



Space allowance during gestation and early maternal separation: Effects on the fear response and social motivation of lambs



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ABSTRACT

A common prenatal stressor in lambs is early maternal separation, which may be aggravated by spatial restriction of ewes during pregnancy. They may influence lambs' development and coping abilities. The aim of this study was to determine how space allowance provided to ewes during pregnancy and early maternal separation affected the fear response and social motivation of lambs. A total of 54 ewes were randomly assigned to three living areas of 1, 2, or 3 m²/ewe (SA1, SA2, and SA3, respectively) during pregnancy. After birth, lambs from each treatment remained with their mothers (MR) or were early separated (ES). In the study 42, 2–3 day old lambs were sequentially subjected to a novel arena, a novel object, and a social motivation test. Information on their behaviour, movement patterns, and vocalizations was collected. Results of the study indicate that ES lambs born from SA1 ewes were more fearful, as indicated by the higher levels of immobility and number of vocalizations during novel arena tests, the latter being also observed during novel object tests ($P < 0.05$). Change of location tended to be less frequent in ES lambs during novel arena tests ($P < 0.10$), while the behavioural response of MR lambs appeared to be more homogeneous and independent from the pre-natal space allowance treatment during the novel object tests. ES SA1 lambs also spent the highest % of time close to other lambs ($P < 0.05$) and performed the highest number of vocalizations during the social test ($P < 0.05$). The fear response of lambs during the novel arena and novel object tests was gender dependent, with females vocalizing more and interacting more frequently with the stimulus during novel object tests ($P < 0.05$). Females also showed higher social motivation than males, but vocalized less ($P < 0.05$). Results suggest that the detrimental effects of early maternal separation might be exacerbated by space limitation during pregnancy, and would limit lambs' coping abilities.

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1. Introduction

The negative effects of stress on pregnant females can extend to the biological functioning and coping abilities of their offspring. Stress challenges during pregnancy

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may lead to an elevation of maternal glucocorticoids, that may cross the placenta (Otten et al., 2004), and alter the normal foetus hypothalamic–pituitary–adrenal (HPA) axis development (Edwards and McMillen, 2002). This will ultimately lead to alterations of the HPA axis responsiveness after birth (Hawkins et al., 2000; Meaney, 2001; Weinstock, 2001; Andrews and Matthews, 2004), inducing behavioural abnormalities. In this sense, the stimulated production of corticotrophin-releasing hormone (CRH) is suggested to increase fear and anxiety levels (Johnson et al., 1994). Elevated glucocorticoid levels have been also associated to depression and impaired cognitive functionality (Seckl and Olsson, 1995; Checkley, 1996). Due to its welfare implications, maternal stress has become a relevant issue in itself (Rutherford et al., 2012). Broom (1986) suggested that welfare is intimately related to animals' ability to satisfactorily handle stressful situations, but the extent to which the coping abilities of the offspring are diminished due to maternal stress remains unknown.

Sheep (*Ovis aries*) may be exposed to different stressful housing conditions and management practices. For example, undernourishment of gestating ewes induced higher emotional reactivity and impaired cognitive flexibility of their lambs at 8 months of age (Erhard et al., 2004) and at adulthood (Hernandez et al., 2010), with results being gender dependent. Repeated social isolation of pregnant ewes led to elevated cortisol levels and altered fear response of lambs at 25 days, and even at 8 months of age (Rousset et al., 2004). Aversive handling during pregnancy tended to increase ewes' cortisol levels (Hild et al., 2011), modifying the gene expression and corticolimbic morphology (Coulon et al., 2013) and impairing the fear response and cognitive abilities of lambs (Coulon et al., 2011). In addition, early post-natal maternal separation determines lambs' ability to cope with environmental and social challenges encountered later in life (reviewed by Brastad, 1998).

The space given to animals is closely related to the fulfilment of basic needs such as resting, eating, drinking, and socialization (Estevez et al., 2007; Petherick, 2007; Petherick and Phillips, 2009). Recent studies in sheep and goats suggest that decreased space allowance during pregnancy is stressful (Vas et al., 2013; Averós et al., 2014a,b) and might have negative implications for their progeny. Likewise, decreased pre-natal space allowances may also have a negative effect on emotionality and cognitive abilities of the lambs. A better understanding of the effects of space restriction on gestating ewes and their offspring would contribute to improve their welfare, fulfilling societal demands and expectations (Averós et al., 2013).

Early separation of lambs after colostrum consumption is a common practice in certain intensive dairy sheep due to economic or technical reasons (Napolitano et al., 2008). The abrupt disruption of the maternal–filial bond is a negative experience that triggers the appearance of altered and redirected behaviours in lambs (Stephens and Baldwin, 1971; Napolitano et al., 1995, 2003), increases cortisol levels, and affects the humoral immune response and performance (Napolitano et al., 1995). Nevertheless, whether reduced space allowance during pregnancy may aggravate the problems of early maternal separation is unknown.

Characterizing the emotional reactivity and social attachment of lambs may be valuable when determining their coping ability, as both are affected by pre- and early post-natal stress. Social isolation is stressful for herbivores (Boissy et al., 1997), and thus exposure to a novel arena is a powerful tool to assess their reactivity (Moberg et al., 1980; Dantzer and Mormède, 1983; Forkman et al., 2007). Assessing sheep's reaction against the sudden appearance of an unknown object is used to characterize their fear response (Vandehede and Bouissou, 1993; Forkman et al., 2007). The disruption of the maternal–filial bond, or the bond established with pen mates after early maternal separation (Veissier et al., 1998), may be used as a social deprivation model to assess the social motivation of lambs (Andersen et al., 2006). Since psychological reactivity depends on the individual perception of stressors (Mason, 1971), emotional reaction of lambs will depend on genotypic traits such as gender (Vandehede and Bouissou, 1993; Dodd et al., 2012).

Little is known about the interactive effects of pre- and early post-natal stress on the emotional reactivity and social attachment in young lambs. This study was designed to determine how pre-natal stress, via decreased space allowance to ewes, and early maternal separation interact and influence the fear response and social motivation of lambs. We hypothesized that there is an interaction between pre- and post-natal treatments, with early separated lambs from ewes subjected to stress during gestation being more fearful and socially dependent.

2. Materials and methods

This study was approved by the NEIKER-Tecnalia Animal Experimentation Committee (Reference AFA_2011.02), and was carried out according to the European Directive 86/609/ECC regarding the protection of animals used for experimental and other scientific purposes.

2.1. Experimental ewes, pre-natal facilities and treatments

The study was conducted between August 2011 and January 2012. Further details about experimental ewes and facilities are provided in Averós et al. (2014a,b). Briefly, 54 1–5-year-old pregnant ewes, randomly selected from the Neiker-Tecnalia flock, were divided into nine groups of six animals, 62 days after artificial insemination. Groups were balanced for body condition score, age and number of viable foetuses, and each group was randomly assigned to 1 of 3 pre-natal treatments: 1 (SA1), 2 (SA2), or 3 m²/ewe (SA3). Group size was kept constant at 6 ewes/pen, with three replicates/treatment. Ewes were kept in these pens until the end of the experiment, 16 days after the beginning of parturitions.

Feed was provided in a conveyor belt, with eight feeding spaces/pen. Further details about feeding are also provided in Averós et al. (2014a,b). Drinking water was available ad libitum through an automatic nipple drinker installed in each pen. From experimental week 4–8 (gestation week 12th to 16th) ewes had free access to salt blocks (TIMAC

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