STRESS SOCIO-ÉCOLOGIQUES LORS DE LA GESTATION ET EFFETS MATERNELS HORMONAUX CHEZ LE SURICATE (*SURICATA SURICATTA*) DU KALAHARI.

par

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Les stress socio-écologiques peuvent compromettre la reproduction des femelles et affecter le développement de leur progéniture. Par exemple, le stress gestationnel peut avoir de profonds effets sur la morphologie, le comportement, la physiologie et ultimement la valeur adaptative des jeunes. De tels effets maternels jouent un rôle particulièrement important chez les mammifères ayant un lien intime et prolongé avec leur progéniture. Les femelles se reproduisant avec l'aide coopérative d'un groupe doivent composer avec les tensions entre femelles reproductrices, qui mènent à la reproduction dans des contextes socio-écologiques plus ou moins stressants. Dans ce contexte, la thèse vise à identifier des indicateurs du stress gestationnel chez le suricate femelle (*Suricata suricatta*) en milieu naturel et investiguer les effets du stress maternel sur l'axe du stress de la progéniture. Le suricate est un mammifère grégaire coopératif chez qui le partage inégal de la reproduction entraîne une forte compétition entre les femelles, dont certaines se reproduisent hors des périodes de bonnes conditions alimentaires. Avant ce travail, le stress prénatal n'avait été étudié qu'en milieu contrôlé, entre autres faute d'une méthodologie adéquate permettant son estimation non invasive durant la reproduction de populations sauvages. Le suricate du Kalahari offrait alors une excellente opportunité d'étudier le lien entre le biais de la reproduction, les conditions socio-écologiques, le stress gestationnel et le phénotype hormonal de la progéniture. En effet, la disponibilité de données d'histoire de vie, de condition corporelle, de comportements sociaux et d'échantillons fécaux permettait l'étude longitudinale d'hormone de stress lors de la reproduction et les premiers moments de vie pour plus de 800 individus sauvages marqués depuis 1997. Le premier chapitre évalue l'effet de l'entreposage de longue durée d'échantillons fécaux sur les concentrations mesurées en hormones de stress (métabolites de glucocorticoïdes, fGC) et de reproduction (métabolites d'estrogènes, fE). Les résultats recommandent la lyophilisation précoce plutôt que l'entreposage d'échantillons frais à -20°C. De plus, l'étude souligne l'importance d'évaluer l'effet d'entreposage pour les différentes hormones étudiées et de contrôler pour le temps d'entreposage. Le deuxième chapitre étudie le lien entre le taux de reproduction, la dominance sociale et les fGC. Les changements en fGC au cours de la reproduction sont influencés par le chevauchement de reproductions, non par la dominance.

L'étude remet ainsi en cause le rôle des GCs dans la suppression de la reproduction chez les subordonnées. Le troisième chapitre étudie les stress socio-écologiques avant et pendant la gestation et identifie la compétition entre femelles et le chevauchement de reproduction lors de mauvaises conditions alimentaires comme facteurs entraînant une augmentation en fGC. Le quatrième chapitre évalue l'effet du stress gestationnel sur l'axe du stress de la progéniture avant le sevrage. Les résultats mettent en évidence l'effet maternel du chevauchement des reproductions en période de stress alimentaire, entraînant une augmentation en fGC de la progéniture, et l'effet, dépendant du sexe de la progéniture, de la compétition entre les femelles sur les fGC de la progéniture après la naissance, entraînant une diminution en fGC chez les fils, non chez les filles. L'étude démontre ainsi l'utilité du suivi longitudinal des fGC pour l'étude des stratégies d'histoire de vie et des effets maternels hormonaux. Les effets du stress alimentaire sur les GCs maternels lors du développement prénatal suggèrent un conflit entre les stratégies permettant de maximiser le succès reproducteur maternel et les effets néfastes sur la santé future des jeunes. Finalement, les effets maternels observés suggèrent un potentiel d'action des GCs gestationnels sur la croissance et le phénotype comportemental de la progéniture. Par exemple, l'effet maternel du stress social dépendant du sexe de la progéniture pourrait aussi modifier leur potentiel physiologique à gérer les stress associés aux stratégies comportementales de coopération et reproduction.

À Louise et Cyrille pour m'avoir donné le goût et la liberté de découvrir.

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"To study individual differences in stress physiology is to study [...] why some bodies [...] deal with stressors better than others" (Sapolsky, 2002).

INTRODUCTION

Hormones stéroïdiennes et réponse au stress

L'endocrinologie comportementale étudie comment les hormones influencent le comportement animal (Becker *et al.*, 2002). Spécifiquement, les causes mécanistiques et fonctionnelles de la réponse au stress ont été étudiées chez une diversité d'espèces animales (Sapolsky, 2002). Chez les vertébrés, l'étude empirique du stress a exploré les causes écologiques (Gesquiere *et al.*, 2008; Pankhurst, 2011; Wingfield, 2003), sociales (Creel *et al.*, 2012) et anthropiques (Van Meter *et al.*, 2009; Wasser and Hunt, 2005), ainsi que les conséquences sur la survie (Romero, 2012; Wada, 2008), la reproduction (Bonier *et al.*, 2009; Rubenstein and Shen, 2009;. Schreck, 2010) et les générations futures (Burton *et al.*, 2011; Haussmann *et al.*, 2012; Henriksen *et al.*, 2011; McGhee *et al.*, 2012; Meylan *et al.*, 2012). Ces études ont révélé l'importance des hormones stéroïdiennes permettant une rapidité et une flexibilité dans la réponse physiologique et comportementale nécessaire à la survie et la reproduction. Les stéroïdes sont des molécules dérivées du cholestérol transmettant les signaux perçus par les organes des sens vers les organes cibles, via les récepteurs cérébraux (Becker et Breedlove, 2002). Spécifiquement, les glucocorticoïdes (GCs) produits lors de la réponse à un stress (Fig. 1), tel que l'attaque d'un prédateur, permettent à la fois de mobiliser l'énergie nécessaire pour la fuite ou la défense et de stimuler la production de molécules anti-inflammatoires et analgésiques (Sapolsky, 2002). Ainsi, les GCs, en concert avec une diversité de composés chimiques du système neuroendocrinien, remplissent des fonctions métaboliques, immunitaires et comportementales vitales au bon fonctionnement de l'organisme et nécessaires à la survie et la reproduction (de Kloet, 1999; Dhabhar, 2002; Magiakou *et al.*, 1997; Whittle *et al.*, 2001; Wingfield et Kitaysky, 2002). Afin de produire une réponse physiologique et comportementale efficace face à un stress, les niveaux en GCs doivent être initialement bas, pour ensuite entraîner une augmentation rapide et brève dans la circulation sanguine et agir aux organes cibles (Fig. 1). Par contre, le stress excessif entraîne des élévations prolongées en GCs et des coûts physiologiques néfastes à la survie et la

reproduction (Goymann et Wingfield, 2004; Sapolsky *et al.*, 2000; Fig. 1). Ces coûts physiologiques, dits allostatiques, perturbent la normalité homéostatique de l'axe du stress (Korte *et al.*, 2005). Afin d'éviter les effets collatéraux du stress chronique sur les systèmes immunitaire, cardiaque, digestif et reproducteur, un individu tendra à respecter ses limites physiologiques en adoptant des stratégies comportementales adaptatives permettant de réduire le stress (Sapolsky *et al.*, 2000).

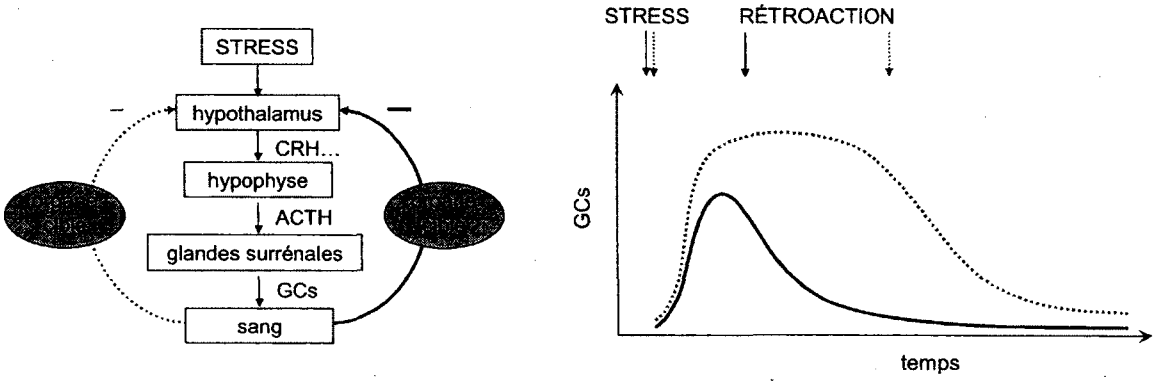

Figure 1. L'axe du stress : lors de l'occurrence d'un stress, l'hypothalamus initie la production en chaîne de corticolibérine (CRH) et hormones associées, d'adrénocorticotrophine (ACTH) et de glucocorticoïdes (GCs). Les GCs, produits en quelques minutes, agiront sur les organes cibles, via la circulation sanguine, puis par rétroaction signaleront à l'hypothalamus l'arrêt de leur production lorsque le stress cesse. Le contrôle par rétroaction de la production de GCs fonctionne adéquatement en réponse à un stimulus ponctuel (lignes pleines). Par contre, le stress chronique compromet la rétroaction causant ainsi une production élevée et prolongée de GCs (lignes pointillées), ce qui nuit à long terme à la santé immunitaire, métabolique et reproductrice. Figure inspirée de Sheriff *et al.* 2011.

Les niveaux de base en GCs, la réponse physiologique au stress ainsi que les stratégies comportementales employées pour éviter et gérer les situations stressantes peuvent varier d'un

individu à l'autre (Sapolsky, 2002). Ainsi, un même niveau en GCs peut indiquer un stress chez un individu et la normalité homéostatique chez un autre. De plus, plusieurs variables intrinsèques (espèce, sexe, age, condition corporelle, reproduction) et extrinsèques (heure du jour, saison, habitat, instabilité sociale) peuvent influencer les niveaux de bases et de stress en GCs. Notre compréhension du lien entre la réponse au stress, le succès reproducteur et le phénotype de la progéniture doit donc se baser sur une connaissance des variations individuelles en GCs.

Stress, socialité et reproduction

La survie et le succès reproducteur sont soumis à divers stress environnementaux, dont l'intensité sera perçue et gérée différemment selon les caractéristiques individuelles. Chez les vertébrés, divers systèmes sociaux ont évolué en lien avec l'intensité de stress écologiques, tels la disponibilité des ressources (Gesquiere *et al.*, 2008; Lynn *et al.*, 2010), le risque de prédation (Clinchy *et al.*, 2011; Eilam *et al.*, 1999) et divers défis climatiques (Boonstra, 2004; Wingfield et Sapolsky, 2003). En soi, la socialité amène aussi son lot de stress (Creel *et al.*, 2012) pouvant entraîner un biais dans le succès reproducteur au sein d'un groupe. Ainsi, on observe une grande variabilité entre les femelles au niveau du nombre de jeunes produits, annuellement et au courant de la vie, dépendamment de l'organisation sociale d'une espèce et des caractéristiques individuelles. Entre autres, la condition corporelle et le statut de dominance influenceront la capacité d'une femelle à se reproduire. Par exemple, chez les ongulés de montagne où la compétition pour la reproduction entre les femelles ne joue pas un rôle sur le succès reproducteur, la plupart des femelles produisent un jeune annuellement, et ce en fonction de leur âge et condition corporelle (Favre *et al.*, 2008; Festa-Bianchet *et al.*, 1998; Festa-Bianchet et King, 2007; Hamel *et al.*, 2010). Par contre, chez les rongeurs, primates et carnivores sociaux où la dominance entre femelles régis l'accessibilité à la reproduction, un biais dans le succès reproducteur permet à un petit nombre de femelles de produire un grand nombre de jeunes par portée et, lorsque les conditions environnementales sont favorables, plusieurs portées annuellement (Chelini *et al.*, 2011; Griffin *et al.*, 2003;

Saltzman *et al.*, 2009). Dans de tels systèmes sociaux, les comportements de dominance (Creel, 2001; Young *et al.*, 2008), ou la seule présence de la dominante (Bennett, 1994), peuvent entraîner l'abstinence ou la suppression du système reproducteur des femelles subordonnées, compromettant ainsi leur conception ou gestation.

Effets maternels chez les mammifères

La biodiversité émane de la variabilité individuelle et de l'hérédité des traits favorables à la survie et à la reproduction (Darwin, 1859). La valeur adaptative d'un animal est ainsi le produit d'interactions complexes entre son génotype et l'environnement prévalant au courant de la vie (Falconer, 1952; West-Eberhard, 1989). Chez les mammifères, l'environnement procuré par la mère, de la conception au sevrage, peut avoir de profonds effets sur le développement et le phénotype adulte morphologique, comportemental et physiologique. Par exemple, des études expérimentales (Edwards *et al.*, 1993; Huck *et al.*, 1986; Seckl, 1997) et démographiques (Lumey *et al.*, 1995; Prentice, 2005; Roseboom *et al.*, 2006; Ross et Desai, 2005) de populations soumises à la famine démontrent les effets néfastes du stress lors de la gestation sur la masse à la naissance et sur la prédisposition à des maladies cardiovasculaires, désordres alimentaires et déséquilibres métaboliques à l'âge adulte. L'environnement périnatal peut aussi affecter le développement fœtal, de façon adaptative ou non, en modifiant l'expression de comportements cognitifs (Meek *et al.*, 2000; Szuran *et al.*, 2000), agressifs (Marchlewska-Koj *et al.*, 2003) ou reproducteurs (Champagne et Meaney, 2006; Meek *et al.*, 2001; Meek *et al.*, 2006). Finalement, l'exposition à diverses hormones provenant de la mère (Brunton et Russell, 2011; Ward et Weisz, 1984) ou de foetus adjacent (Vom Saal, 1981; Vom Saal et Bronson, 1980) peut aussi modifier la physiologie de la reproduction (Unsworth *et al.*, 2005) et de la réponse au stress (Darnaudéry et Maccari, 2008) à l'âge adulte. Les conditions périnatales ont donc le potentiel d'affecter la valeur adaptative de la progéniture en lien avec des effets évidents sur la survie et le succès reproducteur, un départ difficilement contournable à l'âge adulte (Champagne, 2008; Lummaa et Clutton-Brock, 2002).

Transmission maternelle de l'axe du stress

Un facteur déterminant les aptitudes individuelles à gérer les différents stress pouvant compromettre la reproduction et la survie réside dans le tout premier environnement expérimenté lors du développement, avant même la naissance. L'exposition à diverses hormones durant des périodes critiques du développement fœtal ou postnatal programme la sensibilité aux stimuli perçus au courant de la vie (Arnold et Breedlove, 1985; Phoenix *et al.*, 1959). Notre conceptualisation actuelle de ce phénomène d'organisation et d'activation du système neuroendocrinien suggère un continuum d'effets plus ou moins permanents agissant entre autre au niveau des récepteurs hormonaux impliqués dans la réponse au stress (Fowden et Forhead, 2004; Sapolsky, 2002). En raison de la nature lipophile des stéroïdes, un foetus sera en contact avec les GCs produits par la mère et transitant via le placenta (Becker et Breedlove 1992). Une barrière enzymatique placentaire limite toutefois le transfert des GCs, ce qui modulera la programmation de l'axe du stress de la progéniture selon les niveaux de stress perçu par une femelle au courant de sa gestation (Seckl et Meaney, 2004). De plus, l'effet des GCs maternels sur le phénotype de la progéniture peut être collatéral ou adaptatif. Par exemple, des niveaux de stress excessif peuvent entraîner des risques importants pour la survie de la progéniture après la naissance (Seckl, 2004). En contraste, une femelle peut adopter des stratégies comportementales favorisant la valeur adaptative de sa progéniture en fonction du contexte socio-écologique et de l'exposition prénatale au GCs en résultant (Boonstra, 2005; Love et Williams, 2008; Meylan *et al.*, 2012). Par exemple, chez la marmotte à ventre jaune (*Marmota flaviventris*), Monclús *et al.* (2011) suggèrent une modification de l'effort reproducteur des femelles en conditions de forte pression de prédation entraînant des niveaux élevés en fGC gestationnel, ce qui favoriseraient la dispersion des fils et par le fait même leur survie et le succès reproducteur des mères.

Mesures non invasives des glucocorticoïdes

Le développement de méthodes non invasives de mesure d'hormones stéroïdiennes a ouvert la voie au suivi longitudinal de populations animales permettant l'étude de la physiologie de la reproduction et du stress en milieu naturel (Monfort, 2002). Ces méthodes ont d'abord aidé à la conservation d'espèces menacées pour lesquelles l'échantillonnage sanguin n'était pas possible (Millspaugh et Washburn, 2004; Romano *et al.*, 2010) et permettent désormais une meilleure compréhension des variations comportementales individuelles (Adkins-Regan, 2005) et de la dynamique des populations (Berger *et al.*, 1999; Cyr et Romero, 2007; Sheriff *et al.*, 2009).

Les GCs peuvent être mesurés directement dans la circulation sanguine sous forme biologiquement active, ou suite à leur métabolisation et accumulation dans les poils ou excrétion dans les fèces, l'urine, la salive ou le lait (Monfort, 2002; Sheriff *et al.*, 2011). L'avantage principal des mesures non invasives des GCs est d'éviter les variations hormonales liées à la capture et l'anesthésie nécessaire pour obtenir des échantillons sanguins. Les concentrations mesurées dans le sang sont un indice ponctuel de la production en GCs, variant de quelques secondes à quelques minutes selon la durée du stress. Les mesures des métabolites excrétés reflètent une période plus ou moins longue de production en GCs, variant, selon l'espèce et les caractéristiques individuelles, en minutes dans la salive et le lait, en heures ou jours dans l'urine et les fèces, et en semaines ou mois dans les poils (Dobson *et al.*, 1986; Granger *et al.*, 2007; Sheriff *et al.*, 2011). Ces différentes mesures trouvent donc leur utilité selon le type d'information que l'on recherche et l'étendue des phénomènes étudiés, qu'il s'agisse de réponses ponctuelles à un stress ou de variations associées à des stratégies d'histoire de vie ou des climats différents. De plus, la récolte d'échantillons fécaux chez des animaux sauvages est plus praticable que celle d'urine, de salive ou de poil.

La nature moléculaire robuste des stéroïdes confère une résistance à la digestion et aux procédés d'extraction permettant l'estimation non invasive des concentrations en GCs via l'excrétion de leurs métabolites dans les fèces (Wasser *et al.* 2000). La mesure des métabolites

fécaux de glucocorticoïdes (fGC), comme pour tout autre forme de métabolites, nécessite une validation méthodologique et biologique afin de corroborer les concentrations mesurées à une réponse au stress pour une espèce donnée (Palme, 2005). De plus, l'activité microbienne suivant la production des fèces (Woods, 1975) nécessite un protocole d'entreposage adéquat afin de limiter les variations en métabolites pouvant confondre les phénomènes étudiés (Hunt et Wasser, 2003; Möstl *et al.*, 1999; Pappano *et al.*, 2010). Il est donc nécessaire d'évaluer les méthodes d'échantillonnages et d'entreposages avant d'explorer l'étude des différences hormonales intra et interindividuelles.

Objectifs de la thèse

La présente étude vise à identifier des indicateurs du stress gestationnel chez le suricate femelle (*Suricata suricatta*) se reproduisant en milieu naturel et d'ainsi investiguer les effets du stress maternel sur l'axe du stress de la progéniture. D'abord, afin de valider l'utilisation d'échantillons fécaux dans le suivi longitudinal des stéroïdes d'espèces sauvages, l'effet d'entreposage de longue durée sur les concentrations hormonales mesurées devait être évalué chez le suricate (Chapitre I). Par ailleurs, afin d'étudier les sources extrinsèques de stress lors de la reproduction en milieu naturel, les effets de la reproduction sur les fGC devaient aussi être préalablement évalués en quantifiant la variation intra et interindividuelle en fGC, de 35 jours avant la conception jusqu'à la parturition (Chapitre II). Ainsi, l'étude des liens entre la dominance, le taux de reproduction et les fGC lors de la reproduction allait permettre de préciser le rôle des GCs dans le biais de la reproduction (Chapitre II). De plus, l'étude des liens entre le contexte socio-écologique et les fGC lors de la reproduction allait caractériser les stress gestationnels chez les suricates femelles (Chapitre III). Finalement, l'effet du stress maternel lors de la gestation sur l'axe du stress de la progéniture pouvait être évalué en étudiant la variation en fGC de jeunes suricates, de l'émergence au sevrage, en association d'une part avec les fGC maternels au courant de la gestation et d'autre part avec les facteurs précédemment identifiés comme stress gestationnel (Chapitre IV).

Les suricates du Kalahari

Les conséquences néfastes du stress chronique sur la survie et le succès reproducteur ont clairement été démontrées chez des espèces en captivité (Bartolomucci *et al.*, 2005; Blokhuis *et al.*, 1998; Selye, 1936). Toutefois, les causes naturelles du stress lors de la reproduction ont seulement récemment pu être examinées en milieu naturel grâce au suivi à long terme de population sauvage et au développement de technique non invasive permettant l'évaluation de la réponse au stress (Altmann *et al.*, 2010; Ryan *et al.*, 2012; Sheriff *et al.*, 2009). Le suivi longitudinal d'une population sauvage de suricate habitant le désert du Kalahari, en Afrique du Sud, offre une rare opportunité d'investiguer les caractéristiques individuelles ainsi que les conditions socio-écologiques associées à une augmentation dans la production en fGC au courant de la reproduction pouvant entraîner des effets sur le développement de l'axe du stress de la progéniture. L'habituation aux observateurs de la population de suricate de la Kuruman River Reserve a permis de générer, depuis 1997, des données détaillées sur l'affiliation, l'histoire de vie, la condition corporelle et les comportements sociaux de plus de 800 individus marqués, connus depuis la naissance pour la plupart, et répartis dans un quinzaine de groupes sociaux (Fig. 2). De plus, la récolte d'échantillons fécaux offrait l'opportunité de quantifier sur plusieurs générations les variations intra et interindividuelles en hormones de stress. Cet échantillonnage permettait ainsi d'évaluer les patrons en fGC lors de plusieurs événements de reproduction pour une même femelle et pour différentes femelles, au sein d'un même groupe et entre groupes de plus ou moins grande taille, et dans des conditions saisonnières de bonne et mauvaise disponibilité en nourriture. En plus du suivi non invasif de la physiologie de femelles et de leur progéniture, les connaissances acquises sur l'écologie de l'espèce (voir ci-dessous) et le détail de multiples facteurs pouvant influencer la production de GCs permettaient l'interprétation d'analyses complexes des variations en fGC dans des modèles de régressions multiples (Crawley, 2002). Effectivement, peu d'études ont utilisé les fGC lors de la reproduction dû à la difficulté de départager les différentes sources de variation en lien avec la reproduction et les stress socio-écologiques chez des espèces sauvages. Le suricate est un petit mammifère carnivore (<1Kg) grégaire se reproduisant dans un habitat aride avec l'aide coopérative d'un groupe variant de 2 à 40 individus. Un couple dominant monopolise la

reproduction (Clutton-Brock *et al.*, 2001b) et partage les soins aux jeunes et la protection du territoire avec les individus subordonnés du groupe, la plupart apparentés outre quelques males immigrants (Clutton-Brock *et al.*, 2001a; Clutton-Brock *et al.*, 2002). Le partage de la garde des jeunes au terrier pendant le premier mois de vie et le nourrissage coopératif des jeunes permettent d'augmenter grandement le recrutement annuel (Clutton-Brock *et al.*, 2001b; Clutton-Brock *et al.*, 2002). Une augmentation de la taille du groupe permet de sécuriser le territoire des invasions de groupes avoisinants ainsi que de prévenir les attaques de prédateurs aériens et terrestres (Clutton-Brock *et al.*, 1999a). En outre, la vigilance partagée permet d'augmenter l'efficacité d'alimentation de chacun (Clutton-Brock *et al.*, 1999c). L'évolution d'un tel système de reproduction coopérative permet à plusieurs femelles de se reproduire au sein d'un même groupe ainsi qu'à certaines femelles de se reproduire plusieurs fois annuellement, produisant de deux à six jeunes par portée (Russell *et al.*, 2003a). Le suricate habite des zones désertiques caractérisées par deux saisons de six mois ayant un climat très différent au niveau des régimes de pluie et de température et amenant des défis d'alimentation et de thermorégulation. La saison chaude et humide (de novembre à avril) est caractérisée par d'abondantes pluies synchronisant la disponibilité en nourriture avec la majorité des reproductions (Barnard, 2000; Doolan et Macdonald, 1997). En contraste, les événements de reproduction sont plus rares lors de la saison froide et sèche (de mai à octobre) alors que certaines femelles s'y reproduisent afin d'augmenter leur reproduction ou éviter la compétition avec d'autres femelles du groupe pouvant entraîner de violents affrontements culminant à l'expulsion d'une femelle ou à l'infanticide de sa progéniture (Young et Clutton-Brock, 2006). Quoique des niveaux élevés en fGC peuvent entraîner un échec de la reproduction chez les suricates femelles, en lien avec le stress de l'expulsion hors du groupe (Young *et al.*, 2006; Young *et al.*, 2008), plusieurs femelles parviennent à produire des jeunes dans des conditions de forte compétition entre femelles ou de faible disponibilité saisonnière en nourriture. Les suricates à l'étude offrent donc la possibilité d'évaluer l'effet de différent niveau de stress socio-écologiques prévalant lors de la gestation sur la production en GCs de la progéniture.

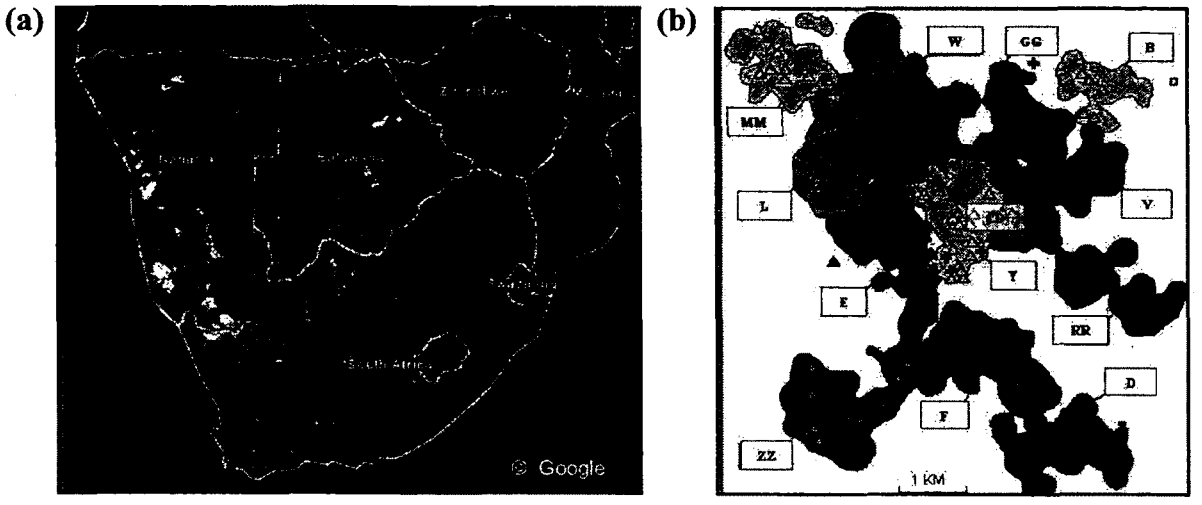

Figure 2. (a) Localisation du site d'étude près de Van Zyl's Rus (26°59' S, 21°50' E) en Afrique du Sud et (b) groupes de suricates de la Kuruman River Reserve. (b) Les initiales réfèrent au nom des groupes et les symboles, aux latrines démarquant chaque territoire (modifié de Jordan, 2005).

CHAPITRE I
ENTREPOSAGE DE MÉTABOLITES STÉROÏDIENS FÉCAUX

Les métabolites stéroïdiens fécaux permettent l'étude non invasive du stress et de la reproduction chez les animaux sauvages (Monfort, 2002). Le suivi longitudinal d'excrétion hormonal au niveau individuel et populationnel nécessite un échantillonnage répété de fèces sur une longue période. Puisque les métabolites stéroïdiens fécaux subissent une dégradation suite à leur production (Millspaugh et Washburn, 2004; Sheriff *et al.*, 2011; Woods, 1975), un entreposage rapide et adéquat est primordial (Hunt et Wasser, 2003; Terio *et al.*, 2002). De plus, une compréhension des changements en concentrations des métabolites étudiés est cruciale dans l'élaboration des protocoles d'échantillonnage et d'entreposage de longue durée. Plusieurs études sur les changements en concentrations de métabolites recommandent la congélation à -20°C (Möstl *et al.*, 1999; Pappano *et al.*, 2010). Cependant, les changements en concentrations mesurées suivant un entreposage de quelques semaines ou quelques mois diffèrent selon le type de métabolites et l'espèce étudiés. En effet, les résultats démontrent selon le cas des augmentations, des diminutions, des changements quadratiques ou simplement aucun changement en concentration d'hormone (Palme, 2005). De plus, nos connaissances demeurent limitées quant aux changements en concentrations au delà d'une année d'entreposage ainsi qu'a l'effet du medium entreposé, soit des échantillons frais, lyophilisé ou extrait. La congélation longue durée d'échantillons fécaux frais est la méthode d'entreposage la plus répandue d'où l'importance de comprendre l'effet des conditions d'entreposage (Palme, 2005). Je propose donc d'étudier l'effet de l'entreposage de longue durée d'échantillons fécaux sur les concentrations en métabolites stéroïdiens afin de justifier l'utilisation d'une base de données longitudinales de 11 ans pour l'étude du stress gestationnel et de ces effets sur l'axe du stress de la progéniture chez le suricate sauvage.

CHAPITRE I

THE IMPACT OF LONG-TERM STORAGE ON FECAL STEROID METABOLITES IN FREE-RANGING MEERKATS.

MARIE-FRANCE BARRETTE, ANDREW J. YOUNG, ET STEVEN L. MONFORT

Description de l'article et contribution

L'article étudie l'effet de différentes conditions d'entreposage de longue durée d'échantillons fécaux sur les concentrations en hormone de stress (fGC) et de reproduction (fE) chez le suricate (*Suricata suricatta*). Les résultats démontrent une diminution des concentrations en fGC suite à quatre ans d'entreposage d'échantillons frais. Une telle diminution n'est pas observée pour les fGC d'échantillons lyophilisés ou extraits, ni pour tout échantillon de fE. Ces résultats recommandent donc la lyophilisation précoce plutôt que l'entreposage d'échantillons frais pour la conservation longue durée à -20°C. De plus, les résultats suggèrent une réduction différentielle des concentrations mesurées en fGC et fE pour les échantillons ayant des concentrations initiales plus élevées. L'étude souligne ainsi l'importance de contrôler pour le temps d'entreposage dans l'étude longitudinale des rôles mécanistiques et fonctionnels des stéroïdes et d'évaluer l'effet d'entreposage pour les différentes hormones et espèces étudiées.

J'ai développé l'idée de l'article avec Andrew Young et Steven Monfort. Andrew Young a récolté les échantillons et effectué la première série d'analyses hormonales. J'ai effectué les séries subséquentes d'analyses hormonales, les analyses statistiques et la rédaction. Andrew Young et Steve Monfort ont commenté une version préliminaire du manuscrit et participé à la subvention du projet.

THE IMPACT OF LONG-TERM STORAGE ON FECAL STEROID METABOLITES IN FREE-RANGING MEERKATS.

par

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Key words: longitudinal monitoring; glucocorticoids; corticosterone; estrogens; feces.

Abstract

Fecal steroid metabolites are effective for noninvasive studies of stress and reproduction in wild animals. Field studies often require long-term storage of fecal samples, yet we know little about the impact of storage on measures of fecal steroid metabolite concentrations. Using samples collected from free-ranging meerkats (*Suricata suricatta*), we examined the effect of storage methods (i.e. dried steroid extract, dried fecal powder or wet feces) across a four-year frozen storage interval (-20°C) on adrenal (fecal glucocorticoid metabolites, fGC, n=44) and reproductive (fecal estrogen metabolites, fE, n=17) steroid hormone metabolite concentrations. We assessed whether storage methods affected (i) mean steroid concentrations and compared (ii) the direction and (iii) slope of changes between steroid concentrations before and after four-year storage. We also (iv) tested the strength of the association between metabolite concentrations before and after four-year storage to quantify confounding variation for each storage method. Mean fGC concentrations derived from wet feces were reduced by four-year storage and although no mean differences were observed from dried fecal powder and dried steroid extract, only the latter showed no differential changes dependant on initial fGC concentrations, i.e. no significant deviation from a regression slope of one. Sex differences in regression slopes were observed from dried fecal powder and dried steroid extract, with male fGC levels after four-year storage being more proportional to initial concentrations in dried steroid extracts than female fGC levels. No mean differences in fE concentrations were observed under all three storage methods. However, only dried fecal powder yielded no differential changes dependant on initial fE concentrations. Four-year storage introduced greater variation in fGC and fE concentrations derived from wet feces than the other storage methods. Furthermore, fGC concentrations derived from dried fecal powder and dried steroid extract varied less than fE under similar storage methods. Our findings suggest that early drying of fecal samples may enhance reliability of steroid metabolite measurements and that pre-storage fecal extraction may be unnecessary. Our study underlines the potential of using fecal steroid metabolite assessments for long-term studies of stress and reproduction in meerkats, but also highlights the need for careful analysis of storage effect on fecal steroid measures.

Introduction

The assessment of physiological stress (Sheriff *et al.*, 2011; Wasser *et al.*, 2000) and reproductive health (Monfort, 2002; Schwarzenberger *et al.*, 1996; Ziegler *et al.*, 1997) in free-ranging populations via noninvasive measures of fecal steroid metabolites is a valuable tool for understanding the proximate regulation of behavior (Adkins-Regan, 2005), population dynamics (Berger *et al.*, 1999; Cyr and Romero, 2007; Sheriff *et al.*, 2009) and to address related conservation issues (Millspaugh and Washburn, 2004; Romano *et al.*, 2010). Fecal hormone monitoring permits tracking individuals and populations in species for which blood sampling is not a viable option, and for species that are easily habituated to close observations, thus enabling regular sampling without the need of capture. Fecal metabolites provide an integrated, indirect measure of biologically active steroids secreted in blood circulation (Monfort, 2002; Sheriff *et al.*, 2010a) over time intervals ranging from less than an hour to more than a day prior to sampling depending on excretion rates (Palme, 2005). Pooled measures therefore provide a valuable proxy for endocrine changes associated with life-history stages, and avoid the pitfalls of assessing blood hormones, which are secreted in a highly variable pattern and provide only a narrow point-in-time assessment of steroid production. Furthermore, noninvasive techniques avoid confounding effects of handling, capture and anesthesia (Sheriff *et al.*, 2011).

Longitudinal studies of hormone excretion in individuals and populations require repeated fecal sampling for extend time intervals (Williams, 2008), which necessitates an understanding of the impact of long-term storage on hormone metabolite concentrations. For a variety of taxa, fecal steroid metabolites degrade over time resulting in the detection of either increased or decreased immunoreactive steroid metabolites (Millspaugh and Washburn, 2004; Sheriff *et al.*, 2011). Such changes in steroid metabolite concentrations could arise from bacterial metabolism (Woods, 1975) in addition to biochemical changes that may affect immunoreactivity and assay reliability (Terio *et al.*; 2002). To reduce the impact of microbial activity, feces should be appropriately stored as soon as possible after excretion (Hunt and Wasser, 2003). Studies showing effects of storage on fecal steroid metabolite concentrations

generally suggest freezing samples (Möstl *et al.*, 1999; Pappano *et al.*, 2010), rather than keeping them refrigerated (Lynch *et al.*, 2003) or in solvents such as alcohol (Khan *et al.*, 2002; Palme, 2005). Whereas plasma and serum steroids can be stable for decades once frozen at -20°C (Stroud *et al.*, 2007), fecal steroid metabolite concentrations may be less stable. For instance, a slight but significant decline in fecal glucocorticoid metabolite (fGC) concentration was observed in samples collected from yellow baboons (*Papio cynocephalus*) after just two weeks of storage at -20°C (Lynch *et al.*, 2003), although Khan *et al.* (2002) identified an initial fGC concentration increase followed by a return to earlier concentrations after four months storage for the same species. In contrast, fecal estrogen metabolite (fE) concentrations fluctuated inconsistently under the same storage conditions (Khan *et al.*, 2002). Furthermore, Pettitt *et al.* (2007) observed no change in Cape ground squirrel (*Xerus inauris*) fE concentrations following storage for three months at -20°C. The diversity of results from these studies reinforce the importance of understanding the impact of storage methods on the detected concentrations of steroid metabolites in feces, and emphasize that storage effects can vary by steroid, species and gender (Palme, 2005). Furthermore, we know little about how concentrations of different fecal steroid metabolites vary over storage intervals exceeding one year, or about the effects of the state of the stored specimen (i.e. frozen steroid extract, dried fecal powder or wet feces). Because such long-term freezing methods are the most common ways of storing samples, it is crucial to assess whether drying and extracting steroids as soon as possible provide better estimates.

Novel approaches that permit samples to be processed, extracted and stored under field conditions or with only rudimentary infrastructure have shown promise (Moss *et al.*, 2010) for field applications, but more work is needed to validate these approaches for other species, and especially to assess the impacts of storage duration and methods on the reliability of endocrine assessments, particularly when samples are stored long-term. We studied the effects of different long-term storage methods on adrenal (fGC) and reproductive (fE) steroid concentrations in meerkats (*Suricata suricatta*) fecal samples. Specifically, we assessed whether fGC and fE (females only) concentrations in frozen (-20°C) dried steroid extract, dried fecal powder and wet feces were consistent after a four-year storage interval: i.e. (i)

whether mean concentrations changed after four-year storage, (ii) whether reductions or increases in mean steroid concentrations where observed and (iii) whether different storage methods led to differential changes dependant on initial steroid concentrations. Furthermore, we assessed (iv) whether four-year storage introduced significant confounding variation on fGC and fE measurements.

Methods

Sample collection and study design

We collected 44 fecal samples in 2000 from 19 male (fGC n=28) and 9 female (fGC n=16; fE n=17) free-ranging meerkats at the Kuruman River Reserve in the South African Kalahari Desert (see Clutton-Brock *et al.*, 1999b for details of study species). Whenever animals defecated, samples were immediately placed on ice in thermos flasks, frozen at -20°C within five hours (mean ca. 2h) and transported frozen to the Smithsonian Conservation Biology Institute in Front Royal, VA, USA. To study changes in fGC and fE concentrations with storage time and methods, we split each fecal sample in two parts in 2001. One portion was dried and crushed into fecal powder from which steroids were extracted and assayed in 2001 (Year 1) to measure fGC (n=44) and fE (n= 17; females only) concentrations. The remaining portion of unprocessed wet feces, along with the remaining dried fecal extract and dried fecal powder, were all kept frozen at -20°C until 2005 (Year 4) when identical extraction and assay protocols were repeated for fGC and fE for all samples (see protocols below).

Fecal steroids extraction

Steroid metabolites were extracted from samples using validated methodologies (Monfort *et al.*, 1997). Briefly, fecal samples were freeze-dried (VirTis XL-70, SP Industries, New York), pulverized and thoroughly mixed. Fecal powder (0.18–0.19 g) was then combined with 6 ml of 100% ethanol, vortexed (10 s) and boiled (20 min) to extract steroid metabolites. After centrifugation (2500 g, 20 min), the supernatant was decanted into a tube and fully dried under a stream of compressed air; during evaporation, the vessel walls were rinsed twice with

ethanol (4 ml, then 2 ml). The residue was then re-dissolved in methanol (1 ml) and placed in an ultrasonic glass cleaner (5 min). A portion of the extractant was then diluted 1:50 in diluent buffer (pH 7.0) and frozen (−20 °C) for subsequent radioimmunoassay (RIA).

Corticosterone radioimmunoassay

Concentrations of fGC were determined using a double-antibody ^{125}I RIA for corticosterone (ICN Biomedicals Inc., Costa Mesa, California, USA), which was validated in meerkats (Young *et al.*, 2006). The antiserum cross-reacts 100% with corticosterone, 0.34% with desoxycorticosterone, 0.10% with testosterone, 0.05% with cortisol, 0.03% with aldosterone, 0.02% with progesterone and <0.01% with all other steroids tested. Fecal extracts (1:50 dilution) were assayed (50 µl) in duplicate according to the instructions provided with the kit, except that all reagent volumes were halved. Assay sensitivity was 25 ng/ml and intra-assay coefficients of variation were <10%. Samples were analyzed in a single assay in 2001 and in another single assay in 2005. Inter-assay variation between 2001 and 2005 values could not be calculated with the use of low and high internal controls.

Estrogens radioimmunoassay

Concentrations of fE were determined using a double-antibody ^{125}I RIA for total estrogens (ICN Biomedicals Inc., Costa Mesa, California, USA), previously validated in meerkats (Moss *et al.*, 2001). The antiserum cross-reacts 100% with both estradiol-17β and estrone, 9.0% with estriol, 7.0% with estradiol-17α, 2.5% with equilin, and <0.01% with all other steroids tested. Fecal extracts (1:40-1:8000, depending on sex and reproductive status) were assayed (250 µl) in duplicate according to the instructions provided with the ICN kit except that all reagent volumes were halved. Assay sensitivity was 4 pg/ml and intra-assay coefficients of variation were <10%. A single assay included all samples analyzed in 2005. Inter-assay variation between 2001 and 2005 values could not be calculated with the use of low and high internal controls.

Steroid data analyses

Statistical analyses were conducted with R (R Development Core Team, 2008). To assess changes in mean steroid hormone concentrations after a four-year storage interval, (i) we used Wilcoxon signed-rank statistics, testing for within-sample differences between metabolite (fGC and fE) concentrations of samples assayed twice, originally in Year 1 and re-assayed in Year 4 after having been stored frozen as either dried steroid extract, dried fecal powder or wet feces. To characterize the direction of any changes in fGC and fE concentrations, (ii) we described how concentrations in Year 1 changed compared to Year 4 concentrations with linear regressions (LMs). Steroid concentrations on Year 1 were fitted as predictor of Year 4 concentrations. To consider non-linear variation with storage time, linear and quadratic regression between Year 1 and Year 4 concentrations were compared with the Akaike information criterion (AIC). To assess whether different storage methods produced differential changes dependant on initial steroid concentrations, we (iii) determined whether LMs regression slopes from each storage method differed from a slope of one and compared regression slopes among all three storage methods by using two-tailed unpaired Welch t-test. To control for any sex differences in initial fGC concentrations, we fitted the animal gender for each samples as a fixed effect in LM analyses. Steroid data are presented with regression lines predicted by respective LMs. To examine the extent to which different storage methods introduced confounding variation into fGC and fE data, we (iv) tested the strength of the association between values originally assayed in Year 1 with those assayed in Year 4 with coefficients of determination.

Results

Effect of storage time and methods on fGC concentrations

Storage time did not significantly affect mean fGC concentrations compared to original concentrations in samples frozen four years as dried steroid extract (Table 1; t-test: W=955, p=0.92) or dried fecal powder (Table 1; t-test: W=777, p=0.11), although significant reductions were observed for wet feces (Table 1; t-test: W=712, p=0.03). The associations

between fGC concentrations in Years 1 and 4 were linear and differed for the two sexes in samples frozen as dried steroid extract (Fig. 1a; LM testing sex and Year 1 data interaction: effect±se=0.17±0.07, p=0.02) or dried fecal powder (Fig. 1b; LM testing sex and Year 1 data interaction: effect±se=0.27±0.09, p=0.004), but there was no significant sex effect in samples frozen as wet feces (Fig. 1c; LM testing sex and Year 1 data interaction: effect±se=-0.10±0.11, p=0.38). The concentrations in fGC from male dried steroid extracts in year 4 were directly proportional to those measured in year 1 (i.e. slope=1; Fig. 1a) while the concentrations in year 4 were less than expected from the first year estimates (slope <1) with all other storage methods for both sex (Table 2; Fig. 1b and c). Within each gender, higher fGC concentrations were more affected than lower ones (lower regression slopes) when derived from dried fecal powder (Table 2; Fig. 1b) than from dried steroid extract (Fig. 1a). Furthermore, higher fGC concentrations were more affected than lower ones (lower regression slopes) when derived from wet feces (Fig. 1c) compared to dried steroid extract and dried fecal powder, although not for females derived from dried fecal powder which yielded similar regression slopes as wet feces (Table 2; Fig. 1b). Finally, fGC concentrations measured in Years 1 and 4 were more weakly associated for wet feces (r^2=0.73) than for dried fecal powder (r2=0.92) or steroid extract (r2=0.97).

Table 1. Meerkat fGC concentrations (ng/g feces) before (Year 1) and after (Year 4) four-year storage at -20°C as dried steroid extract, dried fecal powder and wet feces.

	Mean ± SE	Median	1st- 3rd IQRs
Year 1	132.7 ± 20.0	103.5	58.4 - 141.8
Year 4 dried extract	130.8 ± 20.4	98.7	49.1 - 144.3
Year 4 dried powder	100.1 ± 16.6	66.6	46.4 - 96.4
Year 4 wet feces	85.3 ± 23.5	60.2	49.5 - 80.2

Figure 1. Variation in fGC concentrations in meerkat fecal samples measured after four-year storage as (a) dried steroid extract (n=43), (b) dried fecal powder (n=43) and (c) wet feces (n=44). Regression lines were predicted from respective LMs with sex differences, when significant, presented by black dots and solid lines for males, and clear dots and broken lines for females: (a) male $F_{1,26}$=1326.0, p<0.0001, slope standard error (sse)=0.03, female $F_{1,14}$=256.6, p<0.0001, sse=0.05; (b) male $F_{2,25}$=612.9, p<0.0001, sse=0.04, female $F_{1,14}$=129.3, p<0.0001, sse=0.07; (c) no sex difference: $F_{1,42}$=116.9, p<0.0001, sse=0.07. Samples were collected in 2000 at the Kuruman River Reserve, South Africa, and assayed in 2001 and 2005, referred to as Year 1 and Year 4.

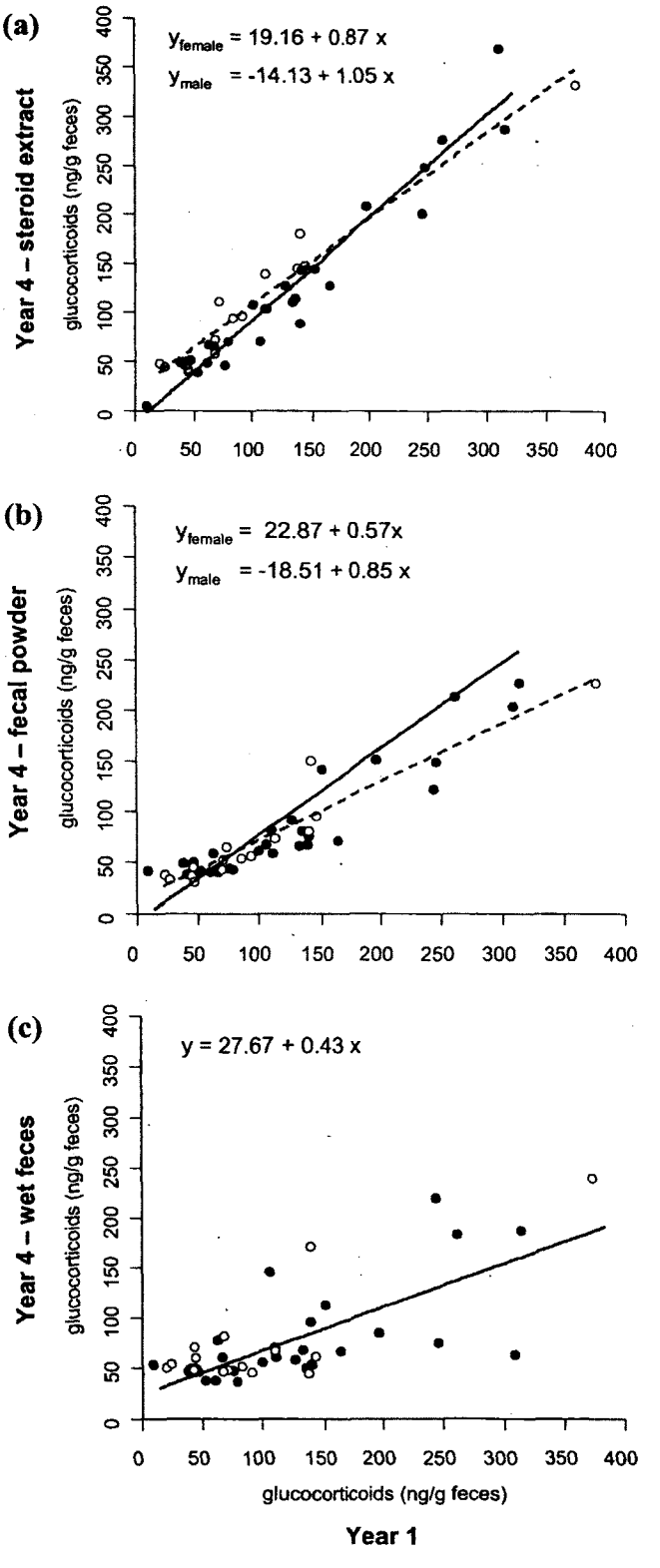

Table 2. Differential changes in fGC concentrations in meerkat fecal samples after four-year storage at -20°C as dried steroid extract, dried fecal powder and wet feces, as predicted by LM regression slopes of Year 4 fGC as a function of Year 1 fGC.

Regression slopes comparisons			gender	figure	df	welch t-test	p value
steroid extract	vs	slope of 1	males	1a	26	1.67	0.11
			females	1a	14	2.60	0.02
fecal powder	vs	slope of 1	males	1b	26	3.75	<0.001
			females	1b	14	8.60	<0.001
wet feces	vs	slope of 1 [a]		1c	42	11.40	<0.001
steroid extract	vs	fecal powder [b]	males		52	4.00	<0.001
			females		28	4.24	<0.001
steroid extract	vs	wet feces [c]	males		68	10.63	<0.001
			females		56	6.22	<0.001
fecal powder	vs	wet feces [c]	males		68	6.56	<0.001
			females		56	1.98	0.05

[a] No sex effect observed on fGC concentrations derived from wet feces. [b] Regression slopes compared within gender. [c] Regression slopes for each gender compared to the single regression slope for wet feces.

Effect of storage time and method on fE concentrations

Storage time did not significantly affect mean fE concentrations compared to original concentrations in samples frozen four years as dried steroid extract (Table 3; t-test: W=120, p=0.33), fecal powder (Table 3; t-test: W=123, p=0.87) or wet feces (Table 3; t-test: W=139, p=0.86). The associations between fE concentrations in Years 1 and 4 were linear under all storage methods (fig. 2). The concentrations in fE derived from dried fecal powder in year 4 were directly proportional to those measured in year 1 (i.e. slope=1; Fig. 2b) while the concentrations in year 4 were less than expected from the first year estimates (slope <1) with all other storage methods (Table 4; Fig. 2a and c). Higher fE concentrations were more affected than lower ones (lower regression slopes) when derived from dried steroid extract and wet feces (Table 4; Fig. 2a and 2c) than from dried fecal powder (Fig. 2b). Finally, fE concentrations measured in Years 1 and 4 were more weakly associated for wet feces (r^2=0.19) than for dried steroid extract (r2=0.49) or dried fecal powder (r2=0.59).

Table 3. Meerkat fE concentrations (ng/g feces) before (Year 1) and after (Year 4) four-year storage at -20°C as dried steroid extract, dried fecal powder and wet feces.

	Mean ± SE	Median	1st - 3rd IQRs
Year 1	324.8 ± 33.4	324.8	203.5 - 425.0
Year 4 dried extract	368.4 ± 26.8	392.0	328.0 - 442.1
Year 4 dried powder	327.2 ± 41.2	324.0	183.7 - 436.8
Year 4 wet feces	313.5 ± 37.8	337.5	181.9 - 450.4

Figure 2. Variation in fE concentrations in meerkat fecal samples measured after four-year storage as (a) dried steroid extract (n=14), (b) dried fecal powder (n=16) and (c) wet feces (n=17). Regression lines were predicted from respective LMs: (a) $F_{1,12}$=11.44, p=0.005, slope standard error (sse)=0.14; (b) $F_{1,14}$=20.43, p<0.001, sse=0.20; (c) $F_{1,15}$=3.45, p=0.08, sse=0.26. Fecal samples were collected in 2000 at the Kuruman River Reserve, South Africa, and assayed in 2001 and 2005, respectively referred to as Year 1 and Year 4.

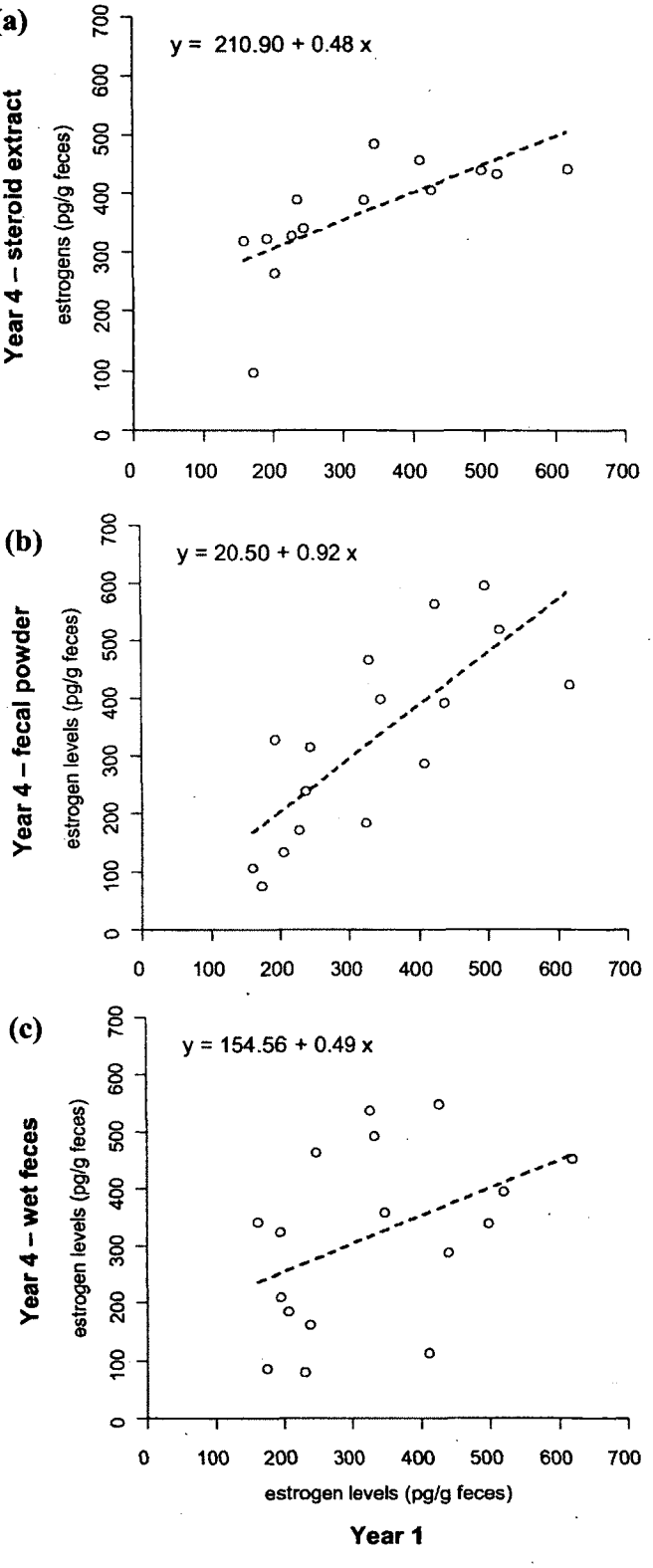

Table 4. Differential changes in fE concentrations in meerkat fecal samples after four-year storage at -20°C as dried steroid extract, dried fecal powder and wet, as predicted by LM regression slopes of Year 4 fE as a function of Year 1 fE.

Regression slopes comparisons			figure	df	welch t-test	p value
steroid extract	vs	slope of 1	2a	12	3.71	0.002
fecal powder	vs	slope of 1	2b	14	0.40	0.70
wet feces	vs	slope of 1	2c	15	1.96	0.07
steroid extract	vs	fecal powder		26	1.80	0.08
steroid extract	vs	wet feces		27	0.03	0.97
fecal powder	vs	wet feces		29	1.31	0.20

Discussion

Our study provides important new insights about the impacts of fecal sample processing and storage methods on our ability to achieve reliable estimates of stress and reproductive status of free-ranging meerkats. Fecal steroid metabolite concentrations varied after four-year storage depending on the storage methods, hormone and sex of the individual. First, in frozen wet feces, mean fGC concentrations declined, whereas mean fE concentrations did not change significantly. In contrast, mean fGC and fE concentrations derived from either dried fecal powder or dried steroid extract did not vary after four-year storage. These results suggest that meerkat feces should be dried and/or extracted as soon as practical following collection. However, short-term frozen storage is also recommended given the well-documented impacts of bacterial degradation on fecal steroid metabolites (Hunt and Wasser, 2003; Palme, 2005; Terio *et al.*, 2002; Woods, 1975). Alternatively, the higher polarity of fGC compared to fE could likely explain our contrasting results for the two hormone types, with more polar fGC metabolites being potentially drawn into water from samples during the lyophilisation process, to a greater extent than less polar fE metabolites. Whether differential metabolite loss through water of more polar steroids in wet samples is an alternative explanation to bacterial degradation responsible for steroid concentration changes with storage time remains to be address in meerkats and other species.

Secondly, our results on differential changes dependant on initial fGC and fE concentrations suggest that drying samples as soon as possible would be best for fE measurements whereas extracting hormones would be best for fGC measurements. Furthermore, that higher steroid concentrations are more affected than lower ones by long-term storage highlight the importance to assess storage effect not only for the species and hormone under study but also in association with characteristics of the animal which may affect the magnitude of steroid concentrations. For instance, we observed differences in fGC concentrations associated with gender, which could likely be associated with individual differences in stress and reproductive status. The generality of this result across life-history stages and species remains to be tested.

Finally, after four-year storage, frozen wet feces introduced higher level of confounding variation in fE than in fGC data as revealed by lower coefficient of determinations. Timely processing of fecal samples is thus likely to reduce variation in the data and improve our ability to explain observed variance in steroid concentrations. Our results confirm that storage method and time can have a major impact on the reliability of fecal hormone assessments and emphasize the necessity to control for storage time when analyzing fGC concentrations at least in meerkats (Barrette *et al.*, 2012, Chapter II). For instance, investigation of hormonal explanations of individual variation in behavioral or life-history strategies would necessitate careful interpretations of subtle hormonal changes which as suggested here would be more susceptible to be confounded by long-term storage of wet feces than dried fecal powder or dried steroid extract. These considerations are valuable for any longitudinal studies investigating the hormonal basis of individual and population variation in meerkats and other free-ranging species.

Alternative explanations for the finding that fGC concentrations varies more over time in wet feces than in dried fecal powder or dried steroid extract may relate to heterogeneous distribution of steroids within feces (Brown *et al.*, 1994; Millspaugh and Washburn, 2003; Wasser *et al.*, 1996) and to inter-extraction and inter-assay variation between Years 1 and 4 data. The weaker association between fGC measurements before and after four-year storage when derived from wet feces may result from our methodology where each fecal sample was originally split in two non homogenized portions. The one sample portion stored as unprocessed wet feces until Year 4 was not homogenized with the second sample portion processed into dried fecal powder and dried steroid extract, stored as such until Year 4. Thereby, steroid concentrations measured on Year 1 originate from the same sample portion used to generate Year 4 data from dried fecal powder and dried steroid extract, but from the different non homogenized portion used to generate Year 4 data from wet feces. This caveat however does not affect our conclusions on the similarities and differences in steroid concentrations observed among dried extract and dried fecal powder. In addition, results in Year 4 from all three storage methods could be confounded to some extent by inter-extraction and inter-assay variation, a bias which we could not control for but consistent across storage

methods. Results derived from dried steroid extract on differential fGC concentrations reduction suggest limited effect of inter-assay variation, with no bias for male samples and some for female samples. In contrast, results derived from dried fecal powder on differential fGC concentrations reduction suggest potential effect of inter-extraction variation for both male and female samples.

Our study confirms the reliability of fecal steroid metabolite assessments for long-term studies of stress and reproduction in meerkats, but also highlights the importance of documenting storage effect for each steroid type, animal gender, life-history stage and species. Our study revealed that early extraction of steroid metabolites from fecal samples may not be necessary for reliable measurements of fGC and fE metabolites, as repeat assays of dried fecal extracts stored frozen after four-year storage did not show stronger associations among measurements compared to those stored as dried fecal powder. However, storage of both dried fecal powder and dried steroid extract could be useful when different storage methods yield best results for different hormones. Validation of field extractions for different hormones and species could thus be beneficial, not only for logistic reasons such as sample transport to laboratory facilities, but also to reduce storage related variation in steroid metabolite concentrations likely to affect our ability to investigate the links between hormones, ecology and evolution in free-ranging populations.

Acknowlegments

We are grateful to the Kotze family and Northern Cape Conservation for allowing us to conduct research in the Kalahari, to Tim Clutton-Brock for providing access to habituated free-ranging meerkats at the Kurman River Reserve, and to N. Presley for guidance with endocrine analyses. This research was funded by the Natural Sciences and Engineering Council of Canada (M-FB) and the Smithsonian Institution (M-FB and SM).

CHAPITRE II
SUCCÈS REPRODUCTEUR ET GLUCOCORTICOÏDES

Chez les espèces sociales se reproduisant avec l'aide coopérative de leur groupe, un biais dans la reproduction des femelles favorise le succès reproducteur des dominantes au détriment des subordonnées (Keller et Reeve, 1994). L'étude de ce biais suggère un rôle des GCs dans la suppression de la reproduction des subordonnées, soit en réponse aux agressions des dominantes (Bennett, 1994; Young *et al.*, 2008), soit par l'abstinence lorsque leur succès reproducteur potentiel est faible (Abbott *et al.*, 1997; Creel, 2005). Cependant, le lien entre le succès reproducteur, la dominance et le stress physiologique demeure discutable en raison de la tendance des individus dominants à montrer des niveaux en GCs plus élevés que les subordonnées chez les espèces à reproduction coopérative (Creel, 2001). Des niveaux en GCs élevés suggèrent ici un coût physiologique associé à la dominance (Rubenstein et Shen, 2009). De plus, le moment où l'on mesure les niveaux en GCs peut influencer le lien avec le statut de dominance. Par exemple, chez le suricate, des niveaux élevés en GCs sont davantage détectables dans le plasma de femelles dominantes en dehors de période de reproduction (Carlson *et al.*, 2004), alors que les femelles subordonnées démontrent des niveaux élevés en fGC lorsqu'elles sont expulsées de leur groupe suite aux agressions de la dominante, lorsque cette dernière est en fin de gestation (Young *et al.*, 2006). La difficulté d'interpréter le rôle des GCs dans le biais de la reproduction émane de nos connaissances limitées des variations intra et interindividuelles en GCs lors de la reproduction de femelles dominantes et subordonnées ainsi que des niveaux spécifiques en GCs pouvant compromettre la conception et la gestation (Koolhaas *et al.*, 1999; Romero et Reed, 2008; Sapolsky, 1994, 1999; Williams, 2008).

Le lien entre succès reproducteur, dominance et GCs a été principalement étudié hors de période de reproduction ou chez des individus non reproducteurs afin d'éviter les effets confondants liés à la production normale en GCs lors de la reproduction (Brunton *et al.*, 2008; Dantzer *et al.*, 2010; Krasnow et Steiner, 2006; Mastorakos et Ilias, 2003; Trainer, 2002). En

effet, les GCs jouent plusieurs rôles primordiaux lors de la reproduction: énergétique lors de la gestation et de la lactation (Atkinson et Waddell, 1995), développemental lors de la maturation fœtale (deM Fencl *et al.*, 1980), et physiologique pour le maintien de la gestation et l'initiation de la parturition (Mastorakos et Ilias, 2000; McLean *et al.*, 1995). Nos connaissances des variations en GCs lors de la gestation chez des animaux sauvages sont toutefois limitées par la difficulté de démêler différentes sources de stress des effets mêmes de la reproduction. Ainsi, l'étude longitudinale non invasive des variations naturelles en GCs, avant et pendant la reproduction, est nécessaire afin de clarifier les différentes sources de variations des patrons observés entre femelles dominantes et subordonnées (Bonier *et al.*, 2009; Dingemanse *et al.*, 2010).

Étant donnée la variation dans le taux de reproduction des femelles dominantes et subordonnées, il est important de démêler les effets de la dominance et du taux de reproduction sur les niveaux en GCs afin de mieux comprendre le rôle des GCs dans le biais de la reproduction chez les espèces coopératives. La reproduction du suricate offre l'opportunité d'investiguer le lien entre le taux de reproduction, la dominance et les GCs. Chez le suricate, la gestation est suivie d'un oestrus postpartum permettant à certaines femelles de concevoir plusieurs portées de suite (Moss *et al.*, 2001; Russell *et al.*, 2003a). De plus, le suivi longitudinal de plusieurs femelles se reproduisant à maintes reprises, annuellement et sur plusieurs années, permet d'étudier les niveaux en fGC en parallèle avec l'âge et la masse corporelle, corrélées à la dominance (Russell *et al.*, 2003a; Russell *et al.*, 2004), tout en contrôlant pour des facteurs méthodologiques et socio-écologiques pouvant confondre les variations en fGC observées. Je propose donc de décrire les variations naturelles en fGC lors de la reproduction des suricates femelles en milieu sauvage afin de déterminer si le taux de reproduction influence les niveaux en fGC, avant et pendant la gestation, puis d'établir si les femelles subordonnées et dominantes ayant un même taux de reproduction varient dans leurs niveaux en fGC.

CHAPITRE II

REPRODUCTIVE RATE, NOT DOMINANCE STATUS, AFFECTS FECAL GLUCOCORTICOIDS IN BREEDING FEMALE MEERKATS.

MARIE-FRANCE BARRETTE, MARCO FESTA-BIANCHET, STEVEN L. MONFORT, TIM H. CLUTTON-BROCK, ET ANDREW F. RUSSELL

Description de l'article et contribution

L'article étudie le lien entre le taux de reproduction, la dominance et les niveaux en fGC chez des suricates femelles, suivant une description de la variation en fGC au courant d'un événement de reproduction, de 35 jours avant la conception jusqu'à la parturition, et entre plusieurs événements de reproduction d'une même femelle ou de différentes femelles se reproduisant dans un même groupe ou dans différents groupes. Les résultats de l'étude démontrent que 87% de la variation totale en fGC s'observe chez une même femelle et révèlent un effet important du taux de reproduction sur les changements en fGC au courant de différentes reproductions. Ces patrons ne sont pas influencés par le statut de dominance des femelles menant à terme leur gestation. Les résultats ne suggèrent donc pas d'effet inhérent à la dominance, avant ou pendant la gestation, et souligne l'importance de connaître la variation intra et interindividuelle en fGC afin d'interpréter les associations avec la dominance et le biais dans le succès reproducteur.

J'ai développé l'idée de cet article avec Andrew Russell. Tim Clutton-Brock a organisé le suivi à long terme de la population de suricates à l'étude. J'ai effectué les analyses hormonales avec le support logistique et sous la supervision de Steven Monfort. J'ai effectué le traitement des bases de données, les analyses statistiques, et la rédaction sous la supervision d'Andrew Russell et Marco Festa-Bianchet. Andrew Russell, Marco Festa-Bianchet, Steve Monfort et Tim Clutton-Brock ont fourni des commentaires sur des versions préliminaires du manuscrit. Andrew Russell, Marco Festa-Bianchet et Steven Monfort ont participé à la subvention du projet.

REPRODUCTIVE RATE, NOT DOMINANCE STATUS, AFFECTS FECAL GLUCOCORTICOIDS IN BREEDING FEMALE MEERKATS.

Par

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Key words: adrenal activity; corticosterone; noninvasive; cooperative breeding; social status; reproductive suppression; reproductive self-restraint; gestation; lactation.

Abstract

Glucocorticoid hormones (GCs) have been studied intensively to understand the associations between physiological stress and reproductive skew in animal societies. However, we have little appreciation of the range of either natural levels within and among individuals, or the associations among dominance status, reproductive rate and GCs levels during breeding. To address these shortcomings, we examined variation in fecal glucocorticoid metabolites (fGC) during breeding periods in free-ranging female meerkats (*Suricata suricatta*) over 11 years. The vast majority of variation in fGC levels was found within breeding events by the same females (~87%), with the remaining variation arising among breeding events and among females. Concentrations of fGC generally tripled as pregnancy progressed. However, females with a high reproductive rate, defined as those conceiving within a month following parturition (mean=9 days postpartum), showed significant reductions in fGC in the final two weeks before parturition. Despite these reductions, females with a high reproductive rate had higher fGC levels at conception of the following litter than those breeding at a low rate. After controlling for the higher reproductive rate of dominants, we found no association between levels of fGC and either age or dominance status. Our results suggest that one should be cautious about interpreting associations between dominance status, reproductive skew and GCs levels, without knowledge of the natural variation in GCs levels within and among females.

Introduction

Individual fitness is typically maximized through independent reproduction. In species that breed cooperatively, however, individuals commonly forego personal reproduction and instead contribute to rearing the offspring of others (Choe and Crespi, 1997; Hager and Jones, 2009; Solomon and French, 1997; Stacey and Koenig, 1990; Wilson, 1971). Given that in such systems dominant breeders gain through the helping actions of non-breeding subordinates, the former should be under strong selection to reduce the reproductive rate of the latter. In some social insects, for example, queens use pheromones to signal their presence and workers are often born sterile, while, those that are not, risk personal injury and egg destruction (Beekman and Ratnieks, 2003). In vertebrates, where all offspring are born with reproductive capabilities, subordinate reproduction is reduced, in part, by evicting them from the group and/or killing their offspring (Cant, 2011; Koenig and Dickinson, 2004). However, whether or not dominants also attempt to physiologically suppress the reproductive system of subordinates or whether subordinates depress their own reproductive system when their chances of success are low, is contentious (Creel, 2001; Koenig and Dickinson, 2004; Young *et al.*, 2006).

In vertebrates, glucocorticoids (GCs) play a key adaptive role in metabolic, immune, behavioral and reproductive functions (de Kloet, 1999; Dhabhar, 2002; Magiakou *et al.*, 1997; Whittle *et al.*, 2001; Wingfield and Kitaysky, 2002). However, at chronic elevations, they can reduce survival and reproductive function (Sapolsky *et al.*, 2000; Selye, 1956). For example, sustained elevations in GCs can render females infertile by suppressing gonadal activity (Bennett, 1994; Nakamura *et al.*, 2008) and have been suggested to lead to increased risk of abortion (Saltzman *et al.*, 2006; Young *et al.*, 2006). The link between stress and female reproductive failure has led to the hypothesis that chronic elevations in GCs can provide a proximate mechanism for understanding skews in the proportion of breeding versus non-breeding group members in some vertebrate societies (Creel, 2005). Hypotheses of reproductive skew based on dominance-mediated physiological suppression versus personally-mediated reproductive restraint have opposing predictions regarding GCs levels in

dominant versus subordinate females. Under the former, subordinates would be expected to have significantly higher GCs levels than dominants, while this should not be the case under the latter (Creel, 2001, 2005; Young *et al.*, 2006; Young *et al.*, 2008).

Creel (2001) rejected a role of physiological suppression in cooperative vertebrates based on findings that subordinates seldom have higher GCs levels than dominants across species. For instance, experimental evidence on the role of social stress in common marmosets, *Callithrix jacchus* (Abbott *et al.*, 1997), and naked mole-rats, *Heterocephalus glaber* (Faulkes and Abbott, 1997), failed to reveal a link between GCs and infertility. Young *et al.* (2006), however, cautioned against premature rejection of the physiological suppression hypothesis, if dominants only induce high levels of stress in subordinates during particular times. In support, subordinate meerkats (*Suricata suricatta*) were shown to have significantly elevated GCs levels when evicted from the group, commonly aborted litters as a consequence of eviction and failed to conceive when evicted despite the presence of unrelated males (Young *et al.*, 2006). Importantly, these results were found despite the finding that there was no difference in GCs levels between dominant and subordinate female meerkats during periods of non-breeding (Young *et al.*, 2008).

One of the problems of interpreting the role of adrenal functions in mediating reproductive skew is that we know little about natural variation in GCs within- and among-individuals and the individual-specific levels required to induce reproductive suppression (Koolhaas *et al.*, 1999; Romero and Reed, 2008; Sapolsky, 1994, 1999; Williams, 2008). This is compounded by the fact that most previous studies have been conducted during non-breeding periods to avoid the confounding effects of increased adrenal activity during breeding (Brunton *et al.*, 2008; Dantzer *et al.*, 2010; Krasnow and Steiner, 2006; Mastorakos and Ilias, 2003; Trainer, 2002). Longitudinal studies making use of noninvasive assessments of GCs excretion across breeding and non-breeding periods are now required to clarify patterns of natural variation (Bonier *et al.*, 2009; Dingemanse *et al.*, 2010). In particular, such studies need to include breeding periods for a full understanding of links among reproductive success, dominance status and GCs levels. For example, it is difficult to judge levels of GCs that might prevent

reproduction or induce abortion, if we do not know natural levels associated with conception and gestation. In addition, GCs levels might not only mediate qualitative differences in breeding propensity, but quantitative differences in breeding success between dominant and subordinate individuals. In this case, we would predict that subordinates have higher GCs levels than dominants during breeding periods and then take longer to return to non-breeding baseline. Whether either are the case has not yet been explored, but would be predicted by the stress-induced reproductive skew hypothesis (Creel, 2001; Young *et al.*, 2006).

The broad aim of this study was to use fecal samples collected from free-ranging cooperative female meerkats across an 11-year period, to quantify within- and among-female variation in fecal glucocorticoid metabolites (fGC) during breeding and to assess the links among reproductive rate, dominance status and fGC (see below). Meerkats are small (<1kg) social carnivores that reproduce cooperatively in groups of 2 to 40 individuals (mean±sd=16.7±8.6) in arid zones of southern Africa. Groups generally consist of a dominant pair and overlapping generations of relatives that help to rear offspring (Clutton-Brock *et al.*, 2001b; Clutton-Brock *et al.*, 2002). Dominant females produce 80% of offspring and subordinate breeding is biased towards older and larger subordinates (Clutton-Brock *et al.*, 2001b; Russell *et al.*, 2004; Young *et al.*, 2008). Gestation lasts ~70 days (Doolan and Macdonald, 1997), with increases in gonadal steroids from 35 days before birth (Moss *et al.*, 2001; Fig. 1). Some mothers can achieve a high reproductive rate by exhibiting a postpartum estrus (Moss *et al.*, 2001); conceiving within days of parturition (Russell *et al.*, 2003a). We refer to these cases as "overlapping breeding events" (Fig. 1, scenario 1). Females breed up to four times a year (mode=2 for dominants), deliver one to six pups per litter (mean±sd=3.73±1.49) and lactate for about 35 days post-parturition (±10 days, Russell *et al.*, 2003a). Meerkats therefore provide an ideal opportunity to test how adrenal activity during breeding varies between dominant and subordinate females, and how this is modified by reproductive rate (Fig. 1).

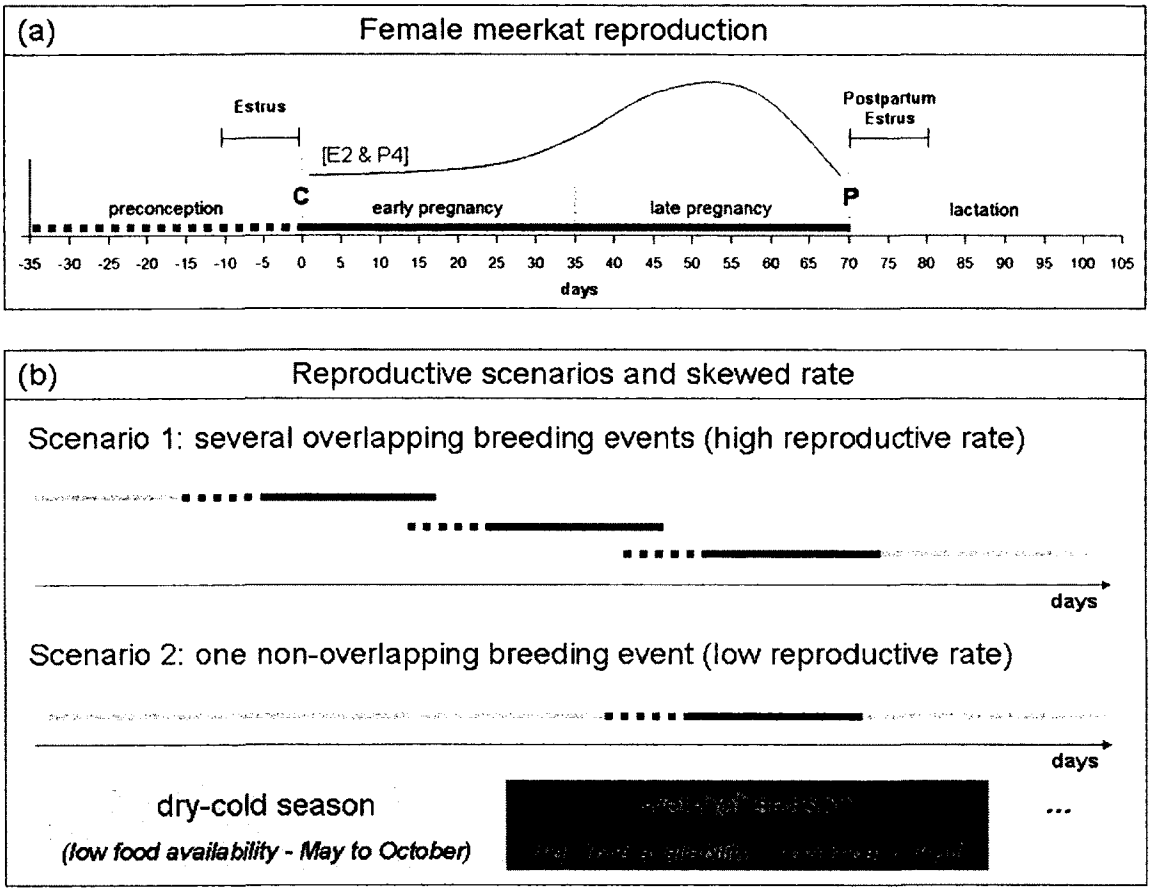

Figure 1. The reproductive cycle of female meerkats. (a) Timing of estrus, conception (C), expected rise in estrogens and progestagens during pregnancy ([E2&P4] curve; Moss *et al.* 2001), parturition (P) and specific phases of each breeding event under study (preconception, dotted bold line, and early and late pregnancy, straight bold line). (b) Reproductive scenarios describing skewed reproductive rate among female meerkats which may or may not conceive immediately after parturition. Grey bold lines refer to non-breeding periods. In Scenario 1, females overlap in at least two consecutive breeding events. We distinguish climatic (yearly dry-cold and wet-hot seasons) from breeding seasons (yearly breeding season specific to each female and varying in duration and timing).

Specifically, we sought to document within- and among- female variation in fGC levels during discrete breeding events, characterized by a preconception and two pregnancy phases (Fig. 1), and to investigate individual characteristics that account for this variation. To this end, we first characterize variation in fGC within breeding events within females, among breeding events within females, among females and among social groups. Second, we describe fGC variation over the course of a breeding event and investigate whether patterns of variation associate with current reproductive rate. Third, we investigate the effect of dominance status and age on fGC levels throughout breeding events. Finally, we test whether individuals were consistent in their relative levels of fGC throughout breeding, such that fGC levels preconception predict levels during gestation and whether levels during early pregnancy predict those in late pregnancy. We controlled for several potential confounding factors, including sampling biases, and the socio-ecology of the group from which samples were collected.

Methods

We studied habituated meerkats from 1997 to 2008 at the Kuruman River Reserve (KRR) in the South African Kalahari Desert (see Clutton-Brock *et al.*, 1999a; Russell *et al.*, 2002 for details of habitat and climate). A total of 15 meerkat groups, numbering two to 40 marked individuals (mean=~16), were followed every one to five days to monitor individual life-history and body mass, and to collect fecal samples, although groups were monitored daily when pregnant females were due to give birth. Body mass was determined before foraging each morning by enticing meerkats onto digital top-pan balances (±1g) using crumbs of hard-boiled egg (e.g. Clutton-Brock *et al.*, 2002). Pregnancy was identified about four weeks after conception by swelling of the abdomen and nipples and by an increase in body mass. Parturition (±1 day) was identified by a combination of sudden female mass loss and the presence of babysitters at the burrow (Clutton-Brock *et al.*, 2001b). Conception date was back-calculated to 70 days before birth (Doolan and Macdonald, 1997; Fig. 1). Based on the timing of increases in gonadal hormones following conception (Moss *et al.*, 2001) and the timing of the main fetal growth phase during pregnancy (Russell *et al.*, 2003a), a breeding event was

defined here by three 35-day phases (Fig. 1): (i) preconception (day -35 to 0); (ii) early pregnancy (day 1 to 35 of gestation;); and (iii) late pregnancy (day 36 to 70). Those three phases allowed us to detail the increased adrenal activity during gestation and to account for the potential effects of consecutive overlapping breeding events on fGC levels (Fig. 1). Females reproducing at a high rate could conceive within 35 days of parturition and thereby would be both in late pregnancy and preconceptive (modal timing between parturition and postpartum conception is 9 days in dominant females; Young *et al.*, 2006). The preconception phase of females known not to be pregnant with the previous litter provided a non-breeding fGC "baseline" level: we found no difference in fGC levels between females not pregnant during the 35-day preconception phase (n=79 samples from 33 females) and those that were not pregnant at other times of the year (n=300 samples from 77 females) (General Linear Mixed Model with normal error structure on log-transformed fGC levels controlling for female identity, collection time of day and storage duration: $F_{1,269}$=1.10, p=0.29, effect±se=-0.13±0.12). Because females experiencing postpartum conception are in early pregnancy and lactation phases at the same time, lactation was not considered as a separate breeding phase in this study but was accounted for by including the presence of unweaned pups at the burrow as a fixed effect in all analyses. All animal-handling protocols were approved by the University of Pretoria, the University of Sherbrooke and the Smithsonian Institution ethic committees.

Sample collection and hormonal analyses

We studied fGC patterns only in females successfully breeding to term to preclude inadvertently including samples from females in the early stages of failed pregnancies. We thus assayed only those fecal samples collected within at least one of the three breeding phases of a given breeding event: preconception (n=191 samples, 102 events, 44 females); early pregnancy (n=188 samples, 113 events, 39 females); and late pregnancy (n=200 samples, 96 events, 36 females). Overall, we assayed 579 fecal samples collected preceding or during 161 breeding events carried to term by 34 dominants (n=473 samples, 132 events; mean±sd samples per event=3.5±2.9) and 22 subordinates (n=106 samples; 29 events; mean±sd samples per event=3.7±5.3).

Fecal GC metabolites provide a pooled measure of adrenal steroids excreted over the past 24 to 48 hours (Young *et al.*, 2006), without the confounding stress of capture. Samples were collected whenever animals defecated, immediately placed on ice in thermos flasks and frozen at -20°C within five hours (mean ca. 2h). Samples were shipped frozen to the Smithsonian Conservation Biology Institute in Front Royal, VA, USA. Steroid metabolites were extracted from samples using validated methodologies (Monfort *et al.*, 1997). Briefly, fecal samples were freeze-dried (VirTis XL-70, SP Industries, New York), pulverized and thoroughly mixed. Fecal powder (0.18–0.19 g) was then combined with 6 ml of 100% ethanol, vortexed (10 s) and boiled (20 min) to extract steroid metabolites. After centrifugation (2500 g, 20 min), the supernatant was decanted into a tube and fully dried under a stream of compressed air; during evaporation, the vessel walls were rinsed twice with ethanol (4 then 2 ml). The residue was then redissolved in methanol (1 ml) and placed in an ultrasonic glass cleaner (5 min). A portion of the extractant was then diluted 1:50 in diluent buffer (pH 7.0) and frozen (−20 °C) for subsequent radioimmunoassay (RIA).

Concentrations of fGC were determined using a double-antibody 125^{I} RIA for corticosterone (ICN Biomedicals Inc., Costa Mesa, California, USA), which had been previously shown to most reliably measure adrenal activity (Young *et al.*, 2006). Immunoreactive metabolites detected by the corticosterone antibody were defined generically as fecal glucocorticoid metabolites (fGC). The antiserum cross-reacts 100% with corticosterone, 0.34% with desoxycorticosterone, 0.10% with testosterone, 0.05% with cortisol, 0.03% with aldosterone, 0.02% with progesterone and <0.01% with all other steroids tested. Assays were conducted according to the instructions provided with the kit except that all reagent volumes were halved. Fecal extracts (1:50 dilution) were assayed (50 µl) in duplicate. Assay sensitivity was 25 ng/ml. Intra-assay coefficients of variation were <10% and inter-assay coefficients of variation for low- and high-dose internal controls were 8.8% and 6.7% for 20 assays performed form December 2005 to September 2008.

Statistical analyses

Analyses were conducted over an entire breeding event (preconception to parturition) and separately for each of the three specified breeding phases (Fig. 1). These separate analyses have three main advantages. First, they facilitate interpretation of non-linear patterns in fGC levels over the entire breeding event. Second, they clarify the relative importance of individual characters for explaining variation in fGC at different times of a breeding event. Third, they permit investigations of the relationships between fGC levels during successive breeding phases.

In all analyses, fGC data were normalized using a natural logarithm transformation and fitted to a normal error structure in a General Linear Mixed Model (GLMM). In order to describe the hierarchical distribution of variation in the samples collected and interpret the data accordingly, we fitted four random terms to the models: (i) samples within a breeding event and within a female (hereafter referred to as inter-sample variation, n = (mean±sd) 3.5±3.2 samples per event); (ii) among events within females (hereafter inter-event variation, n=2.8±2.8 events per female); (iii) among events among females (hereafter inter-female variation, n=4.9±3.2 females per group); and (iv) among groups (hereafter inter-group variation). Random terms followed a nested structure, with samples being the lowest hierarchical level, and with higher levels being retained in final models only if they encompassed significant fGC variation after assessing the importance of fixed effects.

The fixed terms of interest outlined below were considered after controlling for the random terms retained as well as a number of potentially confounding fixed effects. Fixed effects with a potentially confounding influence included: storage duration, collection time-of-day, body mass and socio-ecological factors prevailing at the time of breeding and of sample collection. Because steroid metabolites can degrade over time (Schwartz and Monfort, 2008), we fitted as a fixed co-variate the number of days between sample collection and assay. To control for circadian variation in fGC, sample collection time was coded as morning (am: 06:00–12:00) and evening (pm: 15:00–20:00) and fitted as a two-level fixed co-factor in all analyses. Because female meerkats are more likely to breed and to become dominant when heavy

(Clutton-Brock *et al.*, 2006), we considered the potential association between body mass and fGC levels, although interpretation of mass results is difficult in a pregnant mammal. The potentially confounding socio-ecological factors prevailing at the time of sampling included: climatic season (wet-hot vs. dry-cold); group size (i.e. ≥ 6 months old); number of breeding-aged females in the group (≥ 8 months); and pup presence at the burrow. We also considered the occurrence of concurrent female gestations in the group and the number of females evicted during the entire breeding event or phase in the case of phase-specific analyses. Only those confounding terms that accounted for significant variation in a given model were retained and their retention is indicated through the presentation of their statistical significance in the Results.

All analyses contained four fixed terms of primary interest where relevant. Although fixed terms of interest were also dropped from the final model when not significant, we report their effect when added to the final model individually in all cases. The primary fixed effects of interest in all models included: (1) timing into a discrete breeding event; (2) whether or not the event overlapped with other events; (3) female age; (4) dominance status. Timing of sample collection within a breeding event characterized maternal adrenal activity with days into breeding fitted as linear, quadratic and cubic functions to account for possible non-linearity; days varied from -35 to 70 in the overall analysis, and from 1 to 35 for each of the phase-specific analyses. Whether or not a female was late pregnant during the preconception phase characterized individual reproductive rate and was fitted as a two-level factor in all analyses, except during the late pregnancy phase where we fitted instead whether or not she was also preconceptive. A high reproductive rate resulting from postpartum conception is defined by the reproductive overlap of two phases of reproduction; a low reproductive rate is defined by no reproductive overlap (Fig. 1). Individual age (± 1d), was determined by birth dates in the study population. Dominance status was coded as dominant or subordinate with only one female per group being dominant (Kutsukake and Clutton-Brock, 2006). To disentangle the effect of reproductive rate and dominance status on among female variation in fGC pattern over time, we tested for interactions between timing into breeding and reproductive overlap, timing into breeding and dominance status, and reproductive overlap and dominance status.

Finally, we tested whether fGC levels preconception predict levels during gestation and whether levels during early pregnancy predict those in late pregnancy. A second analysis of fGC levels during early pregnancy was thus performed on a restricted data set where we could fit mean levels during the preconception phase of the same litter. Likewise, a second analysis of fGC levels during late pregnancy was performed on a restricted data set where we could fit mean levels during the preconception and early pregnancy phases of the same litter.

Statistical analyses were conducted with R (R Development Core Team, 2008). Normality was determined using quantile plots, frequency histograms and Shapiro-Wilk normality tests. The explanatory power of models and the individual fixed terms were considered in terms of the proportion of variance explained and was calculated by measuring the difference in residual variance including only random terms versus both random and fixed explanatory terms. The percentage of variation presented reflects the proportion of random variance explained by fixed effects not the proportion of overall total variance explained. Fixed terms and their two-way interactions were retained when they influenced the explanatory power of the model. Effect sizes were calculated by comparing the explanatory power of GLMMs including and excluding a term of interest in an ANOVA. Type III sum of square error structures (i.e. marginal error structure in R) are presented to control for co-variation among fixed terms (Pinheiro and Bates, 2000). Orthogonal polynomials were tested to control for collinearity among polynomial degrees (Shacham and Brauner, 1997). All statistical tests were two-tailed. Means and standard errors when presented in the text are predicted by GLMMs, and back-transformed to provide values in fGC ng/g of dry feces.

Results

Variation in fGC and variance explained

The variation in fGC levels recorded within the same female during a given breeding event was substantially greater than that recorded within females across different breeding events, among females, or among groups. Overall, ~87% of the variation in fGC levels occurred

among samples from within the same female within a breeding event, ~9% occurred among breeding events of the same female, and ~4% occurred among individual females (see Table 1 for proportion of fGC variation explained at each hierarchical level; Fig. 2). The distribution of variation in fGC levels observed within each specific breeding phase closely mirrored the patterns outlined above in the full analysis, with a notable exception (Table 1): during preconception phases, a larger share of the variation, i.e. ~32% *vs.* ~9%, was found among breeding events of the same female.

Table 1. Proportion of variance in fGC excretion within breeding events (inter-samples), among events of a same female (inter-breeding events), and among females across all breeding events (inter-females). Inter-group variance was null in all cases and was thus not presented. Results are presented for analysis of all data (i.e., across a 105-day breeding event) and the phase-specific analyses. These data represent the proportion of variance explained only by random terms (i.e., by hierarchical levels) and the proportion of this variance at each hierarchical level that is explained by fixed effects (in parentheses).

Models	% variance in fecal glucocorticoid metabolites		
	inter-samples	inter-events	inter-females
all breeding data	86.9 (37.1)	8.78 (51.0)	4.3 (0.0)
preconception	68.2 (21.9)	31.79 (57.8)	0.0 (–)
early pregnancy	90.5 (18.1)	4.97 (0.0)	4.5 (99.9)
late pregnancy	89.3 (14.8)	3.86 (99.9)	6.9 (59.0)

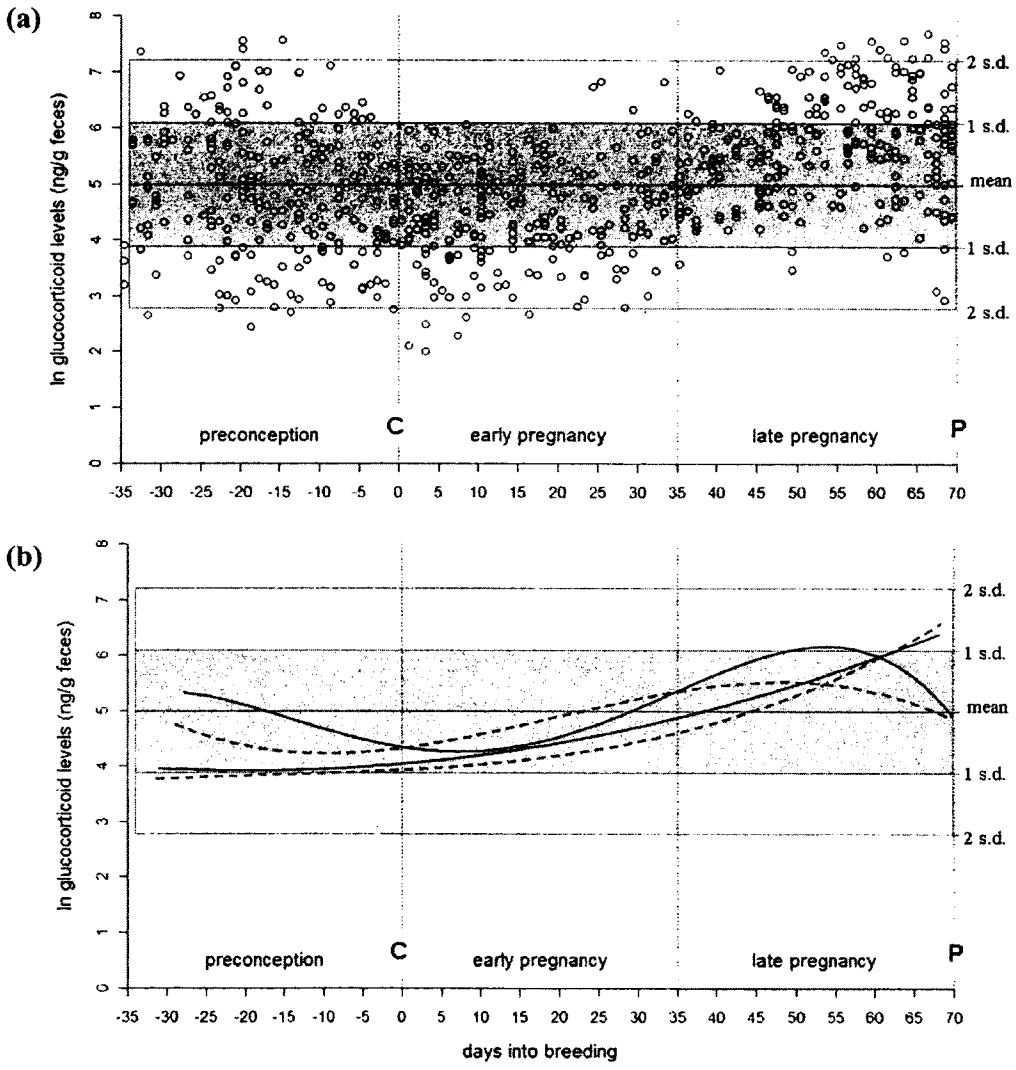

Figure 2. Variation in fGC levels during a discrete breeding event, i.e. from day -35 to +70. (a) Log transformed raw data with mean ±1 and 2 standard deviations calculated for all samples included in this study. (b) Example of fGC variation among female meerkats and among breeding events with fGC profiles represented by respective best fit regression lines for a subset of two females over two separate breeding events each (females selected with most complete fGC profile); one female is illustrated by the continuous line, the other by the dashed line; mean and ±1 and 2 s.d. are calculated for all samples included in this study. Conception and parturition are identified with the letters C and P.

Effect of gestation and reproductive rate

The predicted mean levels of fGC halved during preconception, from ~ 157 to 87 ng/g of feces, then tripled by parturition, reaching ~ 305 ng/g of feces (Table 2; see Fig. 2a for raw data). These predicted mean values were obtained after controlling for positive effects of sample storage time ($F_{1,408}$=14.25, p<0.001) and body mass ($F_{1,408}$=32.94, p<0.001), negative effects of the number of eviction events ($F_{1,108}$=4.34, p=0.04) and differences associated with collection time of day (am<pm: $F_{1,408}$=21.48, p<0.001), climatic season (dry/cold>wet/hot: $F_{1,408}$=12.19, p<0.001) and occurrence of concurrent gestations (no<yes: $F_{1,108}$=3.82, p=0.05). The above average fGC pattern however masked a more complex relationship between timing into a breeding event and fGC levels that was influenced by whether or not females re-conceived within 35 days following parturition (i.e. by reproductive overlap) (Table 2a).

The interaction between reproductive overlap and timing into breeding was clarified by investigating fGC variation within different phases of a breeding event. Females that were pregnant during the 35-day preconception phase of the forthcoming litter, had twice the fGC levels of those that were not pregnant within 35 days of conception (Table 2b). In all females, fGC levels then decreased near conception (Table 2b), although those pregnant preconception continued their decrease in fGC during early pregnancy (Table 2c). Fecal GC levels generally increased during late pregnancy, although females that conceived again postpartum excreted 3.5 times less fGC at parturition compared to females that did not conceive postpartum (Table 2d). In summary, females that were not pregnant during either the preconception or postpartum intervals (low reproductive rate with no reproductive overlap; Fig. 1) excreted low fGC concentrations during preconception and early pregnancy, and then showed an increase in fGC that peaked at parturition (Fig. 3). In contrast, fGC in females that were pregnant preconception and postpartum (high reproductive rate with three consecutive breeding events; Fig. 1) were initially high, declined throughout preconception and early pregnancy, then increased through late pregnancy before declining within 15 days of parturition (Fig. 3).

Table 2. Factors affecting female meerkats fGC during: (a) an entire breeding event (day -35 to 70); (b) preconception (day -35 to 0); (c) early pregnancy (day 1 to 35); and (d) late pregnancy (day 35 to 70). Only terms of interest and their interactions are presented; sampling and socio-ecological confounding effects are presented in the *Results*.

(a) All breeding data	*df*	Effect ± SE	F	p-value
Constant ¥	1, 408	4.03 ± 0.53	57.00	< 0.001
Timing into breeding				
Days	1, 408	-0.063 ± 0.011	30.16	< 0.001
Days^2	1, 408	6.5E-4 ± 1.0E-4	38.19	< 0.001
Reproductive overlap				
Pregnant preconception *(no < yes)*	1, 108	-1.40 ± 0.31	20.61	< 0.001
Pregnant preconception * Days	1, 408	0.039 ± 0.012	10.51	0.001
Pregnant preconception * Days^2	1, 408	-3.2E-4 ± 1.1E-4	9.07	0.003
Age *(days)*	1, 407	-4E-5 ± 6.7E-5	0.36	0.55
Dominance status *(subordinate, dominant)*	1, 407	see Fig. 4	0.005	0.94
(b) Preconception				
Constant ¥	1, 116	5.37 ± 0.20	689.23	< 0.001
Timing into preconception - Days PC	1, 116	-0.025 ± 0.007	13.52	< 0.001
Reproductive overlap		see Fig. 3		
Pregnant preconception *(no < yes)*	1, 57	-0.62 ± 0.19	10.82	0.002
Age *(days)*	1, 115	-7.2E-5±1.1E-4	0.47	0.49
Dominance status *(subordinate, dominant)*	1, 115	see Fig. 4	2.65	0.10
(c) Early pregnancy				
Constant ¥	1, 104	4.41 ± 0.13	1113.77	< 0.001
Timing into early pregnancy - Days EP	1, 104	-0.023 ± 0.006	14.67	0.002
Reproductive overlap		see Fig. 3		
Pregnant preconception *(no < yes)*	1, 73	-0.23 ± 0.23	1.05	0.31
Pregnant preconception * Days EP	1, 103	0.029 ± 0.011	6.87	0.010
Age *(days)*	1, 104	-2.5E-5±6.6E-5	0.15	0.70
Dominance status *(subordinate, dominant)*	1, 72	see Fig. 4	0.43	0.51

Table 2 (Continued)

(d) Late pregnancy	*df*	Effect ± SE	F	p-value
Constant ¥	1, 99	5.23 ± 0.33	252.54	< 0.001
Timing into late pregnancy (LP)				
Days	1, 99	0.031 ± 0.037	0.73	0.39
Days^2	1, 99	2.3E-4 ± 9.8E-4	0.057	0.81
Reproductive overlap		see Fig. 3		
Conceptive postpartum *(no > yes)*	1, 58	-1.20 ± 0.43	7.62	0.008
Conceptive postpartum * Days LP	1, 99	0.15 ± 0.05	7.87	0.006
Conceptive postpartum * Days LP^2	1, 99	- 4E-3 ± 1.4E-3	8.60	0.004
Age *(days)*	1, 98	-1.4E-4±7.9E-5	2.97	0.09
Dominance status *(subordinate, dominant)*	1, 57	see Fig. 4	1.50	0.23

¥ Values presented for the constants are specific to each periods and are calculated in reference to samples collected in the morning (*am*), during the *dry-cold* season, when *no* pups are present at the burrow, when *no* concurent gestation occurs and for females pregnant preconception (*yes*) and *not* conceptive postpartum. Data were collected from 1997 to 2008 at the Kuruman River Reserve, South Africa.

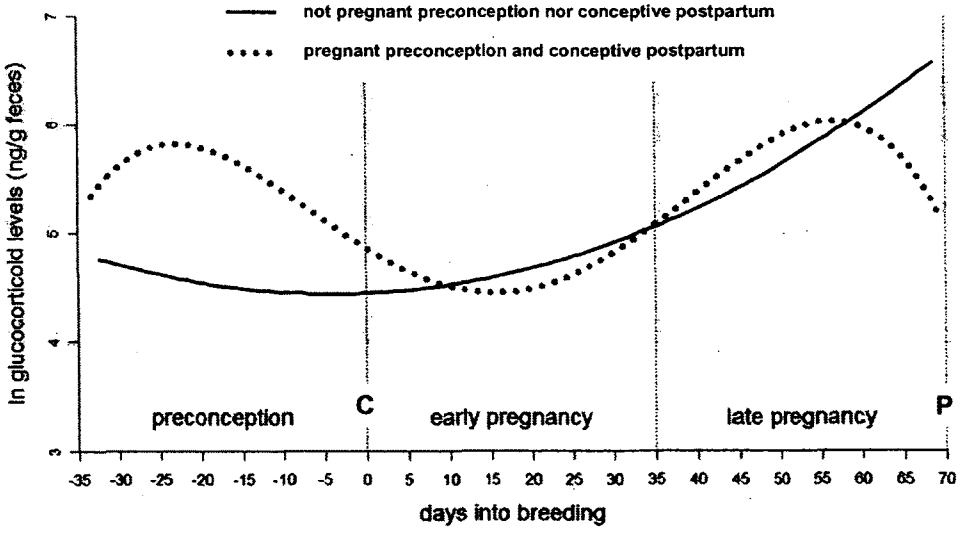

Figure 3. Association between fGC levels and reproductive rates shown over a discrete breeding event as GLMM regression lines for adult female meerkats split into two breeder types: not pregnant preconception and not conceptive postpartum (n=153 samples, 44 events and 20 mothers; $F_{2,40}=12.95$, $p<0.0001$); and pregnant preconception and conceptive postpartum (n=67 samples, 24 events and 23 mothers; $F_{4,101}=14.63$, $p<0.0001$). Days of conception and parturition are respectively identified with the letter C and P.

No effect of age or dominance status

Dominant and subordinate females did not differ in their fGC breeding profiles. We found no association of either age or dominance status with variation in fGC excretion before and during breeding, after controlling for all significant effects outlined above (Table 2; Fig. 4). In addition, we found no interactions between dominance status and reproductive rate or between dominance status and timing into breeding (Table 2). Although dominant females are larger and heavier than subordinates in meerkats (Russell *et al.*, 2004), dominance status failed to reach significance even following the exclusion of significant mass effect (i.e. dominance effect when excluding body mass in GLMM presented in Table 2; overall analysis: $F_{1,407}$=1.35, p=0.25, effect±se=-0.18±0.15; preconception: $F_{1,115}$=0.36, p=0.55, effect±se=-0.13±0.22; early pregnancy: no significant mass effect; late pregnancy: $F_{1,57}$=0.47, p=0.49, effect±se=0.17±0.26).

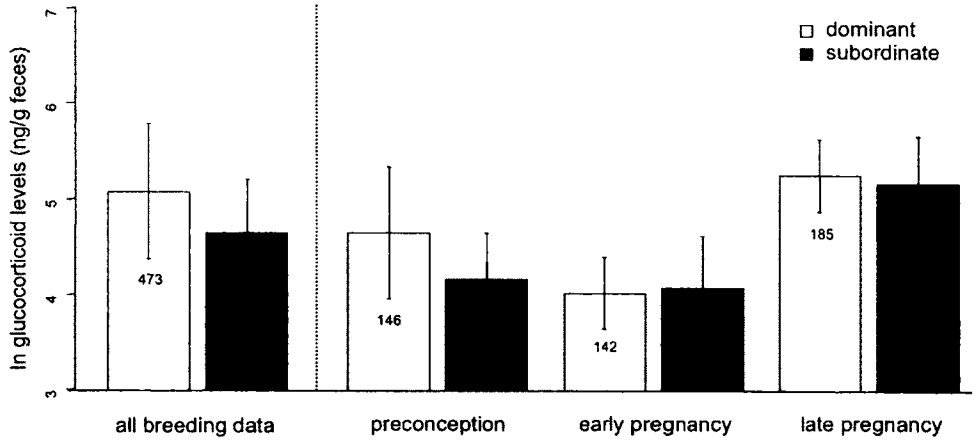

Figure 4. Levels of fGC in subordinate and dominant meerkat females were not significantly different during a discrete breeding event and similarly during preconception and pregnancy phases. Predicted means and standard deviation are presented with sample sizes, after controlling for significant terms of interest and confounding methodological and socio-ecological factors.

Consistency in fGC levels during breeding

Levels of fGC during preconception were positively correlated with levels during early, pregnancy, but not during late pregnancy. Average fGC levels preconception accounted for 8% of variation in fGC levels during early pregnancy, after controlling for all other significant terms (restricted GLMM with preconception and early pregnancy data available for a same breeding event: $F_{1,23}$=9.25, p=0.006, effect±se=0.27±0.09). Specifically, although fGC levels during the 35 days preceding conception did not predict significant fGC variation within early pregnancy phases, preconception levels explained 50% of among early pregnancy phases variation within females (i.e. 4% of overall fGC variation) and 100% of the among females variation (i.e. 4% of overall fGC variation).

Discussion

Longitudinal hormone monitoring of free-ranging female meerkats clarified the interplay between individual variation in fGC during breeding, reproductive rate and dominance status. Levels of fGC were highly variable, encompassing two orders of magnitude, and most of this variation occurred within breeding events from preconception through to parturition. Concentrations of fGC were generally low at conception and increased markedly in the latter half of gestation to parturition, although females with postpartum conception showed significant reductions in fGC levels in the last two weeks of gestation. Our results conform to evidence that generally low GCs levels are required for successful conception and implantation (Douglas, 2010), but suggest that levels during late pregnancy can be very high without litters aborting. Finally, we found no evidence to suggest that dominants and subordinates differ in breeding GCs levels when experiencing the same reproductive rate. Nevertheless, because breeding is associated with higher fGC levels than non-breeding and because dominants breed at a higher rate that subordinates, dominants might often be observed to have higher adrenal activity than subordinates in cooperative breeders (Creel, 2001). We thus propose that studies investigating the role of adrenal activity in mediating reproductive

skew would benefit from long-term sampling within and among individual females throughout non-breeding and breeding periods.

While longitudinal sampling of adrenal activity during periods of breeding is important for the reasons outlined above, care is required when interpreting the results. First, during gestation, GCs can arise from both maternal and feto-placental sources, with the relative contribution by the latter increasing as pregnancy proceeds (deM Fencl *et al.*, 1980; Keller-Wood and Wood, 2001; Mastorakos and Ilias, 2000, 2003; Waddell, 1993). However, it is not unusual for the feto-placental unit to have little impact on maternal GCs levels, as is the case in rodents (Brunton *et al.*, 2008). If feto-placental sources of GCs contribute significantly to maternal fGC levels in meerkats, we would expect fGC to peak at parturition. That fGC levels declined markedly in the last two weeks of gestation in female meerkats with postpartum conception suggests that any feto-placental sources of GCs in maternal feces have limited qualitative impact on maternal fGC levels. Second, relationships between maternal fGC and adrenal activity during lactation might be clouded by the stimulation of maternal adrenal activity by suckling offspring (Casey and Plaut, 2007) and the diffusion of maternal GCs into milk (Brummelte *et al.*, 2010). That fGC levels declined during lactation in mothers with postpartum conception but showed non-significant tendencies to increase in those that were neither pre-conceptive nor early pregnant, suggests that neonatal stimulation/consumption of maternal GCs alone cannot account for maternal fGC levels during lactation (GLMM analysis on fGC levels during lactation in females that were neither pre-conceptive nor early pregnant, controlling for female identity, collection time of day and storage duration, n=93 breeding events by 21 mothers: $F_{1,63}$=1.28, P=0.21; effect±se=1.64±0.21). Thus, our results suggest that the production of GCs from the feto-placental unit or the passive diffusion of maternal GCs into suckling offspring are insufficient to alter our observed patterns of maternal fGC qualitatively. This conclusion is further supported by findings that: (a) dominant females, which deliver and particularly suckle larger litters than subordinates (Russell *et al.*, 2003a), showed no differences with subordinates in fGC levels; and (b) that female mass was positively associated with fGC levels during preconception, suggests that similar correlations during late pregnancy cannot simply be attributed to larger/heavier litters.

That postpartum conception was associated with substantial reductions in fGC during late pregnancy is intriguing because maternal GCs are also expected to rise to parturition to facilitate late fetal development (Atkinson and Waddell, 1995; Mastorakos and Ilias, 2003). This result cannot be explained by a relationship between postpartum conception and small litter sizes, because we have failed to find a relationship between litter size (delivered or weaned) and birth-intervals, and dominants, which show lower post-birth pup mortality than subordinates, have shorter, not longer, birth intervals (Russell *et al.*, 2003a). Indeed, we found little general evidence of a simple relationship between maternal energy requirements and fGC (Krasnow and Steiner, 2006). Maternal fGC levels showed earlier peaks in gestation and declined both late in pregnancy and during lactation in mothers experiencing postpartum conception, when energetic requirements would be at their greatest. The mechanisms underpinning these contrasting patterns in maternal fGC during postpartum and non-postpartum conceptions have yet to be elucidated. One possibility is that reduction in fGC arises from the antagonist effect of increasing estrogens associated with postpartum conception which are known to interfere with the hypothalamic–pituitary–adrenal (HPA) axis (Douglas, 2010; Mastorakos and Ilias, 2003). Whether such potential effects of estrogens arise from a physiological or behavioral strategy on the part of mothers to reduce inter-birth intervals (Breuner, 2008; Meylan and Clobert, 2005; Nguyen *et al.*, 2008), or inter-birth intervals are reduced as a consequence of patterns of fGC during late pregnancy, are currently unclear (Wagenmaker *et al.*, 2009).

Previous research investigating the role of GCs in mediating reproductive suppression has been conducted outside the breeding season or involved non-breeders (Creel, 2001, 2005). The initial conclusion from this research is that in cooperative breeders dominant females do not attempt to suppress the reproductive function of subordinates through inducing chronic elevation in GCs (Creel, 2001). However, Young *et al.* (2006) noted that comparisons of adrenal activity among dominant and subordinate females during periods of non-breeding might be problematic, if pregnant dominants induce chronic stress in subordinates, for example by evicting them from the group. In support, Young *et al.* (2006) found that subordinates had double the levels of fGC when evicted (~200 ng/g feces), and during this

time either failed to conceive or aborted if pregnant. In our study, the majority of conceptions occurred with fGC levels of under 100 ng/g feces, even among those with a postpartum conception, although conception and implantation did appear to be possible with levels as high as ~400 ng/g feces in some instances. These results conform to the general trend that successful conception and implantation require low adrenal activity (Douglas, 2010; Nakamura *et al.*, 2008), and are broadly supportive of the possibility that sustained levels of ~200ng/g feces might preclude conception in most females (Young *et al.*, 2006). However, whether or not high adrenal activity is the mediating factor behind abortions as suggested by Young *et al.* (2006) is less clear, although GCs are proposed to cause abortion in other mammal species (Arck, 2001; Douglas, 2010; Magiakou *et al.*, 1997; Nakamura *et al.*, 2008; Nepomnaschy *et al.*, 2006). In meerkats, identification of pregnancy is difficult until the second half of gestation (i.e. late pregnancy in this study). Even during the first week of late pregnancy, fGC levels already averaged ~200 ng/g feces and levels of up to ~400 ng/g feces were common; with this rising to ~1000 ng/g feces in some females by the start of the final third of gestation. That all our females carried to term despite high levels of fGC, in conjunction with known reduced sensitivity of maternal HPA axis to stressors towards parturition in other species (Brunton *et al.*, 2008; Douglas *et al.*, 2003; Johnstone *et al.*, 2000), suggests that further work is required to test the link between adrenal activity and abortions.

We found no evidence to suggest that dominants and subordinates differ in their levels or patterns of fGC, either during preconception, early pregnancy or late pregnancy. Young *et al.* (2008) failed to find a difference between fGC levels in dominant versus subordinate meerkats during non-breeding periods. That we found no difference between dominants and subordinates during preconception, a period when fGC levels are similar to non-breeding periods, supports this earlier finding of Young *et al.* (2008). In addition, our results that dominants and subordinates reproducing at a similar rate had similar mean fGC levels during preconception and pregnancy phases, and similar slopes with advancement of each phase, suggest that, at least among those that carried successfully to term, there is no indication that dominants and subordinates significantly differ in their adrenal activity. Finally, we found no evidence to suggest that dominants and subordinates differ in their fGC profiles in association

with reproductive rate, suggesting that the low reproductive rate commonly associated with subordinates does not arise due to higher "stress" involved with reproduction. This conclusion has been reached previously, because dominants and sexually mature subordinates do not differ in their foraging success (Russell *et al.*, 2004). Taken together, these results suggest that at least among those females considered in this study, subordinates are not constrained from reproduction due to inferior condition or elevated physiological costs; implying that either dominant suppression or self-restraint, when the probability of breeding successfully is low, mediates the incidence of subordinate reproduction in meerkats.

In conclusion, our study has a number of important implications. First, we suggest that studies of cooperative breeders showing dominants to have higher GCs levels than subordinates might be partly an artifact of high reproductive rates and either reflect increased levels directly involved with aspects of reproduction or the consequences of reproduction in the form of compensatory foraging effort (Russell *et al.*, 2004). Second, increases in adrenal activity during gestation are suggested to be required for successful fetal development (Atkinson and Waddell, 1995; Mastorakos and Ilias, 2003). That female meerkats showed substantial reductions late in pregnancy when followed by a postpartum conception, either calls the generality of this effect into question or suggests that litters followed by postpartum conception should have impaired growth and/or development. Concomitantly, however, females with a postpartum conception conceived with higher fGC levels and higher levels during preconception were associated with higher levels during early pregnancy. The consequences of high reproductive rates for mothers and offspring, and whether mothers might use varying rates to vary offspring phenotype is currently unclear (Russell and Lummaa, 2009).

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CHAPITRE III
STRESS SOCIO-ÉCOLOGIQUES LORS DE LA REPRODUCTION

Les stress sociaux (Creel *et al.*, 2012) et écologiques (Gesquiere *et al.*, 2008; Sheriff *et al.*, 2009) peuvent diminuer le succès reproducteur et la survie en lien avec les effets des GCs (Goymann et Wingfield, 2004; Sapolsky, 2002; Sapolsky *et al.*, 2000). Par exemple, le stress chronique peut compromettre la gestation (Nakamura *et al.*, 2008) et le stress gestationnel peut affecter la valeur adaptative de la progéniture (Braastad, 1998; Seckl et Meaney, 2004). Malgré les conséquences néfastes du stress, les aspects spécifiques du contexte socio-écologique pouvant modifier l'excrétion des GCs en milieu naturel ont rarement été étudiés lors de la reproduction. Cette lacune émane principalement de la difficulté de démêler les sources extrinsèques de stress des effets mêmes de la reproduction sur la production de GCs (Trainer, 2002). En effet, la conception et la gestation peuvent influencer les niveaux de base en GCs et la réactivité de l'axe du stress (Brunton *et al.*, 2008; Wingfield et Sapolsky, 2003). De plus, le taux de reproduction peut modifier les niveaux et les patrons de changements en fGC lors de la gestation (Barrette *et al.*, 2012, Chapitre II). Le suivi longitudinal de populations sauvages ainsi que des méthodes non invasives permettent désormais un échantillonnage hormonal répété pour plusieurs individus au cours de leur cycle de reproduction. Il est ainsi possible d'investiguer les facteurs associés au variations naturelles en fGC lors de la reproduction et de déterminer les moments spécifiques où les patrons de changement en fGC varient en fonction du contexte socio-écologique, en lien ou non avec le taux de reproduction d'une femelle. Ces informations sont primordiales à l'étude des liens entre les GCs, le contexte socio-écologique, le succès reproducteur des femelles et la valeur adaptative de leur progéniture. Je propose donc d'évaluer comment le contexte socio-écologique affecte les fGC, avant et pendant la gestation, chez des suricates sauvages variant dans leur taux de reproduction.

CHAPITRE III
SOCIO-ECOLOGICAL STRESSORS DURING REPRODUCTION IN FEMALE MEERKATS.

MARIE-FRANCE BARRETTE, MARCO FESTA-BIANCHET, STEVEN L. MONFORT, TIM H. CLUTTON-BROCK, ET ANDREW F. RUSSELL

Description de l'article et contribution

L'article étudie les facteurs socio-écologiques associés aux variations naturelles en fGC lors de la reproduction de femelle suricates sauvages sur 11 ans. Le risque de prédation, relié à la taille du groupe, entraîne une augmentation en fGC avant et pendant la gestation, et ce seulement chez les femelles primipares. De plus, la compétition pour la reproduction entre femelles, indépendamment de la parité, et le chevauchement de reproductions d'une femelle multipare lors de faible disponibilité saisonnière en nourriture entraînent une augmentation importante en fGC lors de la gestation. Ces résultats suggèrent un rôle mécanistique des GCs dans la variation individuelle dans le moment et le taux de reproduction. De plus, les niveaux élevés en fGC mesurés lors de la conception et la gestation suggèrent une variation importante dans l'environnement hormonal prénatal pouvant affecter le développement, le phénotype hormonal et comportemental et ultimement la valeur adaptative de la progéniture. L'étude suggère que l'expulsion de compétitrice pour la reproduction pourrait réduire le stress social gestationnel et ainsi favoriser le succès reproducteur des femelles en facilitant la conception postpartum et en épargnant la portée en cours de gestation des effets néfastes du stress prénatal.

J'ai développé l'idée de cet article avec Andrew Russell. Tim Clutton-Brock a organisé le suivi à long terme de la population de suricates à l'étude. J'ai effectué les analyses hormonales avec le support logistique et sous la supervision de Steven Monfort. J'ai effectué le traitement des bases de données, les analyses statistiques, et la rédaction sous la supervision d'Andrew

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SOCIO-ECOLOGICAL STRESSORS DURING REPRODUCTION IN FEMALE MEERKATS.

Par

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Abstract

Stress can impede female reproduction, alter offspring development and reduce future reproductive potential. Despite the well-documented consequences of chronic elevation in adrenal activity, little is known about the socio-ecological factors associated with glucocorticoids during reproduction of wild female mammals. We investigated the socio-ecological context affecting fecal glucocorticoid metabolites (fGC) in free-ranging female meerkats (*Suricata suricatta*) of varying reproductive rate, over 11 years. The effects and the magnitude of stressors varied according to parity, timing of occurrence during breeding and female reproductive rate. Adrenal activity was elevated in primiparous females under high predation risk, in multiparous females when food availability was low, and for all females facing reproductive conflict with other females in their social group. Among multiparous females, high reproductive rate enhanced fGC increases at low food availability. Our study suggests an association between increased adrenal demands around conception and gestation and individual variation in the timing and rate of reproduction and their potential maternal effects on fetal development in meerkats. Furthermore, our results suggest that eviction of pregnant competitors from the group might facilitate postpartum conception and improve growth of the current litter.

Introduction

Glucocorticoids (GCs) are adrenal steroids excreted when internal and external challenges require rapid mobilization of energy, including hunger, fight-or-flight response and social conflict (de Kloet, 1999; Dhabhar, 2002; Whittle *et al.*, 2001; Wingfield and Kitaysky, 2002). When persistent, these energetic challenges can result in prolonged elevations in GCs and in chronic stress (Sapolsky *et al.*, 2000; Selye, 1956). Although chronic elevations in GCs can impede reproduction in females (Bennett, 1994; Nakamura *et al.*, 2008; Saltzman *et al.*, 2006; Young *et al.*, 2006), adrenal function is essential to maintain gestation and fetal development (Brunton *et al.*, 2008; Dantzer *et al.*, 2010; Krasnow and Steiner, 2006; Mastorakos and Ilias, 2003; Trainer, 2002). Furthermore, high GCs levels can be sustained during gestation (e.g. in meerkats; Barrette *et al.*, 2012, Chapter II). Additionally, reduced sensibility of the maternal hypothalamic–pituitary–adrenal (HPA) axis may be an adaptation to breed under extreme environmental conditions (Wingfield and Sapolsky, 2003).

Prenatal stress may modify offspring development in mammals, including humans, leading to reduced neonatal mass, impeded behavioral and hormonal responses to external stimuli and reduced adult survival (Braastad, 1998; Götz and Stefanski, 2007; Kaiser and Sachser, 2009; Michel *et al.*, 2011; Otten *et al.*, 2010; Seckl and Meaney, 2004; Sheriff *et al.*, 2009). Excessive gestational adrenal activity will transfer GCs to embryos, despite the enzyme 11 ß-hydroxysteroid dehydrogenase building up a feto-placental barrier as pregnancy progress (Douglas, 2011; Edwards *et al.*, 1996; Michael and Papageorghiou, 2008). Therefore, socio-ecological stressors inducing increased adrenal activity of the mother during pregnancy could affect offspring development and reduce offspring and maternal fitness (Parker and Douglas, 2010).

Despite the potential fitness consequences of high GCs levels during gestation, we know little about which socio-ecological contexts may induce stress during different phases of the reproductive cycle in free-ranging populations. Socio-ecological stressors in free-ranging mammal females have mostly been studied outside of breeding periods to avoid the

confounding influence of breeding on GCs levels (Gesquiere *et al.*, 2008; Ryan *et al.*, 2012; Sheriff *et al.*, 2009; Starling *et al.*, 2010). Because conception and gestation can modify individual susceptibility to environmental stressors (McEwen and Wingfield, 2010; Wingfield and Sapolsky, 2003), it is essential to assess how socio-ecological contexts both before and during breeding periods can affect GCs levels. Longitudinal noninvasive studies of individual GCs excretion (Wasser *et al.*, 2000) during key stages of the reproductive cycle may explain patterns of natural variation without the complicating effects of capture. To understand how stress during fetal development may affect offspring phenotype, it is crucial to investigate which socio-ecological factors influence variation in GCs during the reproductive cycle, quantify their importance, determine whether they act at specific moments during breeding and assess their interaction with female reproductive rate.

In social species, females may vary in the level of socio-ecological stress they endure during breeding. In meerkats for instance, subordinates generally breed when they are less likely to compete with dominants, such as during periods of low seasonal food availability, to avoid the reproductive cost of infanticide (Clutton-Brock *et al.*, 2001b). Additionally, ecological stressors such as seasonal food shortages (Gesquiere *et al.*, 2008; Lynn *et al.*, 2010) and predation risk (Creel *et al.*, 2009; Eilam *et al.*, 1999; Sheriff *et al.*, 2009) can challenge adrenal homeostasis independently of socially-induced stress. Social stress may be induced indirectly by large numbers of reproductive competitors and directly through aggressive evictions. Furthermore, high annual reproductive rate increase adrenal activity at the time of breeding in meerkat females (Barrette *et al.*, 2012, Chapter II). Here, we used 11 years of data on life-history, behavioral and fecal glucocorticoid metabolites (fGC) to investigate how socio-ecology during breeding affects adrenal activity within and among free-ranging female meerkats breeding at varying rates.

Meerkats are small (<1kg) social carnivores that reproduce cooperatively in groups of 2 to 40 (mean±sd=16.7±8.6) in arid southern Africa. Groups generally include a dominant pair and overlapping generations of relatives that help rear offspring (Clutton-Brock *et al.*, 2001b; Clutton-Brock *et al.*, 2002). Dominant females produce ~ 80% of offspring and subordinate

breeding is biased toward older and larger subordinates (Clutton-Brock *et al.*, 2001b; Russell *et al.*, 2004; Young *et al.*, 2008). Gestation lasts ~70 days (Doolan and Macdonald, 1997), with increases in gonadal (Moss *et al.*, 2001) and adrenal steroids from 35 days before birth (Barrette *et al.*, 2012, Chapter II). Some mothers can achieve a high reproductive rate by conceiving again within a few days of parturition (Moss *et al.*, 2001; Russell *et al.*, 2003a). Reproductive overlap resulting from postpartum conception has two major consequences on fGC levels during gestation: (i) a decrease in the 15 days preceding parturition; and (ii) higher preconception levels than when no reproductive overlap occurs (Barrette *et al.*, 2012, Chapter II). Furthermore, higher fGC levels during the 35 days preceding conception were associated with higher levels during the first 35 days of pregnancy in multiparous females (Barrette *et al.*, 2012, Chapter II). Females may breed up to four times a year, mostly during the wet-hot season (November through April) (Clutton-Brock *et al.*, 1999b). Litters range from one to six pups (mean±sd=3.7±1.5) and lactation lasts about 35 days (±10 days, Russell *et al.*, 2003a). Meerkats therefore provide an ideal opportunity to test how socio-ecological context affects adrenal activity during periods of high and low reproductive rate.

To investigate whether and when adrenal functions during breeding are mediated by the socio-ecological context, we studied fGC patterns from 35 days before conception to parturition. We first investigated fGC levels in primiparous females to examine the effects of socio-ecological stressors independently of previous reproductive experience. We then investigated fGC levels in multiparous females to assess whether socio-ecological stressors act independently of reproductive rate, or whether timing and rate of breeding depend on the association between fGC levels and the socio-ecological context. Ecological factors considered were food availability and group size, while social factors characterized female competition over reproduction (see *Methods*). We controlled for several potential confounding influences, including aspects of the sample collected and traits of the female from which it was collected.

Methods

We studied habituated meerkats from 1997 to 2008 at the Kuruman River Reserve in the South African Kalahari Desert (see Clutton-Brock *et al.* 1999a; Russell *et al.* 2002 for details of habitat and climate). A total of 15 meerkat groups, numbering two to 40 marked individuals (mean=~16), were followed every one to five days to monitor individual life-history and body mass, collect fecal samples and details on each group's socio-ecological context. Groups were monitored daily when pregnant females were expected to give birth. Meerkats were weighed each morning by enticing then onto digital balances (±1g) with hard-boiled eggs (e.g. Clutton-Brock *et al.*, 2002). Pregnancy was identified about four weeks after conception by swelling of the abdomen and nipples and by an increase in mass. Parturition (±1 day) was identified by sudden mass loss and presence of babysitters at the burrow (Clutton-Brock *et al.*, 2001b). Conception date was back-calculated to 70 days before birth (Doolan and Macdonald, 1997). Based on the timing of increases in gonadal hormones following conception (Moss *et al.*, 2001) and of the main fetal growth phase (Russell *et al.*, 2003a), we subdivided breeding events into three 35-day phases: (i) preconception (day -35 to -1); (ii) early pregnancy (day 1 to 35); and (iii) late pregnancy (day 36 to 70). These three phases allowed us to account for increased adrenal activity during gestation (Barrette *et al.*, 2012, Chapter II), and assess whether fGC patterns associated with reproductive overlap are modified by socio-ecological context prevailing at specific times. Because females experiencing postpartum conception gestate and lactate at the same time, lactation was accounted for by including the presence of unweaned pups at the burrow as a fixed effect in all analyses. All animal-handling protocols were approved by the University of Pretoria, the University of Sherbrooke and the Smithsonian Institution ethic committees.

Sample collection and hormonal analyses

Overall, we assayed 734 fecal samples collected from 1997 to 2008 over 193 breeding events by 32 primiparous (n=155 samples) and 50 multiparous females (n=579 samples, 161 events). Fecal GC metabolites provide a pooled measure of adrenal steroids excreted over the past 24 to 48 hours (Young *et al.*, 2006), without the confounding stress of capture. Samples were

collected whenever animals defecated, immediately placed on ice in thermos flasks and frozen at -20°C within five hours (mean ca. 2h). Samples were shipped frozen to the Smithsonian Conservation Biology Institute in Front Royal, VA, USA. Steroid metabolites were extracted and fGC concentrations measured from samples using validated methodologies (Monfort *et al.*, 1997). See Barrette *et al.* (2012, Chapter II) for details on steroid boiling extraction and corticosterone radioimmunoassay methods. Intra-assay coefficients of variation were <10% and inter-assay coefficients of variation for low- and high-dose internal controls were 8.8% and 6.7% for 20 assays performed form December 2005 to September 2008.

Statistical analyses

Analyses were conducted over an entire breeding event and separately for each breeding phase. These separate analyses had two main advantages. First, they facilitated interpretation of non-linear patterns in fGC levels over the breeding event. Second, they clarified the relative importance of socio-ecological factors in explaining variation in fGC at different times of a breeding event. Primiparous and multiparous females were analyzed separately as reproductive overlap occurred only for multiparous females. The association between fGC levels and parity was tested in a preliminary analysis comparing primi and multiparous females not breeding during the 35-day postpartum interval.

In all analyses, fGC data were normalized using a logarithm transformation and fitted to a normal error structure in a General Linear Mixed Model (GLMM). In multiparous females, ~87% of the variation in fGC levels occurred among samples of the same female within a breeding event, ~9% occurred among breeding events of the same female, and ~4% was among individual females; no significant variation occurred among groups (Barrette *et al.*, 2012, Chapter II). During preconception, more of the variation, i.e. ~32% *vs.* ~9%, was found among breeding events of the same female (Barrette *et al.*, 2012, Chapter II). To account for the hierarchical distribution of variation in the samples (Barrette *et al.*, 2012, Chapter II; Hox, 2002), we therefore fitted three random terms: (i) samples within a breeding event and within a female (n=(mean±sd) 3.5±3.2 samples per event); (ii) events within females (n=2.8±2.8 events per female); and (iii) females within groups (n=4.9±3.2 females per group). Random

terms were nested, with samples at the lowest hierarchical level. Higher levels were retained in final models only if they encompassed significant fGC variation after assessing the importance of fixed effects.

The fixed terms of interest outlined below were considered after controlling for random terms and potentially confounding fixed effects. Fixed effects with a potentially confounding influence included: storage duration, collection time-of-day, timing into a breeding event and female characteristics at the time of breeding and of sample collection (Barrette *et al.*, 2012, Chapter II). Because steroid metabolites can degrade over time (Schwartz and Monfort, 2008), we fitted as a fixed co-variate the number of days between sample collection and assay. To control for circadian variation in fGC, collection time was coded as morning (am: 06:00–12:00) and evening (pm: 15:00–20:00) and fitted as a two-level fixed co-factor in all analyses. To account for non-linearity of relations between fGC levels and timing into breeding (Barrette *et al.*, 2012, Chapter II), days into breeding were fitted as linear and quadratic functions in the overall analysis (105 days), as linear functions during preconception (35 days) and early-pregnancy (35 days) and as quadratic functions during late pregnancy (35 days). We included mass in all analyses to account for the association between fGC levels and body mass (Barrette *et al.*, 2012, Chapter II), although interpretation of mass variations is difficult in a pregnant mammal. Female age was determined by birth dates in the study population (± 1 day) and was included in analyses of primiparous females, which were all subordinates. Female age and dominance status were not associated to fGC variation in multiparous females (Barrette *et al.*, 2012, Chapter II) and were thus not included in analyses of those females. Only confounding terms that explained significant variation in a given model were retained.

All analyses contained six fixed terms describing the socio-ecological context, in addition to a seventh term for multiparous analyses, defining current reproductive rate. Although these fixed terms were dropped from the final model when not significant, we report their effect when added to the final model individually in all cases. Ecological factors included: (1) climatic season; (2) pup presence at the burrow; and (3) group size. Food availability varies with season (Barnard, 2000; Doolan and Macdonald, 1997), coded as wet-hot (November 1st

to April 30th) and dry-cold (May 1st to October 31st). Food availability gradually decreases when pups are at the burrow and group foraging range is reduced (Clutton-Brock *et al.*, 2000). Large groups tend to forage over larger ranges, have improved foraging success, reduced predation and reduced workloads (Barnard, 2000; Clutton-Brock *et al.*, 1999a; Clutton-Brock *et al.*, 2002; Russell *et al.*, 2003b). Group size included individuals over six months old, when cooperation increases steeply (Clutton-Brock *et al.*, 2002). As GCs increase with hunger (Busch and Hayward, 2009), we predicted that adrenal activity would increase during the dry-cold season, with pups at the burrow and in smaller groups.

Social factors quantified female reproductive competition and included: (4) the number of breeding-age females in a group; (5) the occurrence of concurrent gestations; and (6) the number of females evicted. Competition for reproductive opportunities was quantified at the time of sample collection by the number of females over eight months old, the minimum age for female reproduction (Young *et al.*, 2006). Actual competition was quantified over a breeding event by the occurrence of concurrent gestations (parturitions within 20 days, mean±sd=5.3±3.1, coded as yes or no) and the number of females evicted from the group, determined from daily observations. Breeders included in analyses were not evicted while pregnant, as most females aborted following evictions of more than a week (Young *et al.*, 2006). In cooperative breeders, individual female reproduction conflicts with reproductive success of others because offspring care is shared among all group members. In meerkats, reproductive competition increases with the number and pregnancy status of adult females in a group (Clutton-Brock *et al.*, 2006; Hodge *et al.*, 2008). We expected fGC to increase with the number of female competitors, especially when other females were pregnant. It is unclear whether evictions would augment stress levels via frequent aggressive interactions (Young *et al.*, 2006) or reduce stress by reducing the number of competitors.

In the analyses of multiparous females, individual current reproductive rate was defined by (7) reproductive overlap of consecutive breeding events. Reproductive overlap was characterized by whether or not a female was pregnant during the 35 days before conception of a litter, and was fitted as a two-level factor in all analyses, except during the late pregnancy phase where

we fitted instead whether or not she was preconceptive. A high reproductive rate was thus defined by the overlap of two phases of reproduction; a low reproductive rate was defined by no reproductive overlap. We predicted the increased adrenal activity associated with reproductive overlap (Barrette *et al.*, 2012, Chapter II) to be exacerbated in females breeding under "stressful" socio-ecological conditions, compared to females breeding at a low rate under similar conditions. To test for a synergistic effect of socio-ecological factors and female reproductive rate on fGC pattern over time, we accounted for the interaction between timing into breeding (in days) and reproductive overlap (Barrette *et al.*, 2012, Chapter II), and tested for interactions between reproductive overlap and socio-ecological factors and between timing into breeding and socio-ecological factors.

Statistical analyses were conducted with R (R Development Core Team 2008). Normality was determined using quantile plots, frequency histograms and Shapiro-Wilk normality test. The explanatory power of models and individual fixed terms was calculated by measuring the difference in residual variance including only random terms versus both random and fixed explanatory terms. Fixed terms and their two-way interactions were retained when significant. Type III sum of square error structures (i.e. marginal error structure in R) were used to control for co-variation among fixed terms (Pinheiro and Bates, 2000). Orthogonal polynomials were tested to control for collinearity among polynomial degrees of days into breeding event and phases (Shacham and Brauner, 1997). All statistical tests were two-tailed. Means and standard errors presented in the text were predicted by GLMMs, and back-transformed to provide values in fGC ng/g of dry feces.

Results

Reproductive experience did not affect adrenal activity (GLMM analysis on fGC levels during non overlapping breeding events, controlling for female identity, collection time of day and storage duration, n=303 samples collected in 32 primiparous and 29 multiparous females: no significant effect of parity; $F_{1,240}$=0.87, p=0.35, effect±se=0.13±0.14). To investigate socio-

ecological stressors in female meerkats breeding at different rates we considered separately adrenal activity in primiparous and multiparous females.

Effect of socio-ecological conditions in primiparous females

Seasonal food availability and group size affected fGC levels during primiparous breeding events (Table 1) after accounting for temporal changes in fGC levels, which tripled from mid-pregnancy through to parturition (positive linear effect of timing into late pregnancy, day 36 to 70, in GLMM analysis on Table 1d; $F_{1,25}$=8.38, p=0.008, effect±se=0.04±0.01). Pup presence at the burrow did not explained significant variation in fGC levels among primiparous females (Table 1). On average, fGC levels were three times higher in females breeding for the first time in the dry-cold season, when food availability is low, than during the wet-hot season (Table 1a). This effect was driven primarily by differences during preconception (Table 1b). Similarly, primiparous breeders excreted ~5% less fGC with every additional helper in their group (Table 1a), an effect driven by differences during both preconception and late pregnancy (Table 1b and d). We found no evidence that ecological factors explained variation in fGC during early pregnancy.

Female competition over reproduction failed to explain variation in fGC over a breeding event, although it had some effects during specific phases (Table 1). During preconception, females excreted ~17% more fGC metabolites with every additional breeding-age female present in their group (Table 1b). In addition, after controlling for timing into late pregnancy, females breeding when concurrent gestations occurred within their group excreted twice as much fGC metabolites than those breeding without competition (Table 1d). Furthermore, females excreted ~25% more fGC metabolites with every additional female evicted from their group (Table 1d). We found no evidence that social factors explained variation in fGC during early pregnancy.

Table 1. Socio-ecological factors affecting primiparous female meerkats fGC during: (a) an entire breeding event (day -35 to 70); (b) preconception (day -35 to -1); (c) early pregnancy (day 1 to 35); and (d) late pregnancy (day 36 to 70). Only terms of interest and their interactions are presented; sampling and individual confounding effects are presented in the *Results*; only significant terms are presented for phase-specific analyses. Effect sizes are log-transformed fGC concentrations.

GLMMs - Primiparous females	*df*	Effect ± SE	F	p-value
(a) All breeding data				
Constant [a]	1, 74	5.36 ± 0.57	194.60	< 0.001
Ecological factors				
Season *(wet-hot < dry-cold)*	1, 74	-1.20 ± 0.48	6.34	0.014
Group size	1, 74	-0.05 ± 0.02	6.25	0.015
Presence of pups at burrow *(yes, no)*	1, 74	-0.28 ± 0.24	1.38	0.24
Social factors				
Number of females > 8 months old	1, 74	0.004 ± 0.07	0.004	0.95
Concurent gestations *(no, yes)*	1, 30	0.28 ± 0.43	0.42	0.52
Number of evictions	1, 30	0.083 ± 0.069	1.46	0.24
(b) Preconception				
Constant [a]	1, 27	5.18 ± 0.44	140.23	< 0.001
Ecological factors				
Season *(wet-hot < dry-cold)*	1, 14	-0.97 ± 0.29	10.83	0.005
Group size	1, 14	-0.12 ± 0.035	12.28	0.004
Social factors				
Number of females > 8 months old	1, 14	0.18 ± 0.08	5.09	0.041
(c) Early pregnancy				
Constant [a]	1, 32	4.45 ± 0.11	1679.08	< 0.001
(d) Late pregnancy				
Constant [a]	1, 25	5.07 ± 0.56	252.54	< 0.001
Ecological factors				
Group size	1, 25	-0.11 ± 0.044	5.77	0.024
Social factors				
Concurent gestations *(no < yes)*	1, 20	0.81 ± 0.33	6.24	0.021
Number of evictions	1, 20	0.30 ± 0.14	4.33	0.051

[a] Values presented for the constants are specific to each periods and are calculated in reference to samples collected during the *dry-cold* season and when *no* concurent gestation occurs. Data were collected from 1997 to 2008 at the Kuruman River Reserve, South Africa.

Interplay of ecological conditions and reproductive rate in multiparous females

Food availability, but not group size, affected fGC levels during multiparous breeding events. Analysis of fGC levels over multiparous breeding events revealed interactions between timing into breeding, reproductive overlap and seasonal food availability (Table 2a). Phase-specific analyses clarified that seasonal variation generally arose during preconception and late pregnancy (Table 2b and d). These results were observed after controlling for positive effects of sample storage time ($F_{1,408}$=14.25, p<0.001, effect±se=1.9E-4±4.6E-5) and body mass ($F_{1,408}$=32.94, p<0.001, effect±se=2.6E-3±5.0E-4), and differences associated with collection time (am<*pm*: $F_{1,408}$=21.48, p<0.001, effect±se=0.36±0.075), and timing into breeding (Table 2a; see Barrette *et al.* (2012, Chapter II) for detailed temporal changes in fGC levels). As with primiparous females, fGC levels during multiparous breeding events were influenced by season (Table 2a): females breeding during the dry-cold season excreted about twice as much fGC metabolites as those breeding during the wet-hot season. Furthermore, multiparous females that conceived within a month of a previous parturition doubled their fGC excretions throughout gestation, primarily because levels excreted when breeding during the harsher dry-cold season were twice those during the wet-hot season (GLMM analysis on fGC levels when pregnant preconception, controlling for terms in Table 2a: $F_{1,264}$=17.39, p<0.0001, effect±se=-0.39±0.12; Fig. 1, dotted lines). In contrast, seasonal differences in fGC excretions were not apparent for females which did not overlap two breeding events (GLMM analysis on fGC levels when not pregnant preconception, controlling for terms in Table 2a: $F_{1,123}$=0.14, p=0.71, effect±se=0.07±0.14; Fig. 1, solid lines). Finally, fGC levels were higher during the dry-cold season only during preconception (Table 2b) and late pregnancy (Table 2d), and showed no interaction with reproductive overlap within each phase.

In contrast with primiparous females, fGC levels during multiparous breeding events were not influenced by group size (Table 2) but were influenced by the presence of pups during early pregnancy (Table 2c). After controlling for the decrease in fGC levels over time in early pregnancy (Barrette *et al.*, 2012, Chapter II), multiparous breeders showed a 1.5-fold increase in fGC when pups were present at the burrow compared with when there were none; no effect of pup presence was observed during preconception or late pregnancy (Table 2b and d).

Table 2. Socio-ecological factors affecting multiparous female meerkats fGC during: (a) an entire breeding event (day -35 to 70); (b) preconception (day -35 to -1); (c) early pregnancy (day 1 to 35); and (d) late pregnancy (day 36 to 70). Only terms of interest and their interactions are presented; sampling and individual confounding effects are presented in the *Results*; only significant terms are presented for phase-specific analyses. Effect sizes are log-transformed fGC concentrations.

GLMMs - Multiparous females	*df*	Effect ± SE	F	p-value
(a) All breeding data				
Constant[a]	1, 407	4.03 ± 0.53	57.00	< 0.001
Timing into breeding[b] (Days B)	1, 407	-0.063 ± 0.011	30.94	< 0.001
(Days B^2)	1, 407	6.5E-4 ± 1.0E-4	39.32	< 0.001
Reproductive overlap[b]				
Pregnant preconception *(no < yes)*	1, 108	-1.40 ± 0.31	20.06	< 0.001
Pregnant preconception * Days B	1, 407	0.039 ± 0.012	10.66	0.001
Pregnant preconception * Days B^2	1, 407	-3.2E-4 ± 1.1E-4	9.29	0.003
Ecological factors				
Season *(wet-hot < dry-cold)*	1, 407	-0.99 ± 0.29	12.06	0.006
Season * Days B	1, 407		6.24	0.01
Season * Days B^2	1, 407	Fig. 2	7.99	0.005
Season * Pregnant preconception	1, 407		13.46	< 0.001
Group size	1, 407	-6.4E-3 ± 9.4E-3	0.46	0.50
Presence of pups at burrow *(yes < no)*	1, 407	-0.13 ± 0.11	1.45	0.23
Social factors				
Number of females > 8 months old	1, 407	-0.13 ± 0.16	0.67	0.41
Concurent gestations *(no < yes)*	1, 108	0.19 ± 0.086	4.93	0.039
Number of evictions	1, 108	-0.039 ± 0.019	4.08	0.045
(b) Preconception[b]				
Constant[a]	1, 116	5.37 ± 0.20	689.23	< 0.001
Ecological factors				
Season *(wet-hot < dry-cold)*	1, 116	-0.37 ± 0.15	6.15	0.015
(c) Early pregnancy[b]				
Constant[a]	1, 104	4.41 ± 0.13	1113.77	< 0.001
Ecological factors				
Presence of pups at burrow *(no < yes)*	1, 104	-0.43 ± 0.13	10.84	0.001

Table 2 (Continued)

GLMMs - Multiparous females	*df*	Effect ± SE	F	p-value
(d) Late pregnancy [b]				
Constant [a]	1, 99	5.23 ± 0.33	252.54	< 0.001
Ecological factors				
Season *(wet-hot < dry-cold)*	1, 99	-0.41 ± 0.15	7.91	0.006
Social factors				
Concurent gestations *(no < yes)*	1, 58	0.89 ± 0.28	10.15	0.002

[a] Values presented for the constants are specific to each periods and are calculated in reference to samples collected in the morning (*am*), during the *dry-cold* season, when *no* pups are present at the burrow, when *no* concurent gestation occurs and for females pregnant preconception (*yes*) and *not* conceptive postpartum. [b] Effects of timing into breeding and reproductive overlap are detailed over a breeding event; see Barrette *et al.* (2012), Chapter II, for details during specific breeding phases. Data were collected from 1997 to 2008 at the Kuruman River Reserve, South Africa.

Independent effect of intrasexual competition in multiparous females

Female competition over reproduction affected fGC levels during breeding independently of current individual reproductive rates. The occurrence of concurrent gestations induced higher fGC levels during multiparous breeding events. However, fGC excretions were independent of the number of breeding-age females and increased with the number of evictions in the group. Controlling for the confounding influences presented above and for reproductive overlap, concurrent gestations were associated with ~19% higher fGC levels in the presence of other reproducing females and fGC decreased by ~5% for every females evicted from the group (Table 2a). The effect of concurrent gestations was restricted to late pregnancy (Table 2d), while the effect of evictions was not significant when restricting our analyses to specific breeding phases (Table 2).

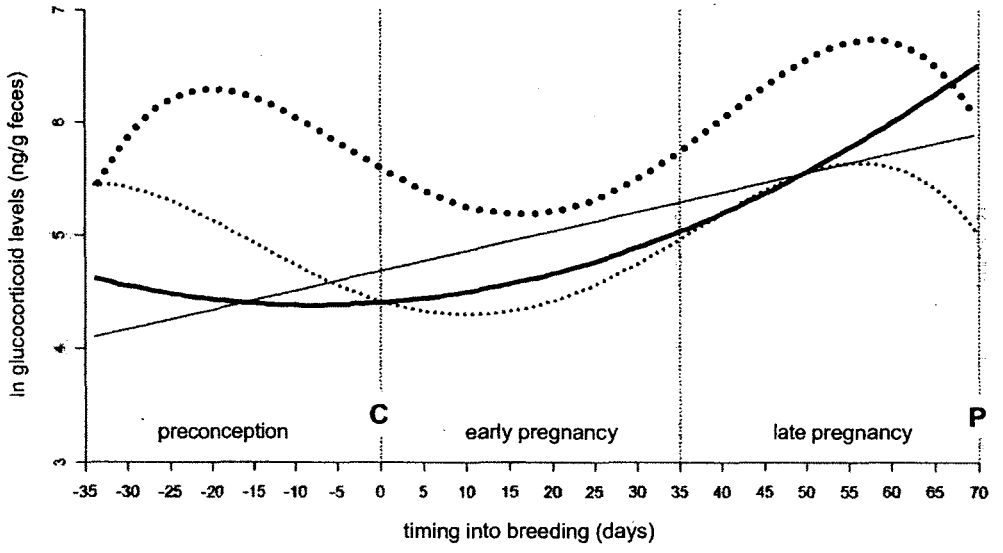

Figure 1. Association among fGC levels, reproductive rates and climatic seasons shown over a discrete breeding event as GLMM regression lines for adult female meerkats split into four breeder types: (1) not pregnant preconception during the dry-cold season (n=117 samples, 49 events and 31 mothers; $F_{2,64}$=33.70, p<0.0001; solid black bold line) or (2) wet-hot season (n=89 samples, 37 events and 29 mothers; $F_{1,51}$=28.92, p<0.0001; solid grey thin line), and (3) pregnant preconception during the dry-cold (n=99 samples, 41 events and 20 mothers; $F_{4,52}$=9.79, p<0.0001; dotted black bold line) or (4) wet-hot season (n=274 samples, 68 events and 31 mother; $F_{4,198}$=13.20, p<0.0001; dotted grey thin line). Days of conception and parturition are respectively identified with the letter C and P.

Discussion

The socio-ecological context during breeding affected fGC levels in female meerkats. Low food availability and reproductive competition affected all females, while small group size increased adrenal activity only in primiparous females. Additionally, when multiparous females reproduced at a high rate, they endured greater fGC increases during the season of low food availability. Our study suggests a proximal mechanism for individual variation in the

timing and rate of reproduction with levels of adrenal activity around conception and gestation being a potential mediating factor. Furthermore, it remains unclear whether this is a by-product of socio-ecological context or an active context dependant reproductive strategy.

High levels of GCs can impede reproduction (Nakamura *et al.*, 2008) and Barrette *et al.* (2012, Chapter II) suggested that a reduction in GCs level is required to allow female meerkats to conceive soon after parturition. It is unclear, however, whether females that show reductions in fGC are simply more likely to conceive (Wagenmaker *et al.*, 2009) or whether females actively reduce fGC levels in order to conceive (Breuner, 2008; Meylan and Clobert, 2005; Nguyen *et al.*, 2008). In meerkats, inter-birth interval is reduced at high food availability and in large groups, where helpers reduce the amount of care required from the mother (Clutton-Brock *et al.*, 2004; Russell *et al.*, 2003a). If the relationship between fGC patterns and conception was simply due to mothers with reduced levels being more likely to conceive, we might expect levels of fGC in multiparous females to be lower in late pregnancy during the wet-hot season when in large groups. We found only partial support for this prediction: during late pregnancy, fGC levels were lower in the wet-hot season but were unaffected by group size. If females reduced levels of GCs to conceive postpartum, we might expect them to adopt behaviors to down-regulate adrenal functions near parturition. Dominant females are more likely to have long successful tenures when reproductive competition is low (Hodge *et al.* 2008) and routinely evict the most competitive subordinates during late pregnancy (Young *et al.* 2006). Interestingly, we found that multiparous females had lower levels of fGC when multiple females were evicted, and had lower levels during late pregnancy when the number of reproductive competitors was low. These results suggest that evicting competitors late in pregnancy might reduce reproductive competition as well as reduce stress, facilitating postpartum estrous (Wagenmaker *et al.*, 2009).

Gestational GCs can also have downstream consequences for fetal development during periods of high sensitivity to elevated adrenal activity (Catalani *et al.*, 2011; Douglas, 2005; Seckl, 2004). Mothers might be able to reduce the deleterious impacts of high GCs on offspring development (Seckl, 2001) or may only breed when their GCs levels are likely to be

low. Food shortages are important stressors which can impede reproduction in many vertebrates (Busch and Hayward, 2009; Lynn *et al.*, 2010; Ross and Desai, 2005), including humans (Bentley *et al.*, 1999). Few studies, however, have shown a direct association between food shortage and elevated GCs in wild mammals (Gesquiere *et al.*, 2008; Goymann *et al.*, 2001). We found that during the wet-hot season, when food availability is greater (Barnard, 2000) and breeding more common (Clutton-Brock *et al.*, 2001a; Russell *et al.*, 2003), fGC levels were lower than during the dry-cold season among primiparous females and among those multiparous females with high reproductive rates. There was less evidence that other ecological factors were associated with fGC levels. Pup presence was significant only during early pregnancy of multiparous females, while group size was significant only in the overall analysis of primiparous females. The dominant ecological effect on fGC variation was season. To reduce fGC, females should not breed at a high rate during the dry-cold season. Accordingly, dominant females rarely breed during this season, when they typically allow subordinates to do so, leading to the production of less competitive offspring (Hodge *et al.*, 2008).

Our study revealed a potential hormonal mechanism underlying the reduction in breeding success and the increase in reproductive senescence rate observed among female meerkats as intrasexual competition intensifies (Sharp and Clutton-Brock, 2011). Our study revealed social factors pertaining to reproductive competition that were associated with high fGC levels, including the numbers of breeding-age females and of evictions, and the occurrence of concurrent gestations. The number of breeding-age females is likely to be of limited importance, and was significant only during early pregnancy in primiparous females. In meerkats, ~80% of reproduction is secured by dominant females (Clutton-Brock *et al.*, 2001b). Reproductive competition, however, should be directly affected by the occurrence of concurrent gestations, which was associated with increases in fGC in late pregnancy of all females. Dominant females may reduce these high levels by evicting pregnant competitors from the group. Reducing stress might not only facilitate postpartum conception, but also improve growth of the current litter (Seckl and Meaney, 2004). Thus, while potential reproductive conflict, quantified by the number of breeding-age females, led to fGC

elevations only in primiparous females, actual conflict, measured by concurrent gestations, elevated fGC levels in all females. It remains to be tested whether elevated adrenal activity associated with social stress during gestation lowers future reproductive potential of female breeders.

Despite numerous studies on the consequences of chronic elevations in adrenal activity for reproductive functions (Breuner *et al.*, 2008; Schoech *et al.*, 2009; Young *et al.*, 2006), it has rarely been possible to identify potential causal socio-ecological stressors in free-ranging populations. Our study revealed aspects of the socio-ecological context affecting fGC levels at specific time before and during breeding, and suggests a mediating role of GCs on reproductive timing and success. Our results raise two important questions. First, whether GCs themselves block the reproductive pathway or whether some other hormone is involved; and second, if GCs are responsible, do individuals vary in the amount of GCs they can tolerate before reproduction becomes unfeasible? Finally, our study identified important differences in fGC profiles during gestation, suggesting that meerkats are a good model species to investigate, in a free-living population, the consequences of GCs levels during pregnancy on litter development, offspring survival and behavior post-birth. Additionally, because high postpartum GCs can interfere with maternal care through transgenerational epigenetic effects (Cameron *et al.*, 2008; Champagne *et al.*, 2009; Champagne, 2008), mothers could strategically time their reproduction in response to the socio-ecological context and the amount of care they will thereby need to provide. Furthermore, it remains to be tested whether the ability of a female to time her reproduction based on current socio-ecological context is in turn maternally derived. Understanding such transgenerational effects would help determine the factors limiting reproductive rate and help understand variability in reproductive skew among social species.

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CHAPITRE IV
EFFETS MATERNELLES HORMONAUX

En raison du lien intime et prolongé entre une mère et sa progéniture chez les mammifères, notre compréhension des liens entre les GCs et la valeur adaptative doit considérer l'environnement hormonal prénatal. Malgré l'évolution de mécanismes permettant la protection du fœtus contre les adversités environnementales, tel la barrière enzymatique placentaire (Burton et Waddell, 1994; Yang, 1997), des niveaux élevés en GCs maternels peuvent modifier le développement de l'axe du stress de la progéniture ainsi que leur croissance, santé et potentiel reproducteur (O'Regan *et al.*, 2001; Seckl, 2004). L'expression appropriée d'une réponse physiologique et comportementale au stress dépendra de la programmation de l'axe du stress (Catalani *et al.*, 2011; Meaney *et al.*, 1994; Seckl, 1998). Cette programmation est fonction de l'intensité des stress et du moment où ils surviennent, de la conception au sevrage, et ses effets peuvent varier selon le sexe de la progéniture et l'espèce (Emack et Matthews, 2011; Kapoor *et al.*, 2006). Malgré plus d'une décennie de recherche, en conditions contrôlées, sur l'effet du stress pré et néonatal, nos connaissances sur ces effets maternels et leurs causes en milieu naturel demeurent très limitées. Le suivi longitudinal non invasif du stress au sein de populations sauvages permet désormais d'étudier les aspects spécifiques de l'environnement naturel causant des stress pouvant affecter le développement de l'axe du stress de la progéniture (e.g. risque de prédation: Sheriff *et al.*, 2010b). Je propose donc d'étudier les liens entre le contexte socio-écologique, les fGC de mère suricates sauvages, avant et pendant la gestation, et les fGC de leur progéniture, de l'émergence du terrier au sevrage.

CHAPITRE IV

MATERNAL STRESS DURING GESTATION AFFECTS GLUCOCORTICOIDS IN UNWEANED FREE-RANGING MEERKATS.

MARIE-FRANCE BARRETTE, MARCO FESTA-BIANCHET, STEVEN L. MONFORT, TIM H. CLUTTON-BROCK, ET ANDREW F. RUSSELL

Description de l'article et contribution

L'article étudie les effets maternels du stress gestationnel sur la variation en fGC de la progéniture avant le sevrage chez le suricate sauvage. Les fGC de la progéniture sont associés positivement avec les fGC maternels. De plus, les jeunes dont le développement prénatal chevauchait la période de sevrage de la portée précédente lors de faible disponibilité saisonnière en nourriture, ont des niveaux en fGC plus élevés que ceux nés d'une gestation non chevauchée ou lorsque la nourriture était abondante. Ces résultats suggèrent un conflit entre le succès reproducteur des mères et la santé de leur progéniture. Au contraire, suite à un développement prénatal en période de compétition pour la reproduction, les fils ont des niveaux en fGC moins élevés que ceux se développant en absence de compétition maternelle, une réduction non observée chez les filles. L'étude suggère des effets maternels hormonaux résultant de stratégies maternelles dépendantes du sexe et du contexte socio-écologique, susceptibles de favoriser le succès reproducteur futur des mères en modifiant le phénotype physiologique et comportemental de la progéniture.

J'ai développé l'idée de cet article. Tim Clutton-Brock a organisé le suivi à long terme de la population de suricates à l'étude. J'ai effectué les analyses hormonales avec le support logistique et sous la supervision de Steven Monfort. J'ai effectué le traitement des bases de données, les analyses statistiques, et la rédaction sous la supervision d'Andrew Russell et Marco Festa-Bianchet. Andrew Russell et Marco Festa-Bianchet ont commenté une version préliminaire du manuscrit. Andrew Russell, Marco Festa-Bianchet et Steven Monfort ont participé à la subvention du projet.

MATERNAL STRESS DURING GESTATION AFFECTS GLUCOCORTICOIDS IN UNWEANED FREE-RANGING MEERKATS.

Par

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Key words: adrenal activity; corticosterone; noninvasive; organizational effect; maternal effect; food availability; reproductive rate; reproductive competition; reproductive strategies; cooperative breeding.

Abstract

Understanding how socio-ecological stress affects fitness in mammals needs to consider the prenatal programming of the offspring neuroendocrine stress axis involving maternal glucocorticoid hormones (GCs). Noninvasive techniques to measure stress in free-ranging populations now provide the opportunity to clarify the extent to which specific aspects of the natural environment induce early-life stress capable of modulating the development of the stress axis. To investigate hormonal maternal effects of socio-ecological stressors, we examined variation in fecal glucocorticoid metabolites (fGC) in free-ranging meerkats (*Suricata suricatta*) over 11 years and documented changes in maternal fGC during gestation and offspring fGC from emergence of the burrow through weaning. Unweaned offspring fGC levels increased with increasing maternal levels during gestation. Females breeding under high reproductive competition within their group (i.e. concurrence of gestations) produced sons with reduced pre-weaning fGC levels compared to daughters. In contrast, mothers increasing their reproductive effort (i.e. conceiving two litters in a row) during the season of poor food availability produced offspring with fGC levels one order of magnitude higher than offspring gestated during good food conditions or from non lactating mothers. Females preparing to conceive in the postpartum interval however showed late gestational reductions in fGC which could act to reduce the deleterious effect of high GCs during late stages of pregnancy on development of offspring stress axis. Our results imply that maternally-derived offspring GCs in meerkats could result from a sex and context dependent maternal strategy to modify offspring phenotype and fitness.

Introduction

In vertebrates, the hypothalamic–pituitary–adrenal (HPA) axis allows individuals to cope with stressors in their environment (Boonstra, 2004; Wingfield and Kitaysky, 2002) and regulates metabolic, immune, behavioral and reproductive functions (Dhabhar, 2002; Landys *et al.*, 2006; Magiakou *et al.*, 1997), which can be compromised by chronic stress (Sapolsky *et al.*, 2000; Selye, 1956). Development of the HPA stress axis is affected by early-life glucocorticoid hormones (GCs) during sensitive periods, from conception through the suckling stage, which can range from a few days to months depending on the species (Catalani *et al.*, 2011; Seckl, 1998). This programming period of the stress axis is crucial for the expression of physiological and behavioral responses to stressors challenging homeostasis throughout the animal's life (Pryce *et al.*, 2002; Richardson *et al.*, 2006). The early-life GCs environment can thereby modulate an individual's later-life GCs baseline and the activation and extent of its stress response (Romero and Reed, 2008; Sapolsky, 1994; Sheriff *et al.*, 2010b), as described by the organizational-activational hypothesis (Seckl, 1998). Elevated GCs during critical period of early development can also modify other offspring traits associated with growth, health, reproduction and survival (O'Regan *et al.*, 2010; O'Regan *et al.*, 2001; Seckl, 2004). Such programming can be adaptive (Boonstra, 2005; Love and Williams, 2008; Meylan *et al.*, 2012), especially when the environment is stable from early development through adulthood (Champagne *et al.*, 2009; Monclús *et al.*, 2011). However, a rise in adrenal activity above a critical threshold during early development can reduce reproductive potential and survival (Adkins-Regan, 2005; Koolhaas *et al.*, 2011; Sheriff *et al.*, 2010b). Despite decades of research on the programming effects of early-life stress, we know little on such effects in free-ranging populations.

In free-ranging mammals, most studies of the socio-ecological causes of stress that can impede female reproduction and survival have been conducted outside breeding periods to avoid the confounding influence of breeding on GCs levels (Brunton *et al.*, 2008). For instance, adult adrenal activity can be generally impeded by prolonged or chronic food stress (Gesquiere *et al.*, 2008; Lynn *et al.*, 2010), predation stress (Creel *et al.*, 2009; Eilam *et al.*, 1999; Sheriff *et*

al., 2009) and social stress (DeVries *et al.*, 2003; Young *et al.*, 2006). Furthermore, studies of socio-ecological stressors with a programming effect on offspring adrenal activity have mostly been conducted under controlled conditions (Braastad, 1998; Kaiser and Sachser, 2005; Michel *et al.*, 2011). However, a few recent studies have examined potential hormonal maternal effects of gestational stress under natural conditions (Monclús *et al.*, 2011; Ryan *et al.*, 2012; Sheriff *et al.*, 2010b). Only with knowledge on the different socio-ecological stressors affecting maternal stress during breeding (Chapter III) can we further clarify the extent to which specific aspects of the natural environment induce early-life stress capable of modulating the development of the HPA axis. Here, we investigated maternal effects of the socio-ecological gestational context on adrenal activity of unweaned offspring in free-ranging meerkats (*Suricata suricatta*).

The long-term study of meerkats in the South African Kalahari Desert (Barrette *et al.*, 2012, Chapter II; Clutton-Brock *et al.*, 1999a; Russell *et al.*, 2002) offer a unique opportunity to examine the role of maternal hormones during gestation on offspring adrenal functions with 11 years of data on life-history, body mass, behavioral, and fecal GCs (fGC). Meerkats are small (<1kg) social carnivores that live in groups of two to 40 individuals (mean±sd=16.7±8.6). Meerkats have a cooperative breeding system with shared offspring care, allowing females to reduce investment in offspring care (Clutton-Brock *et al.*, 2004). Meerkat groups may sustain breeding from more than one female at a time and reductions in parental duties allows postpartum estrus and females to breed up to four times in a year (Clutton-Brock *et al.*, 2001a; Russell *et al.*, 2003a). Stress associated with competition for reproduction among females of a group or high maternal reproductive effort (Barrette *et al.*, 2012, Chapter II; Chapter III) could affect the prenatal development of the HPA axis. For instance, female meerkats breeding at different times of the year show seasonal variation in adrenal activity (Chapter III), but no differences in litter size or survival (Doolan and Macdonald, 1997). Furthermore, maternal adrenal activity during gestation is affected by occurrence of concurrent gestations (Chapter III) and a female's reproductive rate (Barrette *et al.*, 2012, Chapter II).

To investigate how maternal stress during gestation affects adrenal activity in unweaned meerkats, we first aimed to test whether offspring fGC levels varied according to socio-ecological and maternal factors known to affect maternal fGC during gestation (Barrette *et al.* 2012, Chapter II; Chapter III). Gestational social stress was tested with the effect of competition among gestating females: higher gestational fGC levels were measured when concurrent gestations occurred in a group compared to that when a female was the single breeder (Chapter III). Gestational ecological stress included the combined effects of seasonality in food availability and maternal reproductive status at conception: elevated maternal fGC levels were measured when early gestation overlaps lactation of the previous litter, an effect exacerbated during the dry-cold season when food availability is lowest (Barrette *et al.* 2012, Chapter II; Chapter III). Additionally, we examined the effect of maternal reproductive status post-parturition: the increase in fGC excretion observed towards parturition in females not overlapping lactation with gestation of the next litter contrasted with the fGC decrease in the last two weeks of pregnancy in females conceiving postpartum (Barrette *et al.*, 2012, Chapter II). Our second aim was to test whether offspring fGC levels directly varied according to maternal fGC levels during gestation. Our third aim was to assess whether maternal stress during gestation affected adrenal activity in male and female offspring differently based on evidence from other vertebrate species for sex-specific sensitivity of the HPA axis development (Brummelte *et al.*, 2012; Love and Williams, 2008). We controlled for several potential confounding factors, including sampling biases, maternal and offspring attributes and the socio-ecology of the group from which samples were collected during the pre-weaning period.

Methods

We studied habituated meerkats from 1997 to 2008 at the Kuruman River Reserve (KRR) in South Africa (see Clutton-Brock *et al.*, 1999a; Russell *et al.*, 2002 for details of habitat and climate). Individuals from 15 groups were followed every one to five days to monitor their life-history and body mass, and to collect fecal samples. Groups were monitored daily when

females were due to give birth. Body mass was determined before foraging each morning by enticing meerkats onto digital balances (±1g) using crumbs of hard-boiled egg (e.g. Clutton-Brock *et al.*, 2002). Gestation lasts ~70 days (Doolan and Macdonald, 1997) and was identified about four weeks after conception by swelling of the abdomen and nipples and an increase in body mass. Parturition (±1 day) was identified by sudden female mass loss and the presence of babysitters at the burrow (Clutton-Brock *et al.*, 2001b). Pups emerged from the burrow ~ 20 days following birth and were weaned at ~ 40 days, when they started foraging with the group (Clutton-Brock *et al.*, 1998). All animal-handling protocols were approved by the University of Pretoria, the University of Sherbrooke and the Smithsonian Institution ethic committees.

Sample collection and hormonal analyses

We used 185 fecal samples collected from mothers and 74 fecal samples collected from their offspring. Fecal GC metabolites provide a pooled measure of adrenal steroids excreted over the past 24 to 48 hours (adult excretion rate; Young *et al.*, 2006), without the confounding stress of capture. Samples were collected whenever animals defecated, immediately placed on ice in thermos flasks and frozen at -20°C within five hours (mean ca. 2h). Samples were shipped frozen to the Smithsonian Conservation Biology Institute in Front Royal, VA, USA. Steroid metabolites were extracted and fGC concentrations measured from samples using validated methodologies (Monfort *et al.*, 1997). See Barrette *et al.* (2012, Chapter II) for details on steroid boiling extraction and corticosterone radioimmunoassay methods. Intra-assay coefficients of variation were <10% and inter-assay coefficients of variation for low- and high-dose internal controls were 8.4% and 9.4% for 17 assays performed form December 2006 to April 2009.

Statistical analyses

Firstly, we tested maternal effects of the socio-ecological context during gestation on fGC levels of unweaned pups, using 74 fecal samples collected from 29 daughters and 27 sons (mean±sd fecal samples per pup=1.4±0.8) born to 26 mothers in 13 groups over 41 gestations. Secondly, we tested maternal effect of gestational fGC on a restricted dataset with fGC

measures available from both mothers during gestation (185 fecal samples collected from 20 mothers in 12 groups over 27 gestations; maternal fGC levels were averaged over each gestation: mean±sd fecal samples per gestation=3.2±2.3) and offspring during suckling (52 fecal samples collected from 19 daughters and 18 sons). To quantify the hormonal basis of gestational maternal effects, we compared results from the two analyses, conducted on the same restricted data set, using the Akaike information criterion (AIC). All fGC data were normalized using a natural logarithm transformation and fitted to a normal error structure in a General Linear Mixed Model (GLMM). To account for the hierarchical distribution of variation in fGCs, we fitted four random terms to the models: (i) samples within litters; (ii) litters within mothers; (iii) mothers within groups; and (iv) groups. Repeated measures were available for few pups, therefore pup identity was not included as a random term. Random terms were nested, with samples at the lowest hierarchical level. Higher levels were retained in final models only if they encompassed significant fGC variation after assessing the importance of fixed effects.

Fixed terms of interest included maternal effects outlined below and offspring sex (male, female) and were considered after controlling for the random terms retained as well as a number of potentially confounding offspring and maternal fixed effects. Fixed effects with a potentially confounding influence included: storage duration, collection time-of-day, maternal attributes at conception (age, mass, dominance status), offspring attributes (age and mass) and maternal and socio-ecological factors prevailing during the pre-weaning period. Because steroid metabolites can degrade over time (Schwartz and Monfort, 2008), we fitted as a fixed co-variate the number of days between sample collection and assay. To control for circadian variation in fGC, sample collection time was coded as morning (am: 06:00–12:00) and evening (pm: 15:00–20:00) and fitted as a two-level fixed co-factor in all analyses. Individual age (± 1d), was determined from birth dates. Body mass (in grams) was averaged ± 5 days from conception for mothers or from sample collection for offspring. Maternal dominance status was coded as dominant or subordinate with only one female per group being dominant (Kutsukake and Clutton-Brock, 2006). The potentially confounding maternal and socio-ecological factors prevailing at the time of offspring sampling included: average maternal fGC

levels during lactation (on a restricted data set with fGC measures for both lactating mothers and suckling pups); group size (i.e. ≥ 6 months old); litter size; season (wet-hot or dry-cold). Maternal fixed effects during gestation included: occurrence of concurrent gestations within the group (yes or no); season (wet-hot or dry-cold); reproductive status at conception (lactating or not); reproductive status at parturition, (postpartum estrus or not); and average gestational fGC levels.

Statistical analyses were conducted with R (R Development Core Team, 2008). Normality was determined using quantile plots, frequency histograms and Shapiro-Wilk normality tests. The explanatory power of models and individual fixed terms were assessed by the proportion of variance explained, calculated by the difference in residual variance with only random terms and with both random and fixed explanatory terms. Fixed terms and their two-way interactions were retained when they influenced the explanatory power of the model. Type III sum of squares (marginal error structure in R) are presented to control for co-variation among fixed terms (Pinheiro and Bates, 2000). All statistical tests were two-tailed.

Results

Maternal effects of the socio-ecological gestational context

Offspring pre-weaning adrenal activity was associated with reproductive competition among gestating females and maternal reproductive rate, after accounting for offspring age and sex and collection time and season (Table 1). Overall, offspring attributes and the pre-weaning context explained 7% of pup fGC variance, and the gestational context, 34%. The predicted mean levels of fGC decreased in female pups as they aged, from 414 ng/g of feces at emergence to 61 ng/g at weaning, whereas no age effect was observed for males, with predicted mean levels of 61 ng/g throughout the pre-weaning period (Table 1; Fig. 1). Pre-weaning fGC levels were not associated to maternal age, mass and dominance status during gestation, or to pup mass and group and litter sizes during the pre-weaning period (Table 1).

Table 1. Maternal gestational and offspring pre-weaning factors associated with fGC in unweaned meerkat pups.

General Linear Mixed Model	*df*	Effect ± SE	F	p-value	see
Constant [a]	1, 30	4.14 ± 1.29	10.34	0.003	
Sampling confounding factors					
Storage time	1, 29	7.3E-5 ± 1.4E-4	0.27	0.61	
Collection time *(am>pm)*	1, 30	-0.42 ± 0.20	4.58	0.04	
Offspring attributes					
Sex (*m < f*)	1, 30	4.55 ± 1.68	7.28	0.01	
Age *(d)*	1, 30	0.003 ± 0.04	0.006	0.93	
Mass (*g*)	1, 29	0.002 ± 0.003	0.55	0.45	
Sex * Age	1, 30	-0.11 ± 0.05	5.26	0.03	Fig. 1
Pre-weaning context					
Group size *(>6 months old)*	1, 29	-0.004 ± 0.02	0.05	0.83	
Litter size	1, 29	-0.04 ± 0.09	0.16	0.69	
Season *(wet-hot < dry-cold)* [b]	1, 29	0.35 ± 0.20	3.21	0.08	
Maternal attributes					
Age at conception (*d*)	1, 22	-1E-4 ± 1E-4	2.22	0.15	
Mass at conception (*g*)	1, 22	-0.001 ± 0.001	1.21	0.28	
Dominance status at conception (*dom < sub*)	1, 22	0.39 ± 0.26	2.29	0.14	
Maternal gestational context					
Concurent gestations (*yes < no*)	1, 23	0.14 ± 0.26	8.58	0.008	
Season *(wet-hot > dry-cold)*	1, 23	-0.25 ± 0.29	0.74	0.40	
Reproductive at conception (*no > yes*)	1, 23	-0.44 ± 0.27	2.78	0.11	
Reproductive post-parturition (*no > yes*) [c]	1, 22	-0.42 ± 0.23	3.35	0.08	Fig. 4
Season * Reproductive at conception	1, 23	1.30 ± 0.38	12.00	0.004	Fig. 3
Maternal * Offspring effects					
Concurent gestations * Sex	1, 30	-0.75 ± 0.34	4.78	0.03	Fig. 2

Final GLMM explained 41% of the overall variance in pup's fGC. [a] Value presented for the constant is calculated in reference to samples collected in the morning (*am*), from *male* offspring, during the *wet-hot* season, and for offspring born from *dominant* mothers pregnant when concurrent gestations occured (*yes*), during the *wet-hot* season, while being neither reproductive at conception (*no*) nor postpartum (*no*). [b] Pre-weaning season is significant before accounting for gestational factors: $F_{1,29}$=7.19, p=0.01, effect±se=0.58±0.22. [c] Maternal reproductive status postpartum is significant before accounting for two-way interactions in a sequential ANOVA: $F_{1,22}$=5.49, p=0.03, effect±se=-0.42 ± 0.23. Data were collected from 1997 to 2008 at the Kuruman River Reserve, South Africa.

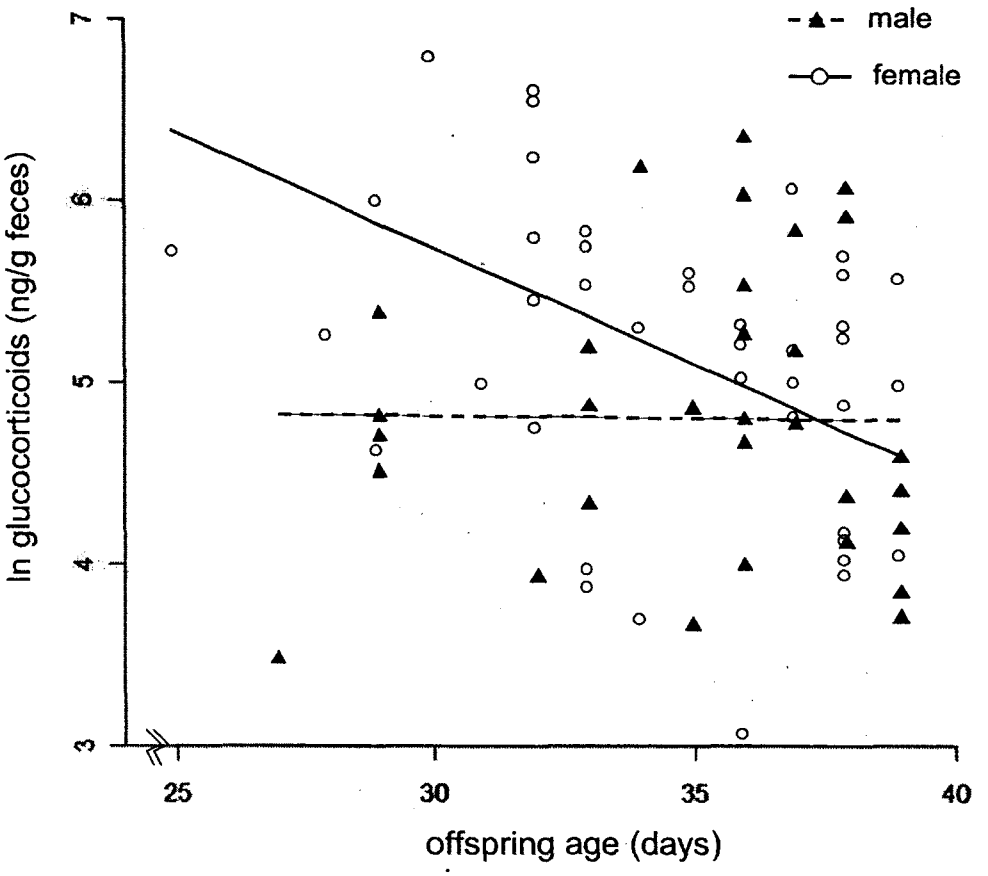

Figure 1. Pre-weaning fGC is associated with sex and age in meerkat pups. Circles and triangles present female and male raw fGC data. Regression lines are predictions from analyses on Table 1 for female (solid line) and male (broken line) pups.

Adrenal activity in unweaned meerkats revealed a sex dependent maternal effect of concurrent gestations. The concurrence of gestations within a group was associated with reduced pre-weaning fGC in sons but not in daughters (Table 1; Fig. 2). In contrast, male and female offspring born when no concurrent gestation occurred did not vary in fGC levels, which levels were similar to those of daughters born when concurrent gestations occurred.

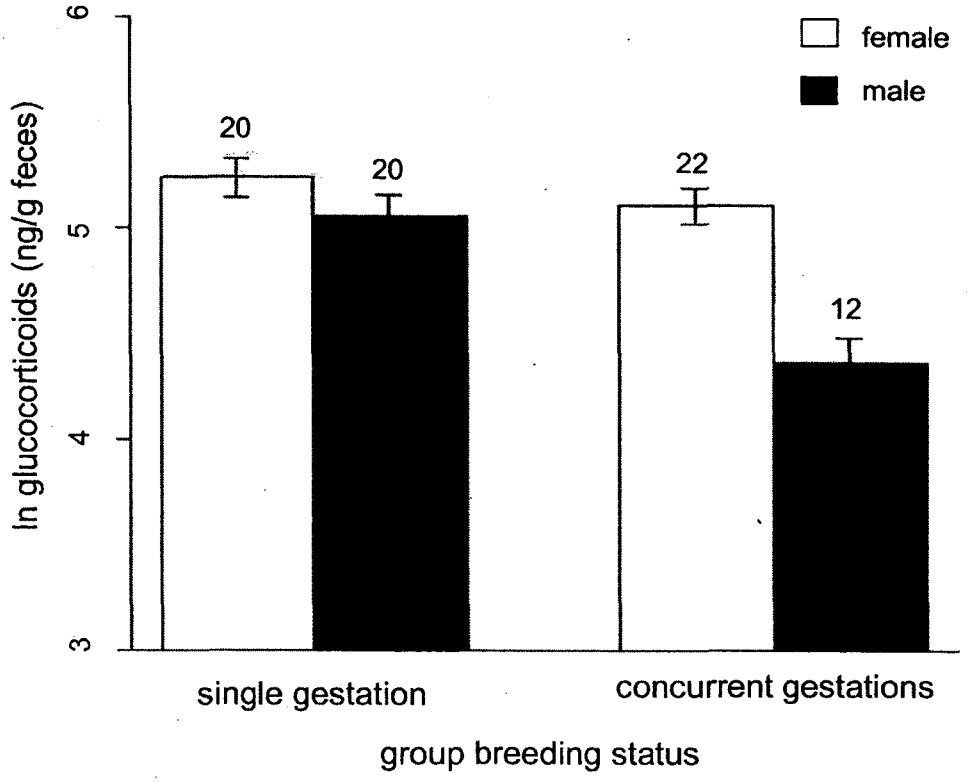

Figure 2. Sex dependent association of fGC levels of unweaned meerkat and occurrence of single vs. concurrent gestations in a social group: significant difference among males (GLMM on restricted data set: p=0.02), not among females (GLMM on restricted data set: p=0.36). Means and standard error bars are predictions from the analysis on Table 1.

Offspring pre-weaning adrenal activity was associated with the seasonality of maternal reproductive status at conception, but not with maternal reproductive status at parturition. Unweaned pups showed elevated fGC levels when conceived during the dry-cold season by a lactating mother, compared to pups conceived either during the wet-hot season or by non-lactating mothers (Table 1; Fig. 3). In contrast, when the seasonal effect of maternal reproductive status at conception was ignored, unweaned pups showed reduced fGC levels when born from conceptive mothers experiencing postpartum estrus and initiating a new gestation during lactation of the current litter, compared to pups born from non-conceptive

mothers (Table 1; Fig. 4). Offspring fGC was not associated with pre-weaning season after accounting for the effect of the gestational season (Table 1).

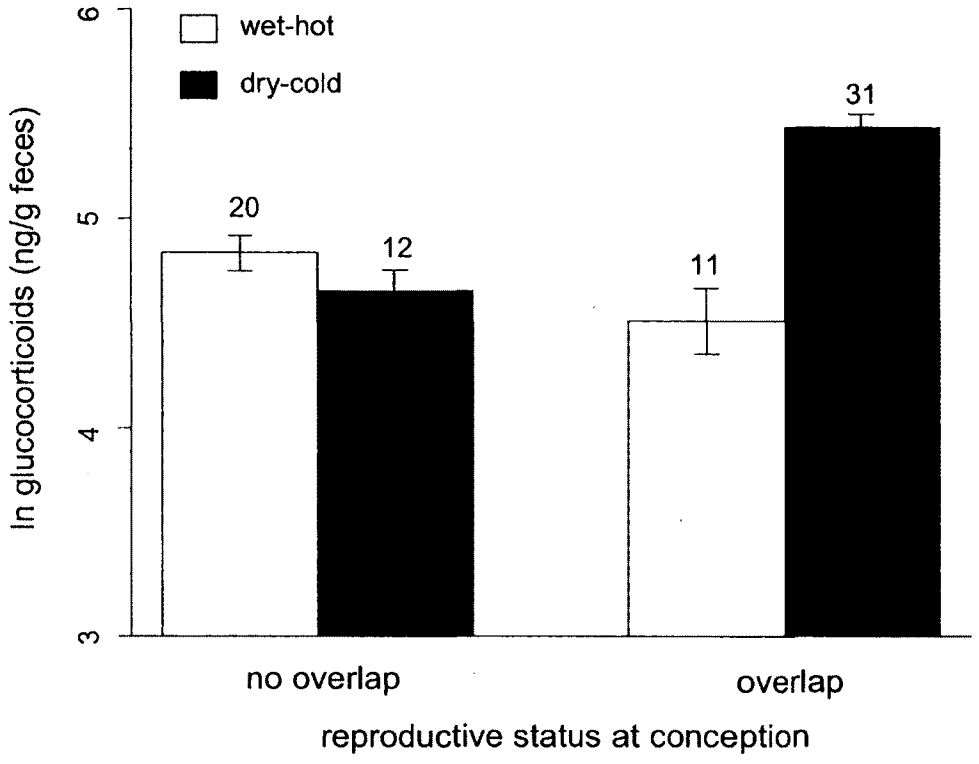

Figure 3. Meerkat offspring fGC levels before weaning is associated with the seasonality of maternal reproductive status at conception: significant difference during the dry-cold season (GLMM on restricted data set: p=0.01), not the wet-hot season (GLMM on restricted data set: p=0.30). Means and standard error bars are predictions from the analysis on Table 1.

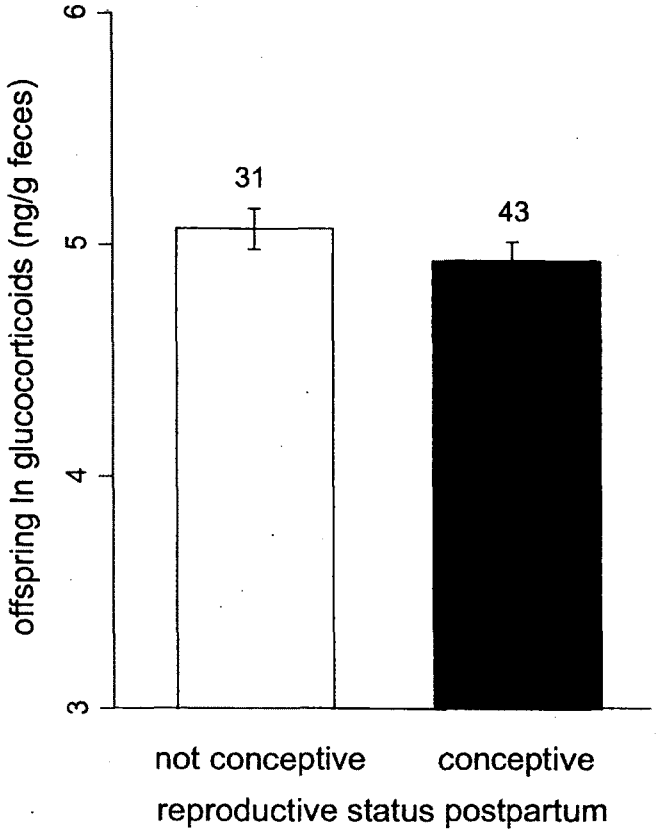

Figure 4. Meerkat offspring fGC levels before weaning as a function of maternal reproductive status at parturition (see GLMM effect in Table 1). Means and standard error bars are predictions from the analysis on Table 1.

Maternal effect of gestational fecal glucocorticoids

Maternal fGC levels during gestation affected unweaned offspring fGC levels (GLMM on pre-weaning fGC, controlling for significant effects of group and litter identity, pup sex and age, gestational season and maternal reproductive status at conception: $F_{1,8}$=11.40, p=0.01), an association modulated by concurrence of gestations (significant interaction: $F_{1,8}$=6.46, p=0.03; Fig. 5). Thereby, a steeper relation between maternal and offspring fGC levels was observed in the absence of concurrent gestation within the group (effect±se=1.41±0.42; Fig. 5)

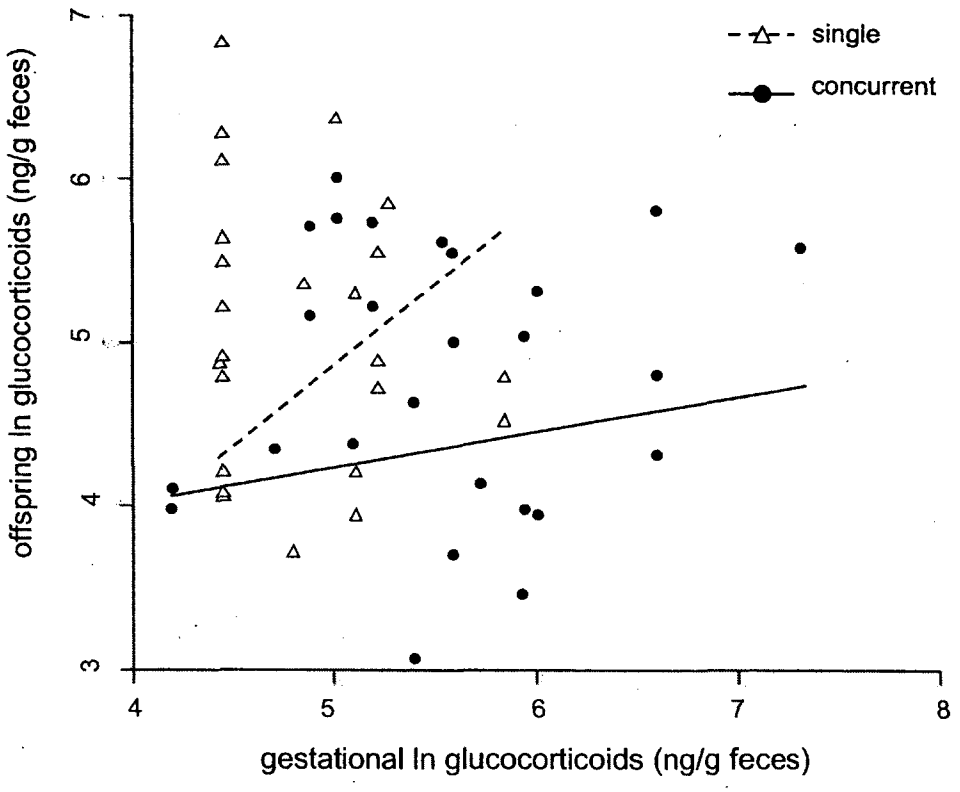

Figure 5. Pre-weaning offspring fGC as a function of maternal gestational fGC when concurrent gestations occurred (circles) or not (triangles) in the social group. Regression lines are predictions from GLMM analysis for offspring conceived when concurrent gestations occurred (solid line) or not (broken line).

compared to that when concurrent gestations occurred (effect±se=1.20±0.47; Fig. 5). After accounting for gestational fGC, both sexes showed reduced fGC levels when concurrent gestations occurred (yes<no: $F_{1,8}$=7.19, p=0.02, effect±se=0.73±0.27; non-significant interaction between pup sex and concurrent gestations: $F_{1,22}$=1.18, p=0.29, effect±se=-0.58±0.53). The effect of maternal reproductive status postpartum was also non-significant after accounting for gestational fGC ($F_{1,7}$=0.001, p=0.98, effect±se=-0.01±0.39). Finally, gestational fGC affected offspring fGC variation only after accounting for the effect of the seasonal timing of reproductive status at conception. Overall, the analysis testing both

the effect of gestational context and gestational fGC explained 52% of pup fGC variance, more than the gestational context alone (ΔAIC=3.8; p=0.01).

Discussion

Our study suggests that unweaned meerkat adrenal activity is affected by gestational stress of reproductive competition among mothers, maternal reproductive rate and seasonal food availability. Our results provide new insights on two alternative explanations of maternally-derived offspring GCs. First, maternal effect of reproductive competition on offspring fGC suggests a sex and context dependent maternal strategy to increase maternal fitness by altering offspring stress response. Second, maternal reproductive effort during season of poor food availability reveals potential deleterious effects of gestational stress underling hormonally-derived mother-offspring conflict.

Our results suggest that concurrent gestations reduce fGC in sons and not in daughters, whereas for both sexes maternal fGC during gestation has a stronger positive effect on pup fGC when no concurrent gestation occurs. Barrette *et al.* (2012, Chapter II) showed that concurrent gestations increase maternal fGC excretions. Here we report that the relation between maternal and offspring fGC levels was dampened by concurrent gestations, regardless of offspring sex. Furthermore, the sex dependent effect of concurrent gestations was not significant after accounting for the effect of gestational fGC levels, suggesting that the downstream effect of concurrent gestations on offspring adrenal activity could be linked to the significant maternal effect of gestational GCs. That sons show reduction in fGC levels in association with social gestational stress, but not daughters, suggest a potential sex and context dependant sensitivity of offspring HPA axis during prenatal development (Brummelte *et al.*, 2012; Love and Williams, 2008), and carrying at least into the suckling stage. Furthermore, mothers could capitalize on such effects of gestational stress in shaping their offspring physiological and behavioral response to social stress (Meylan *et al.*, 2012). In meerkats,

males disperse at adulthood, whereas females remain in their natal group, unless they are evicted when competing for reproductive opportunities (Clutton-Brock *et al.*, 1998). Meerkat offspring thus differ in the level of intrasexual competition for reproduction within their group. The fitness incentive to gain mass and thereby become more competitive breeders has thus been suggested to be greater among philopatric females than dispersing males (Hodge *et al.*, 2008; Russell *et al.*, 2004). Whether males suffer fitness costs of high cohort competition before independence would help elucidate the differential role of GCs at different life-history stages for sons and daughters. It now remains to be assessed whether and how dispersion is associated to reactivity of the stress axis in meerkats, and whether daughters developing in lower gestational GCs in groups with high levels of reproductive competition are more likely to disperse following eviction.

Such maternal effects would have important implications specific to daughters, sons and mothers. First, higher gestational GCs could lead to the production of more reactive female helpers (> six months old) for pup care and the production of female breeders (> eight months old) better prepared to physiologically cope with increased competition for reproductive opportunities. Meerkat groups can grow to 40 individuals before splitting, suggesting that the gestational social environment predicts the environment in later life. Furthermore, that the social environment could be more stressful for daughters than for their mother, if group size and number of reproductive competitors increase over time, would promote the evolution of an adaptive maternal strategy to produce daughters with an efficient stress response, e.g. both quick response to stressors and return to normality (Sapolsky *et al.*, 2000), thereby reducing the deleterious effects of chronic stress. For instance, such a strategy could serve to produce more helpful daughters to care for future generations. In contrast, sons show reduced fGC levels when concurrent gestations occur, perhaps indicating a maternal strategy to reduce responsiveness to pup care in offspring of the dispersing sex in groups increasing in size and in number of adult females providing the most pup care (Clutton-Brock *et al.*, 2002). Supporting this hypothesis, Monclùs *et al.* (2011) suggested an adaptive role of gestational GCs on the stress response and dispersal rate of sons in association with predation pressure and maternal age in yellow-bellied marmots (*Marmota flaviventris*). Young marmot mothers,

with lower body condition and social rank than old ones, are more likely to produce dispersing sons than philopatric daughters under low gestational stress, whereas old mothers show the opposite pattern suggesting they can favor dispersal of their sons when local survival probability is low. It remains to be addressed whether a similar hormonal mechanism would promote the production of dispersing sons by subordinate mothers when reproductive competition among females is high and reproductive success of daughters low, and the production of philopatric daughters by dominant mothers building up group size and favoring their own fitness over that of their offspring.

In our study, the sex independent weaker relation between maternal gestational and offspring pre-weaning fGC levels when concurrent gestations occur argues against a simple by-product of gestational stress. To disentangle a context dependant maternal strategy from non adaptive mechanistic explanations resulting from socio-ecological stress, we need to assess whether sons and daughters obtain fitness advantages or deleterious effects on their health and ability to cope with stress as adults. We also need to assess what level of gestational GCs can reduce offspring fitness. Finally, the high within individual repeatability of helper food provisioning to young in meerkats (i.e. ~50%: English *et al.*, 2010) suggests the potential for gestational GCs in priming helper responsiveness to pup begging, which would be adaptive for mothers and sons, although not for daughters which are indeed more responsive to increased begging rate than males (English *et al.*, 2008).

Our study suggests that mother-offspring conflict could be associated with gestational GCs (Del Giudice, 2012; Fowden and Moore, 2012), with mothers increasing their reproductive success by overlapping several breeding events despite a detrimental effect to the HPA axis and potentially the health of their current offspring (Seckl and Holmes, 2007). During the season of poor food availability, female meerkat with overlapping breeding events produced offspring with fGC levels ~ one order of magnitude higher than those of offspring born to females breeding at a lower rate or when food availability is greater. In meerkats, gestation lasts just over two months, in a habitat with two six-month seasons. To increase annual reproductive rate, dominant mothers breed partly during poor seasonal food conditions,

leading to increased offspring adrenal activity. In contrast, subordinate females typically breed during poor seasonal conditions to avoid infanticide when the dominant female is not reproductively active, which did not incur an elevation in offspring adrenal activity. Because dominant females do not have complete control over subordinate's reproductive timing, dominants might not favour their offspring fitness over their own reproductive success by employing reproductive strategy to reduce GCs levels during gestation. Whether and at what levels gestational GCs affect offspring fitness would help elucidate the potential fitness costs and benefits of such strategy for dominant females. In contrast, subordinate females could make the best of a bad job by strategically time their reproduction when the dominant is not breeding and by not overlapping breeding events during the season of lower food availability, and in doing so avoid the potential deleterious effects of high gestational GCs on their offspring phenotypes. Furthermore, that maternal effect of reproductive status postpartum was not significant after accounting for the seasonal effect of reproductive status at conception suggests that offspring adrenal activity is much more dependent on the mother's previous reproduction than on her next reproduction. We now need to assess whether offspring conceived during lactation of the previous litter when food availability is low show reduced fitness in association with reduced immune function and increased disease susceptibility.

In mammals, the maternal effect of under nutrition on offspring health (Dallman *et al.*, 1993) is linked to the programming effect of maternal glucocorticoids on the offspring stress axis (Almond *et al.*, 2012; Cottrell *et al.*, 2012). Our study shows that the effect of the gestational season was more important than that of the pre-weaning season in explaining pup fGC excretion before weaning. Additionally, maternal fGC during lactation did not associate with offspring fGC before weaning (GLMM on pre-weaning fGC levels in function of mean lactation levels, controlling for maternal and offspring identity, n=40 samples from 26 pups born to 15 mothers: $F_{1,10}$=0.46, p=0.51, effect±se=-0.15±0.22), although the diffusion of maternal GCs into milk is known in other species (Brummelte *et al.*, 2010). These results suggest that the adrenal activity of unweaned pups is dependent upon the gestational season previously shown to also affect fGC in pregnant meerkats (Chapter III). Furthermore, our study provides some support for the hypothesis that high maternal reproductive effort,

associated with lactation of the previous litter during early gestation of the current litter when food availability is low, affects development of offspring stress axis in the womb. The effect of seasonal timing of reproductive overlap at conception on offspring fGC remained significant after accounting for maternal fGC during gestation, which does not support the hypothesis that the downstream effect of maternal reproductive status at conception is hormonally derived. However, we provide some evidence for mothers preparing to conceive postpartum attempting to reduce the deleterious downstream effect of high maternal GCs around conception of the next litter. Barrette *et al.* (2012, Chapter II) showed that maternal fGC levels declined both late in pregnancy and during lactation in mothers experiencing postpartum conception. Furthermore, our study presents evidence that mothers preparing to conceive postpartum produce offspring with lower fGC levels pre-weaning compared to non conceptive mothers. That maternal effect of reproductive status postpartum was not significant after accounting for gestational fGC suggests a hormonally-derived mechanism. However, hormones and nutrition are both likely to affect offspring adrenal functions, mass and health from birth to adulthood (Almond *et al.*, 2012; Cottrell *et al.*, 2012; Edwards *et al.*, 2001; Gokulakrishnan *et al.*, 2012; Whorwood *et al.*, 2001). It thus remains to be tested whether and to what extent the maternal effect of seasonality of reproductive overlap act via gestational GCs compared to the effect of nutritional restrictions associated with lactation of the previous litter during early gestation of the current litter when food availability is low. Furthermore, reductions in GCs during gestation likely reduce glucose mobilization from the mother and transfer to the embryo, and low prenatal glucose is associated with developmental dysfunctions in offspring (Fowden and Forhead, 2007, 2011). The association between gestational GCs and prenatal glucose transfer to offspring would thus need to be considered in further studies of prenatal nutritional stress in meerkats.

Our study raises the question of whether seasonal maternal effects of gestational GCs is an adaptation to produce seasonal phenotypes better geared to deal with seasonal conditions when learning foraging skills and competing for helper's food provisioning (from three to six months old), or at the time of foraging independence (over six months old). For instance, those pups gestated during the dry-cold season would become independent six months later during

the next dry-cold season. Thereby, it could be strategic for female meerkats to time overlapping gestations to favor offspring growth rate and body condition when gestational and post-weaning seasons match. That female offspring show higher fGC levels around 25 days and then decrease to similar levels as males at weaning suggest an association between GCs and suckling in females. For instance, with higher GCs, females could be more competitive at suckling then males, and then showing a decrease in adrenal activity as weaning progress. The role of adrenal activity in the competitive ability among suckling, begging and independent meerkats and how gestational stress can affect such relations would therefore deserve further attention.

In meerkats, body mass is an important predictor of reproductive success especially for females where breeding is dependant on mass at emergence and during reproductive competition (Hodge *et al.*, 2008; Russell *et al.*, 2004). Furthermore, decades of research on hormonal maternal effects have demonstrated the impact of prenatal stress on offspring mass and growth (Seckl, 2004). Our results on the association between maternal stress during gestation and offspring stress axis before weaning suggests a potential impact on offspring mass at emergence. To investigate the role of gestational GCs on a daughter's reproductive success in meerkats, we now need to assess and integrate the effects of prenatal stress on the offspring stress axis, mass, growth rate and competitive ability at different life-history stages. For instance, the maternal reproductive strategy of overlapping breeding events, typical of dominant females in meerkats, could be in turn maternally-derived. It thus remains to be assessed whether high gestational GCs associated with high maternal reproductive rate produce daughters with a stress axis programmed to cope with the adrenal demands of overlapping breeding events in adulthood.

In conclusion, our study suggests that timing of reproduction could be strategic, rather than solely a consequence of current food availability and hormonal priming of reproduction, relative to the amount of care a mother needs to provide during the postpartum interval, which in turn depend on dominance status and group size and composition (Clutton-Brock *et al.*, 2002). Whether and to what extent these gestational effects act via maternal GCs remains

entangle with other components of gestation and lactation not measured in our study and likely to impact on offspring fGC. Whether hormonal maternal effects identified here carry through pup foraging independence and adult life also remains to be assessed. Our study suggests that maternal effect of gestational stress have the potential to produce variability in offspring phenotypes leading to individual variation in fitness. In support of this hypothesis, maternal stress increased offspring fGC and stress responsiveness in snowshoe hare (*Lepus americanus*) when predation density was high at birth, leading to transgenerational effect of gestational adrenal activity modulating female reproductive success (Sheriff *et al.*, 2009, 2010b).

Acknowledgments

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CONCLUSION

Atteinte des objectifs

Cette thèse visait à étudier les liens entre les fGC maternels, le biais de la reproduction, les conditions socio-écologiques et les fGC de la progéniture, en validant préalablement l'utilisation d'échantillons fécaux permettant le suivi longitudinal d'hormones sur 11 ans. Premièrement, nous avons démontré l'utilité des métabolites stéroïdiens fécaux dans l'étude longitudinale du stress et de la reproduction chez le suricate en soulignant l'importance de contrôler pour le temps d'entreposage et en recommandant la lyophilisation précoce plutôt que l'entreposage d'échantillons frais pour la conservation longue durée à -20°C. Deuxièmement, l'étude longitudinale de la variation intra et interindividuelle en fGC révèle comment les stratégies de reproduction des suricates femelles, en lien avec le contexte socio-écologique lors de la gestation, affectent la physiologie du stress des mères gestante et celle de leur progéniture avant le sevrage. Le taux de reproduction des suricates femelles est associé à des changements en fGC lors de la reproduction, des changements non observés auparavant en milieu naturel ou en captivité. Toutefois, la dominance n'est pas associée aux variations interindividuelles en fGC. De plus, le contexte socio-écologique affecte les niveaux en fGC lors de la gestation et des concentrations plus élevées sont mesurées selon le moment de la reproduction chez les femelles cherchant à maximiser leur succès. En effet, l'enchaînement d'événements de reproduction lors de la saison de mauvaise disponibilité en nourriture et la reproduction simultanée de plusieurs femelles du même groupe lors de la saison plus favorable entraînent un stress gestationnel. Les fGC de la progéniture avant le sevrage sont associés aux fGC maternels lors de la gestation. L'effet maternel hormonal lié au niveau de compétition pour la reproduction varie selon le sexe: les filles ont des niveaux en fGC plus élevés que les fils en condition de compétition maternelle lors de la gestation. L'effet maternel associé à un effort reproducteur accru en période de stress alimentaires entraîne aussi des niveaux en fGC plus élevés d'un ordre de magnitude, indépendamment du sexe de la progéniture, en comparaison aux jeunes produits lors de gestations non chevauchées ou lors de bonnes conditions alimentaires.

Originalité et importance des contributions

Ma thèse est la première étude permettant d'identifier des effets maternels hormonaux liés aux stress alimentaire et social lors de la gestation en milieu naturel. Auparavant, les mécanismes et effets du stress prénatal n'avaient pas été étudiés en milieu naturel faute d'une méthodologie non invasive permettant de mesurer le stress de populations suivies sur plusieurs générations. L'étude des effets de l'entreposage sur les stéroïdes fécaux a des implications importantes pour le suivi longitudinal d'espèces sauvages. En effet, la congélation longue durée d'échantillons fécaux frais est la méthode d'entreposage la plus répandue alors qu'une lacune persiste dans la littérature sur l'effet des conditions d'entreposage sur les mesures obtenues pour différentes hormones et espèces étudiées. L'étude des variations intra et interindividuelles en fGC suggère des rôles mécanistiques et fonctionnels pour le succès reproducteur et les stratégies de reproduction des mères, qui pourraient à leur tour affecter la valeur adaptative de la progéniture en fonction du contexte socio-écologique gestationnel. Premièrement, l'effet du taux de reproduction sur les fGC explique les niveaux plus élevés en GCs chez les dominantes d'espèces à reproduction coopérative, de par leur taux de reproduction plus élevé que ceux des subordonnées (Creel, 2001). L'absence d'un effet de la dominance suggère ainsi que le faible succès reproducteur des subordonnées n'est pas associé au « stress » de la reproduction chez le suricate. De plus, les fortes concentrations en fGC observées chez des femelles menant à terme leur gestation remettent en question la suppression forcée du système reproducteur des subordonnées. Deuxièmement, les effets maternels hormonaux de la compétition pour la reproduction suggèrent une stratégie maternelle dépendante du contexte. L'expulsion de compétitrices pourrait réduire le stress social au sein du groupe et ainsi favoriser la conception postpartum d'une nouvelle portée. L'effet de la compétition gestationnelle sur le développement de l'axe du stress de la portée en cours suggère un potentiel à produire des filles mieux équipées physiologiquement à se reproduire sous forte compétition. Troisièmement, les effets maternels hormonaux associés à la reproduction lors de stress alimentaire suggèrent un conflit entre le succès reproducteur des mères et la santé de leur progéniture. L'étude ne permet toutefois pas de démêler les effets du GCs des autres effets pouvant affecter l'axe du stress de la progéniture, telle la malnutrition.

Directions futures

L'étude amène plusieurs questions importantes sur le rôle des GCs dans le succès reproducteur des suricates femelles et l'effet du stress gestationnel sur la progéniture. Premièrement, l'effet du taux de reproduction sur les fGC et l'absence d'un effet de la dominance invitent à tester la généralité de nos résultats avant et pendant la reproduction chez d'autres espèces sociales. De plus, il serait pertinent de détailler le rôle inhibiteur des GCs sur la reproduction chez le suricate, d'évaluer si les femelles varient dans les niveaux en GCs qu'elles peuvent tolérer et si ces niveaux diminuent leur potentiel reproducteur futur. Deuxièmement, il demeure à démontrer si les effets maternels observés avant le sevrage perdurent au courant de la vie de la progéniture, si d'autres effets maternels du stress gestationnel s'opèrent sur le développement physique et comportemental de la progéniture, et si les femelles varient activement le taux et le moment de leur reproduction afin de modifier le phénotype de leur progéniture. Par exemple, un effet du stress gestationnel sur la masse, la croissance, la compétitivité à différents stades de vie et la prédisposition physiologique à enchaîner des reproductions auraient des implications importantes sur le biais de la reproduction chez les suricates femelles et dans le potentiel à devenir et rester dominante (Clutton-Brock *et al.*, 2004; Hodge *et al.*, 2008; Sharp et Clutton-Brock, 2011). De plus, afin d'expliquer l'importante variation individuelle dans la contribution à la coopération (English *et al.*, 2010), il serait pertinent d'évaluer le lien entre le stress gestationnel et les stratégies d'histoire de vie de la progéniture, telle la philopatrie typique des filles ayant une réponse accrue au quémandage des jeunes (Clutton-Brock *et al.*, 2002; English *et al.*, 2008) et la dispersion des fils (Monclús *et al.*, 2011; Young *et al.*, 2005; Young et Monfort, 2009). Troisièmement, afin de départager les rôles adaptatifs de stratégies maternelles dépendantes du contexte socio-écologique et du sexe de la progéniture, des effets collatéraux des GCs, il est nécessaire d'évaluer si les fils et les filles diffèrent dans la valeur adaptative des effets maternels identifiés et dans les coûts associés en terme de survie. Par exemple, il serait intéressant d'évaluer si le stress gestationnel influence la réponse au stress (Champagne *et al.*, 2009; Sheriff *et al.*, 2010b), la réponse immunitaire (Boughton *et al.*, 2011) et la susceptibilité à des maladies chez la progéniture (Drewe, 2010).

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