# Maternal antibodies and immune responsiveness in growing lambs

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## ABSTRACT

In ruminants protection against environmental pathogens in the early post-natal period is largely dependent on passively-acquired immunity from maternal colostrum since evidence suggests placental transfer of antibody does not occur. Our overall aim is to develop antibody based treatments for protection of neonatal animals. In this study we have re-examined aspects of maternal antibody transfer to offspring especially in the light of recent evidence that feeding specific antibodies to neonatal rats decreases subsequent immune responses to the corresponding antigen. In experiment 1, pregnant ewes immunised with model antigen (human growth hormone) were terminated at 135 days gestation and blood samples collected from the ewe and fetus. Analysis of fetal plasma by antibody dilution/tracer binding assay procedures produced no evidence of antibody transfer to the fetal circulation via the placenta. In a further study the kinetics of specific antibodies were examined in lambs from immunised ewes. One group of lambs was permitted to suckle from the ewe for the duration of the trial (40 days) whereas the other group was permitted initial sucklings only, separated from the ewe 24 hours post partum and then fed a milk substitute. Analysis of specific antibody titres revealed a very similar trend in both the amount of antibody transferred to lamb plasma and its persistence with the half-life for both groups calculated at approx. 13 days. In the second experiment lambs from ewes immunised during pregnancy against *E.coli* and fed specific antibodies via maternal colostrum as neonates were compared to a matched control group of lambs derived from non-immunised ewes for subsequent immune responses against the same antigen. Peak antibody titres as analysed by ELISA were lower in lambs from immunised ewes than in control group lambs (mean titre ($-\log_{10}$) $5.29 \pm 0.19$ and $5.57 \pm 0.10$ respectively) although the difference was not significant ($p < 0.39$, unpaired T-test). As a consequence we have found using lambs little evidence to support previous findings in rats that feeding specific antibody to neonatal animals serves to suppress subsequent immune responses to the corresponding antigen.

**Keywords:** Antibodies; immune response; hyporesponsiveness; lamb; sheep.

## INTRODUCTION

In ruminant and other species placental transfer of immunoglobulin to the developing fetus is apparently absent (Brambell, 1970) leaving the neonate predominantly dependent on passively-acquired immunity from maternal colostrum for protection against environmental pathogens in the early post-natal period. Gastrointestinal (GI) pathogens such as some strains of *E.coli* cause severe scouring in newborn livestock and are associated with significant economic losses (Acres, 1985). Adherence of pathogen to the intestinal mucosa and production of enterotoxin are important mechanisms by which *E.coli* exerts its pathologic effects (Tzipori, 1981). Ingested colostral antibodies are known to reduce morbidity associated with *E.coli* infection, acting locally in the GI tract to prevent adhesion and colonisation by the bacterium. Further protection may be obtained by passive absorption of antibody in the period before gut 'closure' for later re-presentation at the level of the gut and the induction of active immunity in the neonate (Banks and McGuire, 1989). Mechanisms of induced immunity are, however, far from certain and it is noteworthy that in some studies feeding specific antibodies to neonatal animals has been found to decrease rather than increase subsequent immune responses to the corresponding antigen (Peppard, 1992). This phenomenon is believed to be discrete from the oral tolerance induced by feeding large amounts of antigen (André *et al.*, 1975; Swarbrick *et al.*, 1979). Owing to the significance of such observations to immunisation practices and eventual animal productivity, we have used specific immunoassays to re-examine aspects of antibody transfer and immune responsiveness using a hyperimmunised sheep model. The long-term aim of this work is to develop improved treatments for the passive protection of neonatal livestock.

## MATERIALS AND METHODS

### Experiment 1: Transfer of maternal antibodies to the fetal and neonatal animal

18 pregnant ewes were randomly divided into three groups (Group 1: pre-term slaughter, Group 2: normal suckling, Group 3: milk substitute) and systemically immunised at multiple intra muscular sites using human growth hormone (hGH; Jones *et al.*, 1979) emulsified in Freunds incomplete adjuvant (FICA; Commonwealth Serum Laboratories) as model antigen. Ewes were immunised 3, 2 and 1 month prior to parturition and blood samples collected before treatment and 2 weeks after each immunisation. At approximately 135 days gestation Group 1 ewes (n=6) were euthanised using sodium pentobarbitol, the fetuses immediately exposed and bled by direct cardiac puncture. Terminal bleeds were also collected from the Group 1 ewes. Group 2 lambs (n=6) were permitted to suckle from the ewes for the duration of the trial. Group 3 lambs (n=6) received maternal colostrum for the first 24 hours post partum only and were then separated from ewes and fed a milk substitute (Anlamb, Riverlea Dairies Ltd). Maternal and neonatal blood and colostrum/milk samples

from Group 2 and 3 animals were collected at 0, 0.1, 0.5, 1, 2, 5, 10, 20 and 40 days post partum. Blood and milk samples were collected onto ice, plasma separated by centrifugation (1350g and 2400g respectively, IEC PR-7000) and stored at -20°C for analysis. Residual fat was removed from milk serum by high-speed centrifugation (30,000g, Sorvall RC5C) to obtain an almost clear supernatant before analysis. hGH antibody levels in serum and milk were assessed by an antibody dilution/tracer binding procedure as described by Chard (1987), using as tracer, radiolabelled hGH prepared by the Iodogen (Pierce, Rockford, IL, USA) procedure (Salacinski *et al.*, 1981) and [$^{125}$I]-NaI (New England Nuclear). Separation of bound and free tracer was achieved by precipitation of the bound fraction using polyethylene glycol (PEG; Sigma 20% w/v) also as described by Chard (1987).

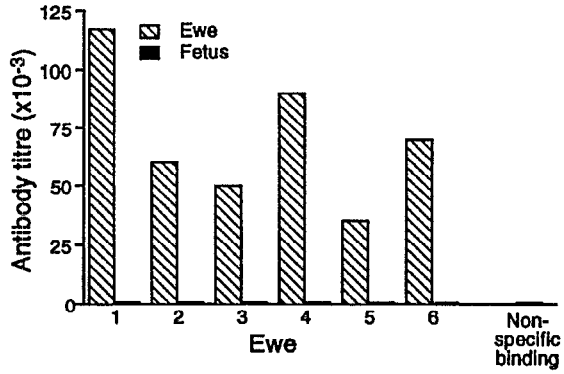
### Experiment 2: Subsequent immune responses of lambs fed specific antibodies as neonates

38 pregnant ewes were randomly assigned to Control and Treatment groups. The Treatment group (n=19) was immunised by multisystemic routes with an *E.coli* immunogen (Suvaxyn Maternafend-4, J & H Pacific Ltd) emulsified in FICA at four weekly intervals for the last three months of pregnancy and the Control group (n=19) received no treatment. Following parturition lambs remained with ewes until weaning (3 months) and then rested until residual *E.coli* antibody titres were undetectable in blood (8 months). Control and Treatment group derived lambs (n=19 per group) then received four intra muscular immunisations at four weekly intervals with the same *E.coli* immunogen emulsified in FICA. Blood samples were collected two weeks after each immunisation for determination of specific *E.coli* antibodies using an in-house ELISA method (Hodgkinson *et al.*, 1995). Briefly, micro titre plates (Maxisorp F-96 immunoplates, Nunc) were coated with antigen then sequentially incubated with sample (primary antibody) dilutions, rabbit anti-sheep IgG, and antibody-enzyme conjugate (goat anti-rabbit/horseradish peroxidase, Dako). Colorimetric endpoint detection employed 3, 3', 5, 5'-tetramethylbenzidine (TMB, Boehringer Mannheim) as substrate and measurement of optical density at 450nm using a microplate autoreader (BioTek EL311).

## RESULTS AND DISCUSSION

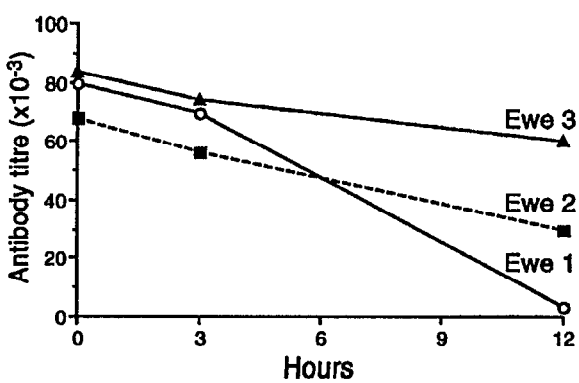
**Experiment 1:** In experiment 1 we re-examined placental transfer of immunoglobulin in the ruminant using hyperimmunised pregnant ewes with high titres of specific antibodies and assay methods allowing for very sensitive determination of antibody in the fetal circulation. Analysis of plasma samples from late gestation fetuses (135 days) of immunised ewes revealed no evidence of specific antibody despite maternal titres in the range 1/30,000 - 1/120,000 (Figure 1). This evidence substantiates earlier reports that placental transfer of antibody from the ewe to the lamb does not occur (Brambell, 1970). The tracer binding/antibody dilution technique used here for titre analysis is suitable for the estimation of specific antibody of all classes (eg IgG and IgA). Since specific antibody was not just low but undetectable in fetal plasma, the possibility that the placenta represents a selective barrier to the passage of the different immunglobulin isotypes is also not supported by the data.

**FIGURE 1:** Comparison of specific antibody titres in paired maternal and fetal sheep plasma collected at 135 days gestation.




Kinetics of antibody transfer from the ewe to lamb and metabolic clearance of antibody were also examined in experiment 1 using the periparturient group 2 and 3 animals. Specific antibody levels in representative colostrum samples are shown in figure 2. Antibody titres were high in first colostrum (~1/80,000) but not maintained, declining steadily with gland emptying (suckling) over the first 12 hours of lactation. In agreement with the fetal data, specific antibody was undetectable in newborn lamb plasma but increased rapidly over the first 3 hours following initial suckling and colostrum intake. Representative ewe and lamb plasma titre data are shown in figure 3. Lamb plasma antibody titres rose to maximal levels of 30-50% of early colostrum (1/25,000 - 1/40,000) within 48 hours of parturition and thereafter slowly declined with an average plasma half-life of approximately 13 days in general agreement with the observation of Stuen and colleagues (1992; 13-21 days). The amount and persistence of antibody in lamb plasma appeared similar whether animals received only an initial bolus of maternally-derived colostrum (Figure 3; Group 3) or continued to suckle throughout the trial (Figure 3; Group 2). These observations are consistent with the milk data (Figure 2) which showed a marked decline in antibody titre in early mammary secretions and developmental changes resulting in gut 'closure' which prevent antibody absorption in the lamb from approximately

**FIGURE 2:** Representative specific milk antibody titres in sheep during early lactation.

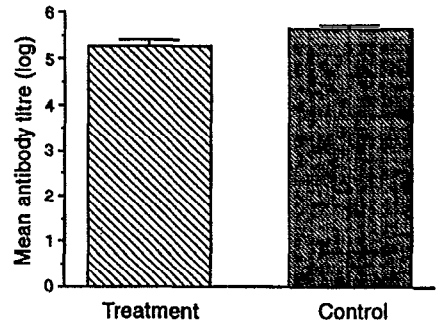


24 - 48 hours post partum (McCarthy and McDougall, 1952). The observations also underscore the key role of initial sucklings for the neonate in acquiring passive immunity.

**Experiment 2:** Good evidence suggests that immune responsiveness in maturing animals may be influenced by factors encountered in the early formative stage of neonatal development and that one such factor is maternal antibody (Peppard, 1992; Peri and Rothberg, 1981; Kindred and Roelants, 1974). In several studies, offspring from immunised animals have shown poorer immune responses to the corresponding antigen than offspring from control animals. However mechanisms of this effect have been difficult to determine and several factors may be involved. The route of antibody transfer may be significant and it is noteworthy that as studies to date have invariably been performed on rats, mice and rabbits, the possibility that direct placental transfer as opposed to oral ingestion of antibody accounts for the observed effects cannot be completely excluded. For this reason and because persistence of maternally-derived antibody into adulthood has often been reported as the causative factor (Jarrett and Hall, 1984), we have examined the phenomenon of maternally-induced immune hyporesponsiveness in lambs.

In experiment 2, specific antibody titres were found to be higher in Control than Treatment group derived lambs (Figure 4; mean titre ($-\log_{10}$) 5.57 and 5.29 respectively) but the difference was not significant ($P < 0.39$, unpaired T-test). The lower response of Treatment group derived lambs is consistent with the concept of maternally-induced immune hyporesponsiveness in sheep although the treatment effect observed here is much less marked than described previously for the rat (Peppard, 1992). Failure to demonstrate a significant difference in specific antibody titres between Control and Treatment group animals may be interpreted as suggesting that an immune response of this kind does not occur in the sheep in contrast to the rat. If this is so, one explanation may be a role for placental transfer of antibody in development of the response. However observations that peak titre responses were much more variable in Treatment (S.E.M., 0.19) than

**FIGURE 4:** Influence of feeding specific antibodies to neonatal lambs via maternal colostrum on subsequent immune responses to the corresponding antigen. The diagram shows log mean and SEM peak antibody titre responses at 12 months of lambs from immunised ewes (Treatment) which received hyperimmune colostrum and a matched group of lambs from unimmunised ewes (Control).




Control (0.10) groups suggest that other factors may be involved. For example the class of ingested antibody, the dose and timing.

In summary: 1. We have re-examined placental transfer of antibody in hyperimmunised sheep and found no evidence for transfer of maternal antibodies into the fetal circulation. 2. The persistence of maternal antibody in neonatal lamb plasma appeared very similar whether animals received only an initial bolus of immune colostrum or continued to suckle for an extended period re-affirming the key role of initial sucklings for the neonate in acquiring systemic immunity. 3. We have critically examined the concept of maternally-induced immune hyporesponsiveness, which suggests feeding specific antibody to neonatal animals serves to suppress subsequent immune responses to the corresponding antigen, and found little evidence to support the phenomenon in the sheep in contrast to earlier reports for the rat.

## ACKNOWLEDGMENTS

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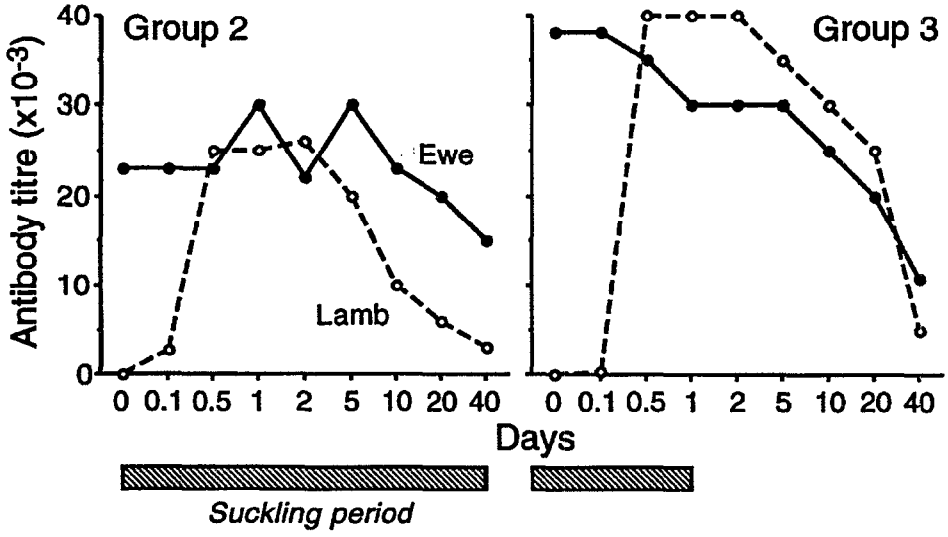
**FIGURE 3:** Plasma antibodies in a representative ewe and lamb pair during the neonatal period; relationship to suckling period.



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