# CAN INDUCTION OF PARTURITION EFFECT THE VAGINAL BACTERIAL FLORA IN EWES?

## PUEDE ¿LA INDUCCIÓN DEL PARTO AFECTAR LA FLORA BACTERIANA VAGINAL EN LAS OVEJAS?

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**ABSTRACT**

Induction of parturition (IP) may have a risk for vaginal infections regarding the higher risk for dystocia and postpartum problems following IP. Thus, this study aims to evaluate vaginal bacterial flora before IP and at postpartum 15 and 30th days (d) in ewes. Twenty-four Kıvırcık breed ewes were divided according to induction methods and then they were induced at 138th gestation d: group I (n=6), control; group II (n=6), dexamethasone sodium phosphate (dexamethasone) 16 miligram (mg), intramuscularly (im); group III (n=6), aglepristone (5mg/kilogram (kg), subcutaneously (sc)] and group IV (n=6), dexamethasone (8mg, im) + aglepristone (2.5 mg/kg, sc). The vaginal samples of the ewes were taken with swaps before IP and this sampling was repeated on postpartum 15 and 30th d. Microbiologically, bacterial culture and antibiotic susceptibility analysis were performed. All parturitions were normal and no complication was observed at postpartum period. *Escherichia coli* were the most isolated bacteria in the samples of all groups, which were taken at 138th gestation and postpartum d. *Acinetobacter* spp. and *Acinetobacter baumannii* were the other isolations in group III at postpartum 15 and 30th d, respectively. Antibiotic susceptibility analysis results pointed out that *E. coli* and *A. baumannii* were 100.0% sensitive to enrofloxacin, and *Acinetobacter* spp. was 100.0% sensitive to trimethoprim/sulfamethoxazole. Even if it was not encountered in this study, it should be considered that IP may affect the vaginal flora and maybe responsible for the postpartum infective vaginal complications.

**Key words:** Induction of parturition; vaginal bacteriology; antibiotic susceptibility; ewe.

**RESUMEN**

La inducción del parto (IP) puede tener un riesgo de infecciones vaginales con respecto al mayor riesgo de distocia y problemas posparto después de la IP. Por lo tanto, este estudio tuvo como objetivo evaluar la flora bacteriana vaginal, antes de la IP y en el posparto 15 y 30 días (d) en las ovejas. Veinticuatro ovejas de raza Kıvırcık se dividieron según los métodos de inducción y luego se indujeron en el d 138 de gestación: grupo I (n = 6), control; grupo II (n = 6), fosfato sódico de dexametasona (dexametasona) 16 miligramos (mg), intramuscularmente (im); grupo III (n = 6), aglepristona (5 mg / kg (kg), subcutáneamente (sc) y grupo IV (n = 6), dexametasona (8 mg, im) + aglepristona (2,5 mg / kg, sc). Se tomaron muestras de las ovejas con hisopos antes de IP y este muestreo se repitió en los d 15 y 30 postparto. Microbiológicamente se realizó cultivo bacteriano y análisis de susceptibilidad a antibióticos Todos los partos fueron normales y no se observó complicación en el período posparto. *Escherichia coli* fue la bacteria más aislada en las muestras de todos los grupos, que se tomaron en el d 138 de gestación y postparto. *Acinetobacter* spp. y *Acinetobacter baumannii* fueron los otros aislamientos en el grupo III en los d 15 y 30 postparto, respectivamente. Los resultados del análisis de susceptibilidad a antibióticos señalaron que *E. coli* y *A. baumannii* fueron 100,0% sensibles a enrofloxacina, y *Acinetobacter* spp. fue 100,0% sensible a trimetoprima / sulfametoxazol. Se debe considerar que la IP, aún si no fue detectada en este estudio, puede afectar la flora vaginal y tal vez puede ser la responsable de las complicaciones vaginales infecciosas posparto.

**Palabras clave:** Inducción del parto; bacteriología vaginal; susceptibilidad a antibióticos; oveja.

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## INTRODUCTION

In ruminants, the parturition process usually occurs in non-steril conditions; thus, an indirect bacterial contamination of the genital tract is an expected episode [27]. The bacterial flora of the genital tracts does not always lead to pathological conditions, because uterine involution, elimination of the bacterial contamination and restoration of the ovarian functions occur at postpartum period to prepare the genital tracts for a new pregnancy [10, 19, 27], which takes about 17-30 days (d) [10, 13, 22, 27].

Although infective reproductive pathologies are rarely encountered in ewes (*Ovis aries*) [5, 9], following parturition, the genital canal (GC) becomes contaminated with bacteria in 80-100% of animals at early postpartum period [15, 29]. For genital canal infections (GCI), retention of the placental remnants or prolonged lochia resulted from weak myometrial contractions are predisposing factors [27]. These infections are often caused by opportunist bacteria (especially *Escherichia coli*), which have frequently been isolated in ewes [23]. Induction of parturition (IP) may cause to dystocia, placental retention, subsequent endometritis and neonatal problems and may also lead to infective postpartum diseases [2, 6, 14, 31]. Fecal origin and the other non-specific bacteria are opportunistic pathogens of the reproductive tracts, and if there is a stress, these opportunist bacteria may cause GCI [23]. There are limited studies about GCI after IP 16, 17, 18. Considering to these limited information, evaluation of vaginal bacterial flora and antibiotic susceptibility results of the obtained bacteria, which may be responsible to postpartum genital tract infections, was aimed before and after IP in ewes.

## MATERIALS AND METHODS

This study received the approval of the Ethical Research Committee at Uludag University (Decision number: 2013-12/02).

### Animals

The material of the study consisted of Kıvırcık breed, healthy 24 pregnant ewes which were at 3rd parturition period. The ewes were randomly separated into 4 groups according to IP methods: group I (GRI) (control) 0.9% NaCl [1milliliter (mL), im); group II (GRII) (n=6) dexamethasone (16 miligrams (mg), im.-4mL); group III (GRIII) (n=6) aglepristone (5mg/kilogram (kg), sc 8,5-10 mL) and group IV (GRIV) (n=6) dexamethasone (8mg, im.-2mL) + aglepristone (2.5 mg/kg, sc 4,5-5mL).

General examinations of the ewes (body temperature, heart and respiratory rate, conjunctival examinations and capillary filling time) were performed to assess the healthy status. Vulva and perineal region of the ewes were inspected in terms of possible vaginal infections. Laboratory analysis (hematological, sero-biochemical values of the blood samples and urine strip tests) and abdominal ultrasonographical (838 VET, Hasvet, İstanbul, Turkey) examinations were performed to evaluate the healthy status of pregnancy in the ewes. And then, parturitions of the ewes were induced at 138$^{th}$ gestation d, which has been reported as early d. for lamb viability [22]. Postpartum involution of the uterus was evaluated at 15 and 30$^{th}$ d ultrasonographically.

### Vaginal sampling

Before sampling, the tail of the ewe was kept up, and the vulva was aseptically cleaned with benzalkonium hydrochloride (Zefirolum®, Kimpa, Istanbul) and then dried with a sterile towel. After opening of the vulvar commissure, the samples were obtained with a sterile swab from the posterior part of the vagina by the same person. Vaginal samples were taken before IP and were repeated on postpartum 15 and 30$^{th}$ d.

### Bacterial culture and identification

The samples were transferred into the transport medium (Stuart's medium, Copan, Italy) and were taken to the microbiology laboratory. For culture, the samples were inoculated in 5% sheep blood agar (Becton-Dickinson, Crystal identification kit, catalog no: 297876), and Eosin Methylen Blue (EMB) agar (Becton-Dickinson, Crystal identification kit, catalog no: 221355) and incubated at 37°C for 24 hour (h). According to colony morphology and Gram color features, isolated colonies were assessed. For the identification of the bacteria, the cultures were analyzed using BBL Crystal (Becton-Dickinson, Sparks, USA) Gram positive and Gram negative ID system kits and its computer program (BD BBL™ Diagnostics, USA).

### Antibiotic susceptibility test

A panel of seven of the most frequently used antimicrobial agents was determined by the disk diffusion method as described previously [3]. The bacteria strains were tested against to antibiotics. The following antibiotic discs on Mueller Hinton agar were applied: penicillin (P) (10U), sulphametoksazol/trimetoprim (SXT) (25µg), cefuroxime (CXM) (30µg), ampicillin (AMP) (10µg), oxytetracycline (OT) (30µg), enrofloksasin (ENR) (5µg), ceftiofur (XNL) (30µg).

### Statistical analyses

Using a statistical package program (SPSS 23.0, IBM Corp. Statistics®, USA), the microbiological culture results obtained for each group according to the sampling times were analyzed by McNemar's test and the comparisons of the sampling times among the groups were also analyzed by the Fisher-Freeman-Halton Exact test. The level of significance was determined as $P < 0.05$.

## RESULTS AND DISCUSSION

Parturitions of the ewes in groups were normal and there were no intra- or pospartum complications such as dystocia and placental retention. In addition, no vaginal discharge was also observed at postpartum 15$^{th}$ d. The uterine involution was well

and there was no luminal abnormality in the ultrasonographic examinations performed at postpartum 30th d.

**Bacteriological results**

All samples had bacterial growth, and 99 isolates were obtained from vaginal samples of ewes. These results showed 24 different types of bacteria. Seventeen of these isolations were environmental contaminants originated from water or soil. The percentages of the isolated aerobic bacteria were 21.21% in group I, 29.29% in group II, 24.24% in group III and 25.25% in group IV. Fourty of 99 isolates were members of Enterobacteriaceae family (34 *E. coli* (34.34%), 3 *Proteus* spp. (2.97%) and 3 *Pantoea agglomerans* (2.97%). Four *Enterococcus faecalis* (3.96%), 2 *Enterococcus faecium* (1.98%), 1 *Acinetobacter* spp. (0.9%) and 1 *Acinetobacter baumannii* (0.9%) were the other pathogen isolations in the groups obtained at different times (TABLE I).

*E. coli* were the most isolated bacteria in the groups at all times (before IP and at postpartum 15 and 30th d). *Proteus* spp. was isolated in all times in GRII. *Pantoea agglomerans* was isolated in a sample of GRIV taken before IP; however, isolation of this bacterium was not obtained at postpartum 15 and 30th d. The other pathogens were isolated either postpartum 15th d or postpartum 30th d samples.

**Antibiotic susceptibility results**

Resistance to the antibiotics was common: all isolates were resistant to at least one tested drug (TABLE II). The majority of the bacteria in Enterobacteriaceae family were resistant to the penicillin, primarily to cefuroxime, and ceftiofur (100% resistance). Resistance to other antibiotics was also observed mainly sulphametoxazol/trimetoprim, ampicillin and oxytetracycline. The most active antimicrobial agents against to Enterobacteriaceae family were enrofloxacin (100% susceptible). *A. baumannii* was resistant to penicillin, cefuroxime, ampicillin, oxytetracycline and ceftiofur, but this bacterium was sensitive to trimethoprim-sulphametoxazol (100% susceptible) (TABLE II).

**Statistical results**

Statistical comparisons of the sampling times in groups showed that there were no significant differences (P=1.000 for GRI, GRII, GRIII and P=0.500 between 0-15th d, P=1.000 between 0-30th and 15-30th d for GRIV). The comparisons of the each groups in terms of the sampling time pointed out that there was no significant difference among the groups (P=1.000: at 0th d, P=0.524: 15th d, P=1.000: 30th d).

This study was planned to evaluate changings of the vaginal bacterial flora before and after IP in ewes, because postparturient complications resulted from the IP may indirectly affect the vaginal bacterial flora. There are many different causes of infertility in farm animals, among which infections play important role. Bacterial microorganisms or fecal flora colonize to genital tracts during manipulations of parturition, and this contamination may lead to some infectious origin postparturient diseases [29]. On the other hand, some predisposing factors such as dystocia, twin birth and placental retention may contribute to bacterial diseases of the genital tracts [1, 8, 11].

Following to IP, the well-known encountered complications are dystocia, placental retention and endometritis that these gynecological problems prevent to the genital canal involution [2, 13, 25]. The clinical investigation of postpartum period is also complicated because uterus cannot be examined at postpartum period by rectal or abdominal palpation, and lochiorrhea ceases shortly because of immediate or almost entire closure of the cervix [13, 18]. However, the presence of intrauterine bacteria does not affect the involution time of uterine due to specific immune defense mechanism of the uterine [25]. Thus ultrasonographic examination at postpartum period should be planned [13]. In this study, clinically, no dystocia and the other postparturient gynecological complications were observed. Moreover, there was no vaginal discharge at postpartum 15th d and the ultrasonographical examinations performed at postpartum 30th d revealed no abnormality to be remarkable for uterine infections.

IP also impairs to the postpartum reproductive performance [16] and delays fertility, which is not a common complication in ewes [5, 9, 31]. There are a few studies reported postpartum complications in ewes induced with different medications [4, 6, 9, 24, 26, 31]. In a study, the reported dystocia rate was 16% after application of 20 mg estradiol benzoate between 142-148th gestation d [6]. In another study, at the 131-133th gestation d, IP with 5 mg diethylstilbestrol (DES) and oxytocin resulted in placental retention in five ewes [24]. After induction of preterm parturition in ewes using dexamethasone, the incidence of placental retention was reported as 52% (31). In the presented study, aglepristone and dexamethasone were administered to the pregnant ewes at 138th gestation time for IP and no dystocia and placental retention were observed following IP.

Since the genital tract is susceptible to infectious diseases after parturition, it is usually able to overcome the nonspecific bacterial contamination [15, 29]. Certain species of Gram-negative bacilli are identified as causative agents in cases with reproductive problem [28]. And anaerobic bacteria are found to be the most common micro-organisms [16]. In a previous study, *E. coli, Clostridial* species, *Staphylococcus aureus, Streptococcus uberis* and *Enterococcus* species were determined in the uterine samples of the ewes, which were assessed as bacterial contamination [25]. In another study, *E. coli, A. pyogenes, S. epidermis, S. faecalis* and *S.* überis were the isolated microorganisms from the ewes without retention of fetal membranes. The main bacteria were *A. pyogenes and E. coli* that they are isolated from the remained fetal membranes of the ewes [29]. *Arcanobacterium* and *E. coli* are also isolated bacteria in animals without any reproductive problem after IP with PGF2α [16]. The opportunistic secondary invaders often cause genital bacterial infections in ruminants. Particularly,

*TABLE I*

**ACCORDING TO SAMPLING DAYS, BACTERIAL CULTURE RESULTS IN GROUPS**

| | GROUP I | | | GROUP II | | | GROUP III | | | GROUP IV | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| BACTERIA | **B-IP** | **PP 15th Day** | **PP 30th Day** | **B-IP** | **PP 15th Day** | **PP 30th Day** | **B-IP** | **PP 15th Day** | **PP 30th Day** | **B-IP** | **PP 15th Day** | **PP 30th Day** | Total |
| *Escherichia coli* | 4 | 3 | 3 | 3 | 4 | 3 | 3 | 2 | 3 | 3 | 1 | 2 | 34 |
| *Acinetobacter baumannii* | | | | | | | | | 1 | | | | 1 |
| *Acinetobacter* spp. | | | | | | | | 1 | | | | | 1 |
| *Enterococcus faecalis* | | | 2 | | | 1 | | 1 | | | | | 4 |
| *Enterococcus faecium* | | | | | | | | | | | 2 | | 2 |
| *Proteus* spp. | | | | 1 | 1 | 1 | | | | | | | 3 |
| *Pantoea agglomerans* | | | | | 1 | | | | | 2 | | | 3 |
| Total | **4** | **3** | **5** | **4** | **6** | **5** | **3** | **4** | **4** | **5** | **3** | **2** | **48** |

**B-IP:** Before induction of parturition, **PP:** Postpartum

*TABLE II*

**ANTIBIOTIC SUSCEPTIBILITY RESULTS OF THE BACTERIA (%)**

| BACTERIA | Liability | ANTIBIOTICS | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | **P** | **SXT** | **CXM** | **AMP** | **OT** | **ENR** | **XNL** |
| | S | - | - | - | - | 59.3 | 100.0 | - |
| *Escherichia coli* | I | - | 55.6 | - | 74.1 | 29.6 | - | - |
| | R | 100.0 | 44.4 | 100.0 | 25.9 | 11.1 | - | 100.0 |
| | S | - | - | - | - | - | 100.0 | - |
| *Acinetobacter baumannii* | I | - | 100.0 | - | - | - | - | - |
| | R | 100.0 | - | 100.0 | 100.0 | 100.0 | - | 100.0 |
| | S | - | 100.0 | - | - | - | - | - |
| *Acinetobacter* spp. | I | - | - | - | 100.0 | 100.0 | - | - |
| | R | 100.0 | - | 100.0 | - | - | 100.0 | 100.0 |
| | S | 100.0 | 100.0 | - | 75.0 | 75.0 | 100.0 | 100.0 |
| *Enterococcus faecalis* | I | - | - | - | - | 25.0 | - | - |
| | R | - | - | 100.0 | 25.0 | - | - | - |
| | S | - | 100.0 | - | 100.0 | - | 100.0 | - |
| *Enterococcus faecium* | I | - | - | - | - | 100.0 | - | - |
| | R | 100.0 | - | 100.0 | - | - | - | 100.0 |
| | S | - | - | 100.0 | - | - | 100.0 | 100.0 |
| *Proteus* spp. | I | - | - | - | 100.0 | - | - | - |
| | R | 100.0 | 100.0 | - | - | 100.0 | - | - |
| | S | 100.0 | - | 66.7 | - | - | 100.0 | - |
| *Pantoea agglomerans* | I | - | 33.3 | 33.3 | 66.7 | 66.7 | - | 66.7 |
| | R | - | 66.7 | - | 33.3 | 33.3 | - | 33.3 |

**S:** Sensitive, **I:** Intermediate, **R:** Resistant, **P:** Penicillin, **SXT:** Trimetoprim-sulphametoxazol, **CXM:** Cefuroxime, **AMP:** Ampicillin, **OT:** Oxytetracycline, **ENR:** Enrofloxacin, **XNL:** Ceftiofur.

*E. coli* is the commonly isolated major pathogen in ewes and cows [15, 23] and this microorganism may affect the reproductive functions [28]. Coliforms and other non-specific bacteria are also opportunistic pathogens in the reproductive tracts [23]. In the presented study, *E. coli* was the most isolated bacterium of the groups before IP and at 15 and 30$^{th}$ d after parturition. Although 99 types of bacteria were isolated in the samples, *E. coli, Proteus* spp.*, Pantoea agglomerans, Enterococcus faecalis, Enterococcus faecium, Acinetobacter* spp. and *A. baumannii* were the cultured pathogen micro-organisms. Moreover, statistical analysis results demonstrated that there was no significant difference in groups according to sampling times and between the groups ($P<0.05$). *Proteus* spp. is an aerobic bacterium that its reported identification rate in vaginal samples is 11% in ewes [23]. *Proteus* spp. may cause to bacteremia, stubborn urinary system infections, wounds infections, meningitis, organ abscess, umbilical remnant infections and sepsis [20]. *Proteus* spp. was isolated at all times in the samples of a ewe in GRII. It was suggested that underlying urinary infection might contaminate the genital canal or *Proteus* spp. might be possibly a dwelling pathogen for this ewe. *Pantoea* spp. is opportunistic bacteria that they can isolate in specific condition of otitis media, keratitis, endophtalmitis, arthritis and peritonitis [30]. In this study, *Pantoea agglomerans* was isolated 3 times from the vaginal samples of ewes, which were taken at 15$^{th}$ d in GRII and before IP in GRIV. This bacterium may casually exist in the vaginal flora of ewes without any specific disorders. As isolated in the uterus samples of ewes [25], in recent years, *Enterococcus faecalis* and *Enterococcus faecium* may cause the nosocomial infections in humans [12]. These bacterium were not isolated in the samples taken before IP; however, they were sporadically isolated either 15 or 30th d samples in all groups. *Acinetobacter* spp. is described as nosocomial pathogens, and particularly, *Acinetobacter baumannii* has increasingly importance due to its isolation in some substantial infective disease [8]. Here, *Acinetobacter* spp. and *A. baumannii* was only determined in samples taken at postpartum 15 and 30$^{th}$ d in GRIII.

The bacteria in Enterobactericeae family resist the many of the antimicrobial agents due to improper usage of the antibiotics; thus, this issue may only overcome with specific antibiotic usage planned after antibiotic susceptibility tests [27]. The bacterium of *A. baumannii* prevents the effective treatment regimen because it resists to both antibiotics and disinfectants [7]. In this study, antibiotic susceptibility results pointed out that Enterobactericeae family bacterium were sensitive to enrofloxacin, while *A. baumannii* was sensitive to trimethoprim-sulphametoxazol.

## CONCLUSION

Even if it was not encountered in this study, it should be considered that IP may disturb the vaginal flora and maybe responsible for the postpartum bacterial vaginal infections. Although the results in this study on ewe showed quite good information about the bacterial species, and no postparturient complication were also reported, as a limitation of the presented study, it might be implied that further experimental or clinical IP studies should be planned to demonstrate the influence of the vaginal flora in ewes with postparturient complications. And the enrofloxacin or trimethoprim-sulphametoxazol can be first option until antibiotic susceptibility results are obtained in ewes with postparturient vaginal infections, which induce with different IP methods.

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