



## RESEARCH PAPER

## OPEN ACCESS

## Studying the effect of ceftiofur on estrogen and progesterone changes in postpartum period in dairy cows in Chahar Mahal and Bakhtiyari

Akram Yar Ahmadi<sup>1</sup>, Amir Shafiei<sup>2</sup>, Forutan Salehi Nezhad<sup>3\*</sup>, Shokooh karimi<sup>4</sup>, Noorallah Sheikh Azadi<sup>5</sup>

<sup>1</sup>National University, Shahre Kord Branch, Shahre Kord, Iran

<sup>2</sup>Islamic Azad University Shahre Kord Branch, Shahre Kord, Iran

<sup>3</sup>Islamic Azad University Karaj Branch, Shahre Kord, Iran

<sup>4</sup>Islamic Azad University, Science and Research Branch, Khoozestan, Ahvaz, Iran

<sup>5</sup>Shahid Chamran National University, Ahvaz Branch, and Ahvaz, Iran

**Key words:** Estrogen, progesterone, ceftiofur, postpartum, cow.

<http://dx.doi.org/10.12692/ijb/4.8.202-208>

Article published on April 22, 2014

### Abstract

Metritis is one of the main factors of postpartum infertility in rows, and as a result reduces reproductive performance in dairy herds. In this study, the uterus of cows were sampled using swap Double Guard after 7 or 8 days of childbirth, the based on the number of counted colonies (more than 10 colonies) are divided into two groups. In the first group (19 cows) were subcutaneous undergone. Ceftiofur for three consecutive days from 10<sup>th</sup> day after birth and second group (16 cows) received no treatment. Blood sampling was performed on 28, 21, 14 and 7. The result showed that estrogen in ceftiofur group reached to the maximum blood level on day 14 in the first month after birth. Also progesterone in ceftiofur group was significantly higher than control group on day 14. Progesterone one was the maximum on day 28 in both groups, which it is higher ceftiofur group than control group. But there was no significant difference. It is generally observed that estrogen production was significantly higher than control group after drug use especially cows with several births showing drug's effect on the group. Also progesterone in ceftiofur group is significantly higher especially in cows with more than one birth. So it can be concluded that ceftiofur by affecting on uterine Bacteria can influence estrogen and progesterone secretion.

\* **Corresponding Author:** Forutan Salehi Nezhad ✉ [forutansalehi@yahoo.com](mailto:forutansalehi@yahoo.com)

## Introduction

Metritis is one of the most important factors of postpartum fertility in cows. These infections can increase the interval between two calving and first estrus. Also it increases number of inseminations resulting in pregnancy and as a result reduces reproductive yield in dairy herds. The resumption of ovarian cycling activity is one of the most important events in dairy cattle to start postpartum maximum reproduction activity. However, ovarian dysfunction is highly prevalent during this period. Most abnormalities are related to cycle or ovulation delay, prolonged luteal phase, and cystic ovaries. The first pre-ovulatory surge of LH may be followed in some cows with a complete cycle of normal luteal phase, and in others it may there is no normal cycle and so the concentration of progesterone is reduced (Gilbert and Schwark, 1992 and Herath *et al*, 2006). Due to the long period the inhibition caused by progesterone negative feedback which is secreted from corpus luteum and placenta during pregnancy, and pituitary becomes resistant after birth, so and doesn't react to transfusion of releasing gonads and Tropic hormones and disappears gradually (Arthur, 2001). Due to the absence or low levels of gonads or Tropic hormones, ovaries were relatively inactive. And the cow will be in N strvs, and this period may be long in high producing lactating cows (Arthur, 2001 and Noakes and Smith, 1989). In the postpartum the ovaries have often large follicles and do not ovulate and diminish, and they are sometimes erroneously called ovarian cysts (Ireland and Roche, 1987). Follicular development continues during pregnancy, but the diameter of the dominant follicle doesn't increase. This is probably due to the decrease of luteanating hormone in late pregnancy and the high concentration of steroids in the period (Ireland and Roche, 1983). Uterus is very vulnerable to infection and trauma after birth. Pathogenic bacteria pass through the cervix and infect uterus and cause endometritis. Uterine infection has harmful effects on reproductive efficiency after delivery, and causes economic losses. The presence of these bacteria causes inflammation of the uterine, delay in cervix bacterial contraction, and endometrium histological

lesions contractive and endometrium histological lesions. Uterine bacterial infection reduces bacteria productions and inflammations, pituitary LH secretion and impairs the growth of ovarian follicles and their function. So uterine disease is associated with lower pregnancy rates, increased calving interval to first insemination and increased pregnancy loss due to the removing. Most bacteria isolated from uterine infection are E.coli and A.pyogen. A.pyogen has been separated in uterine infections alone or in combination with other bacteria such as fusobacterium, Necroforume and Bacillus spp (bonnet *et al*, 1991 and Borsberry and Dobson, 1989). Vulva is loose in delivery or immediately after it, and cervix is open. Thus bacteria are allowed to enter the vagina and then into the uterus. Uterine environment also supports the growth of aerobic and anaerobic bacteria after birth (Sheldon and Dobson, 2004). Uterine disease suppresses ovarian follicular graph growth and impairs their functionality and Estradiol concentration is reduces in the blood (Sheldon *et al*, 2004).

This research aims at studying the effect of ceftiofur on reduction of uterine bacteria and ovarian activity and secretion of Estroids in postpartum period.

## Material and methods

### Place of testing

This study was conducted at a livestock industry in Hashtgerd which benefits from a good reproductive management. In the study, cows with 7 to 8 days of postpartum were separated from the others. After examining their uterus in a hygienic condition and after washing the posterior part of the cow with a mixture of iodine and water by swap double Guard prepared from Minitube Company sampling was completely performed of the uterine body and at two branches of horns. To ensure of sampling, the swab inside the uterine body was gently rotated to be fully smeared into the intra- uterine secretions. When the sampling was taken, the other hand inside the rectum was examining the swap directing and sampling location. After recording the specifications related to the cattle, swap was placed inside the cover to prevent

the contamination and was along with ice to prevent the contamination and was along with ice to prevent the proliferation of bacteria and was transferred to the laboratory within one hour for microbial examination.

#### Test method

In the laboratory, each swab taken in 2 ml nutrient was placed at laboratory of temperature for one hour, so that all bacteria of swap to solved in the environment. Then the environment was centrifuged and its sediment was removed by sampler than diluted and was superficially given on blood agar medium and medium level. Prepared samples were cultivated at blood agar medium for anaerobic bacteria growth and in the anaerobic incubation conditions and after 24 hours the grown bacteria was counted in both aerobic and anaerobic conditions, and the studied cows were divided based on colonies. If the total number of aerobic and anaerobic colonies were less than 10 per swap the cow wasn't classified then, and was excluded from the study. Cows were randomly divided into two groups if more than 10 bacteria colony were isolated from them. In the first group as a treatment group the ceftiofur injection was subcutaneously used for 3 consecutive days (1 mg per kg of weight) as a control group. During the classification we observed that the cows are alike in

terms of calvings and average number of colonies in both groups. In addition to the sampling of cervix using swab, cupping was done on days 28, 21, 14 and 7. The blood samples were centrifuged at 2000 cycles for 10 minutes and serum was removed. Sera were frozen at minus 20 degrees, until all samples were collected and sent to the laboratory to measure estrogen and progesterone using ELISA method. After recording all data related to each cow in both groups by SPSS and T. Test, statistical analysis was done.

#### Results

Estrogen rate was studied in both ceftiofur and control groups. Estrogen is higher in control group than ceftiofur group on day 7. After starting treatment on day 10 it is observed that estrogen is increasing in ceftiofur group, so that estrogen is increasing in ceftiofur group, so that it reaches to its maximum on day 14 in first month after birth. This hormone is higher in ceftiofur group than control group on days 14, 21 and 28 ( $P < 0.05$ ) (Table 1). Progesterone is the same in both groups on day 7, but is higher in ceftiofur group on day 14 and after starting the treatment ( $P < 0.05$ ). Progesterone was the maximum in both groups on day 28. Although it is higher in ceftiofur than control group, there was no significant difference (Table 2).

**Table 1.** Ceftiofur and control estrogen levels in both groups (Mean  $\pm$  SE).

Average estrogen Day 28	Average estrogen Day 21	Average estrogen Day 14	Average estrogen Day 7	Number of cows	of Groups
19 $\pm$ 4.59	16.29 $\pm$ 2.84	17.39 $\pm$ 6.44	23.44 $\pm$ 5.34	16	Control
24.33 $\pm$ 3.75	24.84 $\pm$ 4.24	26.42 $\pm$ 1.38	17.80 $\pm$ 2.34	19	Ceftiofur

**Table 2.** Progesterone Ceftiofur and control groups (Mean  $\pm$  SE).

Average progesterone Day 28	Average progesterone Day 21	Average progesterone Day 14	Average progesterone Day 7	Number of cows	of Groups
2.48 $\pm$ 0.5	2 $\pm$ 0.4	0.25 $\pm$ 0.06	0.19 $\pm$ 0.05	16	Control
2.52 $\pm$ 0.31	2.29 $\pm$ 0.77	0.69 $\pm$ 0.26	0.19 $\pm$ 0.02	19	Ceftiofur

sterogene and progesterone was studied and compared in control groups with cows suffered

various calving. Sterogen is lower in cows with first calving compared to the cows with several calving

cows, at the some time this hormones is higher in cows with first calving in the other days ( $P<0.05$ ). Considering the fluctuations in estrogen, it can be said that ovarian activity begins in primiparous cows on days after 14<sup>th</sup> day and postpartum and increases estrogen secretion, but in cows with higher parity, estrogen increasing becomes slowly after birth (Table 3). Progesterone was studied in control group with

different calving. As it can be seen, progesterone is higher in primiparous cows than multi-parous cows on day 21 ( $P<0.05$ ). Given that progesterone increases after ovulation, it can be concluded that in this time due to a ruptured follicle and formation of luteom in primiparous cows the progesterone increases because high levels of estrone on day 14 reflects graph follicle growth (Table 4).

**Table 3.** Estrogen stomach varies with the number of dairy cows in the control group (Mean  $\pm$  SE).

Average estrogen Day 28	Average estrogen Day 21	Average estrogen Day 14	Average estrogen Day 7	The number of calvings groups Control
34.25 $\pm$ 5.14	20.25 $\pm$ 3.14	31.75 $\pm$ 4.72	12.75 $\pm$ 2.14	Calving 1
14.09 $\pm$ 2.4	14.70 $\pm$ 2.64	11.01 $\pm$ 2.3	27 $\pm$ 5.34	Calving 2, 3, 4
19.85 $\pm$ 4.59	16.29 $\pm$ 2.84	17.39 $\pm$ 6.44	23.44 $\pm$ 5.34	Total

**Table 4.** Progesterone in birth control and a variety of different (Mean  $\pm$  SE).

Average progesterone Day 28	Average progesterone Day 21	Average progesterone Day 14	Average progesterone Day 7	The number of calvings groups Control
2.77 $\pm$ 0.5	2.98 $\pm$ 0.05	0.31 $\pm$ 0.04	0.15 $\pm$ 0.05	Calving 1
2.39 $\pm$ 0.4	1.57 $\pm$ 0.3	0.22 $\pm$ 0.06	0.21 $\pm$ 0.06	Calving 2, 3, 4
2.48 $\pm$ 0.5	2 $\pm$ 0.4	0.25 $\pm$ 0.06	0.19 $\pm$ 0.05	Total

Estrogen and progesterone were compared in ceftiofur group in different births. Estrogen is higher in primiparous cows compared to multiparous cows but there is no significant difference (Table 5). The maximum progesterone was measured in cows with more than 2 calving, on day 21 postpartum and in primiparous on day 28 postpartum. According to the

results it was determined that in primiparous cows the ovulation occurs later and the maximum progesterone in cows with more than calving on day 21 showing rapid growth of follicles following drug's effect, but ovulation takes place later in primiparous cows and progesterone increasing takes places later (Table 6).

**Table 5.** Ceftiofur in increasing estrogen levels in different groups (Mean  $\pm$  SE).

Average estrogen Day 28	Average estrogen Day 21	Average estrogen Day 14	Average estrogen Day 7	The number of calvings groups Ceftiofur
27.80 $\pm$ 4.3	25.60 $\pm$ 3.4	27.50 $\pm$ 2.4	19.75 $\pm$ 3.5	Calving 1
22.60 $\pm$ 2.8	24.38 $\pm$ 4.4	25.63 $\pm$ 1.1	16.38 $\pm$ 2.35	Calving 2, 3, 4
24.33 $\pm$ 3.75	24.85 $\pm$ 4.22	26.43 $\pm$ 1.38	17.80 $\pm$ 2.34	Total

**Table 6.** The amount of progesterone in cows with calving Ceftiofur different groups (Mean  $\pm$  SE).

Average progesterone Day 28	Average progesterone Day 21	Average progesterone Day 14	Average progesterone Day 7	The number of calvings groups Control
0.6 $\pm$ 3.01	0.65 $\pm$ 1.80	0.08 $\pm$ 0.48	0.3 $\pm$ 0.23	Calving 1
0.18 $\pm$ 2.14	0.8 $\pm$ 2.8	0.12 $\pm$ 0.86	0.01 $\pm$ 0.17	Calving 2, 3, 4
0.31 $\pm$ 2.53	0.77 $\pm$ 2.3	0.26 $\pm$ 0.69	0.02 $\pm$ 0.19	Total

Evaluating estrogen in Ceftiofur and control in primiparous and multiparous cows showed that estrogen was higher on days 14 and 28 in control group in primiparous cows compared to other cows ( $P < 0.05$ ) which can be caused by self-improvement and decreasing of uterine bacteria following ovarian function which is evident in primiparous cows. If estrogen is higher in ceftiofur group in more calving cows compared to control group in other cupping times. It is generally determined to control group in other cupping times. It is generally determined that follicle growth and estrogen generation is controlled after using drug, especially in multiparous cows showing drug effect on the group ( $P < 0.05$ ). Progesterone was studied in ceftiofur and control groups and in primiparous and multiparous cows and was determined that the maximum progesterone in primiparous cows is on day 21 after birth and since estrogen is high on day 14 in this group ( $P < 0.05$ ) showing graph follicle growth in this time, these follicles ovulate and increase progesterone on day 21 but follicle growth and then ovulation has been taken place in ceftiofur groups in multiparous cows. Also it is observed that progesterone is higher in ceftiofur group which is due to positive effect of drug on progesterone secretion.

### Discussion

Metritis control ovular graph follicles growth and impedes their function and as a result estradiol concentration reduction is being observed in blood. Bacteria and other pathogenic factors in the uterus control LH secretion from anterior hypophysis, but it doesn't influence FSH secretion. Pathogenic bacteria commonly isolated from metritis are *E. coli* and *A. pyogenes*. *E. coli* may have a positive effect on reproductive activities by producing Lipopolysaccharide, so puts the defense mechanism of the uterus at a lower level, and animal will be more susceptible to endometritis disease (Herath *et al*, 2006). Increasing luteinizing hormone stimulates the maturation of the follicles. When dominant follicle 2 continues to produce estradiol until reaches a threshold limit and stimulates and creates excitement in Luteinizing hormone. When estradiol positive

feedback is established the cow will have a bovine estrus in Luteinizing hormone and that subsequently will ovulate (Hafez and Hafez, 2000). In this study it has been shown in the control group with no treatment estrogen increasing started more late after follicle growth and its rate was in a lower level, but in the groups with treatment estrogen increasing and follicle growth started more rapidly in cows with more than one calving due to a reduction in uterine bacteria and is consistent with other studies showing metritis and uterine bacteria impedes ovarian function (Leblanc *et al*, 2002). This study has also shown that progesterone increasing in Ceftiofur group was on day 14 after birth and followed by treatment showing the positive effect of treatment and ovulation. Also ovulation is later in control group and secreted progesterone level is less than that of treatment group which is consistent with studies by (William *et al*, 2007). First postpartum ovulation is different. On delivery, LH hormone rate is low in both the anterior pituitary gland and blood and increases gradually from birth onwards. Marrow *et al*, (1971) showed that the first ovulation occurs on day 15 to 30 after birth. They also found that the anterior pituitary doesn't react in response to releasing gonads and Tropic hormones until 7 or 8 days after delivery. Uterus containing a large number of postpartum bacteria is of lower luteum growth and progesterone secretion and prostaglandin concentration rises in blood after birth which is similar to the infected uterine and damaged uterine tissue (William *et al*, 2007) using septrin improves cattle breeding in the postpartum period. The studies by Miller *et al*, 1980 didn't report any reproductive improvement of inter-uterus using antibiotics and untreated in postpartum period. According to the researchers efficiency and success of intrauterine antibiotic treatment depends on the effect of antibiotic formulations on pathogens in the uterus, failure to prevent uterine defense mechanism, influencing infectious environment, failure to remain in milk and meat, sufficient concentration of the drug, number of treatments and economic efficiency (Leblanc *et al*, 2002). *E. coli* is one of the most common pathogenic bacteria in cow's uterus. Increasing this bacteria and

(Lip polysaccharide) LPS secretory by these bacteria stimulates prostoglandinins at endometer level or at epithelial cells surface (William *et al*, 2007). There is a relationship between bacterial infection and delayed ovulation in cattle. In a few of endometrit cows, ovulation occurs during the first dominant follicle growth in postpartum period. And this dominant follicle's growth in postpartum period and also estrogen secretion are less in cows with a large number of inter-uterine bacteria (Sheldon and Dobson, 2004). Metritis has no effect on FSH plasma concentration or follicular wave (Sheldon and Dobson, 2004 and Sheldon *et al*, 2006). Metritis influence hypothalamic and anterior pituitary so that the secreted LPS from E.coli can be endometrially absorbed and entered in blood circulation (Mateus *et al*, 2002).

According to the study it can be concluded that using ceftiofur in postpartum period decreases uterine bacteria and followed by follicle's growth and estrogen secretion increasing, also by growing graph follicles and ovulation progesterone hormone increases as well.

#### Reference

**Andrew AH.** 2004. bovine medicine disease and husbandary of cattle, w.b.saundres, 508-513 p.

**Arthur G.** 2001. Veterinary reproduction and obstetrice .s.w.b.saunders 115:161-166 p.

**Bonnet BN, Martin SW, Gannon VPG, Miller RB, Etherington WG.** 1991. Endometrial biopsy in Holstein-Frisian dairy cows.III, Bacteriological analysis and correlation with histopathological findings. Canadian Journal **55**, 168-173.  
<http://dx.doi.org/CJ/55.P168-173>.

**Borsberry S, Dobson H.** 1989. per parturient diseases and their effect on reproductive performance in five dairy herds. Vet rec **124**, 217-219.  
<http://dx.doi.org/R/124.P217-219>.

**Gilbert RO, Schwark DS.** 1992. Management of

per partum condition in the cow.vet. clin. North is. Food Animal **8**, 40-55.

<http://dx.doi.org/FA/8.P40-55>.

**Hafez ESE, Hafez B.** 2000. Reproduction in farm animal, Williams Wilkins, Florida. 216.

**Herath S, Fischer DP, Weling D, William EJ, lilly ST, Dobson H.** 2006 .expression and function of toll-like receptor 4 in the endometrial cells of the uterus. Endocrinology **147**, 562-570.

<http://dx.doi.org/E/147.P562-570>.

**Ireland JJ, Roche JF.** 1987. Hypotheses regarding development of dominant follicles during a bovine estrous cycle. In follicular growth and ovulation rate in farm animal, 1-18 p.

**Ireland JJ, Roche JF.** 1983. Development of non-ovulatory antral follicles in heifers: changes in steroids in follicular fluid and receptors for gonado trophins. Endocrinology **112**, 150-156.

<http://dx.doi.org/E/112.P150-156>.

**Leblanc SJ, Duffield TF, Leslie KE, Bateman KG, Walton WH, Jonson WH.** 2002. The effect of treatment of clinical endometritis on reproductive performance in dairy cos. Journal Dairy sciences **85**, 2237-2244.

<http://dx.doi.org/JDS/85.P2237-2244>.

**Leblanc SJ, Duffield TF, Leslie KE, Bateman KG, Walton WH.** 2002. Defining and diagnosing postpartum clinical endometritis and impact on reproductive performance in dairy cows. Dairy Sciences **85**, 2223-2236.

<http://dx.doi.org/DS/85.P2223-2236>.

**Mateus L, Lopes Da Costa L, Bernardo F, Silva JR.** 2002. Influence of puerperal uterine infection on uterine involution and postpartum ovarian activity in dairy cows. Report. Dom. Animal Journal **37**, 31-35.

<http://dx.doi.org/AJ/37.P31-35>.

**Mateus L, Lopes Da Costa L, Carval H, Serra P, Robolo Silva J.** 2002. Blood and intrauterine leukocyte profile and function in dairy cows that spontaneously recovered from postpartum endometritis. *reprod. Dom. Animal Journal* **37**, 176-181.

<http://dx.doi.org/AJ/37.P.176-181>.

**Noakes DE, Till D, Smith GR.** 1989 . Bovine uterine flora postpartum: A comparison of swabbing and biopsy. *Vet. Rec* **124**, 563-564.

<http://dx.doi.org/R/124.P.563-564>.

**Sheldon IM, Dobson H.** 2004. Postpartum uterine health in cattle. *Animal Reproduce Science* **83**, 295-306.

<http://dx.doi.org/ARS/83.P.295-306>.

**Sheldon IM, lewis GS, leblanc SJ, Gilbert RO.** 2006. Defining postpartum Uterine disease in cattle. *Theriogenology* **65**, 1516-1530.

<http://dx.doi.org/T/65.P.1516-1530>.

**Sheldon IM, Bushnell M, Montgomery J, Rycroft AN.** 2004. Minimum inhibitory concentration of some antimicrobial drugs against bacterial causing uterine infection in cattle. *Vet. Rec* **155**, 383 – 387.

<http://dx.doi.org/155.P.383-387>.

**Williams EJ, Fischer DP, Noakes DE, England GCW, Rycroft A, Dobson H.** 2007. The cow. *Theriogenology* **68**, 549-559.

<http://dx.doi.org/T/68.P.549-559>.

**Younquist RS.** 2007. Large animal theriogenology. 342-343 p.

**Zerbe H, Scheider N, Leibold W, Wensing T, Kruij TA, Schuberth HJ.** 2000. Altered functional and immunophenotypic properties of neutrophilic granulocytes in post partum cows associated it fatty liver. *Theriogenology* **54**, 771 – 778.

<http://dx.doi.org/T/57.P.771-778>.