ARAŞTIRMA

F.Ü.Sağ.Bil.Vet.Derg.
2014; 28 (2): 57 - 60
http://www.fusabil.org

Comparison of Erythrocyte Arginase Activity and Plasma Nitric Oxide Levels in Dystocia and Normal Calving of Cows

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Geliş Tarihi : 22.10.2013
Kabul Tarihi : 10.12.2013

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This study was aimed to investigate erythrocyte arginase activity and plasma nitric oxide (NO) levels in dystocia and normal calving of cows and compare these parameters. The study material consisted of 16 cows, 3–8 years old, brought for parturition at term to the Clinic of Obstetrics and Gynecology at Fırat University. Blood samples of cows normally calving and dystocia were taken into blood collecting tubes with heparin from the jugular vein in the first half hour after delivery and centrifuged at 3000 rpm for 10 minutes. Plasma NO levels and erythrocyte arginase activities were measured. The erythrocyte arginase activity in cows normally calving was significantly increased compared to that in cows with dystocia ($P<0.01$), and the plasma NO level in cows normally calving was significantly decreased compared to that in cows with dystocia ($P<0.05$). It was concluded that the lower erythrocyte arginase activity and higher plasma NO level in cows might play a role in dystocia.

Key Words: *Dystocia, normal calving, arginase, nitric oxide, cow.*

Sığırların Normal Doğum ve Güç Doğumunda Eritrosit Arginaz Aktivitesi ve Plazma Nitrik Oksit Düzeylerinin Karşılaştırılması

Bu çalışma sığırların normal doğum ve güç doğumunda eritrosit arginaz aktivitesi ve plazma nitrik oksit (NO) düzeylerini belirlemeyi ve bu parametreleri kıyaslamayı amaçladı. Çalışma materyali 3 ila 8 yaşında 16 sığırdan oluştu ve Fırat Üniversitesi Doğum ve Jinekoloji Kliniğine gebelik periyodunun sonunda doğum için getirildi. Normal doğum ve güç doğum yapan sığırların kan örnekleri doğumdan sonra ilk yarım saat içinde jugular venden heparinli kan toplama tüplerine alındı, 3000 rpm 10 dakika süre ile santrifüj edildi. Plazma NO düzeyleri ve eritrosit arginaz aktiviteleri ölçüldü. Normal doğum yapan sığırlarda eritrosit arginaz aktivitesi güç doğum yapan sığırlardakine göre önemli düzeyde yüksekti ($P<0.01$) ve normal doğum yapan sığırlarda plazma NO düzeyi güç doğum yapan sığırlardakine göre önemli düzeyde düşüktü ($P<0.05$). Sığırlarda düşük eritrosit arginaz aktivitesi ve yüksek plazma NO düzeyinin güç doğumda rolü olabileceği kanısına varıldı.

Anahtar Kelimeler: *Güç doğum, normal doğum, arginaz, nitrik oksit, sığır.*

Introduction

Dystocia is recognized as difficult calving. Difficult calving takes place when there are failing factors associated with expulsive forces, birth canal and fetal size, and position. Heifers and older cows are affected by all types of dystocia. Difficult calving in heifers is associated with oversized calves, improper fetal position and vulva dilatation failure. Uterine inertia and cervix dilatation failure, in addition to the above mentioned dystocia factors, play a role in calving difficulty in older cows (1).

Labor starts with the beginning of uterine contractions and cervix dilatation. At first, contractions occur about every 15 minutes and then become more powerful and frequent. The cervix enlarges and the uterus and vagina constitute an extended canal (2).

Nitric oxide (NO), which is formed by nitric oxide synthases (NOS) that convert L-arginine to L-citrulline, leads to smooth muscle relaxation by binding to soluble guanylate cyclase and increasing cyclic guanosine monophosphate (cGMP) concentrations (3). NO may act on inhibition of uterine contractility until the end of the normal gestation period, when there is a decrease in the production of NO. An increase in cervix NO production via NOS enzymes may help dilatation of the cervix (4).

Arginase converts L-arginine to urea and L-ornithine (5-8). The common substrate of NOS and arginase is L-arginine (9). NO is produced through the use of L-arginine by NOS. For this reason, arginase functions as a negative control system for the entire NO production (10).

This study was aimed at determining erythrocyte arginase activity and plasma NO levels in dystocia and normal calving of cows and comparing these parameters.

Materials and Methods

The study material consisted of 16 cows, 3–8 years old, brought for parturition at term to the Clinic of Obstetrics and Gynecology at Fırat University. The study included 8 cows normally calving (3 Simmental, 4 Montofon, 1 Holstein) and 8 cows with dystocia (3 Simmental, 4 Montofon, 1 Holstein) and complied with ethical principles (Firat University Ethical Committee Approval Form Number: 2013/56). Cows with dystocia were delivered by traction from the birth canal of the cows. The blood samples of cows normally calving and dystocia were taken from the jugular vein in the first half hour after the delivery. All blood samples were collected into blood collecting tubes with heparin and centrifuged at 3000 rpm for 10 minutes, and the plasma was separated.

Plasma NO levels were measured. Erythrocyte arginase activity was measured after washing the remaining portion 3 times with serum physiologic. The plasma NO level was measured according to the enzymatic Griess method (11). Erythrocyte arginase activity was measured spectrophotometrically with the Thiosemicarbazide-Diacetyl Monoxime Urea (TDMU) method (12). Diacetylmonoxime does not directly react with urea. Previously it was hydrolyzed by temperature action to diacetyl and hydroxylamine. Diacetyl forms diazine, which is a compound with yellow color by condensation reaction with urea in acidic solution. Thiosemicarbazide and Fe^{+2} are used to stabilize the formed yellow color (13). The Drabkin method (14) was used to measure the hemoglobin amount. In this study, 1 unite enzyme is the enzyme amount that forms 1 µmol urea from L- arginine at 37 °C for 1 h, and specific activity is expressed as a µmol urea/hour/g hemoglobin.

SPSS 12.0 program was used. Results were given as mean ± SEM. In the control of difference between groups, Mann-Whitney U test was used. Differences were considered significant when P values were less than 0.05.

Results

The erythrocyte arginase activities and plasma NO levels in cows of normal calving and dystocia are given in Table 1. The erythrocyte arginase activity in cows normally calving was significantly higher compared to that in cows with dystocia ($P<0.01$), and the plasma NO level in cows normally calving was significantly lower compared to that in cows with dystocia ($P<0.05$).

Table 1. Erythrocyte arginase activity and plasma NO level in cows normally calving and dystocia

Parameters	Normal Calving (n=8)	Dystocia (n=8)	P
Erythrocyte arginase activity (U/g hemoglobin)	139.25±13.65	42.50±8.19	<0.01
Plasma NO level (µmol/L)	48.14±0.95	56.15±4.22	<0.05

Results are given as mean ± SEM.

Discussion

The female reproductive system has three isoforms of NOS and synthesizes NO using L-arginine. Accordingly, NO has a role in the physiology of reproduction (15). NO induces smooth muscle relaxation by binding to soluble guanylate cyclase and increasing cyclic guanosine monophosphate (cGMP) concentrations (3). The contractility of the uterus may be inhibited by NO until the end of the normal gestation period. However, an increase in cervix NOS enzymes at the end of the normal gestation period leads to an increase in NO concentrations. Thus, increased NO concentrations in the cervix may help dilatation of the cervix (4). Contractility of the uterus is considered to have a role in cervical dilatation (16).

Previous studies have demonstrated an increase in uterine NO production during pregnancy and a decrease at term (17-21). Sladek et al. (17) reported that NOS activity is high during rabbit pregnancy and progressively decreases by 80% in decidua during the last four days of gestation. Similarly, Buhimschi et al. (4) reported that uterine NO synthesis increases during gestation and decreases at term conversely, cervical NO synthesis is low in the pregnant animals on days 18–22 of gestation but significantly increases at the end of the normal gestation period. Also, Ali et al. (22) suggested that uterine iNOS mRNA is increased during pregnancy and decreased at term in contrast, cervical iNOS mRNA is low until delivery (day 22) when it increases and is significantly increased during labor. In addtion, 3 h after injection with the antiprogestin onapristone, iNOS mRNA is significantly decreased in the uterus (~45%) and increased in the cervix (~245%) when compared with controls, and the changes in bNOS and eNOS during gestation are not significant compared to those in the iNOS. As a result, the changes in iNOS mRNA at the end of pregnancy may play a role in cervical ripening. Furthermore, the changes in iNOS can be resembled during preterm labor following antiprogesterone treatment (22). Momohara et al. (23) reported that NO per se does not directly cause the myometrial relaxation, but NO plays an important role in regulation of myometrium contractions during gestation by modulating ET-1 (endothelin-1) production in the myometrium, and increased endogenous NOS inhibitors and ET-1 production in the myometrium increase myometrial contractions at term and after delivery.

Dilatation of the cervix is important in the regulation of normal labor. Cervical structure is thought to be changed at this time, with increased tissue water content, alteration of matrix glycosaminoglycans, and separation of the connective tissue structure (24). NO exhibits an ultimate metabolic pathway of cervical ripening. It induces local vasodilatation and increases vascular permeability, leukocyte infiltration, and matrix metalloproteinase and other mechanisms, concert with PGE2 (25). The outcome of pregnancy is provided by the suitable timing of cervical ripening. A cervical ripening failure at the end of a normal pregnancy period may

cause dysfunctional labor (26). Shi et al. (16) showed that treatment with NOS inhibitors suppresses ripening whereas NO donors and prostaglandins speed up it; NO and prostaglandins play important roles in the arrangement of cervical ripening. This study showed an important difference between the plasma NO levels of cows with dystocia and those with normal calving ($P<0.05$).

Hirata et al. (27) reported that arginase activity was significantly decreased at the 7^{th}, 14^{th} and 21^{st} day of gestation; on the contrary, the enzyme activity significantly increased at the end of the normal gestation period, and arginase activity in the myometrium had a nonsignificant higher value after delivery. Increased arginase activity at the end of the normal gestation period would be involved in increasing myometrial contractions (27). In this study, it was found that the erythrocyte arginase activity in cows normally calving was significantly increased compared to that in cows with dystocia ($P<0.01$). It was concluded that the lower erythrocyte arginase activity and higher plasma NO level in cows might play a role in dystocia.

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