cases (see below), the post hoc hormone analysis revealed either an additional fetal loss (a previously undetected pregnancy) or a false positive pregnancy (female was not pregnant). Females that disappeared during pregnancy were not included in any analyses except the analysis on the timing of fetal loss across gestation (see below).

For observed (dead infant found) and suspected stillbirths (no dead infant found, but female retained external signs of pregnancy until expected birth date), the loss date was recorded as the observed birth date or the expected birth date, respectively. When blood was observed in the vaginal region of females that eventually miscarried, the loss date was assigned post hoc as the first day the blood was observed. When a fetal loss was not accompanied by blood or a dead infant, the loss date was estimated based on data extracted from BABASE from over 70 pregnancies (from previous years, 1971 to 2004) where blood was observed in conjunction with fetal loss. We used fetal loss data from all pregnancies with unambiguous loss dates (i.e., those accompanied by vaginal bleeding or a dead infant) to calculate the mean number of days between fetal loss and the resumption of sexual swelling (turgescence or 't-date'). We calculated separate means for first trimester (10 days, $N = 13$), second trimester (18 days, $N = 23$), and third trimester (22 days, $N = 35$) fetal losses. The loss date was estimated as t-date minus the mean for that trimester. Using this estimation method, we estimated loss dates for eight pregnancies from which we had hormonal data but no external indicators of fetal loss. We excluded four possible first trimester fetal losses because we did not have any fecal samples during the critical determination period (i.e., 3 weeks after d-date to 10 days before t-date).

Data analysis

Hormone profiles of successful pregnancy

We analyzed fecal estrogen (fE), progestin (fP), and glucocorticoid (fGC) metabolites across all successful pregnancies (pregnancies resulting in live birth). Because hormone measures were not distributed normally, we used a log transformation on all raw hormone values, which produced an approximately normal distribution for each hormone data set. The hormone data set is a mixed longitudinal–cross-sectional one, with sampling across gestation weeks and across females. To avoid any bias of an uneven fecal sample distribution, we divided gestation into 1-week periods and calculated hormone means for each pregnant female per week. Then we calculated hormone means (±SE) for each of the 26 weeks of gestation (mean gestation is 178 days) and 2 weeks postpartum, using only one mean per female per week. Each weekly hormone mean does not necessarily contain a hormone value from all females because fecal sampling was of necessity ad libitum.

We then used multiple regression to determine whether four demographic variables, age (in years), dominance rank, parity (number of pregnancies), and number of females in the group, predicted steroid hormone residuals for fE, fP, or fGCs. We used hormone residuals to control for time into gestation (see below).

Validating visual method of pregnancy detection

Regardless of how females had been scored (pregnant or not), we identified all sexual cycles where the luteal phase (i.e., d-date to menstruation) was longer than the average luteal phase for the population and no menstruation was observed. We then explored these 'possible' pregnancies in more detail by using ovarian hormones to determine whether females were correctly scored. We calculated a threshold value for fE and fP concentrations using hormone samples of 117 known, non-conceptive cycles (only samples from non-swollen females were included in the calculation). This threshold was 61.87 ± 11.49 ng/g for fE and 88.07 ± 4.28 ng/g for fP. Note that the ovarian hormones of conceptive and non-conceptive cycles are indistinguishable until the third week of pregnancy for fE (Fig. 1) and the second week of pregnancy for fP (Fig. 2). Therefore, we examined the hormones of possible pregnancies only during or after the third week of pregnancy. If samples collected from a female with a possible pregnancy were higher than the fE and fP thresholds, we assigned pregnancy to that female.

Detecting fetal loss

We calculated a mean hormone value per female per trimester for four different categories (Table 1): (1) successful pregnancies, (2) early fetal loss (loss in the first trimester), (2) mid-fetal loss (loss in the second trimester), and (4) late fetal loss (loss in the third trimester or stillbirth). We refer to first trimester miscarriages as fetal (rather than embryonic) loss on the assumption that physiological maturation of the placenta has already taken place (the fetal–placental shift in progesterone production in the baboon occurs around 21–25 days into gestation, Castracane and Goldzieher, 1986). Fecal samples collected on or after the estimated loss date were not included in the calculation of loss means. We used one-way ANOVA to compare the hormones of successful pregnancies to those of loss for each trimester.

Predictive value of steroid hormones in detecting loss

To control for time into gestation, we generated residual hormone values using a locally weighted regression procedure (LOWESS) on the full set of raw hormone values. After removing hormone outliers (2 SD above or below the mean for each trimester), we plotted the hormone concentration of each sample as a function of days into gestation (where onset of deturgescence equals day 0). We used a sampling proportion of 0.35 (we lowered the sampling proportion in 0.05 increments from 0.5 until all expected values were non-negative) in Sigma Plot 8.0 (SPSS Inc., 2002). The residuals were calculated for each sample as the ratio of the observed to the predicted values as determined by the LOWESS regression. We then log transformed all residuals to achieve a normal distribution. As such, females with relatively high hormone values for that stage of pregnancy will have positive log residuals, and those with low values will have negative ones. First, we used ANOVA to compare the hormone residuals of fetal loss with those from successful pregnancies. Next, using only the residuals from fetal losses, we calculated mean residual values for each week prior to fetal loss and used a binomial test to assess whether hormones were significantly different from zero for different weeks prior to loss (hormone residuals from successful pregnancies were not significantly different from zero). Where significant differences existed, we conducted a logistic regression analysis with maximum likelihood estimators (SPSS, version 11.0) to determine the likelihood that a female's hormone levels (or hormone residuals, to control for gestation stage) could predict an impending fetal loss. For each pregnancy, one hormone mean was calculated per trimester and entered into the logistic analysis.

Rate and timing of fetal loss

To assess the timing of fetal loss across pregnancies, we constructed Kaplan–Meier failure time curves and conducted a stratified analysis based on the loss dates of fetal losses for pregnancies from two different periods: (1) January 1976–November 1999 and (2) December 1999–December 2004. The first period includes many more pregnancies ($N = 440$), while the second period represents pregnancies from which we had hormone data ($N = 188$) and were thus able to include additional, hormonally detected cases of early fetal loss. Including pregnancies without hormone data from the latter period ($N = 28$), we examined a total of 656 pregnancies. In our analysis, an 'event' was fetal loss, and a 'non-event' was a pregnancy resulting in live birth. Females that disappeared before parturition were included as censored cases (1971–1999: $N = 17$; 1999–2004: $N = 3$).

Results

Hormone profiles of successful pregnancy

Both fE and fP concentrations increased across pregnancy, with the most rapid elevations occurring in the first trimester (Figs. 1a, 2a). By the end of the first trimester, fE had increased nearly 8-fold, and fP was double that of pre-pregnancy. Although fE continued to rise across each trimester, no further elevation in fP occurred after the second

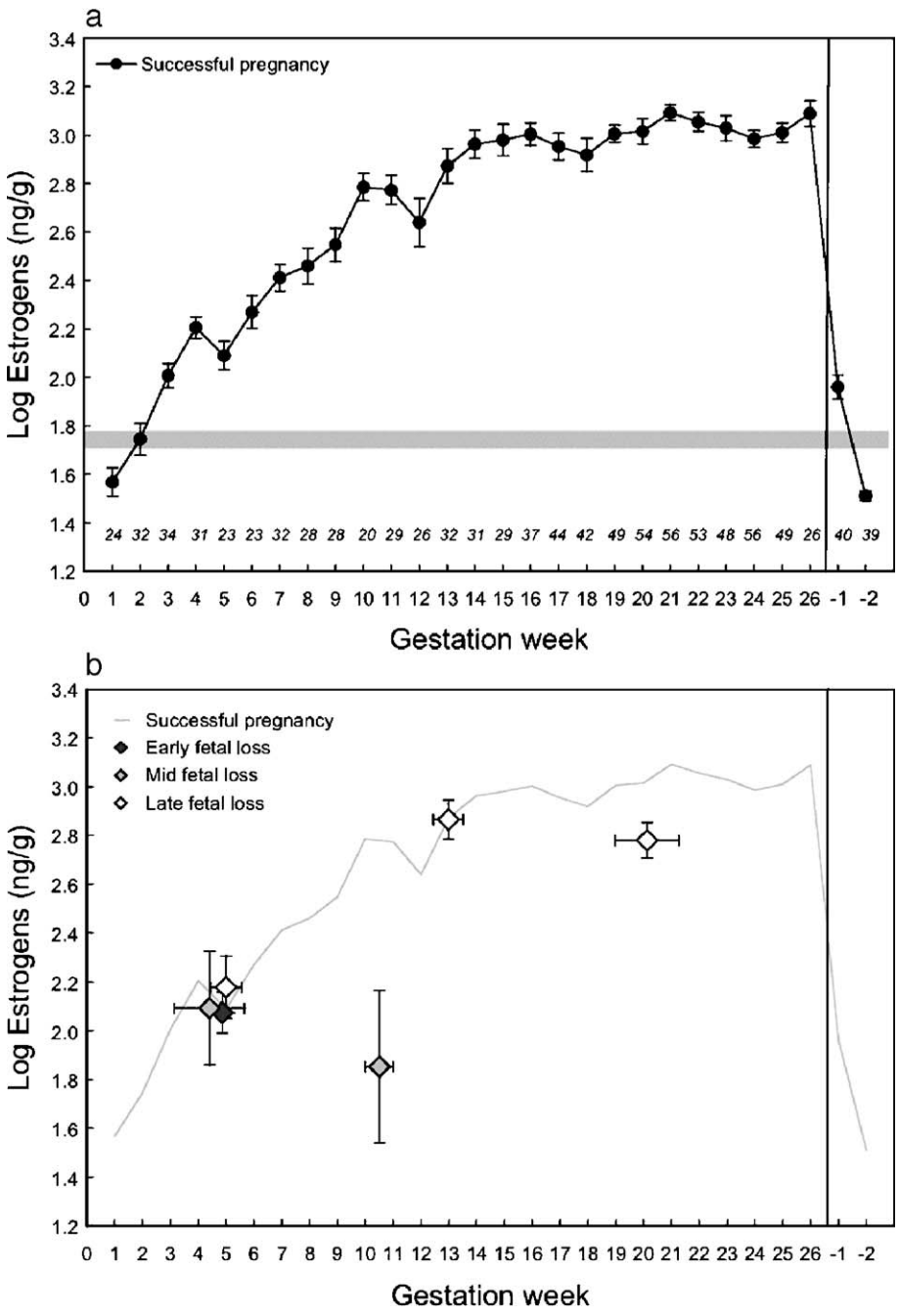

Fig. 1. (a) For successful pregnancies, the black circles represent weekly mean fE concentrations (±SE) throughout gestation. Hormone means begin at ovulation (week 1) and extend to 2 weeks postpartum (weeks −1 and −2). The vertical line indicates parturition, and the horizontal grey bar represents mean fE (±SE) for deturgescent females in the luteal phase of the menstrual cycle. The italicized numbers immediately above the *x*-axis represent the number of successful pregnancies that contributed to weekly hormone means. (b) For fetal losses, the different colored diamonds represent trimester mean fEs (±SE) depending on whether the loss occurred during early- (black), mid- (light grey), or late-pregnancy (white). Fetal loss sample sizes are listed in Table 1.

trimester. At the time of parturition, fE concentrations were approximately 27 times higher than luteal phase levels, while fP concentrations were only 2.5 times higher. Within 2 weeks post-parturition, both fE and fP concentrations returned to pre-pregnancy levels.

During the first trimester, fGC concentrations for pregnant females were within the range of non-pregnant values (Fig. 3a). Fecal GC concentrations exhibited less marked changes than the other steroids we measured, with only moderate rises in the second and third trimester of pregnancy. Within 2 weeks postpartum, fGC concentrations returned to pre-pregnancy levels.

Neither fE nor fP concentrations for full term pregnancies were related to age, dominance rank, parity or number of females in the group (fE: $R^2 = 0.01$, $P = 0.77$; fP: $R^2 = 0.04$, $P = 0.16$). While age, rank, and parity were unrelated to fGC concentrations, the number of females in the group was a significant predictor for fGC levels ($R^2 = 0.08$, $P = 0.01$).

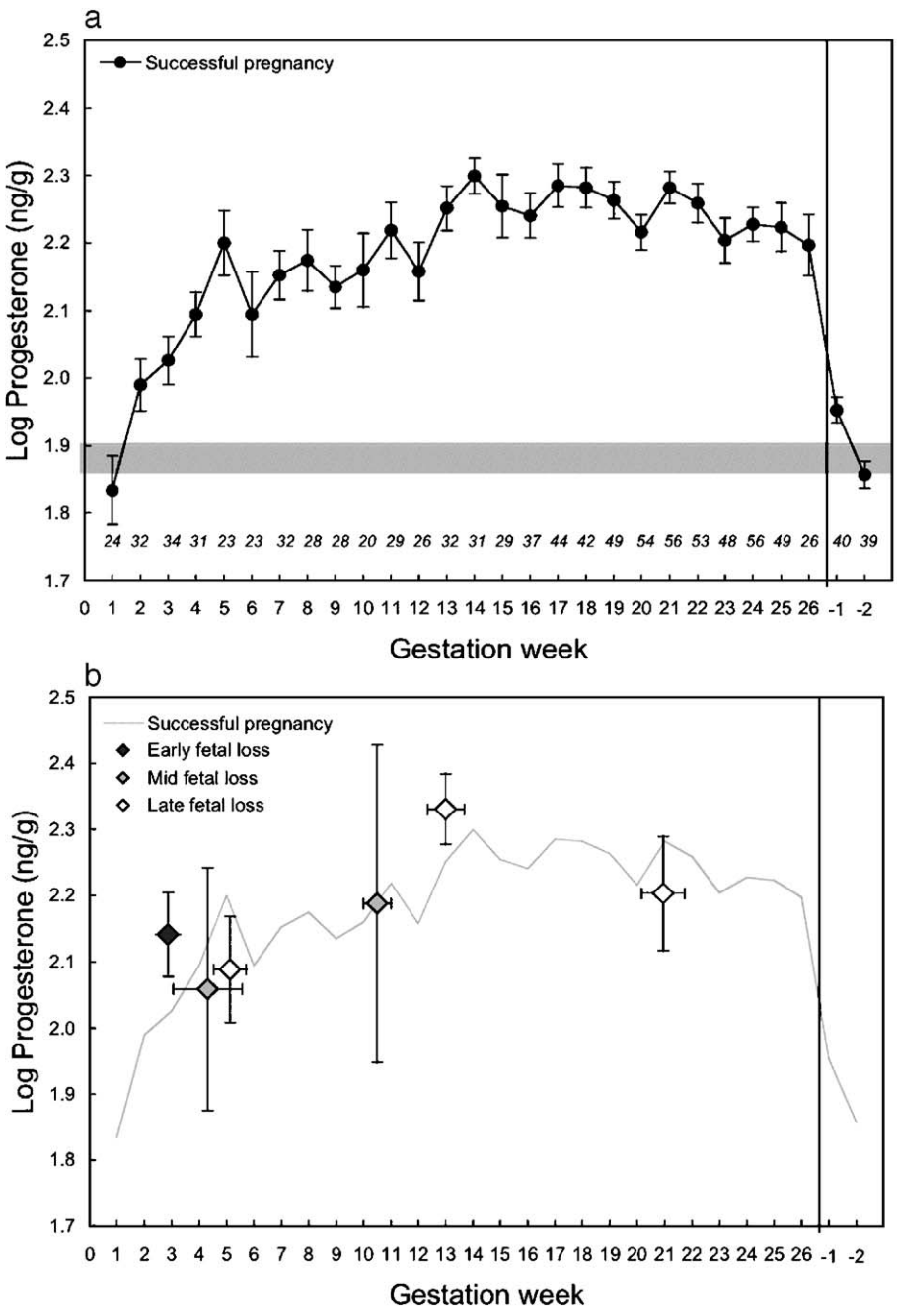

Fig. 2. (a) For successful pregnancies, the black circles represent weekly mean fP concentrations (±SE) throughout gestation. Hormone means begin at ovulation (week 1) and extend to 2 weeks postpartum (weeks −1 and −2). The vertical line indicates parturition, and the horizontal grey bar represents mean fP (±SE) for deturgescent females in the luteal phase of the menstrual cycle. The italicized numbers immediately above the *x*-axis represent the number of successful pregnancies that contributed to weekly hormone means. (b) For fetal losses, the different colored diamonds represent trimester mean fPs (±SE) depending on whether the loss occurred during early- (black), mid- (light grey), or late-pregnancy (white). Fetal loss sample sizes are listed in Table 1.

Controlling for pregnancy stage, individuals in groups with more females had lower GC concentrations (Fig. 4).

Validating visual method of pregnancy detection

Post hoc hormone analysis of possible pregnancies indicated that that our longstanding method of pregnancy detection using external indicators only failed to detect 4 early pregnancies from a total of 257 possible pregnancies where hormone data were available (1.6% false negatives). Post hoc analysis also revealed that 2 assigned pregnancies were, in fact, not pregnancies (0.8% false positives) but rather extended periods of no cycling (cycle 'shut-down'). The duration of these shut-down periods was 55 and 61 days before the normal swelling of cycling resumed.

Detecting fetal loss

Of the 188 pregnancies with hormonal data available, 166 resulted in successful pregnancies and 22 resulted in fetal loss.

Table 1
Comparison of hormone means for successful pregnancies and fetal losses by trimester of loss and by trimester of hormone collection

	Hormone means (ng/g)						ANOVA		
	Successful pregnancy	*N*	SE	Fetal loss	*N*	SE	*F*	*df*	*P*
Fecal estrogens									
1st trimester	217.9	115	16.1	126.6	7	27.3	0.39	1,120	0.54
1st trimester	217.9	115	16.1	198.4	4	80.2	0.12	1,117	0.73
2nd trimester	1005.8	120	57.2	90.7	2	56.0	23.19	1,120	<0.001**
1st trimester	217.9	115	16.1	250.7	9	60.7	0.15	1,122	0.70
2nd trimester	1005.8	120	57.2	843.7	10	120.9	0.01	1,128	0.91
3rd trimester	1257.9	132	58.5	757.0	7	193.1	5.62	1,137	0.019*
Fecal progestins									
1st trimester	151.1	116	6.5	133.0	7	31.0	0.19	1,121	0.66
1st trimester	151.1	116	6.5	156.6	4	45.8	0.04	1,118	0.83
2nd trimester	207.3	120	8.0	178.2	2	89.5	0.28	1,120	0.59
1st trimester	151.1	116	6.5	149.3	9	24.1	0.01	1,123	0.92
2nd trimester	207.3	120	8.0	271.5	10	46.9	2.80	1,128	0.10
3rd trimester	253.3	132	15.3	198.9	7	54.1	2.18	1,137	0.14
Fecal glucocorticoids									
1st trimester	79.2	116	3.4	85.6	7	19.5	0.37	1,121	0.54
1st trimester	79.2	116	3.4	101.6	4	23.0	1.31	1,118	0.26
2nd trimester	87.9	120	3.2	81.1	2	12.3	0.01	1,120	0.94
1st trimester	79.2	116	3.4	74.7	9	8.1	0.01	1,123	0.93
2nd trimester	87.9	120	3.2	82.2	10	9.6	0.08	1,128	0.78
3rd trimester	93.6	132	2.9	83.9	7	6.6	0.23	1,137	0.64

Bold text indicates the trimester of the fetal loss.

Mean fE concentrations from mid- and late-pregnancy fetal losses were significantly lower than those of successful pregnancies, but only during the trimester of loss (Fig. 1b, Table 1). Fecal E concentrations from all previous trimesters as well as those from early fetal losses were not significantly different from successful pregnancy means (Table 1). None of the fetal loss fP concentrations were significantly different than successful pregnancy levels, and the same was also true for fGC concentrations (Figs. 2b, 3b, Table 1).

Predictive value of steroid hormones in detecting loss

Fecal E residuals for fetal losses (controlling for time into gestation) were significantly lower than successful pregnancies (ANOVA: $F_{1,1275} = 4.92$, $P = 0.03$). When all weeks prior to fetal loss were included in the analysis, mean fE residuals did not differ significantly from zero (binomial test: $N = 25$, $P = 0.11$). However, when we evaluated only the weeks leading up to the miscarriage (up to 9 weeks, or approximately one trimester prior to loss), mean fE residuals were significantly lower than zero (binomial test: $N = 9$, $P = 0.02$, Fig. 5). Mean fP residuals for fetal losses did not differ significantly from successful pregnancies (ANOVA: $F_{1,1313} = 2.44$, $P = 0.12$) or from zero when we included all weeks (binomial test: $N = 26$, $P = 0.56$) or just the 9 weeks prior to fetal loss (binomial test: $N = 9$, $P = 0.11$). Similarly, mean fGC residuals for fetal losses did not differ from successful pregnancies (ANOVA: $F_{1,1310} = 0.37$, $P = 0.55$) or from zero when all weeks (binomial test: $N = 26$, $P = 1.00$) or 9 weeks prior to loss were evaluated (binomial test: $N = 9$, $P = 0.75$).

A binary logistic regression model with fetal loss as the dependent variable (loss/no loss) and all trimester means for fE residuals as the predictor variable was not significantly different from a model with only the intercept (model $\chi^2 = 1.12$, $df = 1$, $P = 0.29$, −2 log likelihood = 248.64, Cox and Snell $R^2 = 0.003$). However, when only fE residuals from the trimester of the fetal loss were included in the logistic analysis (fE residuals from other trimesters were discarded for females with losses), the model was significant (model $\chi^2 = 4.15$, $df = 1$, $P = 0.04$, −2 log likelihood = 129.63, Cox and Snell $R^2 = 0.01$); pregnancies characterized by lower fE residuals were more likely to result in fetal loss (fE residuals: $B = -1.35$, SE = 0.60, Wald score = 5.04, $df = 1$, $P = 0.03$, Exp(B) = 0.26; constant: $B = -3.36$, SE = 0.29, Wald score = 133.74, $df = 1$, $P < 0.01$, Exp(B) = 0.04).

Rate and timing of fetal loss

The Kaplan–Meier estimate of the overall probability of pregnancy success is 0.87 and 0.88 for the 1976–1999 and the 1999–2004 datasets, respectively (Fig. 6). Pregnancy success was not significantly different across time periods (Cox

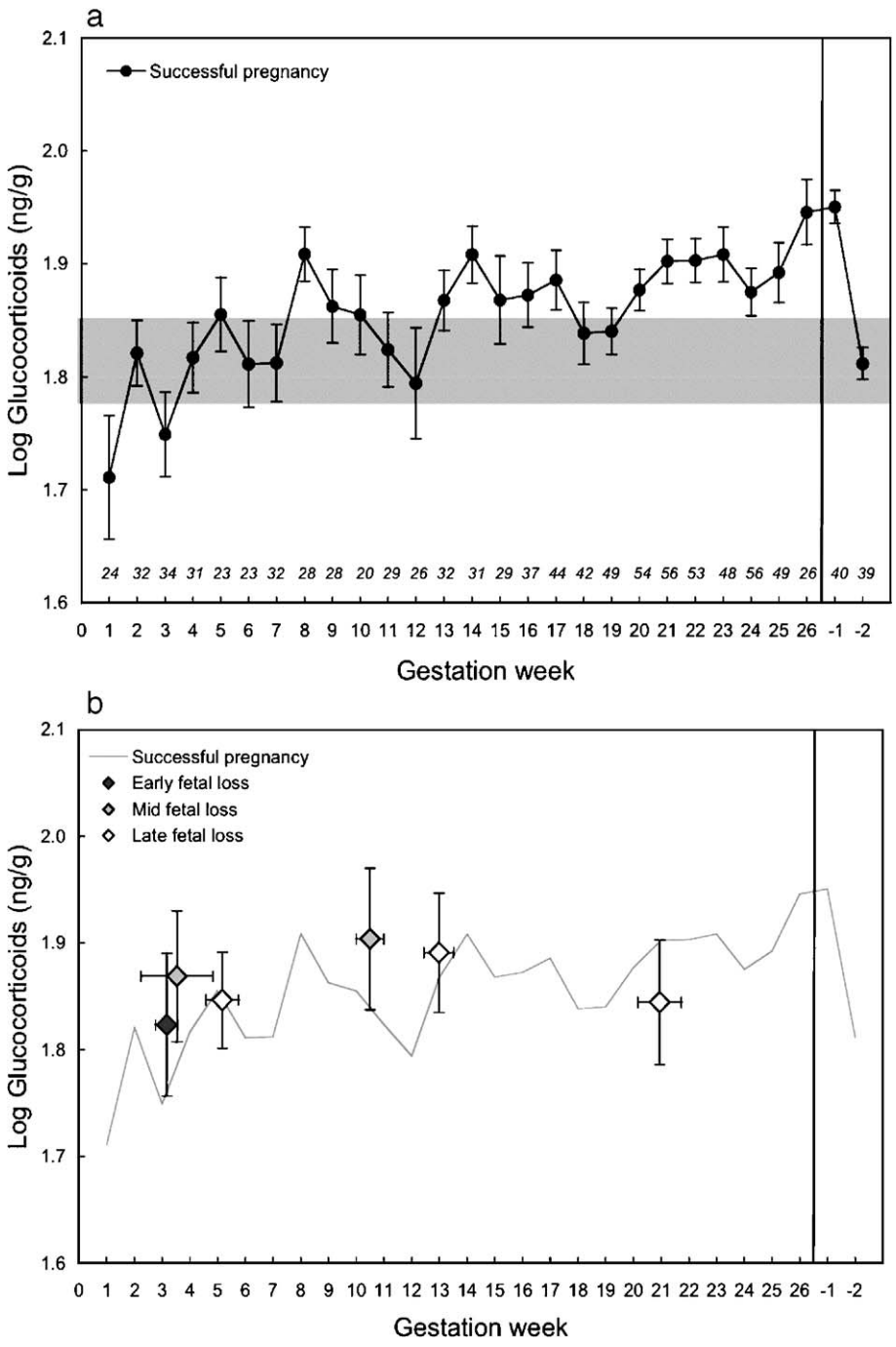

Fig. 3. (a) For successful pregnancies, the black circles represent weekly mean fGC concentrations (±SE) throughout gestation. Hormone means begin at ovulation (week 1) and extend to 2 weeks postpartum (weeks −1 and −2). The vertical line indicates parturition, and the horizontal grey bar represents mean fGCs (±SE) for deturgescent females in the luteal phase of the menstrual cycle. The italicized numbers immediately above the *x*-axis represent the number of successful pregnancies that contributed to weekly hormone means. (b) For fetal losses, the different colored diamonds represent trimester mean fGCs (±SE) depending on whether the loss occurred during early- (black), mid- (light grey), or late-pregnancy (white). Fetal loss sample sizes are listed in Table 1.

regression: $\chi^2 = 0.21$, $P = 0.65$). When both datasets were combined, pregnancy success was significantly different across first and second trimesters and second and third trimesters (Cox regression: $\chi^2 = 9.3$, $df = 2$, $P = 0.01$). Table 2 lists the estimates of fetal survival probability across the stages of pregnancy used to construct these curves.

From 1976 to 2004, we recorded a total of 91 fetal losses in 656 pregnancies for an overall loss rate of 13.9%. Twenty-three fetal losses (3.5%) occurred in the first trimester, 29 losses (4.4%) occurred in the second trimester, and 39 losses (6.0%) occurred in the third trimester. The visual method of pregnancy scoring added 1 early and 1 mid-loss and missed 4 early losses per 188 pregnancies (i.e., 1 additional pregnancy and early loss per 63 detected pregnancies and 1 less pregnancy and mid-loss per 188 pregnancies). If we apply this error rate to the non-hormone dataset, an estimated 30 losses occurred in the first

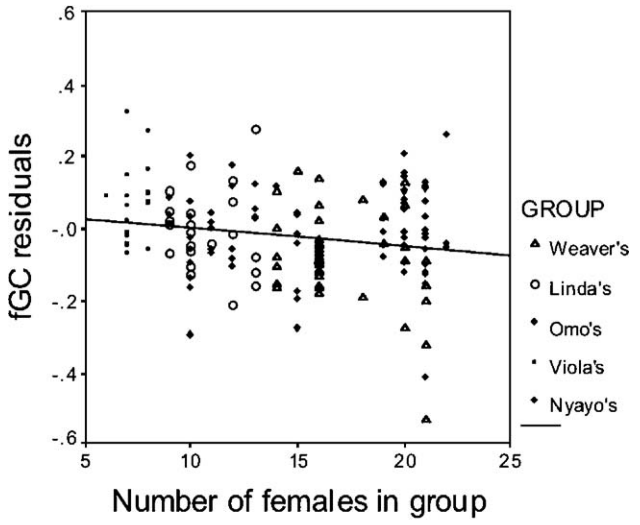

Fig. 4. Fecal GC residuals (controlled for pregnancy stage) as a function of number of females in the group (multiple regression: $R^2 = 0.08$, $P = 0.01$). Different groups are represented by different shapes, and each point represents one pregnancy.

trimester (4.5%), 27 losses occurred in the second trimester (4.1%), and 39 losses occurred in the third trimester (5.9%) for an overall fetal loss rate of 14.5% (96 losses of 661 pregnancies).

Discussion

Ovarian hormones

By monitoring fecal ovarian hormones (fE and fP) of Amboseli baboons, we were able to detect baboon pregnancy by the third week of gestation (or approximately 10 days after expected menstruation). Ovarian hormone profiles for successful pregnancies mirror serum levels reported for captive baboons (progesterone: Albrecht and Townsley, 1976, 1978; Dawood and Fuchs, 1980; estrogens: Townsley, 1974) and closely resemble steroid hormones for human pregnancies (Tulchinsky et al., 1972). The relatively linear increase for estrogens with advancing gestation is a common feature of most primate species (baboons: Albrecht and Townsley, 1978; macaques: Bosu et al., 1973; chimpanzees: Reyes et al., 1975; Townsley, 1974; humans: Tulchinsky et al., 1972). However, published values from baboons as well as humans exhibit an exponential increase in estrogen production in the weeks just before parturition. The fecal estrogen profiles from Amboseli females, however, do not exhibit a marked increase in fecal estrogens in the weeks prior to parturition (Fig. 1). Because the antibody used in this study cross-reacts equally (100%) with estradiol and estrone, the source of this difference is difficult to determine.

Serum progesterone concentrations in captive baboons were reported to rise until approximately day 60 and then level off from mid-gestation to term (Albrecht et al., 1980; Albrecht and Townsley, 1976). Fecal progestins in our subjects continued to rise until about day 90 before reaching a plateau until term (Fig. 2). Among primate species, baboons appear to be intermediate between rhesus macaques, in which no increase in progesterone occurs during pregnancy, and hominoids (apes and humans) in which a marked elevation occurs throughout pregnancy (Albrecht and Pepe, 1990). However, differences in the qualitative patterns and quantities of progesterone among different primate species are not thought to serve different biological actions (Albrecht and Pepe, 1990).

The trimester of fetal loss was associated with a substantial decline in fecal estrogen but not fecal progestin concentrations. As such, fecal estrogens were able to predict pregnancy failure up to 2 months prior to the estimated loss date (Fig. 5). Prior to this period, estrogen concentrations of unsuccessful and successful pregnancies were indistinguishable. Another study on fetal loss in primates (captive common marmosets) also found that estrogen concentrations were significantly lower during the trimester of loss (Tardif et al.,

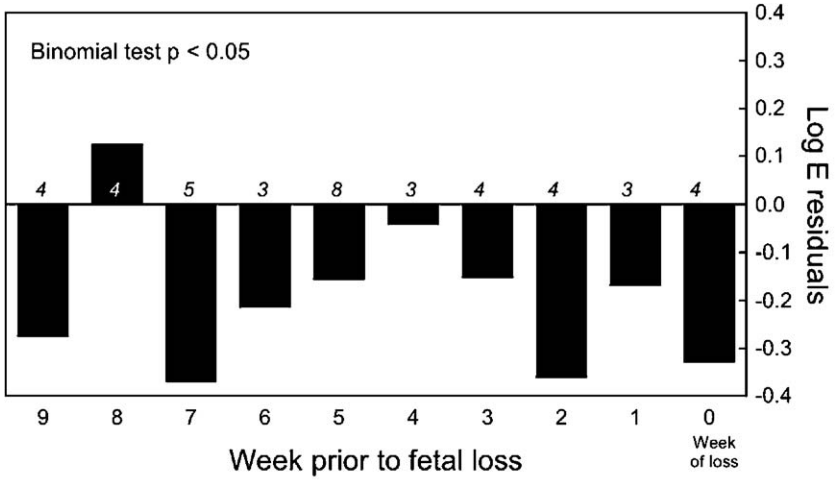

Fig. 5. Mean fE residuals for unsuccessful pregnancies for the 9 weeks prior to fetal loss. Estrogen residuals were determined based on week of gestation (as deviations from a LOWESS regression of all pregnancies), not week of loss. Numbers above the bars represent the number of unsuccessful pregnancies that contributed to each mean.

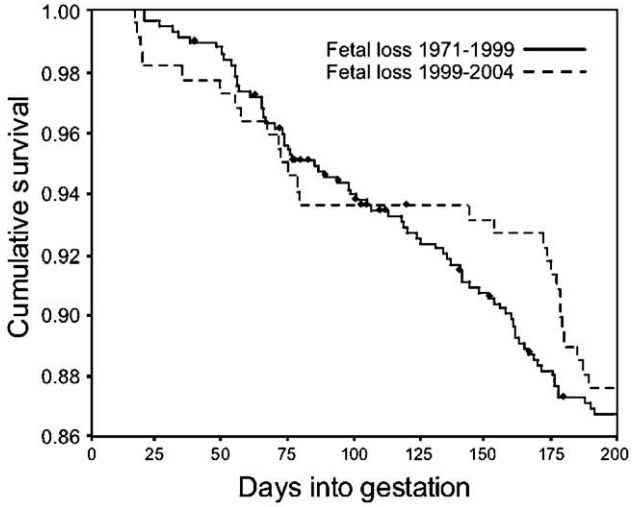

Fig. 6. Kaplan–Meier survival curves for fetal losses between the years 1971–1999 (solid line) and 1999–2004 (dotted line). If the mother disappeared before parturition, the pregnancy was censored (black diamonds). Hormonal data available for the 1999–2004 dataset were used to correct for errors in pregnancy detection. Survival curves for the two periods are not significantly different (Cox regression: $\chi^2 = 0.21$, $P = 0.65$).

2005). The precipitous decrease in fecal estrogens both following parturition and during the weeks prior to fetal loss points to the dependence of estrogen production on a functioning fetus and/or placenta (see also Townsley, 1974). As reviewed by Pepe and Albrecht (1995), estrogen stimulates key steps in progesterone biosynthesis in the syncytiotrophoblasts of the placenta, as well as uteroplacental blood flow. By stimulating progesterone production, estrogens also indirectly promote uterine myometrial quiescence and permit implantation of the placenta and developing embryo—all processes critical for the maintenance of pregnancy. Specifically with respect to baboons, a 50% miscarriage rate was observed among captive baboons in which maternal serum estrogen concentrations were experimentally suppressed (Albrecht et al., 2000), while pregnancy was maintained in all baboons in which the estrogen levels were restored to normal levels.

One of the physiological functions of the primate fetal adrenal cortex is to provide C_{19} substrate to the placenta for the formation of estrogens, a strategy for estrogen formation that is unique to primate species (Diczfalusy, 1964; Novy, 1977). If impaired placental or fetal function occurs during gestation, the biosynthesis of estrogens will thus be compromised. In pregnant baboons, following the removal of the fetus while leaving the placenta in utero (fetectomy), progesterone concentrations decreased by 20–45%, and maternal estradiol concentrations dropped to basal values (Albrecht et al., 1980; Albrecht and Pepe, 1985). Similar results were also found following fetectomy in macaques (Tullner and Hodgen, 1974) and intrauterine fetal death or fetal anencephaly in humans (reviewed in Albrecht and Pepe, 1990). Although the fetus in primate pregnancy is essential to the maternal production of estrogen, it is not a prerequisite for the production of progesterone (reviewed in Albrecht and Pepe, 1990). In primate pregnancy, cholesterol is taken up from the maternal circulation and utilized by the placenta for progesterone biosynthesis (Hellig et al., 1970; Winkel et al., 1980). Nevertheless, estrogens themselves play an indirect role in the stimulation of progesterone production and regulation of progesterone receptors (Castracane et al., 1983; Henson, 1998). Therefore, in the absence of compensatory systems, fetal demise will eventually lead to subnormal levels of progesterone in maternal circulation (Albrecht and Pepe, 1990).

In sum, our results indicate that fecal estrogens, and not progestins, signal the first signs of fetal loss in this population of wild baboons. Although the nature of our data does not permit us to determine whether the decline in estrogens was causal or only symptomatic of fetal loss, the significant decrease in fE but not fP concentrations suggest a fetal and not a placental origin of failure in our subjects.

Glucocorticoids

Fecal glucocorticoid concentrations for pregnant Amboseli females also corresponded to serum and urinary glucocorticoid profiles reported for humans and several non-human primates (e.g., Carr et al., 1981; Smith et al., 1993; Tardif et al., 2005; Wintour et al., 1978). An elevation in glucocorticoid concentrations was observed during the second trimester, followed by only small changes in late gestation until parturition (Fig. 3). In both human and non-human primates, pregnancy results in an activation of the HPA axis, which increases glucocorticoid secretion overall (Keller-Wood and Wood, 2001). In primate pregnancy, increased HPA activity is necessary for the maintenance of intrauterine homeostasis and the maturation of essential organ systems including the lungs, liver, and the gut (Bolt et al., 2001; Mesiano and Jaffe, 1997). Because the fetal adrenal does not produce glucocorticoids until late gestation (Jaffe et al., 1979; Voutilainen and Miller, 1987), hormones secreted by the placenta play a central role in mediating the increased HPA activity. As such, pregnancy should be considered a physiological state in which increased glucocorticoids (over non-pregnant levels) are of adaptive value.

Nevertheless, animal experiments have demonstrated that exposure of pregnant females to stressful conditions can still result in a compromised pregnancy or fetal demise (Clark et al., 1993; reviewed in de Catanzaro and Macniven, 1992; Johnson et al., 1991; see also Wildt et al., 1977). Additionally, well-controlled studies in humans also suggest a direct relationship

Table 2
Kaplan–Meier estimates for survival of pregnancies across trimesters for two data sets, one without (1971–1999) and one with (1999–2004) hormone data for pregnancy validation

	1971–1999			1999–2004		
	Survival probability	SE	No. in risk set	Survival probability	SE	No. in risk set
1st trimester	0.972	0.007	569	0.964	0.013	218
2nd trimester	0.927	0.011	552	0.936	0.017	211
3rd trimester	0.864	0.014	515	0.876	0.022	203

between prenatal maternal stress and a number of pregnancy complications (reviewed in Mulder et al., 2002). The relationship between pregnancy failure and the hormone mechanisms that mediate loss in stressful situations are not completely understood. Although increasing evidence suggests that maternal stress can critically alter ovarian steroids and thus endanger pregnancy maintenance (reviewed in de Catanzaro and Macniven, 1992), many other studies found an association between conventional "stress" hormones and elevated fetal mortality or morbidity across a wide array of taxa (mice: Baumgartner and Chrisman, 1987; rats: Davis and Plotz, 1954; Hansen et al., 1999; La Borde et al., 1992; humans: Nepomnaschy, 2005; sheep: Quinlivan et al., 1998; Yang et al., 1969).

For the Amboseli baboons, pregnancy failure was associated with neither higher nor lower fecal glucocorticoid concentrations. However, pregnant glucocorticoid concentrations were negatively related to the number of females in the group (Fig. 4). As group size increased, females exhibited lower glucocorticoids for their stage of pregnancy than other females, possibly a sign of a compromised pregnancy. For many populations of baboons, including Amboseli, reproductive costs among females have been associated with larger groups (Altmann and Alberts, 2003a; Bulger and Hamilton, 1987; Rhine et al., 1988; Wasser and Starling, 1988). Although we did not find a direct association between glucocorticoids and fetal loss with the current dataset, an association between fetal loss and lower glucocorticoid concentrations may be revealed as more hormone data are collected. In common marmosets, Tardif et al. (2005) demonstrated that food restriction resulted in decreased maternal cortisol and ultimately to fetal demise and pregnancy loss. They suggest that food restriction does not act as a classical stressor with elevated HPA activity, but that endocrine function in the placenta is rapidly impaired by dietary restrictions (Tardif et al., 2005). In wild feeding baboons, larger groups may have decreased food intake, particularly during seasons when food resources are scarce; and drought conditions do indeed result in a higher probability of fetal loss in the Amboseli population (Beehner et al., submitted).

Validating visual method of pregnancy detection

By measuring steroid hormone profiles across reproductive females, we demonstrated that our longstanding external basis for early detection of pregnancies in the baboons closely matches hormonal identification of pregnancy. For decades, the method of pregnancy determination for the Amboseli baboons has of necessity been visual; mainly, one of us (J.A.) has assigned pregnancies when females ceased cycling and failed to menstruate prior to the resumption of cycling. Although this method of pregnancy detection requires frequent monitoring with very few gaps in data, we show here that it identifies 97% of pregnancies. Neither fecal hormone sampling nor visual detection identifies pregnancies that are terminated within the first 3 weeks, although urinary steroids are reported to detect pregnancy as early as day 13 post-ovulation, 1 week earlier (Hodges et al., 1986).

Rate and timing of fetal loss

The results from over 30 years of pregnancy data at Amboseli indicate that the (uncorrected) pregnancy failure rate is 13.9% (91 losses of 656 pregnancies). This loss rate is higher than that reported from two other long-term studies of wild yellow (10% or 17 losses of 170 pregnancies: Wasser, 1995) and anubis baboons (9.6% or 56 losses of 584 pregnancies: Packer et al., 1995), even if we do not adjust for detection error. In most wild baboon populations, reddening of the paracallosal skin is used to indicate pregnancy. However, this change does not occur until approximately mid-gestation and is highly variable. For example, Amboseli females exhibit some reddening of the paracallosal skin at approximately day 61 of gestation ($N = 199$ pregnancies, SE = 1.34 days, range = 10–113 days). Therefore, studies that rely on skin coloration for pregnancy detection may miss early-gestation losses that our method was able to pick up. Alternatively, ecological factors may play a role in this difference. For example, in Gombe, anubis baboons live in rich areas of evergreen forest where food resources are relatively abundant (Packer et al., 2000). In contrast, the Amboseli baboons spend the majority of the day foraging and moving between widely spaced food patches, particularly in dry years (Bronikowski and Altmann, 1996). Ecological factors can produce high variability in female reproduction on the proximate scale because reproductive physiology is sensitive to the availability of oxidizable metabolic fuels (Wade and Schneider, 1992) and on an evolutionary scale because decreased food intake must be balanced by less energy expenditure on activities such as reproduction that are not essential for immediate survival (Bronson, 1989).

Very little information about the timing of fetal mortality across gestation is available for wild baboons. Packer et al. (1995) report that miscarriages among anubis baboons occur at approximately equal rates across trimesters. Similarly our data indicate that fetal mortality in the baboon occurs throughout gestation.

Overall, the rate of miscarriage in Amboseli was remarkably similar whether hormone data were available or not. However, when we evaluated only the hormone dataset (dotted line in Fig. 6), the addition of early-gestation losses combined with the removal of mid-gestation losses revealed three critical periods marked by an increase in the rate of loss (i.e., a steeper curve): 20–25 days, 50–75 days, and 175–180 days into gestation. The first of these periods coincides with placentation in the baboon. Placentation, where the placenta becomes a significant source of progesterone, occurs at approximately days 20–25 of baboon pregnancy (Castracane and Goldzieher, 1986). The extent to which placental development occurs in this period may have a significant influence on subsequent fetal growth and survival. The second period may reflect a problem with the initial fetal adrenal production of C_{19} precursors for estrogen biosynthesis by the placenta (at approximately 40 days, Albrecht and Pepe, 1990). The primate placenta is capable of converting C_{19} steroids to estrogens but cannot produce C_{19}, while the fetal adrenal cortex produces large amounts of C_{19} steroids (reviewed in Mesiano and Jaffe, 1997). Thus, appropriate development

and function of the fetal adrenal cortex are critical for fetal maturation and perinatal survival. The last period marks normal parturition in the baboon (mean gestation in this population is 178 days). As far as these critical periods for fetal mortality in the present study are concerned, it is notable that increased rates of loss coincide with periods of evident changes in steroid synthesis and/or function.

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