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Stillbirths in *Macaca fascicularis*

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Abstract

Background—Stillbirths in nonhuman primates are a major problem and represent failure of the maternal-fetal-placental unit to maintain normal relationships due to various endogenous, undetermined, or environmental factors.

Methods—Records of 236 stillborns and their dams in a *Macaca fascicularis* colony during a 7-year period were reviewed retrospectively.

Results—The 7-year stillbirth incidence was 11.99% (236 stillbirths, 1,968 live births). Most (61.02%, *n*=144) were of undetermined etiology. Fetal causes included trauma (22.46%, *n*= 53), fetal pneumonia (0.85%, *n*=2) and congenital anomalies (0.42%, *n*=1). Maternal causes included dystocia (9.75%, *n*=23), placental abruption (0.85%, *n*=2), and uterine rupture (0.42 %, *n*=1). Forty-nine placentas were available for histologic evaluation; there was placentitis in five and necrosis in five. Most stillbirths occurred close to term. First stillbirths usually occurred in 8- to 12-year-old animals during the first six pregnancies.

Conclusions—Most stillbirths were of undetermined etiology. Fetal trauma was the most common cause.

Keywords

Stillborn; reproduction; cynomolgus; fetus; placenta; dystocia; nonhuman primate

Introduction

The use of nonhuman primates (NHPs) as models for the study of human disease has grown significantly over the past 50 years. They have played a major role in the study of reproductive and developmental processes and remain the model of choice for a variety of applications. The cynomolgus macaque (*Macaca fascicularis*) has been used in many types of reproductive research [4,8].

M. fascicularis exhibits birth seasonality. Sexual maturity in females is reached at 4 years of age and high-ranking daughters begin reproducing before 5.5 years of age, while low-ranking

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daughters begin reproducing after 5.5 years. The normal length of gestation is 155-165 days. Births which occur prior to gestation day (dG) 155 are considered preterm and those which occur prior to 140 dG are considered premature [2].

Stillbirths in NHPs represent failure of the maternal-fetal-placental unit to maintain a normal relationship due to the influence of a variety of endogenous or environmental factors [5,12]. The term stillbirth is used by some to indicate the delivery of a macerated or mummified fetus or for any infant for which there was direct clinical or pathologic evidence that the animal did not breathe at birth [14]. Several authors define fetal loss prior to 140 dG in *M. fascicularis* as abortion; after 140 dG they consider them to be stillbirths because these infants would be capable of survival [5,12,14,18]. Others authors defined stillbirths as all pregnancy outcomes other than live births, including abortion and fetal death of undetermined etiology [10]. Retrospective data on the frequency of prenatal loss in *M. fascicularis* showed the rate of stillbirths increases during late gestation (>150 dG) [12].

The causes of stillbirths remain incompletely understood in humans, and are frequently categorized by presumed etiology. Non-infectious causes of stillbirths include congenital abnormalities, placental abruption, maternal trauma, fetal trauma, and often associated with advanced maternal age [9,11]. Infectious causes of stillbirths include ascending and hematogenous infections [11].

NHPs are a suitable animal model to further our understanding of the specific causes of human stillbirth. Moreover, an accurate knowledge of the historical incidence of fetal loss is essential for management of breeding colonies and for performing developmental reproductive studies in NHPs. The aim of the present study is to evaluate the incidence and causes of fetal loss in a *M. fascularis* colony, including associated maternal risk factors.

Material and Methods

Pathology and clinical records of 236 stillbirths occurring in a *M. fascicularis* breeding colony during a 7-year period were reviewed. Clinical histories of 33 females that had complete records for a 20-year period and with one or more stillborn fetuses were analyzed for maternal age at time of stillbirth and reproductive history. Stillbirths were defined as those fetuses that were delivered without inflation of the lungs, which was generally established by determining if the lungs floated in formalin solution; absence of ingesta in the stomach; and presence of large intestinal content. Complete necropsies were done on all stillborn fetuses. Forty-nine placentas were also evaluated as part of this study set. Gross and histopathologic diagnosis for all stillborn fetuses and placentas were made by two board-certified veterinary pathologists. To determine the gestational age, fetal femur length measurements were used where they were available [4,19].

The *M. fascicularis* colony was maintained by the Southwest National Primate Research Center at the Southwest Foundation for Biomedical Research (SNPRC/SFBR) in San Antonio, Texas, USA. The monkeys were housed in metal and concrete indoor/outdoor enclosures. Breeding groups were composed of one breeding male with up to 15 females and their offspring. They were fed commercial monkey chow (Purina Monkey Diet 5LEO, Purina, St. Louis, MO, USA) and given water *ad libitum*. All procedures were done with approval of the Institutional Animal Care and Use Committee.

The data were analyzed with Excel® software (Microsoft, Redmond, WA, USA) and SPSS® software (SPSS Inc., IL, USA) for ANOVA and chi square analyses.

Results

The incidence of stillbirth in this *M. fascicularis* colony during a 7-year period averaged 11.99% (236 of 1,967) of all births. Of the 1731 live births, 835 were female and 896 were male. All stillbirths and placentas were collected upon discovery, varied in condition and completeness, and were refrigerated until necropsy. The causes of stillbirths are shown in Table 1. Most of stillbirths were of undetermined cause. The three major identified cause categories were fetal, maternal, and placental. The major fetal cause was trauma; trauma was primarily a gross diagnosis and usually presented with hemorrhage in the skin, subcutaneous tissue, meninges, skull, and brain. Fetal pneumonia was generally diagnosed microscopically by the presence of extensive areas of suppurative inflammation. The major maternal cause was dystocia; clinical presentations of dystocia included fetal breech and facial presentation. There were 49 placentas from stillbirths available for histopathologic evaluation. Suppurative placentitis or excessive placental necrosis, infarction and mineralization were most often observed. Of the cases with both fetal and placental tissue available, only one had lesions involving both. One of the two cases of fetal pneumonia was associated with a suppurative placentitis. The placenta was not available for the other case of fetal pneumonia. Femur length measurements were available in 34 fetuses. These stillbirths occurred mostly at term,(>155 dG, 94.12%, 32/34); only 5.88% (2/34) were preterm, 140-155 dG.

Thirty-three maternal histories were reviewed for age and numbers of pregnancies at first stillbirth. The most common age at first stillbirth was 8 to 12 years old (60.6%, 20/33). Younger animals (4 to 7 years old) and older animals (13 to 19 years old) accounted for 21.2% (7/33) and 18.2% (6/33) of first stillbirths, respectively. Parity was unrelated to the occurrence of first stillbirth. Most (84.8%, 28/33) first stillbirths occurred to mothers during their first six pregnancies.

There was a slight predominance of female stillborns (52.54%, 124/236). The proportion of female sex in live births and stillbirths was evaluated using a chi square test, and was higher in the stillbirth group ($p = 0.016$). After stratifying by cause categories, the proportion of female sex was higher in stillbirths of undetermined etiology ($p = 0.023$) and maternal causes ($p = 0.020$). The odds ratio for a stillbirth in the female sex was 1.43 (IC 95% 1.08-1.90) in the total group, 1.55 (1.07-2.23) in the group of undermined etiology, and 2.91 (1.22-6.96) in the group of maternal causes.

Discussion

Previously reported incidences of stillbirths in different macaque species ranged from 4.7 to 39% [10]. The 11.99% incidence of stillbirths in this *M. fascicularis* breeding colony was at the lower end of this range. By a conservative estimate, prenatal mortality in NHPs is generally thought to exceed 15% of all diagnosed pregnancies [4] since many stillbirths and pregnancies are not detected in a group housed environment. The Tsukuba Primate Center for Medical Science reported the stillbirth rate in *M. fascicularis* at 8.9% [7]. Handrie reported the prenatal loss in *M. fascicularis*, including abortion and stillbirth, for a 10-year breeding period in a indoor time-mated colony at the California Regional Primate Research Center as 17.8% [12]. Dukelow described the stillbirth rate in the rhesus monkey (*M. mulatta*) as 10.6% in wild-born and 5.7% in colony-born monkeys [8]. In one report in African green monkeys (*Cercopithecus aethiops*), the prenatal loss was 28% [5].

Determining the etiology of stillbirths continues to be a challenge. In humans, the primary causes of stillbirth at ≥37 weeks of gestation were unexplained (40%), fetal malnutrition (14%), and placental abruption (12%) [9]. King reported a less than 20% success rate in determining

the cause of death for *M. mulatta* stillborns [14]. The 61% of stillbirths of undetermined cause in our study is within these ranges.

Reproductive failure after implantation, including stillbirth, is often divided into three categories; fetal, maternal, and placental [17]. Fetal trauma was the most commonly identified fetal and overall cause of stillbirth in this study. These stillborns typically had extensive hemorrhages in the subcutis of the head and within the meninges. We speculate that some of these may have resulted from trauma during parturition either from the dam or her cagemates.

Dystocia was the most often identified maternal cause of stillbirths in the present study. Cause of dystocia in humans include abnormal pelvic size, large fetal size, unusual fetal presentation [5], and uterine inertia, which can promote long labor and consequent stillbirths [17]. A relationship between fetal position and stillbirths in *M. fascicularis* has been reported. *M. fascicularis* had a 10% incidence of breech position the day before delivery; of these, 65.6% resulted in stillbirths [7]. Dystocia may produce subdural hemorrhage, intracranial hemorrhage or anoxia due to a prolapsed umbilical cord [17], and may account for many of the cases of fetal trauma seen in this study. Other etiologic factors such as short umbilical cords, trauma, uterine anomalies, maternal hypertension, and placental separation are commonly reported in the third trimester in humans and NHPs [6,15].

Placental causes of stillbirths include anomalies of the placenta that cause fetal distress including circumvallate placentas, double placentas, pathology of the umbilical cord (ectopic insertion, hemorrhage, thrombosis, edema, amniotic band stricture and umbilical cord overcoiling), placentitis, and placental insufficiency [2,3,5,17]. Five of the placentas in this study had excessive necrosis, infarction, and mineralization. Buntun described necrosis and calcification of villi in *M. mulatta* in third trimester placentas (124-162 days), and noted that these changes may become significant in correlation with negative pregnancy outcome [6]. Placental causes of stillbirth also include ascending and hematogenous infections [1,11,13]. In the present study there were five cases of suppurative placentitis. The most common cause of fetal death in placental infection is not fetal infection, but fetal asphyxiation due to compromised placental function [1]. Cases of fetal pneumonia due to infection have been described as a cause of stillbirths in humans and non-human primates [11]. In our cases we could not find any particular infectious agent associated with the two cases of fetal pneumonia. Both had no grossly observable lesions and were identified at histopathology. There is a definite need for further evaluation of such questions in non-human primates.

There are several identified maternal risk factors for stillbirth including infection, maternal nutrition, parity, and age [9,10,17]. In the present study, we focused on number of pregnancies and age at first stillbirth. Advanced maternal age in women has been associated with a higher rate of stillbirths [9,16]. In contrast, Gardin found that maternal age and parity of *M. fascicularis* did not correlate with stillbirth incidence [10]. Dukelow [8] described the rate of prenatal loss in *M. mulatta as* the highest at 8 years of age (26%). Although we did not evaluate the overall incidence of stillbirths by maternal age, we found the majority of first stillbirths occurred in animals 8 to 12 years of age. There was no correlation between number of pregnancies and the first stillbirth in this study.

The stillborn female-to-male ratio in this colony was slightly more than one. This was consistent with a prior report that slightly more stillborn fetuses were female [8]. In our animals, this increased risk of stillbirth in females was in the categories of undetermined and maternal causes, there was no sex difference in the categories of fetal and placental causes. The vast majority of stillborns in this study occurred at term, consistent with previous reports that the rate of stillbirths increases during late gestation [12]. Our data can not exclude the possibility

that stillborns at earlier stages are often cannibalized and therefore underrepresented in a group housed environment.

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Table 1

Etiology and sex of 236 *Macaca fascicularis* stillbirths

Etiology	*N*	Incidence of stillbirths (%)	Fetal Sex		
			Female	Male	Undetermined
Undetermined	144	61.02	75	52	17
Fetal causes					
fetal trauma	53	22.46	24	29	-
fetal pneumonia	2	0.85	2	-	-
congenital anomalies	1	0.42	-	1	-
Maternal causes					
dystocia	23	9.75	18	5	-
uterine rupture	1	0.42	-	1	-
Placental causes[a]					
placentitis	5	10.20	2	1	2
placental necrosis	5	10.20	2	3	-
placental abruption	2	0.85	1	1	-
Total	236		124	93	19

[a] A total of 49 placentas were available for histologic evaluation.