EFFECTS OF DYSTOCIA ON PLASMA CORTISOL AND CHOLESTEROL LEVELS IN HOLSTEIN HEIFERS AND THEIR NEWBORN CALVES

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Abstract

The study was designed to determine the effects of dystocia on plasma cortisol, cholesterol, and vitamin levels in heifers (n=22) and their newborn calves (n=22) at delivery. The animals were classified into 4 groups depending on calving difficulty. Plasma cortisol concentrations were significantly elevated in dystocia heifers (P<0.001) and their calves (P<0.019). Similarly, cholesterol levels were also markedly increased in dystocia heifers (P<0.001) and their calves (P<0.007). Vitamin A, β-carotene, and vitamin C levels were significantly decreased in dystocia calves. Overall, plasma glucose, HDL, triglycerides, and creatinine levels were all markedly increased in dystocia heifers as compared to controls. However, glucose, HDL, triglycerides, and creatinine levels were not changed significantly in affected heifers as compared to control group. Calving stress appears to affect several blood parameters including cortisol, cholesterol, and vitamin A in all the groups and β-carotene and vitamin C in the calves. The analyses of these parameters can be practical to improve the health of dystocia-affected mothers and to increase survival of their newborns.

Key words: heifers, calves, dystocia, cortisol, cholesterol, vitamins.

Parturition is a stressful process for cows and their calves. Abnormal parturition further exacerbates the stress (37). Dystocia, difficult calving, has been a long-standing problem in both the beef and dairy industry. Calving difficulty causes trauma for both cows and their offspring, and can lead to increased rates of uterine infections, periparturient disorders such as retained placenta, metritis, longer calving intervals (42), lower milk production, and reduced health of cows and survival of newborn calves (3).

Although there are several studies on the effect of dystocia on uterine infections, periparturient disorders, milk production, and newborn survival (19), limited studies looking at the effect of dystocia on blood biochemical parameters in heifers and their newborn calves are available (22). In addition, the interactions among plasma concentrations of cortisol, cholesterol, and HDL in relation to the difficult labour have not been well established. Therefore, in the present study, we planned to study the effects of dystocia on plasma cortisol and cholesterol levels and characterise the variations in plasma glucose, HDL, β-carotene, and vitamin A and C concentrations at delivery in heifers and in their offspring.

Material and Methods

Animals and samples. Twenty-two Holstein heifers and 22 their newborn calves were used in the study. All the heifers delivered one calf and the calves were born alive. The animals were selected from a private farm (Avsar Agriculture and Stockbreeding Co., Bolvadin/Afyonkarahisar, Turkey) that contained a total of 1,200 Holstein breed cattle, including 480 lactating dairy cows. The heifers and their calves were classified into 4 groups depending on calving difficulty. Ten heifers that required assistance during calving were classified as group 1. While nine heifers from the group 1 calved with forced extraction (a mechanical calf puller) by one or two people (dystocia score is 3), one heifer calved with Caesarean section after unsuccessful forced extraction (dystocia score is 4) (41). Epidural anaesthesia was not used for the heifers, which needed mechanical extraction. The duration of the obstetrical procedures were recorded as 25 to 30 min for nine heifers and 75 min for one heifer with Caesarean. All the animals were able to stand, and any complications were not seen after their delivery. However, three heifers in this group had a retained placenta after birth. In addition, in the group 3, three calves within two days and one calf at the fourth day died after delivery due to the detrimental effect of dystocia stress.
The other 12 heifers, which calved with no assistance, were classified as group 2. The calves of heifers in the group 1 were classified as group 3 and those of heifers from the group 2 were classified as group 4. The heifers in the group 2 had an optimal body condition score (BCS) (2.75-3.50), whereas animals in the group 1 had a higher BCS (3.96 ±0.31). The assessments of the BCS were made by the same individual throughout the experimental period as described by Edmonson et al. (8). It was based on the palpation of the traverse processes of the loin vertebrae, cranial coccygeal vertebrae, and *tuber ischii*. The scores were assigned using a five-point scale (0=very thin to 5=grossly fat).

There was no significant difference regarding the feed intake between groups 1 and 2. The cows were fed the same ration including alfalfa hay (31%), barley hay (52%), and concentrates (18%). The care of the animals and the experimental design of this study were approved by the Local Animal Ethics Committee in Afyon Kocatepe University (No. 113-09.11.2007). The age and BCS of heifers and body weight of calves are shown in Tables 1 and 2.

### Table 1
Age and BCS of the heifers (mean ±SD)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group 1</th>
<th>Group 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (month)</td>
<td>26.15 ±0.50</td>
<td>24.46 ±0.31</td>
</tr>
<tr>
<td>BCS of heifers</td>
<td>3.96 ±0.31</td>
<td>Optimal</td>
</tr>
</tbody>
</table>

### Table 2
Body weight (BW) of the calves (kg)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group 3</th>
<th>Group 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW</td>
<td>49.3 ±3.3</td>
<td>44.1 ±2.8</td>
</tr>
</tbody>
</table>

### Biochemical analyses.

Blood samples were collected from the heifers at the delivery and from their newborn calves immediately after the delivery and prior to feeding colostrum. Blood samples were taken from the jugular vein into heparinised plain tubes and the samples were centrifuged at 3,000 rpm for 10 min at room temperature, and then stored at −20°C until biochemical analysis. Plasma cortisol concentration was enzymatically measured using an EIA kit (Eucardio Laboratory, Inc. USA) according to the manufacturer’s instructions (29). Vitamin A and β-carotene levels were estimated by the method of Suzuki and Katoh (40) using a spectrophotometer. The peaks in the absorption of vitamin A at 325 nm and β-carotene at 453 nm were detected after the reaction of a sample with ethanol: hexane at the ratio of 1 with 1:3, respectively.

Plasma concentrations of cholesterol, triglycerides (TG), HDL, glucose (TECO Diagnostics, USA) and creatinine (Linear Chemicals, S.L. Cromatest, SPAIN) were determined by means of commercially available test kits. Plasma vitamin C was estimated by using a spectrophotometer (23).

### Statistical analyses.

The data were expressed as mean ± standard deviation (±SD) and the statistical analyses were performed using one-way ANOVA test (SPSS Inc.). Pearson’s correlation coefficients were calculated for possible relationships between the groups for the measured parameters. Probability values of less than 0.05 (P<0.05) were considered to be statistically significant.

### Results

Plasma biochemical parameters in dystocia and control heifers are summarised in Table 1. Overall, plasma levels of cortisol, cholesterol, vitamin A, glucose, high-density lipoprotein (HDL), triglycerides, and creatinine were all markedly increased in dystocia heifers as compared to controls (Table 3).

### Table 3
Blood biochemical parameters in dystocia and normal heifers (mean ±SD)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Dystocia heifers (Group 1)</th>
<th>Control heifers (Group 2)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortisol (µg/dL)</td>
<td>22.90 ±5.30</td>
<td>11.95 ±4.05</td>
<td>0.001</td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>59.0 ±14.5</td>
<td>35.90 ±7.93</td>
<td>0.001</td>
</tr>
<tr>
<td>Vitamin C (mg/dL)</td>
<td>0.133 ±0.04</td>
<td>0.183 ±0.14</td>
<td>NS</td>
</tr>
<tr>
<td>Vitamin A (µg/dL)</td>
<td>24.24 ±5.10</td>
<td>18.33 ±3.57</td>
<td>0.010</td>
</tr>
<tr>
<td>β-carotene (µg/dL)</td>
<td>19.95 ±4.82</td>
<td>19.44 ±5.58</td>
<td>NS</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>132.5 ±7.1</td>
<td>68.2 ±5.61</td>
<td>0.001</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>38.77 ±6.14</td>
<td>18.35 ±5.83</td>
<td>0.001</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>4.90 ±0.05</td>
<td>0.70 ±0.05</td>
<td>0.002</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.94 ±0.05</td>
<td>0.70 ±0.05</td>
<td>0.002</td>
</tr>
</tbody>
</table>

NS - not significant.
**Table 4**

Blood biochemical parameters in dystocia and normal calves (mean ± SD)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Dystocia calves (Group 3)</th>
<th>Control calves (Group 4)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortisol (µg/dL)</td>
<td>27.53 ±4.99</td>
<td>22.58 ±3.53</td>
<td>0.019</td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>13.32 ±3.45</td>
<td>8.91 ±2.48</td>
<td>0.007</td>
</tr>
<tr>
<td>Vitamin C (mg/dL)</td>
<td>0.17 ±0.08</td>
<td>0.40 ±0.2</td>
<td>0.003</td>
</tr>
<tr>
<td>Vitamin A (µg/dL)</td>
<td>1.78 ±0.82</td>
<td>14.83 ±3.43</td>
<td>0.000</td>
</tr>
<tr>
<td>β-carotene (µg/dL)</td>
<td>1.53 ±0.31</td>
<td>6.49 ±0.42</td>
<td>0.000</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>117.10 ±5.1</td>
<td>52.10 ±3.3</td>
<td>0.000</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>20.14 ±4.95</td>
<td>24.91 ±3.60</td>
<td>NS</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>7.25 ±1.32</td>
<td>9.57 ±1.84</td>
<td>NS</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>1.33 ±0.21</td>
<td>1.24 ±0.12</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS - not significant.

Dystocia animals in the group 1 exhibited noticeably increased plasma concentrations of cortisol and cholesterol at delivery as compared with those of heifers with normal parturition (P<0.001) (group 2). No significant difference appeared to exist between plasma vitamin C and β-carotene levels in dystocia and control heifers in the groups 1 and 2. Similarly to cortisol and cholesterol, plasma glucose levels were increased considerably in dystocia heifers (P<0.001) and in their calves (P<0.000) (Table 4).

Plasma biochemical parameters in dystocia and normal calves are presented in Table 4. Similarly to dystocia heifers, difficult parturition noticeably increased plasma cortisol (P<0.019) and cholesterol (P<0.007) concentrations in their calves. Cortisol levels in the dystocia heifers were significantly correlated to the concentrations of plasma cholesterol (P<0.01, r=0.490) and HDL. However, there was no significant correlation between plasma cortisol and cholesterol concentrations in the calves (P<0.099, r=0.286).

In addition, plasma vitamin A levels in dystocia calves in the group 3 were significantly (P<0.000) lower as compared to normal calves (group 4) and dystocia heifers (group 1). In contrast, vitamin A levels in the group 2 and group 4 were slightly different. Resembling plasma vitamin A levels in dystocia calves, plasma β-carotene concentrations were also markedly reduced (P<0.000) in the group 3 when compared to the group 1. Similar to this, plasma β-carotene concentrations were also clearly lower (P<0.000) in the group 4 with respect to the group 2.

**Discussion**

The aim of this study was to determine the effect of dystocia on the profiles of plasma cortisol, cholesterol, glucose, HDL, vitamin C, vitamin A, and β-carotene concentrations during delivery in heifers and their newborn calves. Dystocia increases stress of calving and undermines cow’s health and reproduction (9).

The birth weight of a calf is the most important factor leading to dystocia. The calves, which received assistance with calf puller at delivery in the group 3 had 49.3 ±3.3 kg of body weight at their birth. By contrast, the calves in the group 4, which required no assistance at delivery, had the body weight by 5.2 ±0.5 kg lower. This indicates that the body weight at birth is critical for normal parturition. Furthermore, the body weight of heifers at first calving is shown to be decisive for dystocia (13). Smaller and over-conditioned heifers have been demonstrated to experience dystocia more frequently (9). The present investigations confirm these observations by showing that the heifers with higher BCS (3.96 ±0.31) (group 1) experienced dystocia while animals with optimal BCS (2.75 and 3.50) in the group 2 suffered no parturition difficulty. Over-condition and high calf weight seemed to be related with difficult parturition in the group 1 as compared with the group 2.

In dairy farming, there are various stressors including under-nutrition and periparturient disorders (31), and even normal parturition is a natural event that causes stress for a mother (18, 36). Hence, difficult parturition further intensifies this stress and can affect biochemical blood parameters such as cortisol levels.
Cortisol is one of the most important glucocorticoids and its plasma levels have been used as an objective indicator for the quantification of stress in cows (31). Elevated plasma cortisol levels can reflect whether the heifers enduring difficult calving and their calves suffer from the stress. Plasma glucocorticoid concentration is shown to be markedly increased in mammals including humans during parturition (28). Similarly, plasma cortisol levels are elevated during labour in cattle (1). The elevation of cortisol levels around parturition may result from the increased need for glucocorticoids to initiate the lactation and the fact that oestrogen reduces the metabolic clearance rate for cortisol (18). It has been reported that cows with dystocia had plasma cortisol levels higher than cows with normal parturition (13). These authors suggest that dystocia seems to be more stressful for animals than the normal calving would be. Our results are similar to those of Nakao and Grunert (28) who demonstrated that dystocia significantly elevated cortisol level as compared to normal parturition. The rises in the cortisol concentration in dystocia heifers appear to be induced during calving stress. Although, it does not necessarily indicate that cows are in a stressful condition (28), a rise of plasma glucocorticoids may contribute to many metabolic changes, anti-inflammatory activity, and modification of immune competency (4).

Stress elevates cortisol concentration in calves for a longer time. If stress is severe and/or prolonged, the plasma cortisol levels also remain elevated. Elevated cortisol can harm metabolism and immune system (24). Furthermore, dystocia impinges on metabolic process in the calf (30) and affects survival rate in newborn calves by grounding trauma and hypoxia. The adrenocortical response to hypoxia is shown to be a critical component of the adaptation to dystocia stress in newborn calves (33). Several researchers have examined the alterations in plasma cortisol levels in dystocia heifers but little is known regarding alterations in plasma cortisol levels in newborn calves.

Similar to their mothers, plasma cortisol concentrations in calves, which suffered from difficult parturition, were elevated as well. We think that hypoxia, a stress factor that can be induced during difficult calving (3, 25), is responsible for augmentation of cortisol production in calves in the group 3. Stress is known to augment cortisol production through the stimulation of adrenocorticotrophic hormone release (16). Severe stress provoked during dystocia is likely to compromise survival rate of calves that will require extra care (3). Dystocia in heifers is shown to weaken calf’s physiological response and reduce neonatal survival. In fact, around 60% of all perinatal calf mortalities occur within 24 h after birth (25). In the present study, the death of three calves within two days and one calf at the fourth day after delivery in group 3 further designates the detrimental effect of dystocia stress on calf survival.

The steroid hormones including cortisol are all derived from cholesterol. Elevated cortisol levels in the groups 1 and 3 appear to correlate with increased cholesterol levels (38). Dystocia related stress in heifers and their calves increases cholesterol levels (35, 38, 39). Similarly, dystocia in cows and buffaloes is shown to increase total lipid level at parturition (5). The study of Hoffman et al., (11) shows that dystocia-induced hypoxia also profoundly affects lipid stores of the calf. In the present study, HDL and TG levels in the group 3 were only slightly reduced. This decrease appears to relate the timing of blood sampling that was taken prior to colostrum feeding that contains rich essential nutrients including lipids (12, 15).

Severe dystocia is demonstrated to increase serum glucose levels in calves (3) and blood glucose concentrations in cows as compared to normal parturition (28), suggesting that dystocia is more stressful for cows than normal calving. The results of the present study showing the elevated plasma glucose levels in dystocia heifers and in their calves are in line with other studies. Elevated plasma glucose levels are likely to be induced by dystocia-related stress to meet the increased energy demand.

Vitamins C and A, and β-carotene are known to be effective antioxidants in living organisms. Antioxidant vitamins have potential health-promoting properties (34). This indicates that antioxidant vitamins are effective in reducing the effect of dystocia-induced stress on animals (37). Today, scientific community readily embraces the fact that stress impacts most of the physiological processes in the body (6). We found that dystocia-induced stress had more profound effect on calves than on their mothers regarding the reduced levels of vitamin C, vitamin A, and β-carotene in the group 3; and vitamin C in the group 1. These results indicate that calves are more vulnerable to dystocia stress than their mother.

The cortisol is involved in the response to stress (18). Vitamin C is thought to have a role in the production and regulation of corticosteroid (cortisol) production in the adrenal glands (10). Moreover, stress and elevated corticosteroids can reduce the circulating plasma levels of vitamin C (32). In this study, cortisol was significantly correlated with the concentrations of plasma vitamin C in the calves (P<0.007, r=-0.514), whereas there was no correlation between plasma cortisol and vitamin C levels in the heifers. Several factors including stress are shown to affect the effective absorption and utilisation of vitamin A and β-carotene (2). In addition, vitamin A and β-carotene deficiencies in mothers can interfere with the plasma levels of vitamin A and β-carotene in the calves. Hussein et al., (17) show that the plasma levels of vitamin A among the neonates are significantly lower compared to their mothers (27). Likewise, a decrease in plasma retinol (20) and β-carotene levels (42) during parturition in cattle were reported (17). Vitamin A supplementation is shown to reduce dystocia incidences in pregnant cows (21). At the present study, vitamin A levels in dystocia heifers were higher as compared with the group 2. However, the elevation in vitamin A level in the group 1 was between reference ranges for cattle. In cows, normal range of vitamin A level is 25-80 µg/dL, and β-carotene normal level is 300-1,200 µg/dL. (42). Cows fed green fodder have considerably higher serum β-carotene and vitamin
A levels during the summer months (June-July) than in the rest of the year (42). It should be noted that in the present study, the blood samples were obtained in March. The animals used in this study might have had insufficient vitamin A (= 76 IU Vit A/kg/d) and β-carotene intake.

Elevated plasma creatinine levels indicate muscle damage related to difficult parturition (26). The analyses of blood biochemical profiles including plasma levels of cortisol, cholesterol, glucose, vitamin A, β-carotene, and vitamin C in relation to dystocia can be valuable to predict the effect of calving stress in heifers and their newborn calves to provide required medical and husbandry care. Thus, the use of antioxidants (vitamin A, β-carotene, and vitamin C) may be effective for the prevention and treatment of the calving stress in newborn post-dystocia calves.

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References


