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Comparison of fresh to fixed weights of the vervet monkey (*Chlorocebus sabaeus*) placenta and its relation to gestational age

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Abstract

Little is known about the effects of formalin fixation on the nonhuman primate placenta. We analyzed weights of 48 vervet monkey placentas before and after formalin fixation and storage. Most placentas, especially the largest ones, exhibited decreased weight. Fixed placental weight is an excellent predictor of both fresh weight and gestational age. Although the vervet monkey placenta is described as bidiscoid, 14.6% of this sample was fused into a single mass; this did not affect the amount of weight loss due to fixation.

Keywords

placenta; formalin; vervet monkey; tissue banks; fusion; gestational age

Introduction

A successful pregnancy relies on the placenta, and growing evidence implicates placental transport of nutrients as an important mechanism in fetal programming of adult disease [4]. The anthropoid primate placenta shares developmental, physiological, and histological affinities with the human placenta [7, 8]. In research facilities where birth is routinely observed, the placenta may be opportunistically collected, fixed, and stored for future use. The vervet monkey is an important nonhuman primate biomedical model [3], but its placental morphology has not been extensively described in the literature. The purpose of this paper is to characterize placental weight change due to fixation and long-term storage in formalin by studying a rare time-series of 48 vervet monkey (*Chlorocebus sabaeus*) placentas of known gestational age, and to evaluate fixed placental weight as a predictor of fresh weight and gestational age.

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Methods

This study was conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee. Fifty-two vervet monkey placentas were collected throughout August 2009 via timed fetectomy at the St. Kitts Biomedical Research Foundation, on gestational days 85 through 159; 165 days is term. Three placentas were omitted because gestational age was unknown and one was omitted because of highly abnormal fetal size, bringing the final sample to 48 placentas. The vervet placenta is typically bidiscoid; fused placentas in the sample were treated in the same manner as placentas with discrete disks. Untrimmed placentas (i.e. placentas, all membranes, umbilical cord) were gently wiped to remove maternal surface clots. The untrimmed placentas were weighed upon surgical removal of the conceptus; the membranes and cords were not removed and no manual attempt was made to remove blood from the placenta beyond allowing the placentas to passively drain for a few minutes prior to fixation. The untrimmed placentas were immersion-fixed in 500mL 10% neutral buffered formalin for at least one full week at 4°C. Once fixed, the placentas were drained of all but 10 mL of formalin and shipped to the Comparative Primate Biology Laboratory at the University of Illinois at Chicago, where they were stored for 10 months in 10% neutral buffered formalin in 10 mL of fixative per 1 g of tissue. Fixed untrimmed placentas were weighed throughout June 2010. Absolute difference was measured by subtracting fixed weights from fresh weights; percentage change was calculated as the proportion of the difference to the fresh weight.

Correlations describe the relations among gestational age, fresh placental weight, and weight difference. Linear regression models were constructed to evaluate fixed placental weight as a predictor of fresh weight and gestational age. ANOVAs of fixation effects were performed on tertiles of gestational age and fresh placental weight.

Results

Although the vervet monkey placenta is typically bidiscoid, seven of the 48 placentas (14.6%) in this sample were fused into a single mass (Figure 1). Beyond this variant of placental morphology, all placentas in the analyses appeared grossly normal, and preliminary histological examination did not reveal structural or pathological variation outside the normal range. Fresh and fixed weights were significantly and positively correlated with each other and with gestational age; placentas, represented by both fresh and fixed weights, increased in size with gestation (Table 1).

Most of the placentas (87.5%) exhibited reductions in weight; the remaining 12.5% increased in weight (Figure 1). Placental weight, but not gestational age, was significantly correlated with change in weight due to fixation (Table 1). There was a significant difference in loss depending on the initial size of the placenta, with the largest placentas demonstrating the greatest decrease in weight (−24.25% compared to −7.03% in the smallest placentas; Table 2). Weight change did not differ significantly according to gestational age (Table 2). The placentas that decreased in weight had significantly lower fresh weights than did the placentas that reduced; they did not differ in gestational age ($p=0.043$ and $p=0.358$ respectively, not shown).

Fixed weights were significantly predictive of both fresh weights and of gestational age (respectively: $r=1.18$, $p<0.001$; $r=0.78$, $p<0.0001$; Figure 2). Fixed placentas were subsequently trimmed of all membranes and these trimmed fixed placentas retained comparable predictive value for both fresh untrimmed weight ($r=1.35$, $p<0.0001$, not shown) and gestational age ($r=0.87$, $p<0.0001$, not shown).

Discussion

In the vervet monkey, fresh and fixed placental weights were tightly correlated. Yamada et al. [14] also reported a tight correlation between fresh and fixed weights of rat accessory sex organs. The most common response by the vervet monkey placenta to formalin fixation was a reduction in weight. Weight decrease due to immersion in formalin has been reported for the rat heart and spleen [5]. Conversely, increased weight after formalin fixation has been reported in the primate placenta [2, 9]; the rat brain, liver, kidneys, gonads, and lungs [5] and whole salmon [11]; this reaffirms that tissue response to fixation is highly variable, even when the same tissue or fixation method is considered. Six placentas in the current study increased in weight; although differences in fresh and fixed weights were not significantly associated with gestational age, the ones that increased in weight were relatively small for gestational age, a condition that in humans is often associated with impaired villous development [12] and which may contribute to differential fixation effects, although to date we are aware of no other studies that have assessed formalin effects relative to placental maturity or pathology.

Large placentas exhibited the highest rate of weight loss – an average of 25% loss among the largest placentas. As the placenta grows larger and older, the volume of villous tissue relative to overall placental volume increases [Table 28.2 in 1]; much of this is represented by the syncytiotrophoblast, a multinucleated tissue with a high cytosol:membrane ratio. Water loss from this multinucleated tissue compartment due to formalin fixation may represent a significant component of the decrease in overall weight. It must also be noted that the larger the tissue block the less effective immersion fixation may be because of the increased time for diffusion to deeper layers [13], consequently yielding uneven fixation, and potentially contributing to the variation in weight loss exhibited by this sample. However, even the largest placentas were no thicker than 1 cm, and they were fully immersed in formalin for at least one week for the initial fixation. Visual inspection of the tissue under the microscope did not indicate that large placentas were less well-fixed than smaller placentas.

Formalin-fixed vervet placentas in this study were excellent predictors of both fresh weights and of gestational age, and this relationship held when the placentas were subsequently trimmed of all fetal membranes and umbilical cords. This, combined with the observation of a 14.6% incidence of fusion in a bidiscoid hemochorial anthropoid primate placenta, provides important baseline information for future studies of placental variation in anthropoid primates.

There are limits on the applicability of our findings to other samples or species. First, our analyses speak directly only to the untrimmed vervet monkey placenta undergoing formalin immersion and subsequent storage for up to 10 months. Placentas of other primate or mammalian species may behave differently in response to formalin fixation; Rutherford reported a small but insignificant increase in weight between fresh and fixed marmoset monkey term placentas [9] as has also been reported for human term placentas [2, 6]. The placentas in the study were from only the second half of gestation; it is possible that younger placentas respond differently to the effects of formalin fixation. The weights of the fresh placentas included the membranes and cords which adds an unpredictable level of potential error which in turn may explain some of the scatter in these analyses. Fresh weights and volumes should be taken both on untrimmed and trimmed placentas to eliminate this source of error. The loss rates reported here are not meant to be used as universally applied standards but rather as indicative of variation in collection methods, the properties of the fresh placenta, the effects of fixation, and the predictive value of fixed tissue.

Despite these limitations, this study provides useful comparisons for researchers working with banked tissue samples. Further, it is an important contribution to a sparse literature on the vervet monkey placenta. The observation that fixed weights are reliable predictors for fresh placental weights is consistent with previous studies of primate placentas [2, 9]. This provides a rationale for estimating fresh weights and gestational ages from banked placental tissue from vervets as well as closely related species such as guenons, macaques, and baboons. However, the finding that the effects of formalin fixation and long-term storage vary according to the original condition of the vervet placenta is important and reinforces that caution should be exercised when estimating fresh weight from fixed measures. In particular, in stereological analyses of placental structure [10], gestational age and fresh weight (or volume) should be included as covariates to account for differential fixation effects on morphology.

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A. BIDISCOID VS. FUSED PLACENTAS

B. PLACENTAL WEIGHT CHANGE DUE TO FIXATION

Figure 1. Distribution of sample characteristics: A) fused vs. bidiscoid placentas; B) weight change
254x338mm (300 x 300 DPI)

Figure 2.

Fixed placental weight as a predictor of A) fresh placental weight and B) gestational age
 254x338mm (300 x 300 DPI)

Correlations among gestational age, fresh placental weight, fixed placental weight, and difference in weight

Table 1

Gestational age (d)					
Fresh placental weight (g)			Fixed placental weight (g)		
r	p	r	p	r	p
Fresh placental weight (g)	-0.737	<0.00001			
Fixed placental weight (g)	0.870	<0.00001	0.884	<0.00001	
Weight change (%) *	0.152	0.301	-0.401	0.005	0.789

* (Fresh weight – Fixed weight)/Fresh weight

Table 2
Placental characteristics according to gestational age and fresh placental weight categories

	GESTATIONAL AGE (d)			ANOVA RESULTS	
	Tertile I N=17 83 to 122 d	Tertile II N=16 123 to 137 d	Tertile III N=15 138 to 159 d	F	p
	Mean (S.D.)	Mean (S.D.)	Mean (S.D.)		
Fresh placental weight (g)	55.99 (15.55)	94.69 (20.50)	105.71 (25.96)	25.51	<0.00001
Fixed placental weight (g)	47.84 (13.74)	80.56 (11.63)	89.64 (14.01)	45.48	<0.00001
Absolute weight difference (g)	-8.14 (4.86)	-14.13 (13.80)	-16.07 (20.85)	1.34	0.271
Change due to fixation (%)	-14.46 (6.79)	-12.13 (17.62)	-12.10 (17.55)	0.14	0.870
	INITIAL PLACENTAL WEIGHT (g)			ANOVA RESULTS	
	Tertile I N=17 34.46 to 68.2 g	Tertile II N=16 68.6 to 98 g	Tertile III N=15 98.2 to 149.3 g	F	p
	Mean (S.D.)	Mean (S.D.)	Mean (S.D.)		
Gestational age (d)	107.72 (18.91)	132.19 (15.18)	137.56 (8.12)	18.76	<0.00001
Fixed placental weight (g)	49.12 (15.50)	76.30 (12.46)	90.69 (13.23)	38.03	<0.00001
Absolute weight difference (g)	-3.28 (9.03)	-9.97 (9.63)	-32.46 (28.29)	20.78	<0.00001
Change due to fixation (%)	-7.03 (17.31)	-11.54 (11.24)	-24.25 (12.31)	5.99	0.005