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Prenatal androgen exposure and parental care interact to influence timing of reproductive maturation in marmosets

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The timing of reproductive maturation is susceptible to hormonal and environmental influences, and variation in this timing can be partially attributed to the prenatal and post-natal environment. We examined associations between prenatal steroid exposure and the post-natal family environment on the variability in reproductive maturation timing in young marmosets (*Callithrix geoffroyi*). Urine samples from pregnant females were analyzed for cortisol (CORT) and androgens (uA). Post-natal uA was measured in males to determine age (in days) of adult-like levels of androgens associated with spermatogenesis; post-natal pregnanediol glucuronide (PdG) was measured in females to determine age (in days) of first ovulation. Maternal, paternal, alloparental, and total care (carrying, grooming, and rejection/removals) of offspring were observed. Female offspring exposed to lower prenatal uA levels and higher paternal grooming and lower maternal rejection/removals showed later first ovulation, whereas female offspring exposed to higher prenatal uA showed earlier first ovulation. Male offspring showed earlier reproductive maturation as paternal grooming increased, regardless of first trimester uA exposure. Male offspring exposed to low prenatal uA levels showed earlier reproductive maturation as maternal rejections/removals increased. In offspring exposed to low prenatal CORT, high total carrying predicted earlier first ovulation in females, but later reproductive maturation in males. Total carrying duration did not affect timing of reproductive maturation in offspring exposed to high third trimester CORT levels. Our findings expand the evolutionary theory of socialization by demonstrating that the effect of post-natal family environment on timing of reproductive maturation depends on prenatal uA exposure and also influences reproductive maturation timing in male offspring.

KEYWORDS
androgens, cortisol, HPG axis, prenatal steroids, reproductive maturation

1 | INTRODUCTION

The reproductive transition from childhood, adolescence, and adulthood is one of the most critical developmental events, and the timing of this transition is highly sensitive to early-life hormonal and social perturbations. The hypothalamic–pituitary–gonadal (HPG) axis regulates pubertal onset, and the organization of the HPG axis is sensitive to variability in both prenatal steroid exposure and the social and family environment during development. Understanding the influence of prenatal endocrine and post-natal social environments on the timing of reproductive maturation is critical, as accelerations or delays in puberty impact an individual's reproductive opportunities and offspring survival rates (Stearns & Koella, 1986). To date, much of our knowledge regarding the influence of prenatal steroids comes from research utilizing exogenous or experimental means of manipulating steroid exposure (Kosut, Wood, Herbosa-Encarnación, & Foster, 1997; Lunn, Recio, Morris, & Fraser, 1994; Zehr, Van Meter, & Wallen, 2005). Similarly, most studies examining post-natal family environment focus on extreme cases (maltreatment, parental absence, etc.) (Belsky, Houts, & Fearon, 2010; Belsky, Steinberg, Houts, & Halpern-Felsher, 2010; Mendle, Ryan, & McKone, 2015). Yet, little is known about whether timing of reproductive maturation is affected by interactions between natural variability in pre- and post-natal factors.

1.1 | Prenatal steroid exposure

Variability in prenatal steroid exposure has differential effects in male and female offspring. When exposed to exogenous prenatal androgens (via testosterone propionate) in late gestation, adult male rats either show delayed puberty and reduced adult testosterone levels (Cruz & Pereira, 2012) or no differences in pubertal timing (Wolf, Hotchkiss, Ostby, LeBlanc, & Gray, 2002). Insufficient androgen levels during early pregnancy, on the other hand, prevent masculinization of the HPG axis in male offspring, which also impacts pubertal timing. This has been observed in non-human primate (NHP) models. For example, male rhesus macaques exposed to an androgen receptor blocker during early gestation showed accelerated pubertal onset without influencing adult endocrine function or sexual behavior, whereas no differences in pubertal timing were observed in males exposed prenatally to testosterone enathate (an androgen supplement) (Herman, Zehr, & Wallen, 2006). In marmosets, blocking the fetal rise in testosterone during late gestation with a gonadotropin-releasing hormone (GnRH) antagonist did not affect pubertal timing in male offspring, but resulted in decreased testosterone plasma concentrations between 43 and 70 weeks of age (following puberty but prior to adulthood) (Lunn et al., 1994).

While male fetuses require androgens for normal masculinization, elevated prenatal androgen exposure can significantly alter HPG development and timing of first ovulation in female offspring. Female mice exposed to prenatal dihydrotestosterone (DHT) injections during late gestation showed accelerated puberty and irregular estrous cycling (Witham, Meadows, Shojaei, Kauffman, & Mellon, 2012). Similarly, ewes exposed to elevated levels of androgens during early to mid-gestation demonstrated earlier tonic leutenizing hormone (LH) surges that trigger ovulation (Kosut et al., 1997), suggesting a defeminized GnRH system (Foecking, Szabo, Schwartz, & Levine, 2005; Witham et al., 2012). Thus, the influence of prenatal androgens on pubertal timing is sex specific; male offspring require sufficient prenatal androgens for masculinization of the HPG axis, but elevated androgen levels masculinize the same neurosecretory mechanisms in females, resulting in altered HPG axis functioning and subsequent timing of reproductive maturation.

Elevated prenatal cortisol (CORT) can also influence timing of reproductive maturation, as the HPG axis interacts with the hypothalamic–pituitary–adrenal (HPA) axis (Kirby, Geraghty, Ubuka, Bentley, & Kaufer, 2009). Specifically, CORT suppresses GnRH synthesis and decreases activity in the GnRH pulse centers of the hypothalamus (Dubey & Plant, 1985), preventing the release of hypothalamic and gonadotropic steroids via negative feedback. This may result in pregnant mothers with prolonged high CORT producing lower androgen levels. While examining the influence of prenatal androgens on timing of sexual maturation in rhesus macaques, Zehr et al. (2005) found that all female offspring of mothers treated with testosterone enanthate, flutamide (an androgen receptor antagonist), or vehicle control showed delayed first menarche compared with offspring of untreated mothers, possibly due to increased maternal stress during treatment and subsequent prenatal CORT exposure (Zehr et al., 2005). In rodents, both male and female offspring showed delayed puberty onset (Marchlewska-Koj, Kruczek, Kapusta, & Pochroń, 2003; Smith & Waddell, 2000) and abnormal sex steroid levels (Richardson, Zorrilla, Mandyam, & Rivier, 2006) associated with increased prenatal CORT exposure. These findings suggest that increased maternal stress and subsequent increased prenatal CORT exposure may influence timing of reproductive maturity by altering sex steroid concentrations. Other studies have failed to replicate these changes, but have observed impairments in fertility in female offspring exposed to increased prenatal exposure to glucocorticoids (Crump & Chevins, 1989; Herrenkohl, 1979). Thus, exposure to elevated prenatal CORT can negatively impact reproductive function, either through the suppression of onset of puberty or by reducing fertility.

1.2 | Post-natal family environment

The post-natal social environment, including family composition and quality of care, also influences pubertal timing. According to Belsky, Steinberg, & Draper's (1991) evolutionary theory of socialization, a stressful rearing environment (i.e., insecure attachment with parental figures, harsh or neglectful infant care, and unpredictable or scarce resources) promotes early puberty and a shift in reproductive effort that maximizes reproductive opportunities through early sexual behavior, short-term relationships, and reduced parental effort. Human studies support this theory. For example, childhood sexual abuse and emotional harshness from a parent or adult caregiver predicted earlier onset of puberty and development of secondary sex characteristics in females (Mendle et al., 2015). Similarly, maternal harshness (Belsky, Steinberg, Houts, & Halpern-Felsher, 2010) and insecure infant–mother attachment (Belsky, Houts, & Fearon, 2010) were associated with earlier age of menarche. Taken together, these results suggest that quality of parental care influences age of puberty in females (Belsky et al., 2007). Ellis, McFadyen-Ketchum, Dodge, Pettit, and Bates (1999) expanded upon the evolutionary theory of socialization to include effects of paternal presence and quality of care. Specifically, the presence of the biological father and increased paternal care and affection predicted delayed onset of puberty in female children (Ellis et al., 1999). Conversely, puberty onset was accelerated in female offspring raised without the biological father (Matchock & Susman, 2006; Tither & Ellis, 2008), as well as the presence of a step-father (Ellis & Garber, 2000; Quinlan, 2003), or step- or half-brother (Matchock & Susman, 2006). This effect has not been observed in male children (Belsky et al., 2007).

Experimental animal models have produced mixed findings regarding the impact of parental care on pubertal timing. Regarding quality of maternal care, some studies have failed to find differences in pubertal timing in male and female rats based on maternal care (Uriarte, Breigeiron, Benetti, Rosa, & Lucion, 2007), while others have shown delayed puberty onset in female offspring reared by high-grooming mothers (Cameron et al., 2008). Similarly, physical contact with the mother (but not exposure to the mother's urinary chemosignal alone) delayed puberty in female California mice (Gubernick & Nordby, 1992). In relation to paternal presence and timing of reproductive maturation, rodent models have also produced conflicting findings. Male African striped mice showed delayed scrotal development when

living in family groups with the father present (Schradin, Schneider, & Yuen, 2009). However, in prairie voles, paternal presence was associated with more rapid growth in offspring, as well as increased alloparental behavior toward younger litters (Wang & Novak, 1994). There is a dearth of research regarding the influence of alloparental care on timing of reproductive maturation in younger offspring.

Although previous studies have experimentally examined the effect of prenatal exposure to exogenous CORT and T, few have studied the influence of natural variability in CORT and androgen levels on timing of reproductive maturity in offspring. Natural variation exists among mothers and individual pregnancies in concentrations of androgens and CORT, which can influence offspring physiology and behavior (reviewed in Auyeung, Lombardo, & Baron-Cohen, (2013) and Smith, Birnie, & French (2013)). Similarly, significant variability exists in parental effort provided by mothers, fathers, and siblings in marmoset family units (Nunes, Fite, Patera, & French, 2001; Santos, French, & Otta, 1997). Although several studies have separately examined the influences of prenatal steroid exposure or post-natal family environment on pubertal timing, no studies have explored interactions between prenatal androgen and CORT levels and quality of post-natal parental care in influencing timing of reproductive maturation.

Marmosets are an ideal model for exploring pre- and post-natal influences on pubertal timing. Marmosets typically give birth to twins, with both offspring being exposed to the same levels of prenatal steroids, but not necessarily the same level of parental care, thus allowing for both within- and between-litter comparisons. Additionally, marmoset fathers and siblings provide significant care to offspring (Nunes et al., 2000; Nunes, Fite, Patera, & French, 2001). It should be noted that there are differences between marmoset species in timing of peak paternal and alloparental carrying. In *Callithrix geoffroyi* specifically, fathers show significant variability in carrying over the post-partum period, with peak carrying behavior occurring 3–4 weeks after birth (Cavanaugh & French, 2013). We predicted that lower prenatal androgen exposure combined with high maternal, paternal, or alloparental investment (higher carrying and grooming, lower rejection/removals) would be associated with delayed reproductive maturation in female offspring. We also predicted that higher prenatal CORT exposure combined with higher maternal, paternal, and alloparental investment would predict delayed reproductive maturation in female offspring. In male offspring, we did not predict that timing of reproductive maturation would be influenced by prenatal steroid exposure or parental investment.

2 | METHODS

2.1 | Subjects

Sixteen female and 24 male offspring born to eight white-faced marmoset (*C. geoffroyi*) mothers across 26 pregnancies were included in the study. Of the 26 pregnancies and 44 resulting offspring, eight resulted in singleton births and 18 were twin births. Of the twin births, 10 of the litters were comprised of one male and one female, three litters were both females, and five litters were both males. Additionally, 28 of the offspring had older siblings in the family group along with the parents, whereas 16 offspring were not housed with alloparents. Two female offspring were excluded from the study because they died before sufficient hormonal data could be collected. Two male offspring were also excluded: one male did not meet criteria for adult-like levels of androgens; a second male offspring died before sufficient hormonal data could be collected, resulting in a sample size of 40 offspring.

Marmosets were socially housed in wire mesh cages measuring no smaller than $1 \times 2 \times 2\ m^3$ with no less than $1\ m^3$ per animal at the Callitrichid Research Center at the University of Nebraska at Omaha. Cages were furnished with branches, nesting boxes, and various enrichment devices. Marmosets had access to water *ad libitum* and were fed Zupreem® (Zupreem, Shawnee, KS, USA) marmoset chow, along with supplements of fresh fruit, crickets, and mealworms, yogurt, and eggs. Colony rooms were maintained on a 12 hr:12 hr light: dark cycle and at a temperature range of 19 °C and 22 °C. Data were collected between 2004 and 2008. All procedures complied with and were approved by the University of Nebraska Medical Center/University of Nebraska at Omaha Institutional Animal Care and Use Committee (IACUC 07-033-05-FC), and followed all ethical guidelines and principles outlined by the American Society of Primatologists.

2.2 | Urine collection and endocrine measures

Urine collections from pregnant females occurred approximately once or twice per week using non-invasive techniques in which subjects were trained to urinate into hand held aluminum pans for a preferred treat in their home cage. First void urine samples were collected between 0600 and 0830 hr. Samples were transferred to plastic vials, centrifuged at 7000 rpm for 2 min to remove sediments, and the sample was transferred to a clean vial and stored at −20 °C until assayed. Urine collections from offspring occurred in a similar manner beginning as early as 2 months of age. As with the mothers, urine collections from the offspring occurred approximately one to three times per week between the ages of 2 and 24 months.

2.2.1 | Pregnanediol glucuronide (PdG) enzyme immunoassay

Enzyme immunoassay (EIA) for pregnanediol glucuronide (PdG), a urinary progesterone metabolite, was used to determine date of conception in breeding females and date of first ovulation in female offspring. Urinary PdG levels were determined using EIA protocols developed by (Munro et al., 1991) and adapted for marmosets (French et al., 1996). Intra-assay coefficients of variation for high and low pools were 8.1% and 4.3%, respectively. Interassay coefficients of variation for high and low pools were 19.3% and 16.6%, respectively. Urinary creatinine (Cr) concentrations were also measured to adjust for variation in fluid intake and output. The creatinine assays used a modified Jaffé end-point assay, which was previously described and validated for use in marmosets (French et al., 1996). Concentrations of all endocrine measures were adjusted for creatinine content.

2.2.2 | Testosterone (T) enzyme immunoassay

In pregnant females and male offspring, urinary testosterone levels were measured using EIA validated for use in marmosets (Fite et al., 2005; Nunes et al., 2000). Because the T antibody used in the assay also reacts with androstenedione and dihydrotestosterone (Dloniak et al., 2004), results are expressed as urinary androgen level (uA) rather than testosterone concentrations. The extraction and assay procedure are described in (Birnie, Hendricks, Smith, Milam, & French, 2012). The intra-assay coefficient of variance for the high pool was 6.7%, with an interassay coefficient of variance of 13.1%.

2.2.3 | Cortisol (CORT) enzyme immunoassay

Urinary CORT levels were measured in mothers using EIA previously described and validated for marmosets (Smith & French, 1997). The intra-assay coefficient of variation of the high pool was 5.6%, with an interassay CV of 11.1%.

2.3 | Post-natal care

Infant offspring were observed in their family group two to three times per week during the first 2 months (days 1–60) after birth. During the 20-min behavioral observations, a trained observer sat approximately 1 m from the home cage with a laptop computer. Behaviors were recorded using Observer 3.0 software (Information Technology, Leesburg, VA, USA, Noldus, 1991). Behaviors included amount of time the infant was carried by the mother, father, subadult sibling, and total carrying (passive affiliative caregiving behavior), the number of grooming bouts initiated by the mother, father, subadult sibling on the infant as well as total number of grooming bouts (active affiliative caregiving behavior), and the number of times the mother, father, or subadult sibling rejected, removed, or attempted to remove the infant, along with the total number of rejections/removals (avoidant parental behavior) (Birnie, Taylor, Cavanaugh, & French, 2013). Data were converted to rate (grooming bouts and removals/rejections) or duration (carrying) per hour for analysis and data presentation.

2.4 | Data analysis

In pregnant females, date of conception was indicated by a sharp sustained rise in urinary PdG (above 10 μg PdG/mg Cr for at least 30 days). The average gestation for marmosets is 148.6 ± 1.9 days (French et al., 1996; Mustoe, Jensen, & French, 2012). Average CORT and uA levels were determined for the first (days 1–50 of gestation), second (days 51–100 of gestation), and third trimesters of pregnancy (days 101-partruition). These values represented prenatal uA and CORT exposure in each trimester for the offspring. It should be noted that timing of first ovulation in female *C. geoffroyi* offspring is not influenced by the presence of a male co-twin (French et al., 2016), and that maternal gestational androgen levels are not affected by the sex composition of the litter (French, Smith, & Birnie, 2010). Thus, the effects of prenatal androgen exposure are likely associated with androgens produced by the mother, and the prenatal androgen exposure is not influenced by the composition of the litter.

In female offspring, reproductive maturity was defined as age in days of first ovulation, as indicated by the sample immediately prior to three consecutive urine samples with PdG levels above 10 μg PdG/mg Cr. In *C. geoffroyi*, females can achieve first ovulation starting at 1 year of age (Tardif et al., 2003), but may remain anovulatory based on social rank (Abbott, Hodges, & George, 1988; Abbott, McNeilly, Lunn, Hulme, & Burden, 1981). As a result, female marmosets show significant variation in timing of first ovulation. In male offspring, reproductive maturity was defined as age in days in which monthly average urinary androgen levels exceeded 1 μg/mg Cr. In *C. geoffroyi*, androgen levels remain low in males until 8–9 months of age, when androgen levels rise significantly until uA levels reach above 1 μg/mg Cr in breeding adult males (Birnie, Smith, Nali, & French, 2011). This represents a two- to threefold increase in testosterone relative to infant and juvenile levels (Birnie et al., 2011) and coincides with increases in testicular volume (Abbott & Hearn, 1978) and induction of spermatognenesis (Jackson & Edmunds, 1984). There is significant variability in timing of the testosterone increase and testicular development in male marmosets (Abbott & Hearn, 1978; Irfan, Wistuba, Ehmcke, Shahab, & Schlatt, 2015).

Data points for independent variables were excluded from individual analysis if it exceeded three standard deviations from the mean. Based on this criterion, the following data points were removed from analyses: one male and one female (from the same litter) from all prenatal androgen analyses; two females (from the same litter) from all maternal carrying analyses; one female from all paternal grooming analyses. One female was removed from all analyses because the date of first ovulation exceeded three standard deviations from the mean. Linear mixed effects regression was employed to examine the main effects and interactions among offspring sex, parental care received by offspring (separately analyzing maternal, paternal, and alloparental care, plus a cumulative sum of care received by offspring (maternal + paternal + alloparental; all between individual comparisons)) and prenatal CORT and uA in each trimester of pregnancy (between litter comparison) on the timing of reproductive maturation. Analyses of prenatal uA and CORT were examined by individual trimester in order to determine if exposure to uA or CORT in early, mid, or late pregnancy affected pubertal timing. All analyses were run in R Version 3.2.2 (R Core Team, 2015) using the *lmerTest* package (Kuznetsova, Brockhoff, & Bojesen Christensen, 2015), with the intercept entered as a random effect and all other variables entered as fixed effects. Males were coded as 1 and females were coded as −1. The criterion for statistical significance was set at $P < 0.05$ for all analyses and were two-tailed.

3 | RESULTS

3.1 | Sex differences in reproductive maturation

There was a significant difference between male and female offspring in timing of reproductive maturation (regression: $b = -191.73$, $t = -7.56$, df = 29.63, $P < 0.001$), with males (mean days to reproductive maturation = 357.0 ± SD 90.0 days) achieving reproductive

maturation earlier than females (mean days to reproductive maturation = 556.8 ± SD 69.9 days). Prenatal uA or CORT exposure during any trimester did not influence timing of reproductive maturation in either male or female offspring (regression: t's ≤ 1.10, df ≤ 36.97, P's ≥ 0.279). Thus, it appears that neither prenatal uA nor CORT exposure by themselves had a significant impact on timing of reproductive maturation.

3.2 | Carrying behavior

The rates at which infants were carried during early development were associated with differences in timing of reproductive maturation, but these effects depended on the sex of the caregiver. Maternal carrying during the first 2 months of life did not predict timing of reproductive maturation alone, or as an interaction between offspring sex or prenatal steroid (uA or CORT) exposure in any trimester of pregnancy (regression: t's ≤ 1.30, df ≤ 35.60, P's ≥ 0.202). However, paternal carrying duration predicted timing of reproductive maturation in a sex-dependent manner (regression: $b = -0.19$, $t = -2.06$, df = 31.80, $P < 0.05$). Female offspring that received higher paternal carrying showed a later first ovulation, whereas in male offspring, higher paternal carrying predicted earlier reproductive maturation (Figure 1). The influence of paternal carrying on timing of reproductive maturation did not depend on prenatal steroid exposure (uA or CORT) in any trimester of pregnancy (regression: t's ≤ −1.44, df ≤ 34.85, P's ≥ 0.159).

The duration of carrying by alloparents did not predict timing of reproductive maturation in male or female offspring and did not interact with any trimester of prenatal steroid exposure (regression: t's ≤ 1.98, df ≤ 34.98, P's ≥ 0.065). The total carrying time by caregivers influenced timing of reproductive maturation in offspring, and this effect depended on the sex of the infant and third trimester CORT exposure (regression: $b = -0.02$, $t = -2.28$, df = 27.00, $P < 0.05$). In female offspring exposed to lower third trimester CORT, increased total carrying by caregivers predicted earlier first ovulation, whereas male offspring exposed to low third trimester CORT and experienced higher total carrying showed later reproductive maturation. Total carrying duration was not associated with timing of reproductive maturation in offspring exposed to higher third trimester prenatal CORT (Figure 2). The effect of total carrying time did not depend on any trimester androgen exposure (regression: t's ≤ 0.59, df ≤ 34.33, P's ≥ 0.560) or first or second trimester exposure to CORT (regression: t's ≤ 1.48, df ≤ 34.90, P's ≥ 0.149).

3.3 | Grooming behavior

The effect of number of grooming bouts during the first 2 months of life on timing of reproductive maturation depended on caregiver sex. Timing of reproductive maturation did not depend on number of maternal grooming bouts in either male or female offspring, and did

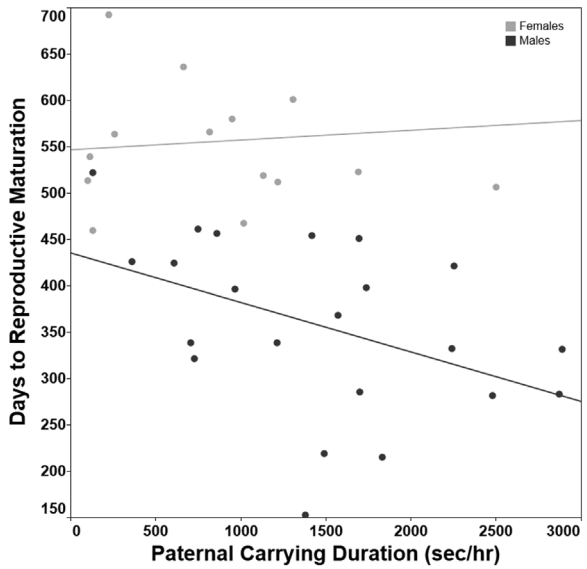

FIGURE 1 Female offspring (gray circles/regression line) that received higher paternal carrying showed later first ovulation, whereas male offspring (black circles/regression line) showed earlier reproductive maturation

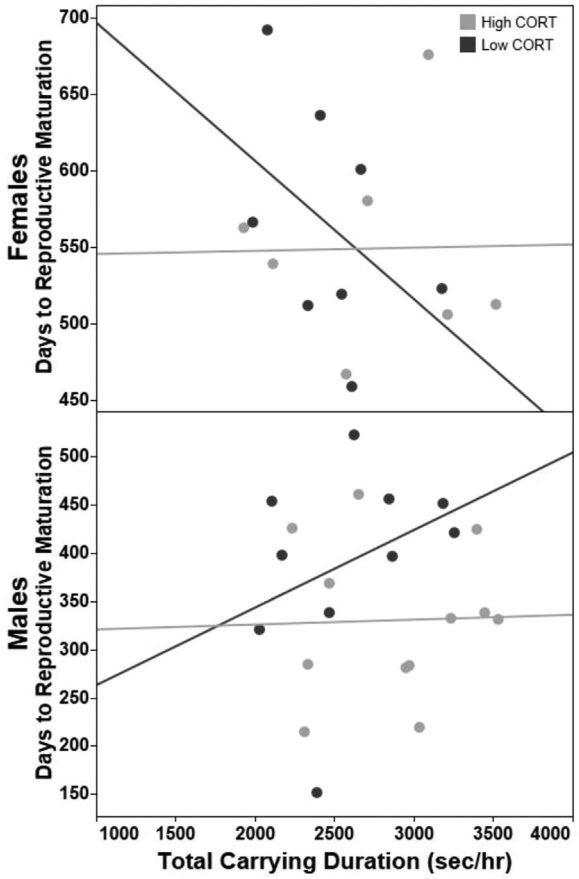

FIGURE 2 Interaction between total carrying and prenatal CORT exposure in females (top) and males (bottom). For illustrative purposes, prenatal CORT exposure data were split at the median for both males and females, with black circles/regression line representing lower third trimester CORT exposure (low CORT), and gray circles/regression line representing higher third trimester CORT exposure (high CORT). In offspring exposed to low prenatal CORT, increased total carrying predicted earlier reproductive maturation in females and later reproductive maturation in males. Total carrying duration did not affect reproductive maturation timing in offspring exposed to high prenatal CORT

not interact with prenatal steroid exposure (uA or CORT) in any trimester of pregnancy (regression: t's ≤ 1.58, df ≤ 34.97, P's ≥ 0.124). Paternal grooming, on the other hand, was predictive of timing of reproductive maturation, but these effects depended on the sex of the offspring and the timing of prenatal uA exposure. Specifically, the effect of paternal grooming on reproductive maturation timing depended on offspring sex and both first trimester uA exposure (regression: $b = 1983.96$, $t = 2.32$, df = 34.88, $P < 0.05$) and second trimester uA exposure (regression: $b = 1075.85$, $t = 2.38$, df = 34.92, $P < 0.05$). In female offspring exposed to lower first and second trimester uA, increased paternal grooming predicted later first ovulation, whereas females exposed to higher first or second trimester uA who received higher paternal grooming showed earlier timing of first ovulation. In male offspring exposed to lower first or second trimester uA, increased paternal grooming bouts predicted earlier reproductive maturation. Male offspring exposed to higher first trimester uA and higher paternal grooming showed earlier reproductive maturation, but male offspring exposed to higher uA in the second trimester and who received higher paternal grooming exhibited later reproductive maturation (Figures 3 and 4). The association between paternal grooming bouts and timing of reproductive maturation did not vary with prenatal CORT exposure (regression: t's ≤ 0.96, df ≤ 34.95, P's ≥ 0.343).

Alloparental grooming bouts did not predict timing of reproductive maturation in males or females, and did not interact with any trimester of androgen or CORT exposure (regression: t's ≤ 1.15, df ≤ 37.01, P's ≥ 0.259). The total number of grooming bouts received by caregivers predicted timing of reproductive maturation, and this effect depended on offspring sex and first trimester uA exposure

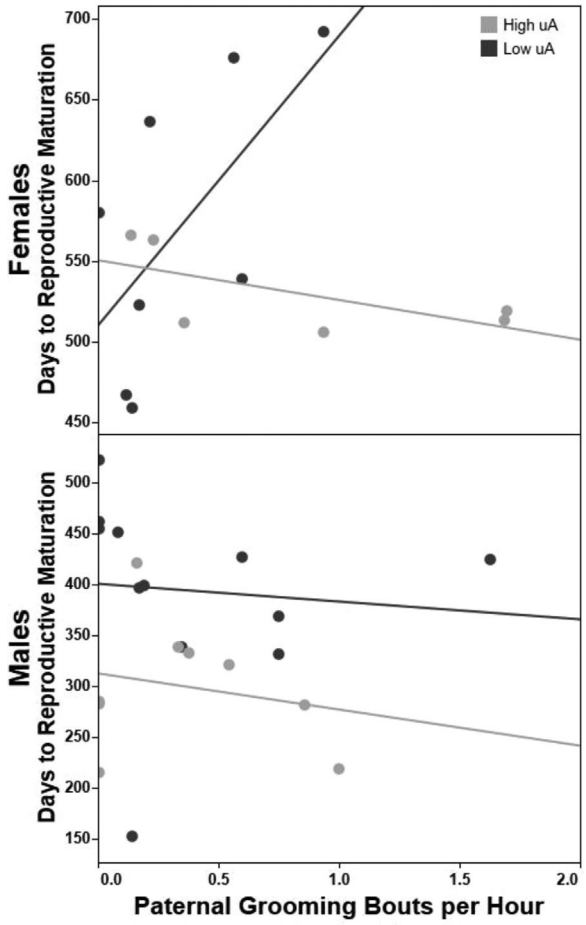

FIGURE 3 Influence of first trimester prenatal uA exposure and paternal grooming differs for females (top) and males (bottom). For illustrative purposes, first trimester uA exposure data were split at the median for both males and females, with black circles/regression line representing lower first trimester uA exposure (low uA), and gray circles/regression line representing higher first trimester uA exposure (high uA). In offspring exposed to low first trimester uA levels, increased paternal grooming was associated with later reproductive maturation in females; females with high uA exposure and increased paternal grooming showed earlier reproductive maturation. Increased paternal grooming predicted earlier reproductive maturity in males, regardless of uA exposure

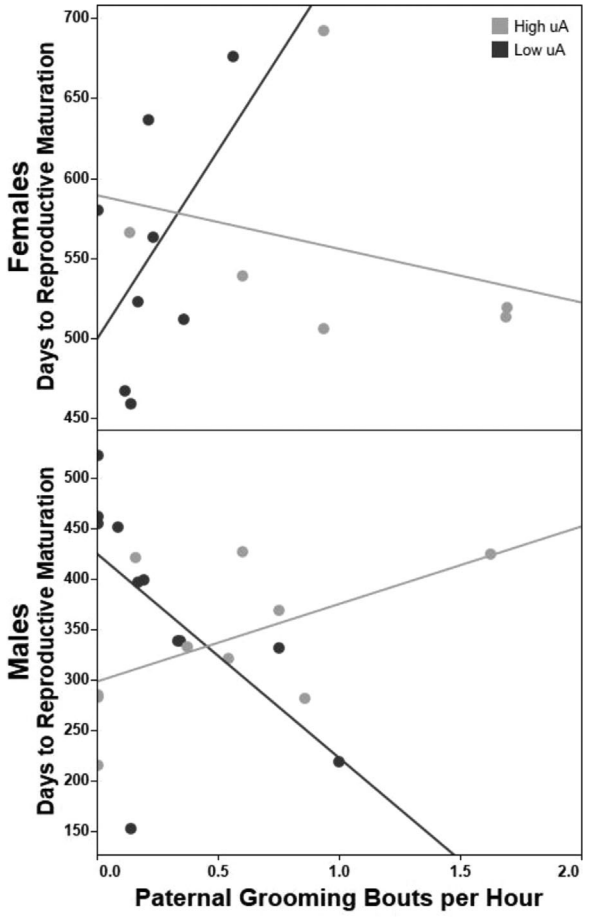

FIGURE 4 Effect of paternal grooming bouts and second trimester uA exposure differs for females (top) and males (bottom). For illustrative purposes, second trimester uA exposure data were split at the median for both males and females, with black circles/regression line representing lower second trimester uA exposure (low uA), and gray circles/regression line representing higher second trimester uA exposure (High uA). Low second trimester uA exposure and increased paternal grooming bouts predicted later timing of reproductive maturation in female offspring and earlier reproductive maturation in male offspring. In male exposed to high second trimester uA, increased paternal grooming predicted later reproductive maturation, whereas female offspring who received increased paternal grooming showed earlier reproductive maturation

(regression: $b = 1185.38$, $t = 2.38$, df = 34.98, $P < 0.05$). Female offspring exposed to lower prenatal uA levels and increased total grooming showed later first ovulation, whereas female offspring exposed to higher prenatal uA levels and increased total grooming showed earlier first ovulation. In male offspring exposed to higher prenatal uA levels, increased total grooming bouts predicted later reproductive maturation, but did not predict timing of reproductive maturation in male offspring exposed to lower prenatal uA exposure (Figure 5). The total number of grooming bouts did not interact with second or third trimester androgen exposure or CORT exposure in any trimester to predict timing of reproductive maturation (regression: t's ≤ 1.54, df ≤ 35.02, P's ≥ 0.133).

3.4 | Rejections/Removals

Maternal rejections/removals were associated with timing of reproductive maturation, but the effect depended on the sex of the offspring and early prenatal uA exposure (regression: $b = -2884.54$, $t = -2.50$, df = 28.91, $P < 0.05$). In female and male offspring exposed to lower first trimester uA, a higher number of maternal rejections/removals predicted earlier timing of reproductive maturation. Maternal rejections/removals were not predictive of timing of reproductive maturation in female or male offspring exposed to higher prenatal uA levels (Figure 6). The effect of maternal rejections/removals on timing of reproductive maturation did not depend on second or third

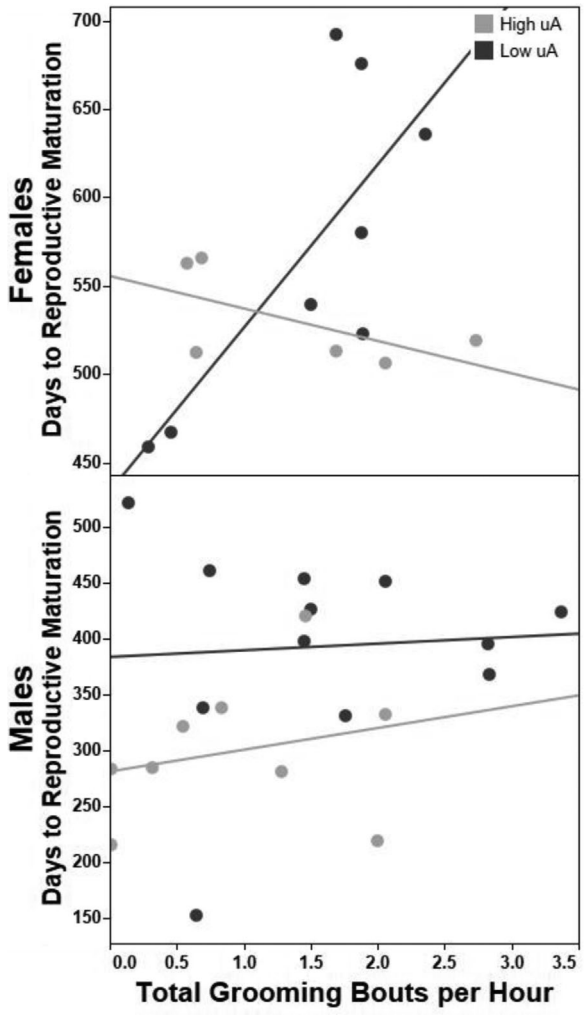

FIGURE 5 Interaction between total grooming by caregivers and first trimester uA exposure in females (top) and males (bottom). For illustrative purposes, first trimester uA exposure data were split at the median for both males and females, with black circles/regression line representing lower first trimester uA exposure (low uA), and gray circles/regression line representing higher first trimester uA exposure (high uA). As total grooming increased, female offspring exposed to low second trimester uA levels showed later first ovulation, whereas female offspring exposed to high prenatal uA showed earlier first ovulation. Male offspring who received higher total grooming showed later reproductive maturation, regardless of first trimester uA exposure

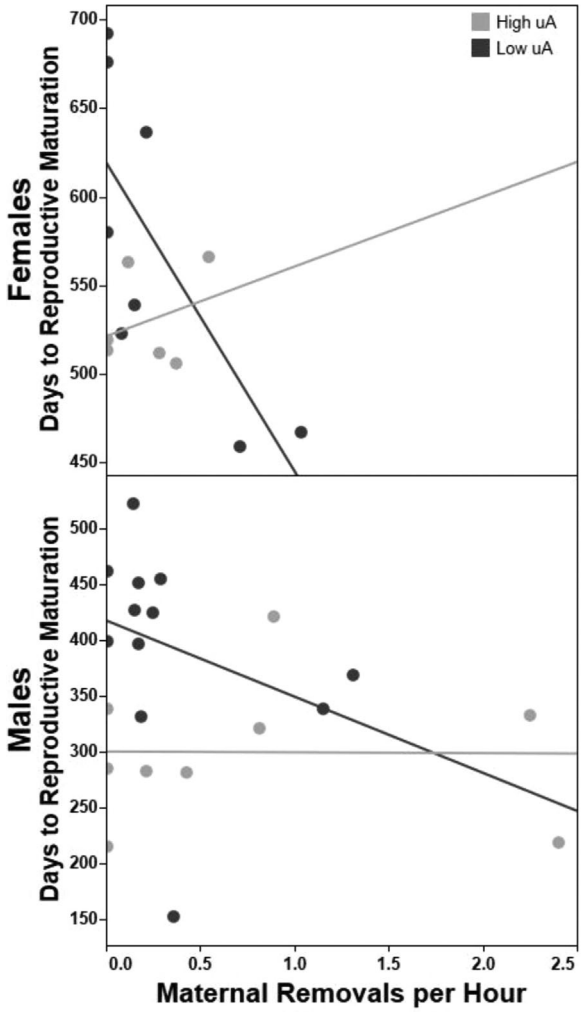

FIGURE 6 Interaction between infant rejections/removals by mother and first trimester uA exposure in females (top) and males (bottom). For illustrative purposes, first trimester uA exposure data were split at the median for both males and females, with black circles/regression line representing lower first trimester uA exposure (low uA), and gray circles/regression line representing higher first trimester uA exposure (high uA). As number of maternal rejections/removals increased, female and male offspring exposed to low second trimester uA levels showed earlier reproductive maturation. Females exposed to high prenatal uA showed later reproductive maturation, but maternal removals/rejections were not associated with timing of reproductive maturation in males with high first trimester uA exposure

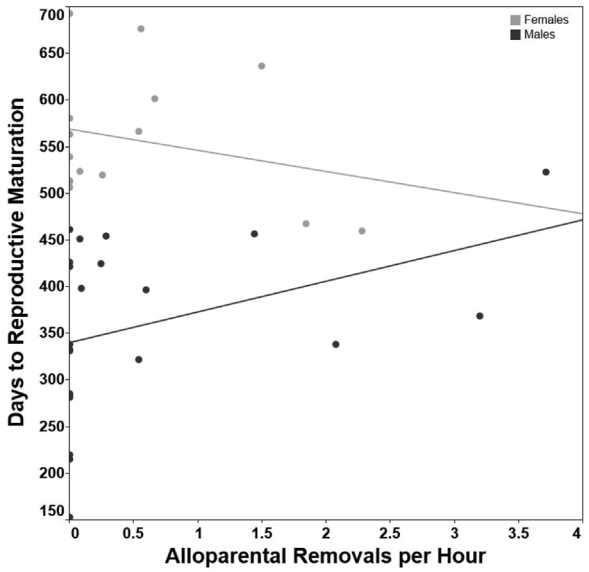

FIGURE 7 Increased alloparental removals/rejections predicted earlier reproductive maturation in female offspring (gray circles/regression line) and later reproductive maturation in male offspring (black circles/regression line)

trimester uA exposure or prenatal CORT exposure in any trimester of pregnancy (regression: t's ≤ 0.76, df ≤ 35.00, P's ≥ 0.451). Paternal rejections/removals were also not associated with timing of reproductive maturation and did not interact with prenatal steroid exposure (regression: t's ≤ 1.33, df ≤ 35.02, P's ≥ 0.202).

The number of alloparental rejections/removals predicted timing of reproductive maturation, and this effect depended on offspring sex (regression: b = 168.74, t = 2.10, df = 19.39, P < 0.05). Female offspring who experienced higher alloparental removals/rejections showed earlier first ovulation, whereas male offspring who experienced higher alloparental rejections/removals showed later reproductive maturation (Figure 7). The number of alloparental rejections/removals did not depend on prenatal steroid exposure (regression: t's ≤ 1.74, df ≤ 33.03, P's ≥ 0.093). The total number of rejections/removals by caregivers was not associated with timing of reproductive maturation and did not interact with offspring sex or prenatal steroid exposure (regression: t's ≤ 1.39, df ≤ 34.21, P's ≥ 0.176).

4 | DISCUSSION

We found that prenatal androgen and cortisol exposure interacts with post-natal care to influence timing of reproductive maturation in male and female marmosets. Consistent with our hypotheses, higher parental investment in the form of increased paternal and total carrying and grooming and reduced maternal and alloparental rejection/removals delayed first ovulation in females exposed to lower prenatal uA exposure. This is consistent with findings in human studies demonstrating earlier reproductive maturation in female offspring raised in a harsh maternal environment (Belsky, Houts, & Fearon, 2010; Belsky, Steinberg, Houts, & Halpern-Felsher, 2010; Mendle et al., 2015). However, contrary to our hypotheses, we also found that female offspring exposed to higher prenatal uA levels and increased paternal grooming showed earlier first ovulation, and female offspring who experienced increased maternal rejections/removals showed later first ovulation. These effects are opposite of those observed in females exposed to lower prenatal uA, and suggest that variations in prenatal androgen exposure renders the neurosecretory mechanisms that control pubertal onset differentially sensitive to post-natal care.

Interestingly, we also observed an effect of increased post-natal paternal investment on timing of reproductive maturation in male offspring. In male offspring exposed to lower prenatal uA levels, we found increased paternal care and maternal rejections/removals to predict earlier reproductive maturation. We observed minimal influence of post-natal care in male offspring with higher prenatal uA exposure. We also observed later reproductive maturation in male offspring as alloparental removals/rejections increased, suggesting a differential influence of maternal versus alloparental post-natal care. The effect of post-natal care on reproductive maturity in males was unexpected given previous findings in humans suggesting no influence of prenatal steroid exposure (Lunn et al., 1994) or parental care (Belsky et al., 2007) on pubertal timing in males. However, we also failed to find a main effect of prenatal steroid exposure on reproductive maturation timing; we found an effect of prenatal steroid exposure only when post-natal care was considered. Our findings thus expand the literature by demonstrating sensitivity of HPG axis mechanisms to interactions between pre-natal and post-natal factors in male offspring. It should be noted that relative to research on female offspring, there is little available research regarding the influence of prenatal steroids or post-natal parental care on reproductive maturation in male offspring. Additional research focusing on male offspring is warranted.

Similar to findings in humans and marmosets (Ellis et al., 1999; Matchock & Susman, 2006; Tither & Ellis, 2008), our results suggest that quality of paternal care affects pubertal timing in female marmosets, with increased paternal carrying and grooming predicting delayed first ovulation. Our findings also expand the evolutionary theory of socialization (Belsky et al., 1991; Ellis et al., 1999) by demonstrating that the influence of the post-natal family environment on reproductive maturation depends on the prenatal environment. Specifically, female offspring exposed to low prenatal uA levels may be more vulnerable to post-natal social effects on reproductive maturation and may demonstrate a shift toward earlier first ovulation in order to maximize reproductive opportunities in less hospitable environments. Females exposed to higher prenatal uA, on the other hand, show a similar response to paternal grooming and maternal removals as male offspring exposed to low uA. This may indicate a degree of masculinization of the HPG axis in females exposed to higher prenatal uA. Our findings also suggest that the evolutionary theory of socialization also applies to male marmoset offspring, with increased parental investment predicting accelerated reproductive maturation in order to possibly allow males to reproduce earlier and thus produce more offspring. Because males have a lower physiological burden associated with reproduction relative to females (Nievergelt & Martin, 1998), reproductive fitness is maximized in males by encouraging early

reproductive maturation. Finally, we also demonstrated that alloparental behavior can influence pubertal timing, as well as the total amount of care an offspring receives. Taken together, our findings show that investment in offspring, regardless of the source, can influence reproductive strategy in offspring.

Exposure to prenatal androgens during early and mid-gestation interacted with post-natal parental care to influence onset of reproductive maturation, which is consistent with other animal models (sheep (Kosut et al., 1997), rhesus macaques (Herman, Jones, Mann, & Wallen, 2000; Herman et al., 2006)) that demonstrate early to mid-gestation as a critical window for organizational effects of steroids. Early prenatal androgen exposure likely influences pubertal timing by altering the HPG axis. Sexual differentiation of neurosecretory mechanisms occurs early in prenatal development. The onset of puberty requires activation of GnRH neurons, which signals the release of LH and follicular stimulating hormone (FSH). In females, androgens defeminize the GnRH neurosecretory system, as evidenced by increased LH pulse frequency (Witham et al., 2012), increased LH secretion, absent estradiol benzoate-induced LH surges, and reduced expression of estradiol-induced progesterone receptors in the preoptic area (Foecking et al., 2005). Abnormalities in LH and FSH signaling, in turn, affects timing of reproductive maturation (Dumesic, Abbott, Eisner, & Goy, 1997; Goy, Uno, & Sholl, 1988; Herman et al., 2000; Kosut et al., 1997). Evidence suggests that post-natal care also influences HPG axis functioning. Female offspring of high grooming mothers show increased estrogen receptor-alpha (ERα) expression in the medial preoptic area of the hypothalamus (Champagne, Weaver, Diorio, Sharma, & Meaney, 2003), reduced ERα expression in the anteroventral periventricular nucleus (Cameron et al., 2008), and reduced methylation of CpG sites in the ERα1b promoter region (Champagne et al., 2006). Thus, early HPG axis programming by prenatal androgens is associated with differential susceptibility to post-natal paternal care and maternal harshness that, in turn, affects timing of reproductive maturation.

We found an effect of prenatal CORT exposure on timing of reproductive maturation in relation to total carrying duration. The sex differences in the interaction between prenatal CORT exposure and total carrying were opposite for prenatal uA exposure. In other words, females exposed to lower third trimester CORT and increased total carrying showed earlier first ovulation, whereas males showed later reproductive maturation. Additionally, unlike the effects observed with early prenatal androgen exposure, late prenatal CORT exposure interacted with post-natal care to influence timing of reproductive maturation. This is similar to other studies demonstrating that prenatal CORT exposure in mid- to late-gestation influences reproductive maturation timing (Cruz & Pereira, 2012; Kanitz, Otten, & Tuchscherer, 2006; Smith & Waddell, 2000). Contrary to our hypotheses, total carrying duration did not affect reproductive maturation in offspring exposed to higher prenatal CORT. We did not find an effect of prenatal CORT in relation to any other care behaviors (grooming or rejections/removals). Additionally, CORT only interacted with total carrying duration and was not specific to maternal, paternal, or alloparental carrying. This was surprising given that prenatal stress affects maternal caregiving, including reduced grooming, nursing, nesting, and pup retrievals (Champagne & Meaney, 2006; Moore & Power, 1986; Patin et al., 2002; Smith, Seckl, Evans, Costall, & Smythe, 2004). Because variability in prenatal CORT exposure results in differential neuroendocrine stress response (Kanitz et al., 2006) and sensitivity to the environment (Maccari et al., 2003), our findings indicate that the amount of carrying offspring receives, regardless of the provider, interacts with organizational effects of CORT to influence HPG axis function.

As with prenatal androgen exposure, post-natal care showed a stronger influence on reproductive maturation timing in offspring exposed to lower prenatal CORT levels. This contrasts with findings demonstrating delayed pubertal onset in offspring exposed to high CORT (Marchlewska-Koj et al., 2003; Smith & Waddell, 2000; Zehr et al., 2005). It should be noted that the literature on the effect of prenatal CORT exposure on pubertal timing is mixed. Some studies demonstrate alterations in timing of pubertal onset and sex steroid concentrations (Richardson et al., 2006), whereas others have failed to find differences (Crump & Chevins, 1989; Herrenkohl, 1979; Kotsampasi et al., 2009). Thus, additional research is necessary to elucidate the relationship between prenatal glucocorticoids and reproductive maturation.

We demonstrated that pre- and post-natal factors interact to influence timing of reproductive maturation in marmosets. It is possible that prenatal steroid exposure predisposes developing marmosets to be differentially sensitive to post-natal rearing environments, with elevated gestational steroids rendering HPG circuitry less sensitive to post-natal effects, and lower gestational steroids allowing HPG neural circuitry to remain labile and hence sensitive to the nature of early post-natal social environments. Dose-response studies may elucidate the level of prenatal androgens and CORT necessary for this to occur, and whether this threshold differs for males and females. It is also unknown whether the combined effects of prenatal steroids and post-natal parental care are unique to biparental species (such as marmosets). In other words, it remains unclear whether maternal carrying and grooming, along with rejections/removals, influences timing of reproductive maturation in species that do not demonstrate biparental or alloparental care. Due to a small sample size, interactions between prenatal uA and CORT could not be explored. As previously mentioned, HPG functioning depends on the HPA axis, and future research should examine interplay between the HPG and HPA axes on pubertal timing. Similarly, we were not able to examine interactions between affiliative and avoidant behaviors to determine which are most critical in influencing pubertal timing.

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Omaha Institutional Animal Care and Use Committee and adhered to all local, state, and national laws regulating research on nonhuman primates, including the principles outlined by the American Society of Primatologists.

CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare.

REFERENCES

Abbott, D. H., & Hearn, J. P. (1978). Physical, hormonal and behavioural aspects of sexual development in the marmoset monkey, *Callithrix jacchus*. *Journal of Reproduction and Fertility*, *53*, 155–166.

Abbott, D. H., Hodges, J. K., & George, L. M. (1988). Social status controls LH secretion and ovulation in female marmoset monkeys (*Callithrix jacchus*). *Journal of Endocrinology*, *117*, 329–339.

Abbott, D. H., McNeilly, A. S., Lunn, S. F., Hulme, M. J., & Burden, F. J. (1981). Inhibition of ovarian function in subordinate female marmoset monkeys (*Callithrix jacchus jacchus*). *Journal of Reproduction and Fertility*, *63*, 335–345.

Auyeung, B., Lombardo, M. V., & Baron-Cohen, S. (2013). Prenatal and postnatal hormone effects on the human brain and cognition. *Pflügers Archiv-European Journal of Physiology*, *465*, 557–571.

Belsky, J., Houts, R. M., & Fearon, R. P. (2010). Infant attachment security and the timing of puberty: Testing an evolutionary hypothesis. *Psychological Science*, *21*, 1195–1201.

Belsky, J., Steinberg, L., & Draper, P. (1991). Childhood experience, interpersonal development, and reproductive strategy: An evolutionary theory of socialization. *Child Development*, *62*, 647–670.

Belsky, J., Steinberg, L. D., Houts, R. M., Friedman, S. L., DeHart, G., Cauffman, E.,... NICHD Early Child Care Research. (2007). Family rearing antecedents of pubertal timing. *Child Development*, *78*, 1302–1321.

Belsky, J., Steinberg, L., Houts, R. M., & Halpern-Felsher, B. L. (2010). The development of reproductive strategy in females: Early maternal harshness→ earlier menarche→ increased sexual risk taking. *Developmental Psychology*, *46*, 120.

Birnie, A. K., Hendricks, S. E., Smith, A. S., Milam, R., & French, J. A. (2012). Maternal gestational androgens are associated with decreased juvenile play in white-faced marmosets (*Callithrix geoffroyi*). *Hormones and Behavior*, *62*, 136–145.

Birnie, A. K., Smith, A. S., Nali, C., & French, J. A. (2011). Social and developmental influences on urinary androgen levels in young male white-faced marmosets (*Callithrix geoffroyi*). *American Journal of Primatology*, *73*, 378–385.

Birnie, A. K., Taylor, J. H., Cavanaugh, J., & French, J. A. (2013). Quality of maternal and paternal care predicts later stress reactivity in the cooperatively-breeding marmoset (*Callithrix geoffroyi*). *Psychoneuroendocrinology*, *38*, 3003–3014.

Cameron, N., Del Corpo, A., Diorio, J., McAllister, K., Sharma, S., & Meaney, M. J. (2008). Maternal programming of sexual behavior and hypothalamic-pituitary-gonadal function in the female rat. *PloS ONE*, *3*, e2210.

Cavanaugh, J., & French, J. A. (2013). Post-partum variation in the expression of paternal care is unrelated to urinary steroid metabolites in marmoset fathers. *Hormones and Behavior*, *63*, 551–558.

Champagne, F. A., & Meaney, M. J. (2006). Stress during gestation alters postpartum maternal care and the development of the offspring in a rodent model. *Biological Psychiatry*, *59*, 1227–1235.

Champagne, F. A., Weaver, I. C., Diorio, J., Dymov, S., Szyf, M., & Meaney, M. J. (2006). Maternal care associated with methylation of the estrogen receptor-α1b promoter and estrogen receptor-α expression in the medial preoptic area of female offspring. *Endocrinology*, *147*, 2909–2915.

Champagne, F. A., Weaver, I. C., Diorio, J., Sharma, S., & Meaney, M. J. (2003). Natural variations in maternal care are associated with estrogen receptor α expression and estrogen sensitivity in the medial preoptic area. *Endocrinology*, *144*, 4720–4724.

Crump, C. J., & Chevins, P. F. D. (1989). Prenatal stress reduces fertility of male offspring in mice, without affecting their adult testosterone levels. *Hormones and Behavior*, *23*, 333–343.

Cruz, C. D., & Pereira, O. C. (2012). Prenatal testosterone supplementation alters puberty onset, aggressive behavior, and partner preference in adult male rats. *Journal of Physiological Sciences*, *62*, 123–131.

Dloniak, S. M., French, J. A., Place, N. J., Weldele, M. L., Glickman, S. E., & Holekamp, K. E. (2004). Non-invasive monitoring of fecal androgens in spotted hyenas (*Crocuta crocuta*). *General and Comparative Endocrinology*, *135*, 51–61.

Dubey, A. K., & Plant, T. M. (1985). A suppression of gonadotropin secretion by cortisol in castrated male rhesus monkeys (*Macaca mulatta*) mediated by the interruption of hypothalamic gonadotropin-releasing hormone release. *Biology of Reproduction*, *33*, 423–431.

Dumesic, D. A., Abbott, D. H., Eisner, J. R., & Goy, R. W. (1997). Prenatal exposure of female rhesus monkeys to testosterone propionate increases serum luteinizing hormone levels in adulthood. *Fertility and Sterility*, *67*, 155–163.

Ellis, B. J., & Garber, J. (2000). Psychosocial antecedents of variation in girls' pubertal timing: Maternal depression, stepfather presence, and marital and family stress. *Child Development*, *71*, 485–501.

Ellis, B. J., McFadyen-Ketchum, S., Dodge, K. A., Pettit, G. S., & Bates, J. E. (1999). Quality of early family relationships and individual differences in the timing of pubertal maturation in girls: A longitudinal test of an evolutionary model. *Journal of Personality and Social Psychology*, *77*, 387.

Fite, J. E., French, J. A., Patera, K. J., Hopkins, E. C., Rukstalis, M., & Ross, C. N. (2005). Elevated urinary testosterone excretion and decreased maternal caregiving effort in marmosets when conception occurs during the period of infant dependence. *Hormones and Behavior*, *47*, 39–48.

Foecking, E. M., Szabo, M., Schwartz, N. B., & Levine, J. E. (2005). Neuroendocrine consequences of prenatal androgen exposure in the female rat: Absence of luteinizing hormone surges, suppression of progesterone receptor gene expression, and acceleration of the gonadotropin-releasing hormone pulse generator. *Biology of Reproduction*, *72*, 1475–1483.

French, J. A., Brewer, K. J., Schaffner, C. M., Schalley, J., Hightower-Merritt, D., Smith, T. E., & Bell, S. M. (1996). Urinary steroid and gonadotropin excretion across the reproductive cycle in female Wied's black tufted-ear marmosets (*Callithrix kuhli*). *American Journal of Primatology*, *40*, 231–245.

French, J. A., Frye, B., Cavanaugh, J., Ren, D., Mustoe, A. C., Rapaport, L., & Mickelberg, J. (2016). Gene changes may minimize masculinizing and defeminizing influences of exposure to male cotwins in female callitrichine primates. *Biology of Sex Differences*, *7*:1.

French, J. A., Smith, A. S., & Birnie, A. K. (2010). Maternal gestational androgen levels in female marmosets (*Callithrix geoffroyi*) vary across trimesters but do not vary with the sex ratio of litters. *General and Comparative Endocrinology*, *165*, 309–314.

Goy, R. W., Uno, H., & Sholl, S. A. (1988). Psychological and anatomical consequences of prenatal exposure to androgens in female rhesus. In: T. Mori, & H. Nagasawa (Eds.), *Toxicity of hormones in perinatal life* (pp. 127–142). Boca Raton, FL: CRC Press, Inc.

Gubernick, D. J., & Nordby, J. C. (1992). Parental influences on female puberty in the monogamous California mouse, *Peromyscus californicus*. *Animal Behaviour*, *44*, 259–267.

Herman, R. A., Jones, B., Mann, D. R., & Wallen, K. (2000). Timing of prenatal androgen exposure: Anatomical and endocrine effects on juvenile male and female rhesus monkeys. *Hormones and Behavior*, *38*, 52–66.

Herman, R. A., Zehr, J. L., & Wallen, K. (2006). Prenatal androgen blockade accelerates pubertal development in male rhesus monkeys. *Psychoneuroendocrinology*, *31*, 118–130.

Herrenkohl, L. R. (1979). Prenatal stress reduces fertility and fecundity in female offspring. *Science*, *206*, 1097–1099.

Irfan, S., Wistuba, J., Ehmcke, J., Shahab, M., & Schlatt, S. (2015). Pubertal and testicular development in the common marmoset (*Callithrix jacchus*) shows high individual variation. *Primate Biology*, *2*, 1–8.

Jackson, M. R., & Edmunds, J. G. (1984). Morphological assessment of testicular maturity in marmosets (*Callithrix jacchus*). *Laboratory Animals*, *18*, 173–178.

Kanitz, E., Otten, W., & Tuchscherer, M. (2006). Changes in endocrine and neurochemical profiles in neonatal pigs prenatally exposed to increased maternal cortisol. *Journal of Endocrinology*, *191*, 207–220.

Kirby, E. D., Geraghty, A. C., Ubuka, T., Bentley, G. E., & Kaufer, D. (2009). Stress increases putative gonadotropin inhibitory hormone and decreases luteinizing hormone in male rats. *Proceedings of the National Academy of Sciences*, *106*, 11324–11329.

Kosut, S. S., Wood, R. I., Herbosa-Encarnación, C., & Foster, D. L. (1997). Prenatal androgens time neuroendocrine puberty in the sheep: Effect of testosterone dose 1. *Endocrinology*, *138*, 1072–1077.

Kotsampasi, B., Chadio, S., Papadomichelakis, G., Deligeorgis, S., Kalogiannis, D., Menegatos, I., & Zervas, G. (2009). Effects of maternal undernutrition on the hypothalamic-pituitary-gonadal axis function in female sheep offspring. *Reproduction in Domestic Animals*, *44*, 677–684.

Kuznetsova, A., Brockhoff, P. B., & Bojesen Christensen, R. H. (2015). lmerTest: Tests in linear mixed effects models. R package version 2.0–29. Available from: http://CRAN.R-project.org/package=lmerTest

Lunn, S. F., Recio, R., Morris, K., & Fraser, H. M. (1994). Blockade of the neonatal rise in testosterone by a gonadotrophin-releasing hormone antagonist: Effects on timing of puberty and sexual behaviour in the male marmoset monkey. *Journal of Endocrinology*, *141*, 439–447.

Maccari, S., Darnaudery, M., Mroley-Fletcher, S., Zuena, A. R., Cinque, C., & Van Reeth, O. (2003). Prenatal stress and long-term consequences: Implications of glucocorticoid hormones. *Neuroscience & Biobehavioral Reviews*, *27*, 119–127.

Marchlewska-Koj, A., Kruczek, M., Kapusta, J., & Pochroń, E. (2003). Prenatal stress affects the rate of sexual maturation and attractiveness in bank voles. *Physiology & Behavior*, *79*, 305–310.

Matchock, R. L., & Susman, E. J. (2006). Family composition and menarcheal age: Anti-inbreeding strategies. *American Journal of Human Biology*, *18*, 481–491.

Mendle, J., Ryan, R. M., & McKone, K. M. (2015). Early childhood maltreatment and pubertal development: Replication in a population-based sample. *Journal of Research on Adolescence*, DOI: 10.1111/jora.12201/full

Moore, C. L., & Power, K. L. (1986). Prenatal stress affects mother-infant interaction in Norway rats. *Developmental Psychobiology*, *19*, 235–245.

Munro, C. J., Stabenfeldt, G. H., Cragun, J. R., Addiego, L. A., Overstreet, J. W., & Lasley, B. L. (1991). Relationship of serum estradiol and progesterone concentrations to the excretion profiles of their major urinary metabolites as measured by enzyme immunoassay and radioimmunoassay. *Clinical Chemistry*, *37*, 838–844.

Mustoe, A. C., Jensen, H. A., & French, J. A. (2012). Describing ovarian cycles, pregnancy characteristics, and the use of contraception in female white-faced marmosets, *Callithrix geoffroyi*. *American Journal of Primatology*, *74*, 1044–1053.

Nievergelt, C. M., & Martin, R. D. (1998). Energy intake during reproduction in captive common marmosets (*Callithrix jacchus*). *Physiology & Behavior*, *65*, 849–854.

Noldus, L. P. (1991). The observer: A software system for collection and analysis of observational data. *Behavior Research Methods, Instruments, & Computers*, *23*, 415–429.

Nunes, S., Fite, J. E., & French, J. A. (2000). Variation in steroid hormones associated with infant care behaviour and experience in male marmosets (*Callithrix kuhlii*). *Animal Behaviour*, *60*, 857–865.

Nunes, S., Fite, J. E., Patera, K. J., & French, J. A. (2001). Interactions among paternal behavior, steroid hormones, and parental experience in male marmosets (*Callithrix kuhlii*). *Hormones and Behavior*, *39*, 70–82.

Patin, V., Lordi, B., Vincent, A., Thoumas, J. L., Vaudry, H., & Caston, J. (2002). Effects of prenatal stress on maternal behavior in the rat. *Developmental Brain Research*, *139*, 1–8.

Quinlan, R. J. (2003). Father absence, parental care, and female reproductive development. *Evolution and Human Behavior*, *24*, 376–390.

R Core Team. (2015). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Vienna, Austria: R Foundation for Statistical Computing. Available from: https://www.R-project.org/

Richardson, H. N., Zorrilla, E. P., Mandyam, C. D., & Rivier, C. L. (2006). Exposure to repetitive versus varied stress during prenatal development generates two distinct anxiogenic and neuroendocrine profiles in adulthood. *Endocrinology*, *147*, 2506–2517.

Santos, C. V., French, J. A., & Otta, E. (1997). Infant carrying behavior in callitrichid primates: *Callithrix* and *Leontopithecus*. *International Journal of Primatology*, *18*, 889–907.

Schradin, C., Schneider, C., & Yuen, C. H. (2009). Age at puberty in male African striped mice: The impact of food, population density and the presence of the father. *Functional Ecology*, *23*, 1004–1013.

Smith, T. E., & French, J. A. (1997). Psychosocial stress and urinary cortisol excretion in marmoset monkeys. *Physiology & Behavior*, *62*, 225–232.

Smith, J. T., & Waddell, B. J. (2000). Increased fglucocorticoid exposure delays puberty onset in postnatal life. *Endocrinology*, *141*, 2422–2428.

Smith, A. S., Birnie, A. K., & French, J. A. (2013). Prenatal androgens affect development and behavior in primates. In K. B. H. Clancy, et al. (Eds.), *Building babies: Primate development in proximate and ultimate perspective* (pp. 103–131). New York: Springer.

Smith, J. W., Seckl, J. R., Evans, A. T., Costall, B., & Smythe, J. W. (2004). Gestational stress induces post-partum depression-like behaviour and alters maternal care in rats. *Psychoneuroendocrinology*, *29*, 227–244.

Stearns, S. C., & Koella, J. C. (1986). The evolution of phenotypic plasticity in life-history traits: Predictions of reaction norms for age and size at maturity. *Evolution*, *40*, 893–913.

Tardif, S. D., Smucny, D. A., Abbott, D. H., Mansfield, K., Schultz-Darken, N., & Yamamoto, M. E. (2003). Reproduction in captive common marmosets (*Callithrix jacchus*). *Comparative Medicine*, *53*, 364–368.

Tither, J. M., & Ellis, B. J. (2008). Impact of fathers on daughters' age at menarche: A genetically and environmentally controlled sibling study. *Developmental Psychology*, *44*, 1409.

Uriarte, N., Breigeiron, M. K., Benetti, F., Rosa, X. F., & Lucion, A. B. (2007). Effects of maternal care on the development, emotionality, and reproductive functions in male and female rats. *Developmental Psychobiology*, *49*, 451–462.

Wang, Z., & Novak, M. A. (1994). Alloparental care and the influence of father presence on juvenile prairie voles, *Microtus ochrogaster*. *Animal Behaviour*, *47*, 281–288.

Witham, E. A., Meadows, J. D., Shojaei, S., Kauffman, A. S., & Mellon, P. L. (2012). Prenatal exposure to low levels of androgen accelerates female puberty onset and reproductive senescence in mice. *Endocrinology*, *153*, 4522–4532.

Wolf, C. J., Hotchkiss, A., Ostby, J. S., LeBlanc, G. A., & Gray, L. E. (2002). Effects of prenatal testosterone propionate on the sexual development of male and female rats: A dose-response study. *Toxicological Sciences*, *65*, 71–86.

Zehr, J. L., Van Meter, P. E., & Wallen, K. (2005). Factors regulating the timing of puberty onset in female rhesus monkeys (*Macaca mulatta*): Role of prenatal androgens, social rank, and adolescent body weight. *Biology of Reproduction*, *72*, 1087–1094.