ABSTRACT

Foetal fluids are important in preventing mechanical shock to the developing foetus during entire gestation. Amniotic and allantoic fluids are actively involved in constant exchange of biochemical substance between foetus and maternal circulation. Hence the knowledge regarding changes in the composition of the foetal fluid is important for understanding foetal metabolism and diagnosis of pathological conditions during gestation. A study was conducted in sixty gravid sheep uteri. The four stages of gestation as Stage - I (30-60 days), Stage - II (61-90 days), Stage - III (91-120 days) and Stage - IV (121 to term) were identified based on crown anus length of the embryo/foetus. The amniotic and allantoic fluids collected from the gravid uteri of each group were subjected to biochemical analysis of glucose, protein, urea and creatinine. The levels of glucose, total protein, urea and creatinine were increased in the amniotic fluid with gestation. The concentration of glucose and total protein were increased with pregnancy in the allantoic fluid, while those of urea and creatinine were decreased. Allantoic fluid had significantly higher concentration of total protein and creatinine in all the four stages of gestation than amniotic fluid.

Key words: Amniotic fluid, Allantoic fluid, Glucose, Total protein, Urea, Creatinine

INTRODUCTION

The foetoplacental unit is a dynamic system, with water and fluid constituents in constant exchange between foetal fluid compartments and maternal circulation (Baetz el al., 1976). The amniotic and allantoic fluids contain metabolic constituents, electrolytes, proteins, enzymes, hormones, cells and other statures (Hafez, 1980). The composition of foetal fluids of mammals is influenced by the excretion of foetal urine. The changes in the concentration of number of components during the late gestation may indicate the major developmental changes reflecting the metabolic activity occurring in the foetus (Aidasani el al., 1992). A broad knowledge of amniotic fluid is of the utmost importance in understanding foetal metabolism and identifying pathological conditions during pregnancy (Prestes et al., 2001).

MATERIALS AND METHODS

Sixty gravid uteri of Madras Red breed of sheep of different parity were collected from Chennai Corporation slaughter house and brought to the laboratory in ice. The gravid uteri were washed and cut open along the dorsal curvature without damaging the foetal membrane. The foetal sacs were exposed. Amniotic and allantoic fluids were aspirated separately in 15 ml screw capped
specimen tubes, centrifuged at 50 x g for 10 minutes to remove the cellular debris and stored in the deep freezer at - 400C for biochemical analysis. The embryo/foetus was removed from the uterus and the crown anus length of the foetus from the vertex of the skull to the anus was measured using a measuring tape. The stages of gestation and age of the foetus were determined by applying the Richardson formula (1980). Based on the age of the foetus the course of gestation was divided into four stages as Stage - I (30-60 days), Stage - II (61-90 days), Stage - III (91-120 days) and Stage - IV (121 to term). Each experimental group consisted of fifteen gravid uteri.

The following biochemical profile in the amniotic and allantoic fluids was estimated

<table>
<thead>
<tr>
<th>Biochemical Parameter</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>O-Toluidine method (Kaneko, 1997)</td>
</tr>
<tr>
<td>Total protein</td>
<td>Biuret method (Doumas et al., 1978)</td>
</tr>
<tr>
<td>Urea</td>
<td>DAM method (Finco, 1997)</td>
</tr>
<tr>
<td>Creatinine</td>
<td>Alkaline picrate method (Toro and Ackermann, 1975)</td>
</tr>
</tbody>
</table>

Statistical analysis was done by complete randomized design and the comparison between the amniotic and allantoic fluids was made by paired t-test (Snedcor and Cochran, 1989).

**RESULT AND DISCUSSION**

The concentrations of glucose, total protein, urea and creatinine are presented in the table.

Glucose revealed the lowest and highest concentrations in the amniotic fluid in the first stage of gestation. The observation of this study differ from that of Bradley and Mistretta (1973), Reddy et al. (1995), Tangalakis et al. (1995) and Prestes et al. (2001) in sheep. The difference may partly due to the gravid uteri being obtained from slaughterhouse wherein the animals were of different nutritional status. The high level of glucose in the second stage might probably be related to the increased glucose content in the foetal plasma (Mellor and Slater, 1973). Until about 130 days of gestational age in sheep, exchange of glucose occurred between the amniotic sac and foetal blood. Changes in the foetal fluid had an orientation of foetal adrenocortical activity towards glucose homeostasis (Mellor and Slater, 1974). Elevated levels of glucose in the amniotic fluid might be associated with concurrent cortisol secretion by the foetus (Williams et al., 1993). In normal pregnancy, amniotic fluid glucose levels correlated with simultaneous maternal glucose concentrations (Archimaut et al., 1974) and rose acutely in response to maternal glucose load (Greco et al., 1980). Glucose from the maternal circulation was the main energy source to the foetus during normal pregnancy (Jainudeen and Hafez, 1980).

The level of glucose declined relatively insignificant from second to fourth stage of gestation. The glucose concentration in the allantoic fluid was significantly higher (P< 0.01) in stage IV and III when compared to stage I and II of gestation, while the glucose levels between fourth and third stages did not differ significantly. This finding is different from the report of Mellor and Slater (1973) in sheep.

The glucose concentration in the allantoic fluid was significantly higher in stages III and IV when compared to stages II and I. This finding differed from the report of Mellor and Slater (1973) in sheep. The increased glucose content of the allantoic fluid during late gestation might reflect glucogenesis occurring in sheep foetus triggered by thyroid hormone (Fowden et al., 2001). The relatively greater permeability of the chorioallantoic membrane suggested that foetal blood in the chorioallantoic and maternal blood in the endometrium were potential source of glucose in the allantoic fluid. However, the parallel changes and relative equality of the glucose concentrations in both fluids and the delay in the response of allantoic fluid glucose to changes in maternal plasma
levels, suggested that foetal plasma in the chorioallantoic might be the major source of glucose (Mellor and Slater, 1973).

The amniotic fluid contained significantly higher (P< 0.05) level of glucose than allantoic fluid in the second stage of gestation, where as there was no significant difference in the glucose levels of the amniotic and allantoic fluids during the other three stages of gestation. The findings in the second stage of gestation are in agreement with the observations of Wintour and McFarlane (1993) in sheep. This could be due to increased demand for glucose by the foetus during stage II of gestation.

The level of total proteins in the amniotic fluid showed a relatively insignificant increase from the first to the second stages of gestation. Significantly higher (P < 0.05) concentrations of total proteins were recorded in the third and fourth stages in comparison with the first stage of gestation. However, there was no significant variation between the second, third and fourth stages. This is in agreement with the observation of Tuboly et al. (1979) in sheep. In contrast Reddy et al. (1995) reported a decline in total protein content at fourth month of gestation. According to Reddy et al. (1995) the lower concentration of amniotic total protein could be attributed to the absence of the fibrinogen and other proteins due to foetal immunity.

Amniotic fluid proteins were apparently derived from both maternal and foetal sources. The maternal contribution was principally the globulin fraction, while the foetus contributed prealbumin, albumin, ? -fetoprotein and sex hormone binding globulin. Passage of proteins across the amnion might have occurred in either direction; the net quantity remaining in amniotic fluid also bears upon apparent concentration (Dito, 1970). The foetus synthesized all its proteins from the amino acids derived from the mother, proteins were used mainly for synthesis rather than oxidation or gluconeogenesis (Jainudeen and Hafez, 1980).

The highest concentration of protein was observed in the allantoic fluid at the fourth stage of gestation, followed by the second and third stages, though there was no significant difference between stages IV, II and III. Significantly higher (P < 0.01) total protein concentration was recorded in stage II when compared to stage I. On contrary Tuboly et al. (1979) concluded that the total protein concentration in the allantoic fluid of sheep showed no change between less than three months of gestation. Areolar secretions were likely source of the allantoic fluid proteins (Bazer, 1975).

The allantoic fluid contained highly significantly higher total protein (P < 0.01) than amniotic fluid at the first, third and fourth stages and significantly (P < 0.05) more protein than amniotic fluid in the second stage of gestation. This finding went along with that of Wintour and McFarlane (1993) in sheep.

The lowest urea concentration was recorded in the amniotic fluid in the first stage of gestation. Significantly increasing trend was observed from stage I to stage III, though the levels of urea in the amniotic fluid did not differ significantly between the second, third and fourth stages of gestation. This finding coincided with the report of Anderer and Schinder (1975) who stated that high levels of urea in amniotic fluid are primarily determined by foetal micturition in the cavity. There was an insignificant difference between the urea concentrations in the first and fourth stages of pregnancy.

In early pregnancy, the concentrations of urea were the same in amniotic fluid as in interstitial fluid elsewhere; leading support to the view that amniotic fluid might be an ultrafiltrate of maternal serum at that stage. Foetal urine was the main contributor of urea to amniotic fluid in the second half of gestation (Benzie et al., 1974).
The urea concentrations of the allantoic fluid increased significantly from second to third stage of gestation. Where as third and fourth stages indicated no significant variation in the urea concentration. The allantoic fluid contained significantly (P < 0.01) higher level of urea than the amniotic fluid in all the stages except the third stage, which showed an insignificant difference between the fluids. This observation is in agreement with those of Wintour and McFarlane (1993) and Tangalakis et al. (1995) in sheep. Entry of foetal urine into fluid sac had increased the urea concentration of the allantoic fluid (Mellor and Slater, 1972, 1973). In sheep until day 75 of gestation, foetal urine drained exclusively into the allantoic sac and in the final third of pregnancy, foetal urine drained equally into both amniotic and allantoic sacs (Tangalakis et al., 1995).

In amniotic fluid, significantly higher (P < 0.01) levels of creatinine were recorded in stages III, IV and II of gestation when compared to stage I. However there was no significant difference between these three stages. This finding is in agreement with those of Prestes et al. (2001) in sheep. As gestation advanced, the level of creatinine raised, which might indicate increased protein metabolism in the foetus. Creatinine in amniotic fluid was derived by the conversion of creatine in foetal muscle and was a reflection of increasing foetal muscle mass and foetal glomerular filtration (Chez et al., 1964).

The allantoic fluid had significantly higher (P < 0.01) creatinine concentration in the first stage, which declined significantly in stage II and III, but showed a relatively insignificant increase in the fourth stage of gestation. This finding is in contrast to the report of Anderer and Schinder (1975). This could be due to dilution resulting from increase in the allantoic fluid volume during advancement of gestation. The allantoic fluid contained significantly more (P < 0.01) creatinine than the amniotic fluid in all the four stages of pregnancy. Wintour and McFarlane (1993) and Tangalakis et al. (1995) reported the same findings in sheep. The lack of exchange of solutes from the allantoic fluid might have increased the creatinine level in comparison with amniotic fluid (Stanier, 1965).

REFERENCES


Biochemical characteristics of amniotic and allantoic fluid in late gestational mares. Theriogenology 40: 1241-1257.


Table

<table>
<thead>
<tr>
<th>Biochemicals</th>
<th>Foetal fluid</th>
<th>Stage - I</th>
<th>Stage - II</th>
<th>Stage – III</th>
<th>Stage - IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dl)</td>
<td>Amniotic</td>
<td>17.09a ± 3.52</td>
<td>75.25b ± 12.46</td>
<td>60.85b ± 5.69</td>
<td>57.29b ± 4.31</td>
</tr>
<tr>
<td></td>
<td>Allantoic</td>
<td>21.50a ± 2.19</td>
<td>34.33a ± 6.05</td>
<td>62.37b ± 12.23</td>
<td>67.00b ± 5.70</td>
</tr>
<tr>
<td>Total Proteins (g/dl)</td>
<td>Amniotic</td>
<td>0.51a ± 0.08</td>
<td>0.91ab ± 0.30</td>
<td>1.02b ± 0.09</td>
<td>1.17b ± 0.08</td>
</tr>
<tr>
<td></td>
<td>Allantoic</td>
<td>0.91a ± 0.09</td>
<td>2.01b ± 0.35</td>
<td>1.54ab ± 1.45</td>
<td>2.71b ± 0.26</td>
</tr>
<tr>
<td>Urea (mg %)</td>
<td>Amniotic</td>
<td>26.61a ± 1.97</td>
<td>38.99b ± 4.01</td>
<td>38.75b ± 2.47</td>
<td>34.45ab ± 0.49</td>
</tr>
<tr>
<td></td>
<td>Allantoic</td>
<td>71.99b ± 3.99</td>
<td>64.98b ± 6.87</td>
<td>40.65b ± 3.23</td>
<td>42.43a ± 0.53</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>Amniotic</td>
<td>8.32a ± 0.90</td>
<td>12.37b ± 1.20</td>
<td>13.53b ± 0.49</td>
<td>12.57b ± 0.70</td>
</tr>
<tr>
<td></td>
<td>Allantoic</td>
<td>57.21b ± 7.13</td>
<td>31.33a ± 2.07</td>
<td>36.33a ± 2.12</td>
<td>44.57ab ± 1.67</td>
</tr>
</tbody>
</table>

Means in the same row bearing different alphabets differ significantly (P < 0.01 and P< 0.05)