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The physiology of cooperative breeding in a rare social canid; sex, suppression and pseudopregnancy in female Ethiopian wolves ☆

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HIGHLIGHTS

- We studied the reproductive physiology of cooperatively breeding wild female Ethiopian wolves.
- Fecal samples were collected and analyzed for progesterone, estradiol and glucocorticoids.
- Dominant females had increased E2 concentrations during the mating season, but subordinates did not.
- Both dominant and subordinates females had increased P4 levels during the dominant's pregnancy.
- Glucocorticoid levels did not differ significantly between dominant and subordinate females.

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ABSTRACT

Ethiopian wolves, *Canis simensis*, differ from other cooperatively breeding canids in that they combine intense sociality with solitary foraging, making them a suitable species in which to study the physiology of cooperative breeding. The reproductive physiology of twenty wild female Ethiopian wolves (eleven dominant and nine subordinate) in Ethiopia's Bale Mountains National Park was studied non-invasively through the extraction and assaying of estradiol, progesterone and glucocorticoids in collected fecal samples using enzyme and radioimmunoassays. All dominant females showed increased estradiol concentrations and/or mating behavior during the annual mating season. In contrast, none of the subordinate females showed increased estradiol concentrations or mating behavior during the mating season. However, two subordinate females came into estrus outside of the mating season. Both dominant and subordinate females had higher average progesterone concentrations during the dominant female's pregnancy than at other times of the year, and two subordinate females allosuckled the dominant female's pups. No statistically significant differences in glucocorticoid concentrations were found between dominant and subordinate females. These results suggest that subordinate females are reproductively suppressed during the annual mating season, but may ovulate outside of the mating season and become pseudopregnant. No evidence was found to suggest that reproductive suppression in subordinate females was regulated through aggressive behaviors, and no relationship was found between fecal glucocorticoids and dominance status.

1. Introduction

Cooperative breeding can be defined as a breeding system in which more than a pair of individuals exhibit helping behavior towards young from a single litter [1]. In most cooperative breeders reproductive rates are lower for subordinates than for dominants, and reproduction in subordinates may be completely suppressed [2]. Cooperative breeding has been described in a range of taxa including fish [3], birds [4], and mammals [5,6].

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Cooperative breeding requires the reproductive suppression of subordinates, which may occur through hormonal mechanisms. One hormonal mechanism involves increased glucocorticoids, which are released in response to adverse situations, and as such are seen as indicators of stress [7]. Chronic elevation of glucocorticoids may cause reproductive failure and disease [8,9].

Early studies on dominance rank and stress in captive rodents and primates found that, following an aggressive interaction, both winners and losers had increased glucocorticoid concentrations, but the stress response was higher in losers, leading to the 'stress of subordination' theory (for a review see [2]). However, dominants in several cooperatively breeding species including dwarf mongoose, *Helogale parvula* [10], African wild dogs, *Lycaon pictus* [6] and gray wolves, *Canis lupus* [11] were found to have higher average glucocorticoid concentrations than subordinates. It has now been suggested that aggression is stressful to both the instigator and the recipient [10], and that, in the wild, subordinates may be able to avoid aggression, but dominants may have to act aggressively more often to maintain their status [10], which may be stressful.

Reproductive suppression can also be achieved through behavioral mechanisms. For example, dominant gray wolves have been observed aggressively preventing subordinates from mating [12,13] and reproductive suppression may be mediated post-partum through infanticide [14].

Ethiopian wolves are the world's rarest canid [15] and a greater understanding of their reproductive physiology would provide the basis for future reproductive management initiatives for the conservation of this species [16]. Although there are no Ethiopian wolves in captivity [17], due to advances in fecal hormone assays it is possible to study the reproductive physiology of wild populations non-invasively through the collection of feces [18].

Ethiopian wolves are medium-sized, territorial canids, endemic to the Ethiopian highlands [17,19]. They live in family packs of up to 20 individuals [20], and breed cooperatively. Ethiopian wolves breed once a year, and reproduction is seasonal. Pups are born at the end of the rainy season (October–January), and become independent at the end of the dry season, coinciding with the period of highest prey availability [21]. In addition, breeding is synchronized among adjacent packs, with litters in adjacent packs born within weeks of each other [21]. Gestation lasts approximately 60–62 days and litters consist of one to six pups [17]. All pack members help rear pups through den-guarding, regurgitating prey to pups [17], and subordinate females may allosuckle pups [20]. Within a pack, there is a dominance hierarchy and usually only the dominant pair breeds. However, subordinate females do mate and give birth on rare occasions [20,22]. In addition, there may be extra pair copulations between the dominant female and extra-pack males [20], leading to multiple paternity litters [22,23].

Ethiopian wolves specialize in hunting rodents and usually forage solitarily [24]. The fact that Ethiopian wolves forage solitarily yet live in packs and breed cooperatively make them somewhat unusual, since other pack-living, cooperatively breeding canids such as African wild dogs [25] and gray wolves [26] hunt cooperatively. This unusual social system makes Ethiopian wolves an interesting species in which to study the physiology of cooperative breeding.

Using fecal samples from wild female Ethiopian wolves, we aimed to study seasonal patterns in estradiol and progesterone and the mechanism by which subordinates are reproductively suppressed. We predicted that dominant females would show an increase in estradiol concentrations associated with estrus, and increased concentrations of progesterone during pregnancy. Subordinate females rarely mate or breed, although they may allosuckle pups [20], suggesting that they may become pseudopregnant [27,28]. As pseudopregnancy in canids follows an infertile ovulation [28], we predicted that some subordinate females would come into estrus, and that this would be evidenced by increased estradiol concentrations and/or observations of mating behavior. We hypothesized that average glucocorticoid concentrations would be higher in dominant females than in subordinate females, as was found in other cooperatively breeding canids [6,11].

2. Materials and methods

2.1. Study area and population

This study took place in two Afroalpine areas in the Bale Mountains National Park (BMNP, 7°N, 39°40′ E) of Southern Ethiopia; the Web Valley (WV, 3450 to 3550 m a.s.l.) and the Sanetti Plateau (SP, 3800 to 4300 m a.s.l). The climate in BMNP is characterized by an eight month wet season, with rainfall maxima of 1150 mm per year, and a four month dry season. Temperatures in the dry season range from −15 °C at night to +26 °C during the day, with more modest temperature fluctuations during the wet season [29]. Wolves occur at densities of up to 1.2 adult wolves/km^2 [17], with marked periodic fluctuations due to rabies outbreaks [30,31].

Nine packs were selected (six in WV, three in SP) and, between 2008 and 2010, 20 female Ethiopian wolves (11 dominant, 9 subordinate) were studied. All were at least 22 months of age when they were first included in the study, the age at which Ethiopian wolves start to show reproductive behavior [17]. Packs were studied from August to February each year, with the exception of females SOD02b and MEG06b who were also observed between March and July 2009 as part of extra monitoring in WV following the outbreak of rabies [30]. The number of weeks for which each female was studied differed due to the premature death of seven females from rabies. Packs were visited twice a week, with the aim of collecting at least one fecal sample per female per week. However, due to field conditions this was not always possible so the average sampling frequency ranged from 3.4 to 9 days (Table 1).

2.2. Field observations and sample collection

Ethiopian wolves in the BMNP are well habituated to humans due to long term monitoring by the Ethiopian Wolf Conservation Programme (EWCP) and associated researchers, and as such could be easily observed. Individual wolves were identified through ear tags (n = 5) or individual markings (n = 15). Dominance hierarchies were established by observing interactions between wolves of the same pack. Signs of submission in Ethiopian wolves are similar to those in domestic dogs

Table 1
Females sampled as part of this study. The average sampling frequency was calculated as the average of the number of days between samples, and not the total number of samples divided by the number of weeks a female was observed.

Year	Pack	Female	Status	# of weeks observed	# of fecal samples	Avg. # of days between collected samples
2008–2009	Addaa	SOD06	Dominant	20.7	30	5.18
	Darkeena	DAR02	Dominant	23.6	37	4.53
	Darkeena	DAR10	Subordinate	16.4	21	5.75
	Darkeena	DAR12	Subordinate	17.3	18	7.12
	Darkeena	DAR14	Subordinate	19.4	21	6.80
	Kotera	KOT30	Dominant	16.9	21	5.90
	Megity	MEG02	Dominant	29.4	61	3.40
	Megity	MEG06a	Subordinate	29.4	47	4.48
	Mulamo	DAR06	Dominant	30.14	45	4.80
	Mulamo	DAR08	Subordinate	21.9	21	7.60
	Sodota	SOD02a	Dominant	28.7	55	3.81
2009–2010	BBC	BBC32	Dominant	23.6	24	7.22
	BBC	BBC42	Subordinate	21.9	18	9.00
	Dumal	DUM02	Dominant	23	27	6.19
	Dumal	DUM04	Subordinate	25.3	28	6.48
	Nyala	NYA36	Dominant	23.6	28	7.17
	Nyala	NYA32	Subordinate	26.9	25	5.74
	Sodota	SOD02b	Dominant	49	80	3.64
	Sodota	SOD04	Subordinate	25.9	46	5.62
	Tamara	MEG06b	Dominant	49.7	48	5.36