Introduction

The importance of dexamethasone is demonstrated by its wide uses in human and veterinary medical practices [1-4]. Clinically, dexamethasone is administered to animals for the treatment of disease conditions such as canine distemper, meningitis, necrotizing enterocolitis [5-10]. In humans, dexamethasone is most notably, used for the treatment of various complications, such as preterm complications to stimulate surfactant production by the lungs [3,11], intrahepatic cholestasis [12] and inflammatory conditions during pregnancy [13]. These actions are critical to prepare the fetuses for extrauterine life. Although this treatment greatly improves fetal and neonatal survival [3,14], they are not without adverse effects. Dexamethasone use has been associated with intrauterine fetal growth restriction (IUGR), decrease birth weight and placental weights in some animal models as well as humans [15-22].

The mechanisms underlying inhibitory effects of dexamethasone have largely not been elucidated. However, dexamethasone may probably exert its effect through fetal fluid medium. Amniotic and allantoic fluids protects the fetus by forming a protective physical cushion around it and allows the developing fetus to move freely inside the uterus and prevent teratogenicity due to adhesion and maintains an environment of constant temperature [23-25]. Fetal fluids are usually produced by placenta and other placentomes or fetal membrane during gestation until kidneys take over the task [23,24]. Other sources of fetal fluids include secretions from amniotic epithelium, fetal saliva, and nasopharyngeal secretions and skin, excretions of fetal kidneys and allantoic epithelia [26]. The biochemical constituents of amniotic fluid and allantoic usually provide valuable information for the assessment of fetal physiology and...
metabolism [24]. The objective of this study was to evaluate and compare the effects of dexamethasone on fetal fluid volume and biochemical parameters in Yankasa sheep and Sahel goats.

Materials and Methods

We adopted some aspects of the methods of Yahi et al. [22, 27] in our methodology.

Ethical consideration

All procedures involving animals were reviewed and approved by the Faulty Post graduate board committee of the Faulty of Veterinary Medicine, University of Maiduguri and cleared by School of Post graduate Studies, University of Maiduguri, and done according to ethical standards concerning animal welfare and the rules, regulations, and laws for the humane treatment of animals as spelt by Consensus Guidelines on Animal Ethics and Welfare for Veterinary Journals (International Association of Veterinary Editors, Geneva, Switzerland, 2010).

Animals and management

Twelve adult Sahel goats comprising 10 does and 2 bucks and 12 Yankasa sheep comprising of 10 ewes and 2 rams were used for this study. The animals were sourced from main livestock markets within Maiduguri metropolis, Nigeria. The ages of the female ranged between 2½ and 3 years, while that of the males were between 3 to 3½ years. The does weighed between 20 to 25kg and the bucks 30kg and 34kg. The ewes weighed between 30 to 35kg; the weights of the rams were 40 to 45kg. The body condition score (BCS) between 3.0 and 3.5 was maintained during the period of the experiment in all the animals. Breeding history, abdominal palpation and ballottement, nature of mammary secretions, conditions of udder were used in the initial selection of the non-pregnant ones [28].

They were managed intensively in the University of Maiduguri Livestock research Farm and were acclimatized for six weeks before the commencement of the experiment. The feed rations consisted of wheat offal, beans husks and hay from groundnut leaves. Mineralized salt licks and water were provided ad libitum. During the stabilization period, the animals were treated with oxytetracycline LA (Introxin-200®, Interchiemie, Venray, Holland) at 20mg/kg body weight I/M and ivermectin (Acon Labs.Inc., San Diego, USA). Fetuses harvested during the experiment in all the animals. Breeding history, abdominal palpation and ballottement, nature of mammary secretions, conditions of udder were used in the initial selection of the non- pregnant ones [28].

Estrus synchronization

The animals were synchronized at the end of the acclimatization period using cloprostenol (Estrumate®, Schering Trough Animal, Germany) at 250µg given intramuscularly 11-day interval, as reported previously [29]. The females were teased with apronned males daily and all those that came into estrus after the second treatment were allowed to be served naturally by the male. Days of estrus were recorded and considered as day 0 of the gestation. After successful synchronization and fertile mating, the animals were randomly separated into 4 groups of 5 each. Accordingly, the groups were as follows: DTT (Dexamethasone treated) Sheep, NDT (Non-dexamethasone treated) Sheep (Control), DTT (Dexamethasone treated) goat, and NDT (Non dexamethasone treated) goat (Control).

Dexamethasone treatment

The animals in the dexamethasone treated group were treated with dexamethasone injection (Dexaphan®, Pharma Pharmaceuticals, Swede-Egypt) intramuscularly at 0.25mg/kg body weight on days 1, 3 and 5 during first trimester and day 51, 53 and 55 during second trimester and keenly observed daily for possible clinical changes throughout the period of the study. Their initial body weights, rectal temperatures, pulse rates and respiratory rates were measured and recorded. This was continued at two weeks interval during the course of the experiment. Pregnancies were later confirmed by failure to return to estrus and by ultrasonographic examination.

Sample collection

The pregnant animals from both the control and treatment groups were selected and the fetal fluids were harvested through Caesarian Section (CS) at day 78 of gestation. Caesarian Section was performed under anesthesia using left flank incision approach according to standard procedure as described by Freeman [30].

Fetal fluids (amniotic and allantoic fluids) from each compartment were collected using 50ml syringe with 18G needle aspirated gently, and the volumes measured using 100ml measuring cylinder and stored at -20°C until analyzed. Some biochemical parameters (alkaline phosphatase (ALP), glucose, Total protein (TP), urea, creatinine and electrolytes (calcium (Ca2+), potassium (K+)) of each fluid sample were analyzed using standard methods. The pH and specific gravity of the samples were also determined using Veri-Max® reagents Strips (Acon Labs.Inc., San Diego, USA). Fetuses harvested during the operation were used for other investigations. Post operatively, 5% aceticaminophen injection (10mg/kg intramuscular) (Cadence Pharmaceutical Inc., Ireland) was administered for 3 days after surgery to take care of postoperative pain. Long acting 15% amoxicillin injection at 20mg/kg (Vetrimoxin) was also administered once after the operation.

Statistical analysis

Data collected were expressed as Means ± S.D. The Significant differences between the dexamethasone treated and non dexamethasone treated groups were compared using Student’s t-test. Significant differences were considered at p < 0.05. Statistical Software package, GraphPad InStat® version 3.0 [31] was used for the analyses.
**Results**

Table 1: Effects of dexamethasone on fetal fluid volume and some biochemical parameters in Yankasa sheep and Sahel goats at mid gestation.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups*</th>
<th>Amniotic Fluid</th>
<th>Allantoic Fluid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Sheep</td>
<td>Goat</td>
</tr>
<tr>
<td>Volume (ml)</td>
<td>NDT</td>
<td>400.10±1.39</td>
<td>335.71±1.20</td>
</tr>
<tr>
<td></td>
<td>DTT</td>
<td>397.10±1.30b</td>
<td>332.80±1.50</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>NDT</td>
<td>8.50±0.40</td>
<td>7.60±0.39</td>
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<tr>
<td></td>
<td>DTT</td>
<td>7.50±0.20b</td>
<td>7.50±0.40</td>
</tr>
<tr>
<td>TP (g/dl)</td>
<td>NDT</td>
<td>6.80±0.28</td>
<td>6.15±0.10</td>
</tr>
<tr>
<td></td>
<td>DTT</td>
<td>6.42±0.30</td>
<td>6.20±0.40</td>
</tr>
<tr>
<td>ALP (IU/L)</td>
<td>NDT</td>
<td>63.70±0.32</td>
<td>58.60±0.40</td>
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<tr>
<td></td>
<td>DTT</td>
<td>60.80±0.30b</td>
<td>57.70±0.20</td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td>NDT</td>
<td>54.90±0.50</td>
<td>58.70±0.09</td>
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<tr>
<td></td>
<td>DTT</td>
<td>54.80±0.50</td>
<td>58.5±0.10</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>NDT</td>
<td>0.32±0.02</td>
<td>0.50±0.03</td>
</tr>
<tr>
<td></td>
<td>DTT</td>
<td>0.32±0.01</td>
<td>0.50±0.01</td>
</tr>
<tr>
<td>Calcium (mmol/L)</td>
<td>NDT</td>
<td>2.40±0.02</td>
<td>2.70±0.4</td>
</tr>
<tr>
<td></td>
<td>DTT</td>
<td>2.40±0.01</td>
<td>2.80±0.2</td>
</tr>
<tr>
<td>Sodium (mmol/L)</td>
<td>NDT</td>
<td>116.70±1.21</td>
<td>130.12±1.09</td>
</tr>
<tr>
<td></td>
<td>DTT</td>
<td>115.80±1.30</td>
<td>130.15±1.10</td>
</tr>
<tr>
<td>K+ (mmol/L)</td>
<td>NDT</td>
<td>4.10±0.01</td>
<td>5.70±0.29</td>
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<td></td>
<td>DTT</td>
<td>4.09±0.5</td>
<td>5.80±0.31</td>
</tr>
<tr>
<td>Specific gravity</td>
<td>NDT</td>
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<td>1.00±0.002</td>
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<tr>
<td></td>
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</tr>
<tr>
<td>PH</td>
<td>NDT</td>
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<td>7.50±0.22</td>
</tr>
<tr>
<td></td>
<td>DTT</td>
<td>7.35±0.31</td>
<td>7.50±0.23</td>
</tr>
</tbody>
</table>

NDT = Non dexamethasone treated (control); DTT = dexamethasone treated

b = Significant decrease compared to respective control at (P<0.05)

ALP = Alkaline Phosphatase

TP = Total protein

*N = 5 in each group in both species

The volume of amniotic and allantoic fluids and their biochemical parameters (alkaline phosphatase (ALP), glucose, Total protein (TP), urea, creatinine) and electrolytes (calcium (Ca<sup>2+</sup>), potassium (K+) and sodium (Na+)) values in treatment and control groups are presented in Table 1. The volumes of both the amniotic and allantoic fluids were significantly (P<0.05) decreased in dexamethasone treated sheep (397.10±1.30 ml) compared to that of the control (400.10±1.39 ml). In goats the decrease was mild and insignificant (P>0.05) in dexamethasone treated group compared to control. Also the concentration of glucose and alkaline phosphatase (ALP) in amniotic (7.50±20 mg/dl, 6.80±0.30 IU/L) and allantoic fluids (7.11±0.20mg/dl, 18.0±0.10 IU/L) in dexamethasone treated sheep were significantly (P<0.05) decreased compared to control group (8.50±0.40mg/dl, 63.70±0.32 IU/L, 7.80±0.36mg/dl, 18.90±0.20 IU/L, respectively). There was no variation (P>0.05) in the mean values of total protein (TP), urea, creatinine, Ca<sup>2+</sup>, Na+, and K+ concentrations in both amniotic and allantoic fluids between dexamethasone treated and control groups in both species. Also, the pH and specific gravity (S.G) of amniotic and allantoic fluids did not vary between dexamethasone treated groups and their controls in both species.
Discussion

The decrease in amniotic and allantoic fluids volume associated with prenatal dexamethasone treatment in pregnant sheep in this study could be due to defects in placental functions. Dexamethasone has been reported to decrease placental weight and placentl efficiency in rats, humans, sheep and goats [16,17,19,22]. The observed decreased fetal fluids in this study is in contrast with the report of Wintour et al. [32] who reported significant increase in allantoic fluid but no change in amniotic fluid volume in merino ewes after maternal dexamethasone treatment. The difference could be due to breed variation.

Low amniotic and allantoic fluids volume as observed in dexamethasone treated sheep in this study can lead to oligohydramnios which increases the chances of pregnancy complications, fetal defects or fetal teratogenicity and subsequent abortion. During gestation fetal fluids are majorly produced by placenta and other placentomes, fetal membrane, amniotic and allantoic epithelia, fetal saliva and nasopharyngeal secretions until kidneys take over the task [26,24,27]. Amniotic and allantoic fluids protects the fetus by forming a protective physical cushion around it and allows the developing fetus to move freely inside the uterus and prevent teratogenicity due to adhesion and maintains an environment of constant temperature [24-27]. Similarly, the decrease in amniotic and allantoic fluids glucose in dexamethasone treated sheep could be due to impaired placental function associated with decreased placental weight and placental efficiency. The placenta has two principal glucose transporters, GLUT1 and GLUT3, that are not dependent on insulin [33] and the abundance of which tends to increase in more efficient placentas and decrease in less efficient ones [34].

The decrease in mean ALP value in dexamethasone treated sheep indicates that dexamethasone suppresses fetal fluids ALP secretion. In the same vein, this may be related to decrease placental function. Alkaline phosphatase (ALP) concentration has been correlated with placental function Lind et al. [23]. On the other hand, the lack of effect on amniotic and allantoic fluids calcium(Ca++,), potassium (K+) and sodium (Na+) between dexamethasone treated groups and the control in both species suggests that pre natal dexamethasone treatment does not have profound effect on fetal fluids electrolytes. This may be due to the lack of mineral corticoid effect of dexamethasone [9,10]. Also dexamethasone did not have any effects on fetal fluid pH and specific gravity (S.G) as well as total protein (TP), urea and creatinine as the data on these parameters did not vary significantly (P>0.05).

Conclusion

In conclusion, the findings suggest that dexamethasone exposure during pregnancy negatively affects fetal fluids by decreasing allantoic and amniotic fluid volume and some biochemical constituent, ALP and glucose, but has no effect on the fluid electrolytes in sheep. In goat, dexamethasone influence on these parameters was low. Therefore sheep is more susceptible to the inhibitory effects of dexamethasone and carries more risk factor compared to goat. It is recommended that in advanced pregnancy, dexamethasone should be used with caution in sheep.

References


