FACTORS AFFECTING IgG CONCENTRATION IN DAY-OLD LAMBS

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SUMMARY

Blood and colostrum were collected from 28 ewes at parturition and blood from their 49 lambs at 0, 8 hr and 24 hr postpartum. Serum and colostral IgG were determined by radial immunodiffusion. Ewes at parturition averaged 21.3 ± 1.5 mg/ml of IgG in serum and 115.1 ± 10.1 mg/ml in colostrum. Prior to nursing, the lambs had .07 mg/ml of serum IgG. At 8 hr after nursing, the lambs serum IgG levels had increased to 25.3 mg/ml and by 24 hr to 35.6 mg/ml. Associated with this increase in serum IgG was a decrease (P<.01) in hematocrit. The correlation (r) of colostral IgG concentration with the lamb serum IgG level at 8 hr and 24 hr was .37 and .34. After nursing, there were no differences (P>.05) in serum IgG levels at 24 hr postpartum between male lambs and female lambs (35.7 mg/ml vs 32.6 mg/ml) and between first born lambs and second born lambs (30.5 mg/ml vs 32.9 mg/ml). Despite 11/49 lambs having <15 mg IgG/ml at 24 hr postpartum, only two lambs died during the first month of life.

(Key Words: IgG, Lambs, Colostrum, Blood, Hematocrit, Immunoglobulins.

INTRODUCTION

The principal immunoglobulin in the colostrum of species that transmit only small or no amounts of immunoglobulin to their offspring in utero is IgG (Larson, 1974). In sheep, it constitutes 92% of the total while approximately 6% is IgA and 2% is IgM (Smith et al., 1975). Ingestion and absorption of IgG via colostrum are extremely important to the survival and well being of the newborn calf, lamb and pig (Hemmings, 1976). Many thousands of dollars are lost each year as a result of unthrifty hypogammaglobulinemic animals which are many times more susceptible to infection than their “normal” counterparts (Fey, 1971; Campbell, 1974; Halliday, 1974b). Therefore, failure of the newborn to receive passive immunity through ingestion and absorption of immune globulins is a major concern to the food-animal producing industry.

Although much is understood today concerning conference of passive immunity to the newborn bovine, little research has been devoted to the ovine species. The objectives of this study were to determine the relationship between the level of IgG in the ewe's serum, colostrum and that in the day-old lamb; to determine whether the IgG concentration in the newborn lamb is influenced by litter size, order of birth or sex and to determine whether survival of the newborn lamb is dependent on the concentration of its passively acquired IgG.

MATERIALS AND METHODS

Colostrum and Serum Samples. Postpartum colostrum and blood serum samples were collected from 26 Columbia and two Hampshire ewes at the University Experimental Station in St. Paul, Minnesota. Prior to suckling, equal amounts of colostrum were manually withdrawn from each quarter and frozen immediately. A blood sample was obtained at the same time.

Blood samples were taken from lambs at birth and at 8 hr and 24 hr postpartum via the tail vein in the following manner. Small animal clippers were used to remove the wool from the tip of the tail. The capillary bed was then arterialized by placing the shaved tail in a small beaker of hot water (80 C). After disinfecting thoroughly with 70% alcohol, a small incision was made approximately 7 mm from the end of the tail and 10 to 12 capillary tubes of blood were collected. The samples were centrifuged in a microhematocrit centrifuge for 10 minutes. The capillary tubes were scored with a three-cornered file at the cell-serum interface, broken, and serum was blown into small plastic vials and frozen.

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Preparation of Ovine IgG. The isolation of ovine IgG was accomplished as outlined in Figure 1. Purity of the IgG was established by ultracentrifugation (Schachman, 1959) and by immunoelectrophoresis (Weime, 1959) using rabbit anti ovine serum.

Antiserum to IgG. Normal serum was obtained from a rabbit before immunization. Purified ovine IgG (5 mg) was suspended in .5 ml of saline. The immunization scheme consisted of intradermal injections of .5 ml of IgG mixed with .5 ml of Freund's complete adjuvant into five sites in the scapular region. This was followed by four weekly injections of .5 ml of IgG mixed with .5 ml of Freund's incomplete adjuvant. One week after the fourth injection, the animal was bled by venipuncture. Serum was recovered and frozen until used.

Quantitation of Ovine IgG. Blood samples and colostrum samples were assayed for IgG concentrations by modification of the radial immunodiffusion gel procedure (Fahey and McKelvey, 1965; Mancini et al., 1970). The monospecificity of the rabbit anti ovine IgG was established by immunoelectrophoresis using ovine serum and purified ovine IgG.

A standard curve was prepared for each using six known concentrations of purified ovine IgG. The correlation coefficient (r) was .99 for precipitin ring diameter and IgG concentrations between .5 mg/ml and 16 mg/ml. All samples were diluted to fall in that range. Duplicate samples were run and repeated if their ring diameters deviated by more than .8 millimeters.

The Relationship between the Level of IgG in Ewe Serum and Colostrum. Table 1 presents the IgG concentration in ewe's serum and colostrum. Twenty-eight ewes averaged 21.3 mg/ml of IgG in the serum and 115.0 mg/ml in the colostrum. In all cases, colostral IgG concentration was higher than that of serum. However, as indicated in Table 1 there was great variation between individuals. The average difference between colostral and serum IgG levels was five-fold. This value corresponded reasonably with that reported for the pig, horse and cow (Larson, 1974).

The serum IgG level and the colostral IgG level were not correlated (r = -.03). Therefore, there are separate physiological factors which regulate the concentration of IgG in these fluids. The predominant immunoglobulin in sheep colostrum is IgG1 (Cuperlovic, 1970). It is a blood transudate (Lascelles and McDowell, 1974). Cripps and Lascelles (1974) demonstrated that the 'half-life' of serum IgG was reduced during colostrum formation, whereas there was no significant reduction in that for serum IgG2. They suggested that transfer of serum derived IgG1 across the glandular epithelium of the sheep mammary gland was a selective process requiring the existence of specific receptor sites located on the basal membrane of the epithelial cell.

Antiserum for IgG1 will cross-react with IgG2. Therefore, no distinction has been made in this study between relative amounts of IgG1 and IgG2 in either colostrum or serum samples. However, IgG1, which is selectively concentrated into ewe's colostrum from the serum during late pregnancy, is the main component absorbed by the lamb (Osebold et al., 1965; Cripps and Lascelles, 1974).

Factors Affecting the IgG Concentration in Ewe's Serum and Colostrum

Table 1. IgG Concentrations in Ewe's Serum and Colostrum

<table>
<thead>
<tr>
<th>No. of ewes</th>
<th>IgG (mg/ml) a in Serum</th>
<th>IgG (mg/ml) a in Colostrum</th>
</tr>
</thead>
<tbody>
<tr>
<td>28</td>
<td>21.3 ± 1.5</td>
<td>115.1 ± 10.1</td>
</tr>
<tr>
<td></td>
<td>(9.2 – 37.2)</td>
<td>(43.1 – 260.3)</td>
</tr>
</tbody>
</table>

a Mean ± SE with range in parentheses.

300 ml Ovine colostrum
30,000 Xg 2 Hr
Fat and sediment
Discard
Supernatant
1:2c
H2O
pH 4.6
e in HCl
Casein
Whey, pH 7
50% (NH4)2SO4 ppt
8 ml:25 mg/ml
Sephadex G-200
5 cm X 73.5 cm eluted c 5% NaCl
Peak 3
150,000 M.W.

Breakthrough
IgG, Lyophy.

Other peaks

Figure 1. Scheme for isolation of Ovine IgG.
TABLE 2. LAMB IgG LEVELS AND HEMATOcritS AT 0, 8 HR AND 24 HR AFTER BIRTH

<table>
<thead>
<tr>
<th>Time hr</th>
<th>Serum IgG mg/ml</th>
<th>Hematocrit %</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.07 ± 0.00 (0 - 0.64)</td>
<td>45.10 ± .88 (36 - 62)</td>
</tr>
<tr>
<td>8</td>
<td>25.34 ± 2.70 (0 - 77.71)</td>
<td>42.14 ± .84 (31 - 57)</td>
</tr>
<tr>
<td>24</td>
<td>35.56 ± 3.27 (0 - 102.0)</td>
<td>39.89 ± .88 (28 - 53)</td>
</tr>
</tbody>
</table>

aMean ± SE with range in parentheses for 48 lambs.

Newborn Lambs. Table 2 shows the serum IgG change in 48 newborn lambs during the first 24 hr of life. Seven of the 48 lambs (14.5%) had measurable amounts of IgG present at birth. This finding was consistent with Klaus’s et al. (1969) work in which they found trace amounts (.1 to 2.0 mg/ml) of IgG in fetal calf sera. At 8 hr after feeding, the lamb’s serum IgG levels had increased to 25.3 mg/ml and by 24 hr to 35.6 mg/ml. This increase in serum IgG levels was due to absorption of intact IgG from ingested colostrum. Lambs absorb maternal immunoglobulins mainly during the first day after birth, although they may absorb small amounts during the second day (McCarthy and McDougall, 1953; Lundqvist, 1962; Halliday, 1971).

Associated with this increase in serum IgG was a marked decrease (P<.01) in hematocrit over the period from birth to 24 hr old (table 2). Several workers (McEwan et al., 1968; McCance and Widdowson, 1959; Pownall, 1970; Pownall and Dalton, 1973) observed that after the consumption of colostrum by bovine and porcine neonates there was a marked decrease in hematocrit. This was accompanied by a rise in plasma protein and plasma volume between birth and 72 hr of life. The expansion in plasma volume appeared to be accounted for by a dilution effect since plasma osmotic pressure, plasma Na concentration and plasma K concentration decreased. The increase in plasma protein was accounted for by absorption of albumin and gamma globulin from the colostrum.

The decrease in hematocrit may be explained both by the dilution effect of increased plasma volume and by the normal physiological hemolysis of red blood cells experienced in neonates shortly after birth (Crabo et al., 1970). There exists an inverse relationship between hematocrit and gamma globulin levels (McEwan et al., 1968) in newborn calves which received colostrum. The results presented in table 2 on lambs supported that finding. Thus, hematocrit determinations may be a useful clinical tool for the quick appraisal of the day-old ruminant’s passive immunity status.

The amount of colostral immunoglobulin is limited and decreases rapidly after the first milking. Hence large litters may obtain inadequate immunoglobulin if the amount of colostrum secreted is small. Since twinning in the ovine species is common and economically desirable, the question as to whether twin lambs would absorb less IgG and thus have less immunological protection than singleton lambs was studied. Table 3 shows that singleton lambs had absorbed more IgG at 8 hr and 24 hr after birth than twin lambs but this difference was not significant (P>.05) since there were only seven singleton lambs born in this study vs 42 twins.

At 24 hr of age, the twin lambs had an IgG serum level that was 72% of that for singleton lambs. Halliday (1974b) also reported a similar but significant difference (75%). The difference between triplets and single lambs was even more marked (Halliday, 1974a,b). However, the effect of litter size is much less marked in

<table>
<thead>
<tr>
<th>Age</th>
<th>n</th>
<th>IgGa</th>
<th>n</th>
<th>IgGa</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 hr</td>
<td>7</td>
<td>33.6 ± 9.4</td>
<td>42</td>
<td>23.4 ± 2.7</td>
</tr>
<tr>
<td>24 hr</td>
<td>7</td>
<td>47.5 ± 6.1</td>
<td>42</td>
<td>34.2 ± 3.8</td>
</tr>
</tbody>
</table>

aMean ± SE in mg/ml.
Finnish sheep, which have unusually large litters, than in other breeds (Halliday, 1968, 1973, 1974a,b, 1976).

In same sex twins, males had higher (P<.05) concentrations of IgG than females (43.42 ± 12.33 mg/ml vs 19.57 ± 7.13 mg/ml). However, when singleton and twin data were combined, the serum IgG levels in 22 male and 23 female lambs were 35.7 ± 4.1 mg/ml and 32.6 ± 4.4 mg/ml, respectively. This was not a significant difference (P>.05). These IgG sex associated findings agreed with immunoglobulin findings reported by Halliday (1974b).

Twins are not born at the same time. In this study, based on data from 19 ewes, 36.5 min ± 1.6 min (SE) elapsed before the second lamb was born. It was hypothesized that the first-born of a twin pair might have an immunological advantage over its siblings. However, a comparison of IgG levels in first-born lambs vs second-born (table 4) revealed no difference (P>.05). These results confirmed a previous study by Halliday (1974b).

However, first access perhaps would be a more critical factor in low producing or young ewes where colostrum supply was limited. The ewes in this study were all multiparous and colostrum supply could not be accurately measured. However, subjective estimates of colostrum supply (volume) were made at parturition by one observer. The volume estimates were based on the size of the udder, the feel of the udder (to rule out edema), and the ease with which 5 to 10 ml of colostrum could be withdrawn from the udder. Based on these estimates, the ewes were grouped into three categories: "small", "medium" and "large" potential colostrum suppliers.

Examination of table 5 shows the lambs' which nursed the three ewes with "small" amounts of colostrum, had considerably less serum IgG than the other lambs at 8 and 24 hours. The second-born lambs from the ewes having a "small" amount of colostrum were practically hypogammaglobulinemic. This suggests a limited colostrum supply for multiple offspring. However, two singleton lambs nursing ewes classified as having "small" amounts of colostrum, obtained enough IgG to raise serum values to 56.5 mg/ml by 24 hours. Therefore, there was adequate colostrum for singletons. The lambs from ewes classified as having "medium" amounts of colostrum, absorbed the most IgG. This may be explained on the basis of concentration. Table 5 shows the IgG concentration in ewes with "large" amounts of colostrum, was less than those in the "small" and "medium" categories. Therefore, for the lambs of these ewes to absorb an equal amount of IgG they must consume larger amounts of colostrum.

In the present study, the correlation (r) of initial colostral IgG concentration with the lamb serum IgG level at 8 hr and 24 hr was .37 and .34. Halliday (1974b) reported that the serum immunoglobulin concentrations in lambs were not affected by variations in the colostrum immunoglobulin concentrations. Bush et al. (1971) reported approximately 68% of the variation in blood serum immunoglobulins in calves at 24 hr postpartum was due to differences in immunoglobulins consumed per unit of weight. They (1973) found that the absolute amount of IgG consumed, but not its concentration in colostrum had a significant effect on blood concentration of IgG. However, it should be noted that, even though IgG is ingested, it may not be fully absorbed through the intestinal lining.

It is realized that the data presented in table 5 because of its qualitative nature and small numbers are quite vulnerable to criticism. However, the authors are including it here as an interesting observation and to emphasize a need for more research in this area.

**Table 6** presents this data. Though all lambs received colostrum two lambs were agammaglobulinemic at 24 hours. One of these survived. Nine lambs were hypogammaglobulinemic (<15 mg/ml) and eight survived. Thirty-eight lambs had IgG levels greater than 15 mg/ml.

**Table 4. IgG Levels in First Born vs Second Born Lamb**

<table>
<thead>
<tr>
<th>Age</th>
<th>Sets of twins</th>
<th>First born IgG (mg/ml)</th>
<th>Second born IgG (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 hr</td>
<td>20</td>
<td>23.1 ± 3.9</td>
<td>24.5 ± 4.2</td>
</tr>
<tr>
<td>24 hr</td>
<td>20</td>
<td>30.5 ± 5.0</td>
<td>32.8 ± 4.2</td>
</tr>
</tbody>
</table>
TABLE 6. SERUM LEVEL OF IgG IN LAMBS AT 24 HR POST BIRTH AND SURVIVORS AT 1 MONTH OF AGE

<table>
<thead>
<tr>
<th>Serum IgG (mg/ml)</th>
<th>Lambs born at 24 hr</th>
<th>Alive at 1 month</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>&lt;15 mg/ml</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td>&gt;15 mg/ml</td>
<td>38</td>
<td>38</td>
</tr>
<tr>
<td>49</td>
<td>47</td>
<td></td>
</tr>
</tbody>
</table>

than 15 mg/ml and all survived. Passive immunization and neonatal nutrition are closely related—immunoglobulin concentrations are a measure of both—and their relative importance depends on the environment at birth (Halliday, 1974a). The lambs in this study were born in a very clean, dry, heated lambing barn. After 1 week they were moved with their dams to an unheated sheep shed but had access to heating lamps. These conditions were no doubt ideal compared to range conditions where lambs are born in the open and probably account for the survival of all of our hypogammaglobulinemic lambs.

The dividing line between hypogammaglobulinemic and “normal” serum IgG levels was arbitrarily set at 15 mg/ml and is perhaps unique to this study. However, more work is needed under various environmental conditions to establish what constitutes a “normal” concentration of serum IgG in lambs.

Harker (1973) found that lambs with lighter birth weights had lower levels of serum immunoglobulins. More deaths occurred in the lighter lambs but the mortality increased again in those groups of lambs with birth weights 4.5 kg or more. Halliday (1974b, 1976) found the 48 hr postpartum immunoglobulin concentrations were significantly higher in lambs which subsequently survived for at least 6 months after sampling, than in lambs which died earlier. However, many lambs also survived with low to non-detectable serum immunoglobulin concentrations. Campbell (1974) concluded that agammaglobulinemia or hypogammaglobulinemia due to colostral deprivation should be considered as a possible primary cause of the animals’ demise in lambs dying during the first week of life of colisepticemia, pasteurellosis or other generalized bacterial infections.
LITERATURE CITED


