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The Effect of using Ceftiofur drug on estrogen (E2) and progesterone (P4) concentrations during post parturition period in dairy cattle with Bacterial Endometritis

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Abstract:

Postpartum infertility is caused by a range of different conditions. In female cattle, uterine infections are one of the most common causes. As a result, the affections of uterine infections will reduce in dairy herds fertility. In this investigation, samples were taken by using double guard swaps from the uterus of cattle that had passed nine to ten days after calving. They were divided into two groups depending on the number of colonies which were over 12 colonies.

Twenty cows in the first group were treated with Ceftiofur hydrochloride (injection) subcutaneously, and the treatment was carried out twelve days after the calving process, and it took four days consecutively. The second group has remained as the control group as long as it represented 18 untreated cattle. After calving, the procedure of collecting samples began, which took place on days 5, 12, 19, and 26. The results indicated that the level of estrogen in the Ceftiofur treated group had reached its peak (maximum level) on the twelfth day after delivery. On day 12, the level of progesterone in the ceftiofur-treated group was greater than the control group significantly. Furthermore, in both of the groups, the level of progesterone had reached its maximum level on day 26, however it was higher in the ceftiofur-treated group than in the control group, but no significant distinguish was observed (<0.05). Conclusively, according to the results, it was observed that after using ceftiofur, the amount of estrogen production, especially in cow with multiple births, was significantly higher than the control group (<0.05), which reveals the effect of the drug on this group. Also, the level of progesterone in the Ceftiofur treated group was significantly higher, especially in cattle with several calvs. As a result, it is possible to deduce that ceftiofur can influence estrogen and progesterone secretion by reducing the number of uterine bacteria.

Key Words: Estrogen, Ceftiofur, Progesterone, Postpartum, Cattle.

الملخص:

عدوى الرحم ما بعد الولادة في الابقار يعد واحد من الأسباب الأساسية لخفض أداء الإنجابي. يهتم هذا البحث بدراسة رحم الابقار بعد مرور ٩ الى ١٠ أيام من الولادة، واخذت عينات منهما والتي تم تقسيمهما الى مجموعتين معتمدا على عدد المستعمرات المتكونة (أكثر من ١٢ مستعمرة). المجموعة الأولى متكونة من ٢٠ بقرة والتي عُملت ب Ceftiofur hydrochloride حُقنت تحت الجلد. المعاملة استمرت لمدة ٤ أيام بعد ١٢ يوماً من الولادة. المجموعة الثانية متكون من ١٨ بقرة والتي بقيت كمجموعة السيطرة دون اخذ العلاج. تمت اخذ العينات من الدم في اليوم ٥، ١٢، ١٩ و٢٦. النتائج أظهرت ان مستوى الاستروجين في المجموعة المتعاملة ب Ceftiofur وصلت اعلى مستوى في يوم ١٢. مستوى البروجسترون في المجموعة المتعاملة ب Ceftiofur في اليوم ١٢ كانت

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اعلى مقارنة بمجموعة السيطرة. في كلا المجموعتين مستوى البروجسترون وصلت الى اعلى مستوى في اليوم ٢٦، مع ذلك كانت بمستوى اعلى في المجموعة المتعاملة ب Ceftiofur ولكن لم تلاحظ أي فروقات معنوية (<0.05). استنادا الى النتائج لوحظ ان بعد استعمال ال Ceftiofur كمية انتاج الاستروجين وخاصةً في البقار المتعددة الولادة كانت اعلى في المجموعة المتعاملة ب Ceftiofur معنويا (<0.05)، هذه انعكاس تأثير الدواء على تلك المجموعة. كذلك مستوى البروجسترون في المجموعة المتعاملة ب Ceftiofur كانت اعلى خاصةً في الابقار المتعددة الولادة. كنتيجة ممكن ان نستنتج بان Ceftiofur يؤثر على افراز البروجسترون والاستروجين بتقليل اعداد بكتريا الرحم.

الكليمات المفتاحية: الإستروجين، السيفتيوفور، البروجسترون، ما بعد الولادة، الماشية.

پوختە:

هۆکارەکانی هەوکردنی منداڵدان بە یەکێك لە سەرەکیترین هۆکارەکانی نەزۆکی دادەنترێت لە دوای وەچە خستن لە مانگادا، لە ئەنجامی ئەمەشدا، بەرهەمهێنان کەمتردەبێت. لەم توێژینەوەیەدا، نمونە لە منداڵدانی ئە و مانگایانە وەگیراوە بە ٩ یان ١٠ ڕۆژ دوای وەچە خستنەوەیاندا تێپەریوە. بۆ دو کۆمەڵە دابەش کراون بە پشت بەستن بە ژمارەی کۆلۆنیەکان (زیاتر لە ١٢ کۆڵۆنی) . لە کۆمەڵەی یەکامدا، ٢٠ مانگا لە ژێر پێستەوە چارەسەریان بۆ کرا بە Ceftiofur hydrochloride بۆ ماوەی ٤ ڕۆژ بە بەردەوامی لە ١٢ ەم رۆژی پاش وەچە خستنەوە، وە لە کۆمەڵەی دووەم ١٨ مانگا چارەسەر نەکران کە وەك گروپی کۆنترۆڵ دانراوە. هەروەها نمونە لە خوێنیان وەگیرا لە رۆژانی ٥ و ١٢ و ١٩ و ٢٦. ئەنجامەکان دەریانخست کە هۆرمۆنی ئیسترۆجین لە کۆمەڵەی Ceftiofur دەگات بە لوتکە (بەرزترین ئاست) لە رۆژی ١٢ یەمی پاش وەچە خستنەوە. لەگەڵ ئەوەشدا، لە رۆژی ١٢ یەم، ئاستی پرۆجسترۆن لە کۆمەڵەی Ceftiofur زۆر بەرز ترە لە کۆمەڵەی کۆنترۆڵ کراو. لە هەردو کۆمەڵەکەدا، ئاستی پرۆجسترۆن لە رۆژی ٢٦ دا دەگاتە لوتکە، کە ئەمەش بەرزترینە لە کۆمەڵەی Ceftiofur وەك لە کۆمەڵەی کۆنترۆڵ کراو، بەڵام جیاوازیەکی بەرچاوی دروست نەکرد (<0.05). بە شێوەیەکی گشتی، تێبینی ئەوە کرا کە پاش بەکارهێنانی دەرمانی Ceftiofur ، ڕێژەی بەرهەمهێنانی هۆرمۆنی ئیسترۆجین، بە تایبەتی لە ئەو مانگایانەی کە چەند جارێك وەچەیان خستۆتەوە،بەراورد بە کۆمەڵەی کۆنترۆڵکراو زۆر بەرزترە (<0.05). ئەمەش کاریگەری دەرمانەکە لەسەر ئەو کۆمەڵەیە دەردەخات. وەهەروەها، ئاستی پرۆجسترۆن لە کۆمەڵەی Ceftiofur بە ڕێژەیەکی بەرچاو بەرزترە، بە تایبەتی لە ئەو مانگایانەی کە زیاتر لە یەك جار وەچەیان خستۆتەوە. لەبەر ئەمەش دەتوانرێت بوترێت کە Ceftiofur ئەگەری کاریگەری لەسەر دەردانی هۆرمۆنی ئیسترۆجین و پرۆجسترۆن هەیە لە ڕێگەی کارکردن لەسەر کەمکردنەوەی بەکتریا لەناو منداڵداندا.

1. Introduction

In order to begin the next cycle and prepare for the next pregnancy, the cow's reproductive system must recover both physically and physiologically after calving. However, difficulties known as post-partum disorders might occur during this period. One of the leading causes of postpartum infertility in cows is uterine infections. The distance between the two calving and the first estrus is increased by these infections. It also increases the number of fertilizations, leading to pregnancy, which in turn reduces reproductive performance in dairy cattle herds. One of the most critical events in dairy cattle following calving is the resumption of ovarian cycle activity (Galvao et al., 2009). The role of postpartum uterine infections in dairy cows in explaining the reasons for ovarian dysfunction is critical. Ovarian function is harmed by uterine infections, which results in lower conception rates and significant infertility. Uterine bacterial infections cause the overy's dominant follicles to develop slowly, limiting the ovary's capacity to ovulate, and eventually leading to infertility, so it can be said that the most common disorder is delayed menstruation o during this time, there occurs ovulation, a protracted luteal phase, and cystic ovaries. In some cows, the initial wave of luteal hormone (LH) before ovulation is followed by a whole cycle with a normal luteal phase, and in others, they may not have a normal cycle in which the progesterone concentration is reduced (Williams's *et al.*, 2007). The pituitary gland becomes resistant after birth due to a lengthy period of suppression caused by the

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negative feedback effect of progesterone, which is released by the placenta and corpus luteum throughout pregnancy, and does not respond to injections of gonadotropin-releasing hormone (Cheong *et al*., 2016). As a result of the low number of gonadotropins, poor reproduction will occur in the ovaries and the female cow will be in the state of infertility, which can be continuous lactating and great-yielding female cattle (Sicsis *et al.*, 2018). Multiple large follicles in the ovaries that do not ovulate and degenerate quickly in the postpartum stage are incorrectly referred to as ovarian cysts (Ireland & Roche, 1987; Bromfield & Sheldon, 2011). The dominant follicle diameter does not expand during pregnancy, presumably due to a drop in luteinizing hormone in late pregnancy and a high concentration of steroids during this time (Ireland & Roche, 1987). Infection and postpartum trauma are highly dangerous to the uterus. endometritis is caused by pathogenic germs that enter the uterus through the cervix. The impact of a postpartum uterine infection on reproductive efficiency and economic losses are both negative. Inflammation, endometrial histological lesions, and delayed uterine contractions are all symptoms of these bacteria present in the uterus. Cows' reproductive cycles are controlled by a variety of events after parturition, including the establishment and growth of the ovaries and the management of harmful bacteria in the uterine tissues. In dairy cattle, pathogenic bacteria frequently induce uterine illness, which reduces fertility and output (Walker *et al.*, 2015).

Endometritis is caused by mixed bacterial infection of the uterus, which is common in post-partum dairy cows and is associated with impaired reproductive performance shown in reduced first service conception, increased abortion across the breeding period (Bromfield *et al.*, 2015). *Escherichia coli and Aeromonas Pyogenes* are common pathogenic bacteria recovered from endometritis patients, and they have been found alone or in conjunction with other bacteria such as *Fusobacterium necrophorum* and species with involvement in uterine infections (Foster *et al.*, 2019). During and immediately after delivery, the vulva is loose and the cervix is open, allowing bacteria to enter the vagina and enter the uterus. Both aerobic and anaerobic bacteria are supported by the postpartum environment (Pascottini *et al.*, 2020). The size and function of ovarian graph follicles are suppressed by uterine illness, and the concentration of estradiol in the blood is reduced (Iain & Sain, 2017). Antibiotics, on the other hands, must be taken on a regular basis for at least five days. Ceftiofur is effective against gram-positive and gram-negative bacteria (Witte *at al.*, 2011).

Ceftiofur is a third-generation cephalosporin antibiotic that was first used in veterinary medicine in 1987. Excenel is the active ingredient in Zoetis' Specramast LC (lactating cow formula) product and is marketed by the pharmaceutical company Zoetis. It is resistant to beta-lactamase hydrolysis and shows antibacterial action against Gram-positive and Gram-negative bacteria. Ceftiofur-resistant *E. coli* strains have been discovered. Because the metabolite desfurolyceftiofur has antibiotic activity as well, the two chemicals are combined to assess antibiotic activity in milk. Ceftiofur has a broad antibacterial spectrum and kills Gram positive and Gram-negative bacteria as well as some anaerobic bacteria. *Escherichia coli*, *Pasteurella Multifidi*, *Actinobacillus pleuropneumonia*, *Haemophilus*, and *Salmonella Spp* are all susceptible to it (Pomorska *et al.*, 2015). It is also recommended for the treatment of mastitis in animals due to its broad-spectrum action (Owens et al., 1990) Because of its broad-spectrum, typically bactericidal properties, ceftiofur has considerable uses in both human and veterinary medicine (Dołhań *et al.*, 2014; Foster *et al.*, 2015). The sodium version of ceftiofur, Naxcel®, was introduced into veterinary medicine by the Upjohn Company in 1988. The

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hydrochloride salt of ceftiofur Excenel®, which is a more stable version, was then introduced. It has an oxyiminoaminothiazolyl group and a furoic acid thioester at position 3.

The goal of this study was to see how effective Ceftiofur was at reducing the uterine bacteria's number, ovarian activity, and steroid hormone release (E2 and P4) in postpartum dairy cows.

2. Material and Methods

This study was conducted in 2019 in one of the private industrial farms in Bardakar in Sulaimani, Iraq, which has good reproductive management. In this study, calves that were 9 to 10 days old were separated. After the examination of uteri of selected cattle in completely hygienic conditions and after washing the back of the caw with a mixture of water and betadine, a double guard swab (Babio abiotechnology co. LTD, China) was used for sampling from the uterine body site and at the place of the bifurcation of the branches. To ensure of the correct sampling, the swab was gently rotated inside the uterine body to be completely impregnated with intrauterine secretions. During the sampling, the swap path and the sampling site were examined by rectal palpation. The tubes labeled, and the swab was placed in a cool place to prevent contamination and placed next to the ice to prevent bacterial multiplication, and was transferred to the laboratory for microbial testing within an hour.

The swab was placed in 3 ccs of broth nutrition and left at room temperature for an hour in the laboratory. The sediment was removed with a sampler from the centrifuged media, which was subsequently diluted and cultured on blood agar medium and surface culture medium. The prepared samples were cultivated in a blood agar medium and incubated under anaerobic conditions for 24 hours at 37 degrees Celsius. The number of bacteria growing in aerobic and anaerobic environments was recorded after 24 hours (Rosmini *et al.*, 2014).

The cattle were separated into groups based on the number of colonies counted. The animals were eliminated from the trial if the total number of aerobic and anaerobic colonies per exchange was less than 12. Cattle having more than 12 bacterial colonies in 24 hours were allocated into two groups at random. Ceftiofur was administered subcutaneously for four days in the first group, which was the treatment group (1 mg per kg body weight). The second group received no therapy and was classified as a control group. The cattle were divided into groups based on the number of calves born and the average number of colonies. Blood samples were taken on days 26, 5, 12, and 19 in addition to swab sampling from the uterus. Blood samples were centrifuged for 8 minutes at 4000 rpm. Serums were kept frozen at -20 ° C until all samples were collected and delivered to the lab for ELISA testing to determine estrogen and progesterone levels. SAS 9.4 M5 software 2017 was used for statistical analysis, using T-Test and probability tests (0.05).

3. Results

Estrogen levels were assessed in both Ceftiofur and control groups. The level of estrogen in control group on day 5 was higher than in the Ceftiofur group (Table 1). The amount of estrogen in the Ceftiofur group increased after starting treatment (for 3 days) on day 8, reaching its highest level on day 12 in the first month after delivery. The levels of this hormone on days 12, 19, and 26, the Ceftiofur group are higher comparing to the control group ($p<0.05$) (Table 1). The levels of progesterone in the two groups were same on day 5, but on day 12 and after starting treatment, the

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level of progesterone was altered in the Ceftiofur group ($p<0.05$). In both groups, the level of progesterone reached its maximum on the 26th day, despite the fact that it was greater in the Ceftiofur group than in the control group, there was no statistically significant difference (Table 2).

Emplacement of Table 1
Emplacement of Table 2

Estrogen in the first calving cows was lower than in multi-lactating cows on day 5, while on other days the level of this hormone was getting higher at the same time in the first calving cows ($p<0.05$). Due to changes in estrogen levels, it can be concluded that in the 12 days after calving, ovarian activity began in cows in the first pregnancy and increased E3 secretion, but in cows with multiple pregnancies, estrogen increased slowly after calving (Table 3). P4 levels in the control group in different deliveries were also assessed. As can be seen, the amount of progesterone on day 19 became higher in first-calf cows than in multi-calf cows.

Emplacement of Table 3
Emplacement of Table 4

Estrogen and progesterone levels in the Ceftiofur group were also compared in different calves and it was observed that the amount of estrogen in first calving cows was higher than in multi-calving cows, the deference was no significant (Table 5). On day 19 postpartum, maximum progesterone levels in cattle with more than two calves were assessed, and on day 26 postpartum, maximum progesterone levels in first-calf cows were measured.

Emplacement of Table 5
Emplacement of Table 6

Examination of estrogen levels in Ceftiofur and control groups and first and cattle with multiple pregnancies showed that estrogen levels were higher in the control group in 12-day-old cows than in other cows ($p<0.05$).

The level of progesterone in the Ceftiofur and control groups, as well as the first and multi-lactating cows, were examined, and it was found that the maximum level of progesterone in the control group is on the 19th day after calving. Estrogen in this group was high on day 12($p<0.05$).

4. Discussion

Ovarian follicles' development and function are hampered by uterine disease, resulting in a decrease in the concentration of estradiol in the blood. Bacteria and other pathogens in uterus inhibit the LH secretion from the pituitary glands anterior. However, they do not affect on the secretion of follicle stimulating hormone (FSH). Commonly the isolated pathogenic bacteria from uterine infections are *Escherichia coli, Aeromonas Pyogenes. E. coli* can affect reproductive activity by producing lipopolysaccharide. In this case, the mechanism of defense of the uterus is lowered and makes the animal extra susceptible to endo-metritis (Sheldon *et al.*, 2014). In this study, The control group was shown, the increment in estrogen levels following follicular growth started later and its level was low, but in the treatment group, due to the decrease in the number of uterine bacteria, growth Follicular and estrogen levels increased rapidly, particularly in cows that have had multiple calves,

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which is consistent with other studies that have shown that uterine diseases and bacteria impair ovarian function (Le Blanc *et al.*, 2002; Potter *et al*., 2010). Also, the present study, it was shown that the increment in progesterone in the Ceftiofur group occurred earlier and on the 12th day post_ parturition and after treatment, which indicates the treatment had positively effect on ovulation. Also, in the control group, both ovulations are delayed and the level of progesterone secretion is lower than in the ceftiofur group, which is agree with the results of William *et al.* (2007). Furthermore, the high estrogen level in the control group indicates the follicle graph growth at this time, because on day 19, the same follicles ovulate and produce more progesterone, but in the ceftiofur group cow have more fertile follicle growth which followed by ovulation. In the ceftiofur group the higher level of progesterone was also observed, that is due to the positive effect of the treatment (drug) on the growth and progesterone secretion.

The timing of the first post_ parturition is different. in vet, the level of LH hormone in the pituitary gland anterior and the blood is low and gradually elevates from the time of delivery onwards. A study by Borsberry and Dobson (1989) showed that the first ovulation occurs 15 to 30 days after delivery. They also found that the anterior pituitary gland did not respond to the gonadotropin-1-releasing hormone until day seven or eight after delivery. A uterus that contains a high quantity of bacteria after delivery has a lower rate of corpus luteum development and secretion of progesterone, and postpartum increases the concentration of the blood prostaglandin, that is assembly to the function of the infected uterus and uterine tissue injury (Opsomer *et al.*, 2000; Williams *et al.*, 2005). Considering that progesterone levels rise after ovulation, it's reasonable to assume that at this time, due to the rupture of the follicle and the corpus luteum formation in the cows of the first pregnancy, because of the high level of estrogen on day 12, the progesterone level has increased, indicating follicular development. According to the results, ovulation occurs later in the first calving cows, and the maximum level of progesterone in cows lying higher than 2 on day 19, which indicates faster growth of follicles following the effect of the drug. But in the first abdomen ovulation occurs later and progesterone increases later.

The use of Ceftiofur in the postpartum period in cows improves the reproductive status of the animal. In studies by Iain & Sain, (2017**)**, no improvement in reproductive status was reported during intrauterine antibiotics and no treatment in the postpartum period. Depending on researchers, the success and effectiveness of using intrauterine antibiotics depends on the effect of antibiotic formulation on pathogens present in the uterus, inability to inhibit the defense or protect mechanism of the uterus, affecting the infectious environment, not remaining in milk and meat, sufficient concentration drugs, number of treatments and economic returns (Zerbe *et al.*, 2000). In cows, there is a relationship exists between bacterial delayed ovulation and infection. The reduction of uterine, bacteria follows the function of the ovaries, which is evident in cows in the first pregnancy. However, the level of estrogens in the Ceftiofur group is greater in cows with multiple calving than in the control group at other times of blood collection. In general, it was found that after using the drug, follicle growth and estrogen production, especially in cows with multiple births, is higher than the control group, which indicates the effect of the drug on this group. In a small number of endometrial cows, ovulation happens when the dominant follicle grows for the first time in the postpartum period, and the growth rate of this dominant follicle is slow. The follicular waves or concentration of plasma FSH

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does not affected by uterine infection (Iain & Sain, 2017). The hypothalamus and anterior pituitary gland are both affected by the uterine infection. Lipopolysaccharide secreted by E. coli can be absorbed from the uterine mucosa and enter the bloodstream (Magata *et al*., 2015). This study's findings indicate that, the using of ceftiofur in the postpartum period reduces the number of uterine bacteria, followed by the growth of follicles and increased estrogen secretion. Progesterone also increases with the growth of follicles and ovulation.

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Table 1: Effect of Ceftiofur on E2 in concentration (pg/ml) in both Ceftiofur and control groups (Mean ±SE)

Groups	Number of cattle	Average of estrogen in 5 days	Average of estrogen in 12 days	Average of estrogen in 19 days	Average of estrogen in 26 days
Ceftiofur	21	18.7 ± 3.3	27.33 ± 2.4	25.71 ± 5.11	25.32 ± 4.65
Control	18	24.54 ± 6.2	18.3 ± 7.3	17.25 ± 3.9	20.8 ± 5.1

*The E2 concentration are significantly higher (P<0.05) between Ceftiofur and control groups on day 5, 12, 19, and 26.

Table 2: Effect of Ceftiofur on P4 concentration (pg/ml) in both Ceftiofur and control groups (Mean ±SE)

Groups	Number of cattle	Average of progesterone in 5 days	Average of progesterone in 12 days	Average of progesterone in 19 days	Average of progesterone in 26 days
Ceftiofur	21	0.21 ± 0.03	0.8 ± 0.35	3.3 ± 0.78	3.6 ± 0.42
Control	18	0.21 ± 0.06	0.36 ± 0.07	3 ± 0.5	3.5 ± 0.61

No significant (NS) different in the concentration of P4 (P<0.05) between Ceftiofur and control groups on day 5, 12, 19, and 26.

Table 3: Effect of Ceftiofur on E2 concentration (pg/ml) in both Ceftiofur and control groups on cows with different calving number (Mean ±SE)

The number of calving in control group	Average of estrogen in 5 days	Average of estrogen in 12 days	Average of estrogen in 19 days	Average of estrogen in 26 days
1	13.84 ± 3.15	32.8 ± 5.61	21.33 ± 4.11	35.11 ± 6.21
2,3 and 4	27.1 ± 5.4	12.05 ± 3.5	15.83 ± 3.62	15.1 ± 3.31
Total	24.56 ± 5.41	18.5 ± 7.49	17.44 ± 3.91	20.21 5.61

*The E2 concentration are significantly higher (P<0.05) in control group on day 5,12,19, and 26 on cows with different calving number

Table 4: Effect of Ceftiofur on P4 concentration (pg/ml) in both Ceftiofur and control groups on cows with different calving number (Mean ±SE)

The number of calving in control group	Average of progesterone in 5 days	Average of progesterone in 12 days	Average of progesterone in 19 days	Average of progesterone in 26 days
1	0.27 ± 0.06	0.42 ± 0.05	3.1 ± 0.6	3.65 ± 0.61
2,3 and 4	0.32 ± 0.06	0.34 ± 0.07	2.31 ± 0.35	3.51 ± 0.49
Total	0.28 ± 0.05	0.33 ± 0.05	3.1 ± 0.45	3.62 ± 0.63

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*No significant (NS) different in P4 concentration in day 5, 12, 19, and 26. P4 concentration was significantly higher in day 19 for first –calf cows than in multi-calf cows($P<0.05$)

Table 5: Mean of estrogen in the Ceftiofur group in cows with different calving number (Mean ±SE)

The number of calving in Ceftiofur group	Average of estrogen in 5 days	Average of estrogen in 12 days	Average of estrogen in 19 days	Average of estrogen in 26 days
1	20.9 ± 4.47	28.1 ± 3.12	26.1 ± 4.1	27.18 ± 5.44
2,3 and 4	17.4 ± 3.31	26.4 ± 2.1	25.41 ± 5.1	23.37 ± 3.15
Total	18.9 ± 3.21	27.5 ± 2.11	25.91 ± 5.12	24.42 ± 4.81

No Significant (NS) different in P4 concentration. P4 concentration was significantly higher ($P<0.05$).

Table 6: Mean of progesterone in the Ceftiofur group in cows with different calving number (Mean ±SE)

The number of calving in Ceftiofur group	Average of progesterone in 5 days	Average of progesterone in 12 days	Average of progesterone in 19 days	Average of progesterone in 26 days
1	0.24 ± 0.31	0.51 ± 0.09	2.1 ± 0.75	4.12 ± 0.71
2,3 and 4	0.19 ± 0.02	0.91 ± 0.16	3.33 ± 0.92	3.18 ± 0.26
Total	0.21 ± 0.04	0.71 ± 0.33	3.22 ± .85	3.43 ± 0.47

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