BABY ON BOARD: OLFACTORY CUES INDICATE PREGNANCY AND FOETAL SEX IN A NONHUMAN PRIMATE

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Running headline: ***Olfactory cues of pregnancy in lemurs***

ABSTRACT

Olfactory cues play an integral, albeit underappreciated, role in mediating vertebrate social and reproductive behaviour. These cues fluctuate with the signaler's hormonal condition, coincident with and informative about relevant aspects of its reproductive state, such as pubertal onset, change in season and, in females, timing of ovulation. Although pregnancy dramatically alters a female's endocrine profiles, which can be further influenced by foetal sex, the relationship between gestation and olfactory cues is poorly understood. We therefore examined the effects of pregnancy and foetal sex on volatile genital secretions in the ring-tailed lemur (*Lemur catta*), a strepsirrhine primate possessing complex olfactory mechanisms of reproductive signaling. Whilst pregnant, dams altered and dampened their expression of volatile chemicals, with compound richness being particularly reduced in dams bearing sons. These changes were comparable in magnitude to other, published chemical differences among lemurs that are salient to conspecifics. Such olfactory 'signatures' of pregnancy may help guide social interactions, potentially promoting mother-infant recognition, reducing intragroup conflict, or counteracting behavioural mechanisms of paternity confusion; cues that also advertise foetal sex may additionally facilitate differential sex allocation.

Keywords: olfactory communication, reproductive signal, gestation, sex allocation, hormone, chemosignal

1. INTRODUCTION

Female animals routinely broadcast information about their reproductive state. In general, the research emphasis in reproductive signaling has been on cues associated with fecundity, ovulation or mate quality; however, there are also potentially potent cues associated with gestation. The physiological changes underlying pregnancy are complex and, compared to ovulation, are more enduring. The corresponding changes to endocrine concentrations, that may guide reproductive cues of pregnancy, can be extreme. Notably, in comparison to maximum concentrations outside of pregnancy, concentrations of certain sex steroids during pregnancy can increase >1,600% [1] and vary by foetal sex (reviewed in [2]). Moreover, pregnancy cues may serve important functions. In primates, for instance, gestational cues may reduce intragroup conflict in species that compete intensely for mates [3] or may prepare cooperatively breeding males for the energetic and behavioural changes necessary for paternal care [4]. Historically, the conveyance of reproductive information, particularly in primates, has been evaluated primarily through visual and behavioural channels (e.g. [5,6]); nonetheless, there is growing recognition of reproductive signaling via olfactory channels [7]. As in several non-primate species [8–10], olfactory cues of pregnancy occur in humans, possibly facilitating mother-infant recognition [11]; otherwise, the potential for olfactory gestational cues in primates has been ignored. Here, we investigate pregnancy effects on olfactory cues in a promiscuous, nonhuman primate, the ring-tailed lemur (*Lemur catta*).

Recently, the ring-tailed lemur has emerged as a useful model for examining the form and function of olfactory signals relevant to reproduction. Among females, that use scent to demarcate birthing sites [12] and for competitive overmarking [7], variations in odorant profiles (whether in compound type, number, or relative concentration, see e.g. diversity indexes in [13]) correspond to natural [14,15] and experimentally induced [16] variations in reproductive hormones. Through behavioural bioassays, genetic profiling and chemical analyses, lemur chemosignals have been shown to convey information about sex, breeding condition and individual identity, as well as genetic quality and relatedness (reviewed in [7,16]). By contrast, dominance status within the sexes, which in lemurs can be nonlinear, non-transitive, and highly variable [17], is recognizable via scent in known individuals only [18], but is not chemically encoded [18,19]. Using a within-subjects sampling design, we extend these prior studies by characterizing chemical changes in lemur genital secretions between preconception and pregnancy, whilst also considering the potential effects of other concurrent physiological or environmental changes, such as age, season, litter size, and foetal sex.

2. MATERIAL AND METHODS

The subjects were 12 captive, sexually mature (1.5-21 years), female ring-tailed lemurs, housed in semi-free-ranging, mixed-sex groups at the Duke Lemur Center in Durham, NC, USA. We monitored reproduction over a six-year period (2004-2010), including during 14 pregnancies ([2]; see Electronic Supplementary Material, ESM). To illustrate any potential olfactory-endocrine relationship, we present accompanying sex steroid concentrations in female subjects, recalculated from [2]. Our animal procedures were approved by the Institutional Animal Care and Use Committee of Duke University (see ESM).

We collected samples of labial secretions near conception (mean ± SEM: 17.3 ± 3.7 days preconception) and mid-pregnancy (mean ± SEM: 88.8 ± 4.6 days of a 135-day gestation period) for analysis by gas chromatography-mass spectrometry (see [20]). We used richness as a proxy of chemical complexity [13], retaining only compounds that eluted at 8-43 minutes and comprised ≥ 0.01% of the chromatogram [20]. We assigned ‘foetal sex’ following [2], grouping dams carrying singleton or twin females as ‘females bearing daughters’ (FBD; n=8) and dams carrying at least one male (singleton male, twin males, or mixed-sex twins) as ‘females bearing sons’ (FBS; n=6; ESM).

We first addressed and dismissed any potential effects on a female’s chemical richness of her age, the seasonal timing of her pregnancy, and her litter size (see ESM). We then tested for effects of reproductive condition (preconceptive versus pregnant) and foetal sex (FBD versus FBS) on her chemical richness and endocrine concentrations, using two-factor ANOVAs (univariate repeated-measures model, JMP PRO 11.0, SAS; ESM). To contextualise the main effect of reproductive condition on chemical complexity, we also calculated the average differences in chemical richness between several previously studied groups from this population ([13,14,16,19]; see ESM). Having decided *a priori* to compare FBD and FBS, we resolved the significant interaction using F-tests for simple effects. These data can be accessed at Dryad [21].

3. RESULTS

The odorant profiles of female ring-tailed lemurs changed with pregnancy, relative to preconception, as revealed by differences in the gas chromatograms of their genital secretions (Figures 1*a*,*b*): pregnancy was associated with changes in the relative proportions of the volatile compounds expressed and with a significant decrease in the total number of compounds ($F_{1, 10}$ = 14.41, $P < 0.01$; Figure 1*c*). The change in chemical richness (mean ± SEM: 9.67 ± 6.62 compounds) was comparable in magnitude to the behaviourally salient, chemical differences observed between other groups of individuals, including those found between intact and hormonally contracepted females (Table 1).

Remarkably, a dam’s volatile chemical profile during pregnancy also varied with the sex of her foetus. A significant interaction between reproductive condition and foetal sex ($F_{1, 10}$ = 5.21, P < 0.05) owed primarily to a greater loss of chemical richness during pregnancy in FBS than in FBD ($F_{1, 12.34}$ = 5.51, P < 0.05; Figure 2*a*). Because there was no effect of a female’s eventual foetal sex on the chemical richness of her preconceptive odorants ($F_{1, 12.34}$ = 0.74, P = 0.40, *n.s.*), the differences by foetal sex during pregnancy could not be explained by the scent signatures (see [19]) of individual females. Instead, these findings may relate to underlying differences, by foetal sex, in the concentrations of maternal sex steroids, which increase during pregnancy (Figure 2*b*; Table 2).

4. DISCUSSION

We show that odorant expression in lemurs is altered during pregnancy and is further differentiated in accordance with the sex of the dam’s developing offspring. The chemical effects induced by pregnancy were comparable in magnitude to other differences in scent that, as revealed by behavioural bioassays, are salient to ring-tailed lemurs [16,18,22]. The degree to which compounds are lost during pregnancy and influenced by foetal sex appears to be inversely related to the female’s changing sex steroid concentrations. Given the established link between sex steroids and olfactory profiles [7], these findings suggest possible endocrine involvement (or competing energy allocation) in the production of olfactory gestational cues.

Gestational cues could serve various functions across primate species, from promoting social cohesion [3] to engendering parental investment [4] or kin recognition [11]. To the extent that olfactory cues of pregnancy occur in other primates, our findings could be relevant to these existing hypotheses, as well as to theories on the functionality of multiple mating. Notably, a female's efforts to confuse males about their potential paternity ('paternity confusion') is commonly invoked to explain a female primate's engagement in multiple mating during pregnancy, particularly at times when there are no overt behavioural cues of pregnancy or when visual cues of pregnancy are not yet apparent [23,24] – in other words, when males presumably lack knowledge about female reproductive state. Yet, we have no information about potential scent cues of pregnancy in these species, whose males are nevertheless known to engage in olfactory investigation of vaginal secretions prior to mating [7,25,26]. We must therefore consider the possibility that olfactory cues could provide males with a means of detecting a proceptive dam's reproductive state. Discerning males could possibly thwart the female's purported tactics, either by not mating with her or by mating with her without becoming confused about paternity. If so, one might only expect multiple mating to be an effective means of paternity confusion in species in which either the female does not produce olfactory pregnancy cues or the male is unable to detect them.

To the extent that olfactory advertisement of foetal sex might also occur more broadly than in *Lemur*, our findings might help elucidate a potential mechanism of differential sex allocation. Under certain conditions, animals increase fitness by preferentially investing in offspring of a particular sex [27]. Although theories addressing sex allocation typically implicate mechanisms operating at conception [28] or post-parturition [29], mechanisms operating during gestation would allow animals to respond to important social or environmental changes whilst the ongoing costs of investment are still considerable. An expectant dam could potentially use her own olfactory cues as a self-referent to inform her investment decisions; alternately, conspecifics could use these cues as predictors of imminent sex ratios to guide their own sex-allocation strategies. Although opportunities to study a putative gestational mechanism of sex allocation will likely remain limited in this endangered, long-lived species, such limitations do not preclude investigations of potential sex-specific gestational cues or gestational sex allocation in more tractable study systems that share key life history characteristics (e.g., [30]).

The observation that pregnancy alters scent profiles in lemurs (this study) and in humans [11] suggests that olfactory cues of pregnancy may be widespread or highly conserved among primates. Such findings highlight the importance of considering multiple sensory modalities when examining reproductive signals, even in taxa historically thought to rely relatively little on olfaction [7,31,32].

Figure 1. Volatile chemicals varied among the genital secretions of preconceptive and pregnant ring-tailed lemurs: gas chromatograms depict variation in the relative proportions of compounds present in the labial secretions of a representative ring-tailed lemur before conception (*a*) and whilst pregnant with twin daughters (*b*). Numbers denote: 1, internal standard (hexachlorobenzene); 2, squalene; 3, cholesterol. Bar graphs represent differences by reproductive condition in the chemical richness (mean ± SEM) derived from the chromatograms of all female subjects (*c*).

Figure 2. Mean ± SEM chemical richness (*a*) and serum steroid concentrations (*b*) varied by reproductive condition and foetal sex in female ring-tailed lemurs. T, testosterone; A_4 androst-4-ene-3,17,dione; OHP, 17α-hydroxyprogesterone; E_2, 17β-oestradiol.

Table 1. Differences in the chemical richness of ring-tailed lemur genital secretions between groups or conditions for which existing behavioural data support the salience of the olfactory signal.

Table 2. Differences in the endocrine profiles of female ring-tailed lemurs in relation to reproductive condition and foetal sex. Notably, serum concentrations during gestation were significantly different between females bearing daughters (FBD) and females bearing sons (FBS) for all measured steroids.

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