ASPECTS OF SOCIAL BEHAVIOR AND REPODUCTION IN THE WILD RABBIT – IMPLICATIONS FOR RABBIT BREEDING ?

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ABSTRACT

Further knowledge on aspects of social behavior in the wild rabbit (*Oryctolagus cuniculus*) including the link to reproduction could possibly point to new ways how to improve housing and breeding conditions in rabbit farming. In our long-term study on a 2-hectare field enclosure population of wild rabbits, we explored group-level and individual-level differences in agonistic behavior of females and their potential associations to reproductive traits including offspring survival. The frequency of agonistic behavior in which females were involved increased with increasing group size, and was lower in groups with a more heterogeneous age-structure. At the individual level, reproducing females were involved in more agonistic interactions when groupmates gave birth and thus built their burrows and nests at around the same time, and higher-ranking mothers were particularly aggressive when other females approached close to their nursery burrows. Associations between females' social environment and reproduction were evident as the numbers of litters and offspring per female were lower at higher female densities, high-ranking females produced more offspring and had a lower offspring mortality than low-ranking ones, and cases of infanticide were lower in more stable groups which we quantified by the more heterogeneous age structure of the females' rank hierarchy in such groups. Furthermore, perinatal offspring mortality was increased in females with a delayed burrow and nest building activity, i.e. which dug their nursery burrow and built their nest only during the last 24 h pre-partum, possibly driven by the more unfavorable social environment experienced by such females. Most importantly, our studies highlight the importance of the presence of litter siblings in improving an individual's social environment, which resulted in an earlier onset of breeding in such females. Higher levels of positive social interactions with litter siblings were also associated with lower stress hormone (corticosterone) levels and with a better health status in terms of lower loads with an intestinal nematode. These findings on ameliorating effects of litter sibling presence in growing rabbits as well as in reproducing females may be a promising starting point worth further exploration in the context of group housing of domestic rabbits.

Key Words: affiliative behavior, agonistic behavior, density effects, group stability, infanticide, sibling effects

INTRODUCTION

The use of traditional single cage house systems for rabbit farming is increasingly discouraged by national recommendations or legislation in favor of alternative rearing systems in which the animals are kept in groups (reviewed in Szendrő *et al.*, 2019). Although keeping a group-living animal such as the rabbit *Oryctolagus cuniculus* (cf. Myers and Poole, 1961; Cowan, 1987; von Holst *et al.*, 2002) in an environment allowing the animals to interact with conspecifics complies with animal welfare standards at first sight, group-housing in spatially confined environments of smaller scale can have negative consequences for their wellbeing. Studies on domestic rabbits, making use of different housing systems showed that group living, in particular in breeding does but also in growing rabbits, is associated with the occurrence of aggressive interactions (e.g., Szendrő *et al.*, 2009; Szendrő and

McNitt, 2012; Buijs *et al.*, 2015; Gerencsér *et al.*, 2019). Such conditions can result in the infliction of sometimes severe wounds, higher disease risk and increased kit mortality, and in lower growth rates or even weight loss, thus negatively affecting animal welfare and productivity (Pérez-Fuentes *et al.*, 2000; Szendrő and McNitt, 2012; Dal Bosco *et al.*, 2019). This highlights the need for further research providing the basis to rethink, improve and possibly develop new rabbit housing systems (Trocino and Xiccato, 2006; Szendrő *et al.*, 2019; Villagrá García, 2020). In this context it may prove useful to take a closer look at the European (wild) rabbit, either in the wild or under semi-natural conditions. Such studies may lead to a better understanding of the drivers of agonistic interactions including their link to reproduction, and thus may possibly indicate ways how to improve the social environment in rabbit groups kept under human care. In addition, detailed and individual-based information on social behavior and reproduction of the wild rabbit may also be useful for improving the management of captive colony systems in which rabbits are reared for restocking of wild populations for conservation or for hunting purposes (Moreno *et al.*, 2004; Letty *et al.*, 2008).

This paper reviews parts of our work which was carried out on animals from a wild rabbit (*Oryctolagus cuniculus cuniculus*) colony living in a 2-hectare field enclosure in a temperate zone habitat, at the campus of the University of Bayreuth in Germany (49.92°N, 11.59°E, elevation 359 m a.s.l.). The paper particularly will focus on conditions driving group-level and individual-level differences in social behavior and the association of these with female reproductive performance and offspring survival. The animals of this population, which were studied by our group from 1987 to 2011, were descendants from rabbits caught in the wild in South Germany (details in von Holst *et al.*, 2002). Vegetation in the enclosure consisted of grassland (mainly *Perennial ryegrass* and *Festuca rubra*) interspersed with groups of trees and a pond in the middle. The green pasture was the animals' only food source during the breeding season and thus there was seasonal variation in food quality. Additional hay, harvested from meadows of the surrounding farmland, with similar vegetation than inside the enclosure, was provided only during the winter. The enclosure contained 16 artificial warrens made of concrete, with interconnected chambers and removable lids. These were evenly distributed in the enclosure, with at least one per social group. Females used them for breeding, although they also dug their own, natural burrows.

A major strength of this project was that the animals lived under minimum human disturbance in a natural environment of larger scale and thus could show their natural social and reproductive behavior, including their self-organization into distinct social groups. By regular observations from two outlook towers, we collected individual-based behavioral data, as all animals could be identified from the distance by their colored aluminum ear tags (details in: von Holst *et al.*, 2002; Rödel *et al.*, 2008c). Furthermore, since we found virtually all litters on postnatal day 1 during our daily check walks, and since we could determine maternities (by direct observations together with coloring mothers' abdominal hair, see Rödel *et al.*, 2008b), we obtained individual reproductive histories of all females over lifetime, including information on survival of their offspring (details in: von Holst *et al.*, 2002; Rödel *et al.*, 2009b).

RESULTS AND DISCUSSION

Brief summary of the wild rabbit's social organization and seasonal reproduction

The European rabbit ('wild rabbit') is organized in social groups which can vary in size, with around 1–5 adult males and 1–8 adult females (Parer, 1977; Cowan, 1987; von Holst *et al.*, 2002). Within groups, adult rabbits are maintaining a sex-specific linear rank order (Myers and Poole, 1961). During the onset of the breeding season, and related to the acquisition and maintenance of high(er) social rank positions, an annual maximum in intra-sexual competition can be observed. This includes chasing and even fierce fights in both sexes, particularly pronounced in males (von Holst *et al.*, 1999). As a consequence, during the early breeding season, adult males typically show an on average higher number of wounds inflicted by such intra-sexual aggression compared to females (von Holst *et al.*, 1999). Later in the breeding season, agonistic interactions become increasingly ritualized such as by

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the display of displacements rather than chasing events, mainly evident among females of the same social group with established and stable rank hierarchy (von Holst *et al.*, 1999).

Since males predominantly compete for the access to females and females for the access to high quality sites to build their nursery burrows, obtaining a high social rank provides priority of access to these resources (Mykytowycz and Fullagar, 1973; von Holst *et al.*, 1999). Priority of access to adult females of the group inevitably leads to a high reproductive success in dominant males. Also, females which obtained a higher (dominant) social rank early in life, showed a higher lifetime reproductive success including better offspring survival (von Holst *et al.*, 2002; Rödel *et al.*, 2009a).

Natal dispersal in the rabbit tends to be sex-specific, i.e. young males usually disperse to other groups whilst young females often remain in their group of birth (Parer, 1992; Künkele and von Holst, 1996). This typically results in a high degree of relatedness amongst the adult females of a social group (Webb *et al.*, 1995; Richardson *et al.*, 2002).

The breeding season of the rabbit considerably varies in onset and in duration, mainly depending on the climatic conditions (Thompson and King, 1994; Tablado *et al.*, 2009). In Mediterranean habitats with dry summers, the breeding season usually lasts from autumn/winter until late spring/early summer. In temperate zone habitats with colder winters, i.e. under the conditions where our field enclosure study was carried out, it lasts from early spring until summer/autumn (von Holst *et al.*, 2002; Rödel *et al.*, 2008a). However, also in temperate zone habitats, the rabbit's reproductive activity can be decreased or paused during hot and dry summer periods (Rödel and von Holst, 2008).

Depending on the climatic conditions, the age at first reproduction can vary strongly. The wild rabbit, like domestic breeds, can potentially reach sexual maturity at an age of 3-4 months (Tablado *et al.*, 2009). For example, in populations in South Spain, some females already start reproducing at this age, i.e. within their season of birth (Rogers *et al.*, 1994). In temperate zone habitats with colder winter periods, such as under the conditions of our field enclosure study in South Germany, rabbits usually started to reproduce in spring following their year of birth, thus at an age of 8-12 months (von Holst *et al.*, 2002).

Mainly depending on the length of the breeding season, the average number of litters per female and season can vary considerably between populations, ranging from an average of 2.3 to 7.0 (Tablado *et al.*, 2009). There is an even stronger variability at the individual level within populations, as for example females of our study population produced between 0 and up to 8 litters (up to 25 kits) per season, on average 2.5 litters and 11.8 kits (Rödel *et al.*, 2009b). The litter size in the wild rabbit is smaller than in domestic breeds used for rabbit farming and shows a clear seasonal pattern (Eccard and Rödel, 2011). Our long-term study revealed that litters born during the early breeding season are usually the smallest ones, with an average litter size of 2.9 kits ($CI_{95\%} = [2.7, 3.1]$; min. = 1, max. = 5), then increasing to on average 4.2 kits/litter ($CI_{95\%} = [4.0, 4.4]$; min. = 1, max. = 8) in the mid breeding season, and decreasing again to on average 3.2 kits/litter during late season ($CI_{95\%} = [2.9, 3.5]$; min. = 1, max. = 7). Furthermore, first-season breeders (under the climatic conditions of our study: one-year-old females) typically show a lower reproductive performance than older mothers, as for example our study over 9 years revealed that one-year-old mothers produced on average 4.1 offspring less per season than older ones (Rödel *et al.*, 2004b).

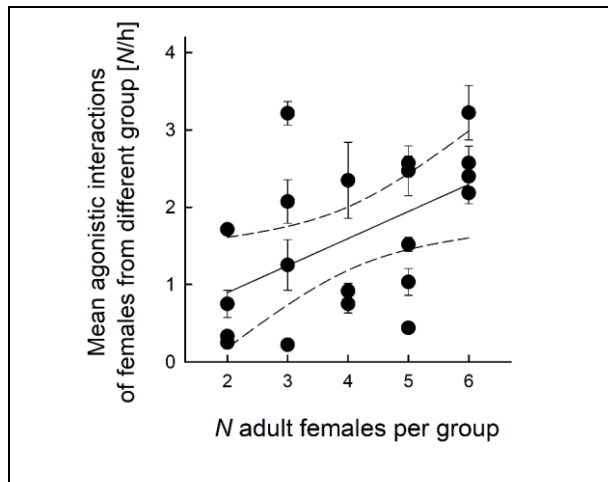### *Effects of density on reproduction and on agonistic behavior*

Our results demonstrate clear effects of the density of adult females on their reproductive performance (Rödel *et al.*, 2004b). The higher the female density was, the lower was the average number of litters born per female during the breeding season (linear model LM: $R^2 = 0.576$, $P = 0.007$; Figure 1a). As a consequence, the average offspring number per female under higher density conditions was also significantly decreased (LM: $R^2 = 0.581$, $P = 0.006$; Figure 1b). This density-dependent pattern is in accordance with the results obtained in other studies on wild rabbits describing a decrease in annual birth rates when population density increased (Brambell, 1944; Myers and Poole, 1962). Interestingly, our long-term study did not support any density-dependent differences in litter size or in offspring body mass (details in: Rödel *et al.*, 2004b).
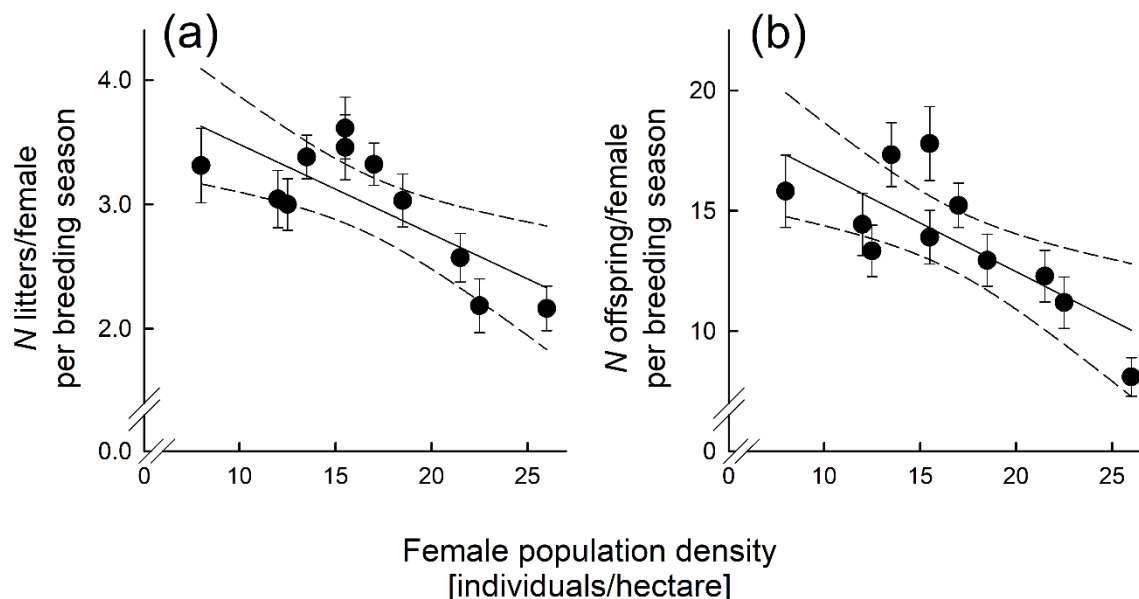
Figure 1: Associations between the annual breeding density (per hectare) of adult female European rabbits and (a) the mean litter size and the (b) mean number of offspring/females during the breeding season (modified after Rödel *et al.*, 2004b). Data were averaged (± SE) over females present until the end of the particular breeding season (*N* = 11 years); only females older than one year were considered. See text for statistics.

These results, although obtained at a very different spatial scale than under breeding conditions (8–26 adult females per 10,000 m^2; Figure 1), indicate the existence of mechanisms compromising the reproductive performance of individual females when at higher density (Rödel *et al.*, 2004b). Under the climatic conditions of our field enclosure study, green pasture was always available, and thus density effects due to scramble competition for food appear rather improbable. We suggest that social mechanisms, such as an increased competition for breeding resources among females like for high-quality burrows or sites for burrows (cf. Myers and Poole, 1961; Mykytowycz and Fullagar, 1973) could be the main driver of the observed density-dependent pattern in reproductive performance. An increased level of aggressive interactions at high density may cause chronic social stress including an increased adrenocortical activity. This, in turn, can result in a decreased secretion of gonadotropins and thus in a depression of reproductive function (Bronson *et al.*, 1973; von Holst, 1998). Interestingly, our study demonstrated that density-driven changes in reproductive performance affect both high-ranking (dominant) and lower-ranking (subordinate) females in a similar way (Rödel *et al.*, 2004b), even though subordinate rabbit females typically show higher stress hormone (corticosterone) levels compared to dominant ones (von Holst *et al.*, 1999).

An increase in agonistic interactions among females along with an increasing population density has been shown in a wild rabbit enclosure study in Australia (Myers and Poole, 1961). Furthermore, the authors reported that in contrast to the results obtained in females, agonistic behavior in males was always high, either at higher or lower population densities (Myers and Poole, 1961). We could confirm this positive density-dependence in female agonistic interactions by our behavioral observations during the early breeding season, based on data collected from different social groups over three years (LM: R^2 = 0.406, P = 0.024; Figure 2). In groups including only two adult females, the average frequency of agonistic behavior observed per female (offensive or defensive displacements and chasing events, and fights in rare cases) was 0.8 interactions/h, whereas in groups including 6 females the average frequency of interactions/h was 2.6, thus around 3.3 times higher. During the later breeding season, such agonistic interactions among females usually decrease in frequency and become more ritualized (von Holst *et al.*, 1999). That is, later in the season chasing events between females and fights among them can be hardly observed since the social rank order usually (but not always) stabilizes. Furthermore, note that in our field enclosure study, the average territory size occupied and

Figure 2: Association between the number of adult females per social group and the mean agonistic behavior (offensive and defensive interactions) averaged per female of each group. Behavioral observations of females from a total of $N = 20$ groups (5-9 different groups per year, data from 3 years), carried out during the first 4 weeks after the occurrence of the first annual parturition around early April. See text for statistics.

defended by a social group was around 2,500 m^2 (details in Seltmann *et al.*, 2017). Thus, females, even within groups, were able to keep larger inter-individual distances, although aggregations of groupmates feeding in close proximity over longer spans of time were frequently observed (e.g., Monclús and Rödel, 2008)

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Such increases in female intra-sexual aggression at higher stocking densities have been also observed in studies on domestic rabbits kept in pens or small colonies (Di Meo *et al.*, 2003; Szendrő *et al.*, 2009; Buijs *et al.*, 2016; Zomeño *et al.*, 2017). Furthermore, studies in the domestic rabbit have reported negative consequences of high density or larger group size, for example with respect to an increased occurrence of wounds inflicted by agonistic interactions and in some studies by a compromised weight gain or even weight losses (Di Meo *et al.*, 2003; Villalobos *et al.*, 2008; Szendrő *et al.*, 2009; Bhattacharjya *et al.*, 2015; Trocino *et al.*, 2015). The latter findings are in accordance with our results obtained in the wild rabbit, as a high population density had a negative, although only slight impact on the body mass the animals reached until autumn of their year of birth, i.e. when around 4-7 months old (Rödel *et al.*, 2004a).

Further drivers of differences in agonistic behavior among females

*Competitive interactions among females are affected by their reproductive timing*

Within a population, wild rabbit females often show a certain degree of synchronization in the timing of parturition. Since the onset of the annual breeding activity in this species is triggered by the prevailing weather conditions (and thus by the resulting food availability, affecting female body condition: Bell and Webb, 1991; Rödel *et al.*, 2005), the rabbit's post-partum estrus (Brambell, 1944) can lead to the occurrence of rather distinct breeding pulses along the breeding season. Our long-term studies showed that concurrent reproduction of different females within a group can enhance

competition among them and can entail negative consequences for offspring survival (Seltmann *et al.*, 2017).

That is, females which experienced concurrent parturitions of groupmates within 2 days prior to or after their own parturition date were involved in significantly more (offensive and defensive) agonistic interactions than female with no such concurrent birth events within their social group (linear mixed-effects model LMM: $\chi_1^2 = 5.11$, $P = 0.024$; Figure 3a). Our findings further indicate that a high degree of involvement in such competitive interactions around the time of parturition can constrain females in constructing their nursery burrow including the nest consisting of hay and her own abdominal hair. Usually, the pregnant rabbit digs the nursery burrow and starts building the nest with dry grass several days before giving birth (Mykytowycz, 1958; Myers and Poole, 1961). During the last night, she usually lines the nest with hair pulled from her ventrum (González-Mariscal and Rosenblatt, 1996). However, we sometimes found cases of females which accomplished all these tasks during the last 24 h pre-partum (Seltmann *et al.*, 2017). This accumulation of different energy-demanding activities within such a short time possibly was the main reason why such females showed an increased probability of perinatal offspring mortality (including stillbirths), affecting around 36% of their litters (see a detailed discussion of possible mechanisms in Seltmann *et al.*, 2017).
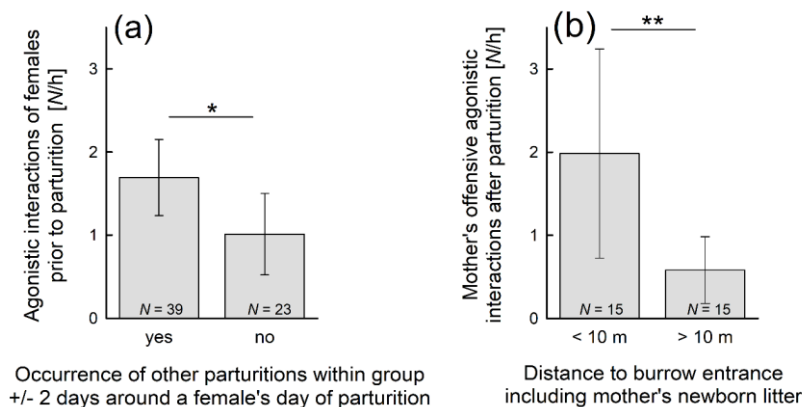
Figure 3: (a) Comparison of agonistic interactions (offensive and defensive) of females experiencing or not experiencing concurrent parturition of group mates within 2 days prior to or after giving birth. Behavioral data were collected during the last 2 weeks prior to parturition of the focal females. (b) Comparison of offensive agonistic interactions (chasing, displacements) of high-ranking mothers (ranks 1, 2) towards other adult females occurring in close or farther distance to the entrance of mother's nursery burrow. Data were collected during post-partum days 1–20. For both graphs, mean values with 95% confidence intervals are given. Sample sizes inside the bars provide (a) *N* reproductive events from a total of 41 females, and (b) individual-based repeated measurements; see text for statistics. Modified after (a) Seltmann *et al.* (2017) and (b) Rödel *et al.* (2008b).

Agonistic behavior in the context of offspring defense

For this study, we observed the agonistic interactions of high-ranking females (social ranks 1 and 2) shortly after parturition (Rödel *et al.*, 2008b). Such high-ranking mothers showed significantly increased levels of agonistic behavior towards other adult females, thus displaced or chased them away when these were approaching the area close to the entrance of the burrow including the offspring of the high-ranking mother in focus (LMM: $\chi_1^2 = 7.79$, $P = 0.005$; Figure 3b). A further indication that rabbit mothers were particularly attentive in defending their burrow during the first few days after parturition was given by the fact that mother's average distance to her burrow entrance during the first 10 days post-partum was significantly lower (on average 17 m, $CI_{95\%} = [12\ m, 22\ m]$) than during the later period from postpartum day 10 until the emergence of the kits above ground at around day 20 (mother's distance to burrow of on average 23 m, $CI_{95\%} = [18\ m, 27\ m]$; LMM: $\chi_1^2 = 10.37$, $P = 0.001$) (more details in Rödel *et al.*, 2008b).

Occurrence of infanticide

An increased aggressiveness of females around parturition is a well-known behavioral phenomenon in mammals, with a clear hormonal basis (Svare, 1981; Caughey *et al.*, 2011). Such 'maternal aggression' is an important component of maternal care behavior to adaptively protect offspring from harm. In the rabbit, patterns of maternal offspring defense against approaching conspecifics, as described above, may have evolved as a response to the occurrence of infanticide. Under natural conditions, nest mortality in the rabbit is high, approximately 40% mostly affecting the whole litter and driven by manifold factors and conditions (von Holst *et al.*, 2002; Palomares, 2003; Rödel *et al.*, 2009a). Among these factors, infanticide plays a small but non-negligible role, and our field enclosure studies revealed the occurrence of cases of infanticide in around 2–6% of the litters per season, although during some years this phenomenon was not observed at all (Künkele, 1992; Rödel *et al.*, 2008b; Rödel *et al.*, 2009a). The perpetrators of infanticide in the wild rabbit are adult females other than the mother. The nursery burrow constitutes an important and energetically costly-to-build breeding resource, and thus females sometimes attempt to take over the burrow of another female. In doing so, a female may scrape out an existing nest of another mother including the new-born kits, frequently before starting to construct her own nest inside this burrow. In most cases, infanticide happens during the first few days post-partum. Kits are typically killed (or severely hurt) by bites and sometimes by the claws of the perpetrator. Even if some kits may survive the removal from the nursery burrow by the infanticidal female, these are quickly taken by predators such as magpies (*Pica pica*) or carrion crows (*Corvus corone*) (Rödel *et al.*, 2008b; Rödel *et al.*, 2009a).

Our studies further revealed that rabbit mothers responded strongly to distress calls of kits (Rödel *et al.*, 2013). Rabbit kits, even when only a few days old, are capable of emitting trains of high-pitched calls under conditions of distress (see sonogram in Rödel *et al.*, 2013). We hypothesized that such distress calls, which are expectantly emitted by the kits while taken out of the nursery burrow by an infanticidal female, might serve to alert the mother. Indeed, during playback experiments in the field, mothers quickly ran towards their burrows when recordings of kit distress calls were played close to the burrow entrance (Rödel *et al.*, 2013). Mothers then carefully checked the entrance area and remained close to the burrow for some time. In addition, we found some anecdotal support that maternal offspring defense triggered by the kits' distress calls can also occur later on. Shortly after the emergence of the kits from the nursery burrow (i.e. after postnatal day 20, see Rödel *et al.*, 2015), we observed two cases during which the mother readily came running to defend her young against the attack of a magpie.

Group stability as a potential driver of infanticide

Given the large variation in infanticide rates among different years and social groups (see above), we aimed to identify factors driving the occurrence of this phenomenon. Surprisingly, we did not find any support that offspring from lower-ranking or younger mothers were more prone to infanticide than offspring from higher-ranking or older ones. Furthermore, in our field enclosure study, infanticide rates were not higher during higher population densities (Rödel *et al.*, 2008b).

We hypothesized that instabilities in female social rank hierarchy associated with an increased intra-group competition may be a possible driver of infanticide within a group (see Rödel *et al.*, 2008b). Usually, the social rank position of females is closely related to their age (i.e., to their year of birth, Rödel *et al.*, 2004b). Young adult females (under the conditions of our enclosure: one-year-old females) typically start occupying the lowest social rank positions once they are recruited into a social group at the onset of their first breeding season. When getting older, females are climbing up in social rank, and thus usually the oldest ones within a social group will occupy the highest (dominant) rank positions. In this way, when only single or very few (young) females are integrating a social group during consecutive seasons, the rank hierarchy remains maximally age-structured (heterogeneous) and thus intra-sexual competition should be expectantly low. In contrast, we predicted that the occurrence of clusters of same-aged females within a social group, i.e. a low degree of heterogeneity in age structure, *(a)* may lead to a more unstable social situation characterized by more competitive

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interactions among female groupmates, and *(b)* as a consequence, this may lead to increased infanticide rates within such groups.

These assumptions were supported by our findings. *(a)* Observations of high-ranking females prior to their parturition revealed that individuals whose lower-ranking female groupmate had the same age (i.e., the same year of birth) were involved in significantly more agonistic interactions than females with a younger groupmate on the following (lower) rank position (LMM: $\chi^2_1 = 6.97$, *P* = 0.008; Figure 4a). At the group level, this finding suggests lower levels of intra-sexual aggression in groups with a more age-structured female rank hierarchy. *(b)* Furthermore, by a study of all social groups from our population over 5 years, we found that indeed the rate of infanticide depended on the age structure of the social rank hierarchy within groups. That is, in groups with a more heterogeneous age structure, the proportion of litters with infanticide was significantly lower, by 6.9%, compared to groups including clusters of same-aged females occupying consecutive social ranks (LMM: $\chi^2_1 = 4.06$, *P* = 0.043; Figure 4b).
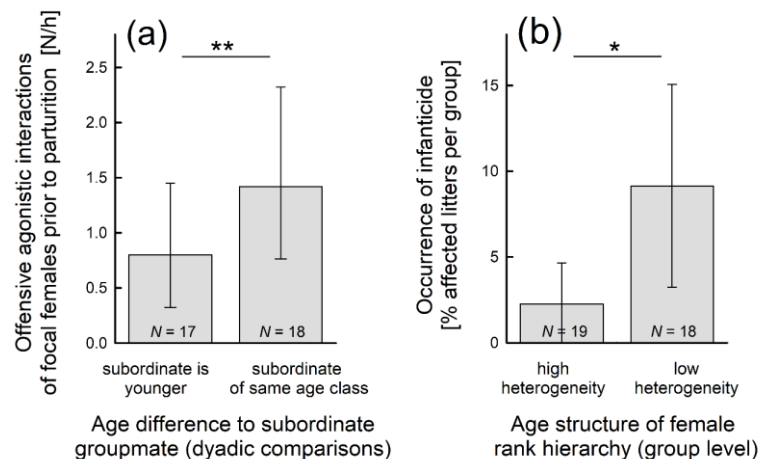
Figure 4: (a) Offensive agonistic interactions (chasing and displacement) displayed by higher-ranking females (social ranks 1-3) during 3 weeks prior to parturition. Comparison between females which had the same age (in years) as their female groupmate with subsequent lower social rank score and females whose groupmate with subsequent social rank was different (in all cases: younger) in age. Only higher-ranking females (ranks 1-3) were considered for analysis; the number of females is given in the bars (data from 3 years). (b) Comparison of the percentage of litters in which infanticide occurred (single kits or whole litter) on the total number of litters born per breeding season between groups with high or low heterogeneity in the age structure of the females' rank hierarchy (modified after Rödel *et al.*, 2008b). High and low heterogeneity were determined based on the age (in years) of the 3 highest ranking females per group (social rank 1-3); the number of social groups is given in the bars (data from 5 years). In (a) predicted values with 95% confidence intervals based on parameter estimates of a multifactorial model (including the effects of age difference to the lower ranking group mate as well as of the social rank of the female in focus) are given, and in (b) mean values with 95% confidence intervals are provided; see text for statistics.

These results underline the potential of an age-structured rank hierarchy in keeping potentially stressful competitive interactions within-group as well as infanticide-driven offspring mortality at lower levels. It should be noted that in our study, groups with a more heterogeneous age structure were naturally emerging over consecutive years, but were not artificially designed by mixing together females of different age classes. Thus, it remains unclear to what extent the observed link between female age structure within groups, its link to intra-sexual aggression and the potential consequences on offspring survival may find applications in the context of domestic rabbit breeding.

Ameliorating effects of sibling presence

The presence of positive social interactions with bonding partners, for example with close kin, can be beneficial as these can effectively increase an individual's ability to cope with challenging situations. Such effects, termed as 'social buffering' or "social support' have the potential to reduce (chronic) stress including its negative consequences on health and wellbeing (e.g., Henry and Stephens, 1977; Rodriguez and Cohen, 1998; DeVries *et al.*, 2003).

Social support lowers stress hormone levels and loads of an intestinal nematode

We investigated the existence of such social support effects on stress hormone levels and endoparasite loads in juvenile and subadult rabbits from our field enclosure population. Therefore, we quantified the level of positive social interactions with potential bonding partners (close kin), i.e. the percentage time an individual spent in close contact with its litter siblings and mother during early life. This was done by regular observations, starting on postnatal day 30 (i.e., around weaning) until around day 90. During summer, we caught the animals and determined their stress hormone levels from blood (serum corticosterone concentrations after ACTH challenge). In autumn, when the animals were on average 6 months old, we caught and dissected them and quantified their loads with gastro-intestinal nematodes (Rödel and Starkloff, 2014).

Individuals which experienced a more favorable social situation in terms of more positive social interactions with siblings and mother during early life showed lower stress hormone (corticosterone) levels in summer (LMM: χ_1^2 = 13.85, $_{marginal}$ R^2 = 0.250, P < 0.001; Figure 5a). Furthermore, more positive social interactions with close kin during the period after weaning were associated with lower loads with the intestinal nematode *Passalurus ambiguus* (and also with *Trichostrongylus retortaeformis* with a similar statistical trend) during later life, when the animals had reached an age of on average 6 months (LMM: χ_1^2 = 8.28, $_{marginal}$ R^2 = 0.117, P = 0.004; Figure 5b). These results strongly suggest effects of social support by siblings and mother. Juveniles/subadults experience various sources of social stress, for example because resident adults typically start chasing them out of the group territories during late summer and autumn, in particular at higher population densities (Myers and Poole, 1961; Cowan, 1987). Positive social interactions with siblings and mother might have lowered resulting effects of social stress, such as by buffering its potential immunosuppressive effects (cf. von Holst, 1998). In turn, immunosuppression in more stressed animals experiencing lower levels of social support could have led to their increased endoparasite loads.
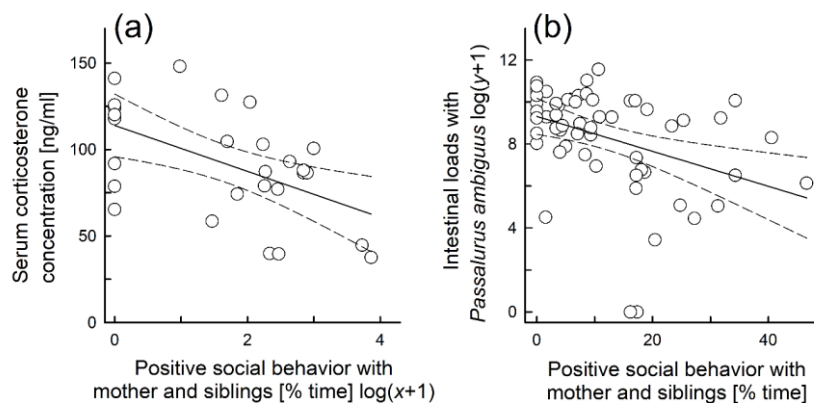

Figure 5: Associations between the level of positive social behavior of juvenile rabbits with their mother and litter siblings during the following 2 months after weaning by the mother (around postnatal day 28) and (a) their serum corticosterone concentrations (after challenge with synthetic adrenocorticotropic hormone ACTH; *N* = 27 subadults, both sexes) during summer, and (b) their loads of the intestinal nematode *P. ambiguus* in autumn (*N* = 58 subadults, both sexes). Positive social behavior was quantified as the percentage observation time spent in close proximity to mother and litter siblings, within a distance of less than 1 body length. Regression lines with 95% confidence bands are given; see text for statistics. Modified after Rödel and Starkloff (2014).

Sibling presence and reproduction

We also explored potentially ameliorating effects of the presence of a female's litter sisters within the same social group on her social situation and reproduction during her first year of life and her first breeding season (Rödel *et al.*, 2008c). For this study, we managed to observe 4 pairings of litter siblings (3 twins, 1 trio) which stayed together in the same social groups, from their early juvenile period until their first reproduction in spring during the following year. During all periods of this study, in summer/autumn when the animals were around 3–6 months old (LMM with permutation test: $P = 0.008$; Figure 6a), in winter at an age of 8–10 months ($P = 0.026$; Figure 6b), and during the following reproductive season when the animals had reached an age of around 12–14 months ($P = 0.002$; Figure 6c), females with present litter sisters were involved in significantly more positive social interactions compared to other females of the same age but without sisters in their group. Most importantly, these higher levels of positive social behavior experienced by females with litter sisters was not only attributed to affiliative interactions with their sisters but also to interactions with other female groupmates. This suggests that the presence of litter sisters generally improved the social environment for such females, with potential positive effects for their welfare.

Furthermore, our results showed positive consequences of sibling presence on an important reproductive trait, the annual onset of breeding. Females with litter sisters in their group started to reproduce significantly earlier during the breeding season, by on average 2 weeks, compared to females without present sisters (details in Rödel *et al.*, 2008c). Under natural conditions, an early onset of reproduction is advantageous, as offspring born early in the season will reach a higher body mass until autumn, thus this will increase the chance that the offspring will survive the winter season (Rödel *et al.*, 2004a; Rödel *et al.*, 2009b). However, there were no significant effects on other reproductive traits such as on litter size or offspring body mass. We suggest that due to social support, in particular by litter sisters, young females may have been better capable to successfully cope with challenge, such as with stressful competitive intra-sexual agonistic interactions during the early breeding season, as outlined in the chapters above. Such social buffering might have reduced potentially negative consequences of stress on reproductive function (Henry and Stephens, 1977; Sapolsky, 1992).
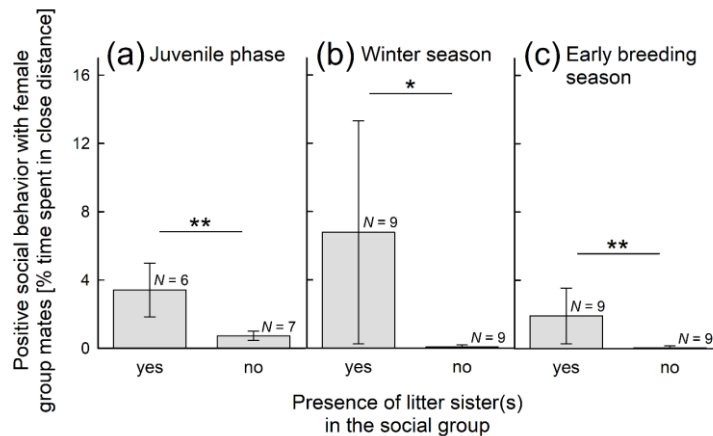
Figure 6: Mean percentage of time (with 95% confidence intervals) that females with litter sisters (one or two) or without sisters in their social group spent in close proximity (as a measure of positive social behavior) to (a) other juvenile/subadult females during August to October, and to other females (same age or older) during (b) the winter (January/February) and (c) the early reproductive season (March–April). Numbers of females studied are given in the graphs, see text for statistics. Modified after Rödel *et al.* (2008c).

SUMMARY AND CONCLUSIONS

The findings of our long-term studies on wild rabbits highlight various factors affecting the frequency of agonistic interactions among adult females. At the group level, agonistic interactions were increased when the group size was larger. Furthermore, a more age-structured rank hierarchy within a group led to a higher group stability in terms of an overall lower level of agonistic interactions among females. At the individual level, reproductive activity triggered the occurrence of agonistic interactions. First, higher-ranking mothers were particularly aggressive towards other females when these approached closer to their nursery burrows (Rödel *et al.*, 2008b), and second, reproducing females were involved in more agonistic interactions when other females of their group reproduced at around the same time (Seltmann *et al.*, 2017).

Our findings also indicate links between the females' social environment and traits of their reproduction, for example as the seasonal reproductive output (litters and number of offspring per female) was lower at higher female densities (Rödel *et al.*, 2004b), and as dominant mothers produced more offspring over lifetime and had a lower offspring mortality than lower ranking ones (von Holst *et al.*, 2002; Rödel *et al.*, 2009a; Rödel *et al.*, 2009b). Moreover, offspring mortality in terms of infanticide was higher in more unstable groups as characterized by a less heterogeneous age structure (Rödel *et al.*, 2008b, and results in Figure 4). Finally, perinatal offspring mortality was increased in females with a delayed burrow and nest building activity, i.e. which dug their nursery burrow and built their nest all within 24 h pre-partum, possibly due to the more unfavorable social environment experienced by such females (Seltmann *et al.*, 2017).

Most importantly, our study highlights the importance of the presence of litter siblings (or litter sisters) in improving an individual's social environment and, possibly as a consequence, by bringing forward the timing of first reproduction (Rödel *et al.*, 2008c). Furthermore, a higher level of positive social interactions with litter siblings led to a lower physiological stress response in terms of lower serum corticosterone levels and to a better health status in terms of lower loads with an intestinal nematode during later life (Rödel and Starkloff, 2014). These results could have applied implications, since the latter parameters reflect a better welfare status in animals with higher levels of affiliative interactions with litter siblings.

However, the applicability of these findings with regard to domestic rabbit housing and breeding has certain limitations. First, the results reported here were obtained on a far larger spatial scale compared to the usual housing practice of domestic rabbits, with an average group territory size of around 2,000–2,800 m^2 under the conditions of our field enclosure study. Second, the pairings of litter sisters, which we observed in different social groups, joined together voluntarily. This was evident as in some cases litter sisters even left their natal group together and dispersed into the same neighboring group, although in other cases litter sisters split up and joined different social groups (Rödel *et al.*, 2008c). Thus, housing a group of litter sisters together in a pen or a small enclosure may not inevitably imply that the relationships among them are always affiliative. But still, our findings regarding the ameliorating effects of litter siblings may be a promising starting point worth further exploration in the context of group housing of domestic rabbits.

Declaration of competing interest
None.

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