The effectiveness of combined preventive treatment with Ceftiofur, Oxytocin and PGF$_{2\alpha}$ on fertility parameters in cows

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SUMMARY

The present study is aimed at evaluating the effectiveness of combined preventive treatment with Ceftiofur Hydrochloride (CHR), Oxytocin (OXT) and PGF$_{2\alpha}$ on fertility parameters and puerperal disorder rates in postpartum (pp) dairy cows. A total of 191 Holstein-Friesian cows were divided into four groups. The cows in group I (GI; n=37) and II (GII; n=69) were treated on a daily basis with CHR and OXT for five and three days pp, respectively. Group III (GIII; n=52) was designed as the PGF$_{2\alpha}$ group, and group IV (GIV; n=33) served as a control group. The animals in GI, GII, and GIII were given a PGF$_{2\alpha}$ analogue on days 15 and 26 pp. Each group was further divided into subgroups of normal parturition (np) and dystocia (d) when the study results were evaluated. It was found that the first service pregnancy rates for the subgroup of dystocia were significantly higher (P<0.05 and P<0.01) in subgroup GI (76.9%) than in GI (30.0%) and GIV (37.5%). Furthermore, the median time to first service and the median time to pregnancy were longer for cows in subgroup GIId as compared with subgroups GLd, GIId, and GIIId (P<0.001; P<0.01). Group II was found to have the lowest endometritis rates after treatment of subgroups-d and -np. No statistically significant difference was found between the groups with regard to cystic ovary rates after treatment (P>0.05). As the treatment with CHR-OXT combined with PGF$_{2\alpha}$ on days 15 and 26 was associated to improved uterine involution patterns and reproductive performance, our results suggest that administration of this preventive treatment would be useful, especially for cases of dystocia.

Keywords: Postpartum cows, preventive treatment, fertility.

RÉSUMÉ

L’objectif de la présente étude était d’évaluer l’efficacité d’un traitement préventif, associant le Ceftiofur Hydrochloride (CHR), l’ocytocine et les prostaglandine F2-alpha (PGF$_{2\alpha}$) sur les paramètres de fertilité et l’incidence des troubles puerpéraux chez les vaches laitières en post-partum (pp). Cent quatre vingt onze Holstein-Friesian vaches ont été réparties en quatre groupes. Les vaches du groupe I (n = 37) et II (n =69) ont été traités quotidiennement avec le CHR et l’ocytocine pendant 5 et 3 jours post-partum, respectivement. Le CHR et l’ocytocine n’ont été administrés au groupe III (n = 52) ou au groupe IV (n = 33) qui a servi de groupe contrôle. Dans tous les groupes de l’étude, un analogue de la PGF$_{2\alpha}$ a été administré par voie intramusculaire au 15ème et au 26ème jour postpartum. Chaque groupe a été divisé en 2 sous-groupes selon les conditions normales (np) ou dystociques (d) de la parturition. Le taux de gestation au premier service (FSPR) du sous-groupe da été significativement plus élevé dans le groupe III (76,9 %) que pour les groupes GI (30,0 %) et GIV (37,5 %). En outre, les valeurs médianes du temps de latence au premier service et à la gestation ont été supérieures pour les vaches du sous-groupe GIId par rapport aux sous-groupes GIld, IId et IIIId. L’incidence des troubles puerpéraux après les traitements n’a pas différé entre les sous-groupes d et np. Le groupe II a présenté le plus faible taux d’endométriose après le traitement des sous-groupes np et d. L’incidence des kystes ovariens n’a pas différé entre les groupes. Étant donné que le traitement avec le CHR, l’ocytocine et les PGF$_{2\alpha}$ a été associé à de meilleurs résultats en termes d’involution utérine et de performances de reproduction, nous suggérons que l’administration de ce traitement préventif pourrait être bénéfique, en particulier pour les cas de dystocie.

Mots clés : Vaches, postpartum, traitement préventif, fertilité.

Introduction

In the postpartum (pp) period there productive tract returns to its non-pregnant state and is prepared for a new pregnancy [48]. Several post parturient factors such as retained foetal membranes (RFM), dystocia, abortion, uterine prolapse, clinical/subclinical endometritis and pyometra can cause delayed uterine involution [22, 49]. These factors are associated with decreased conception rates and increase in the mean number of days to first service and to conception, all of which result in economic loss [23, 30].

The reported lactational incidence of endometritis varies from 7.5% to 61.6% [23]. This difference in value is highly confounded by the diagnostic method (observation or odor of discharge, palpation, vaginoscopy, bacteriologic culture, biopsy, or cytology), as well as the postpartum interval at which diagnosis is made [31].
Dystocia is another important risk factor for the development of uterine infection, which is often associated with delayed uterine involution and luteal activity and irregular progesterone secretion [14]. Manual assistance during dystocia and damage of the uterine tissue might lead to greater bacterial invasion [13, 43]. The median incidence of dystocia varies from 2% to 10% [37]; however, the rate of manual assistance at calving might be more than 50% [26].

Several studies have reported different treatment methods for enhancing fertility or preventing uterine infections. The postpartum application of GnRH and intrauterine antibiotic infusion [2] or homeopathic agents as pulsatilla miniplex [4] can improve the pregnancy rates and decrease the incidence of endometritis.

A variety of broad spectrum antibiotics has been used for parenteral administration in cows with uterine infections. Cefalosporins are used worldwide both in human and veterinary medicine, and have been recommended for both parenteral as well as local treatment of uterine infections in cattle [11]. It has been found that intrauterine antibiotic treatment is not sufficient for raising the therapeutic concentration of antimicrobials in other parts of the genital tract such as ovaries, oviducts, and the uterine wall. Alternatively, parenteral antibiotic treatment might provide adequate therapeutic concentrations to all parts of the female genital tract. Owing to its association with general illness, parenteral antibiotic treatment is indicated for the treatment of acute metritis [10]. On the other hand, some authors have found intrauterine cephalosporin (cephapirin) treatment to be beneficial for reproductive performance [30, 36].

Ceftiofur hydrochloride (CHR) is a third generation cephalosporin with broad spectrum activity, and in contrast to many other antibiotics, CHR does not cause antibiotic residues in milk at levels detectable by commonly used commercial tests [46, 47]. Further, it is quickly metabolized to desfuroylceftiofur and furoic acid after parenteral administration. Only about 0.1% of the applied dose was found to be excreted in the milk after the parenteral administration of ceftiofur in lactating dairy cattle. The major milk residue was desfuroylceftiofur bound to protein and parent ceftiofur was not detected [29].

The preventive effect of cefquinome [1] and ceftiofur hydrochloride [46] against metritis has also been shown previously.

The administration of uterotonic drugs during the puerperal period lead to the evacuation of the uterus by intensifying contractions, and also support the process of involution [6]. Prostaglandin F2α and oxytocin (OXT) could be used for this purpose. Although the mechanism is not clear, exogenous PGF2α is known to enhance the ability of the uterus to resist to bacterial infections. In fact, these effects are independent of its luteolytic function and of progesterone concentrations [33]. The uterus is sensitive to OXT under estrogenic influence during the early puerperal period [44]. Exogenous OXT injections increased the frequency of myometrial contractions during this period [6].

The present study was aimed at determining the efficacy of the combined preventive treatment with CHR-OXT and PGF2α on puerperal disorder rates and fertility parameters in puerperal cows with normal parturition and dystocia as well as the practical suitability of this preventive treatment, particularly after cases of dystocia.

**Material and Methods**

**ANIMALS AND STUDY DESIGN**

This study was performed on Holstein-Friesian cows (n=191) acquired from a commercial dairy farm. All of the cows in the study were 3-4 years old and had a milk yield of 5612.63 ± 1462.11 kg/cow-per lactation, and were fed with the same type of rations (Total Mix Ration, TMR) twice daily. The body condition score was ranging from 2.5 to 3.5. The cows were divided into four groups. In group I (GI; n=37) and group II (GII; n=69), Ceftiofur Hydrochloride (Excenel® RTU, 1.0 mg/kg, i.m., Pfizer, Germany) and oxytocin (Faromoksin®, 2 ml/cow, i.m., Aydın İlaç, Turkey) were daily administered for the first five and three days pp, respectively. In groups I, II, and III (GIII; n=52) synthetic PGF 2α analogue (Dalmazin®, 2 ml/cow, i.m., Vetaş, Turkey) was injected 15 and 26 days after parturition. No treatment was given to the control group (GIV; n=33). Postpartum examinations were performed daily for 45 days after parturition.

**Figure 1:** Schematic design of the study.
performed in all groups from day 30. Figure 1 presents the schematic design of applications for each study group.

The groups were divided into two subgroups as dystocia (d) and normal parturition (np). In GI, the subgroups with dystocia and normal parturition were designed as GId (n=20) and GInp (n=17). Similarly, the other subgroups were designed as GIIId (n=32), GIInp (n=37), GIIId (n=18), GIIInp (n=34), GIVd (n=14), and GIVnp (n=19). Dystocia was defined as parturition that required assistance and had an extended parturition period [38]. All cases of dystocia in this study required veterinary assistance; however, there was no evaluation of the degree of assistance required. Normal parturition was described as parturition without any assistance. The animals were grouped randomly, which is the reason for the number of animals being different in the subgroups.

POSTPARTUM EXAMINATION AND EVALUATION OF FERTILITY PARAMETERS

Postpartum involution controls were performed on day 30 pp by transectal palpation and vaginoscopic examination and on day 45 by ultrasonographic examination (Agroscan L, 5 MHz, ECM, Angoulême, France) in all animals.

The cases of chronic endometritis and cystic ovaries were noted during the evaluation of puerperal disorders. Owing to the on-going administration of CHR-OXT (GI and GII), animals with RFM were removed from the study.

As reported by SHELDON et al. [49] clinical endometritis is characterized by the presence of purulent or mucopurulent discharge in the vagina during vaginoscopic examination without signs of systemic illness 26 days or more after parturition. Furthermore, the location of the uterus and the presence of asymmetry were investigated during transrectal palpation. During the ultrasonographic examination, the diameter of the horns was measured on the transversal cross-sectional area that was nearest to the corpus uteri based on the external diameter of the horns from serosa to serosa.

Ovarian cysts were diagnosed as a firm, thick-walled structure (luteal cyst) or a soft, thin-walled structure (follicular cyst) during pp examinations on day 30. The ovaries of each cow were then examined by ultrasonography. Cows with a follicular structure having an antrum diameter ≥25 mm in the absence of a corpus luteum [35] in two ultrasonographic examinations performed on day 30 and 45 were considered to have an ovarian cyst.

An experienced veterinarian performed inseminations of all cows that had estrus from day 45 pp, and the diagnosis of pregnancy was done by ultrasonography 30 - 45 days after artificial insemination.

In the study, the overall pregnancy rates (OPR: Pregnancy rates after a maximum of three consequent inseminations, %), the first service pregnancy rates (FSPR: Pregnancy rates after first insemination, %), the pregnancy index (PI: number of services per pregnancy), the number of days to first service (D-FS: Time from calving to first service), and the number of days to pregnancy (D-PR: Time from calving to pregnancy) were used as the fertility parameters. The rate of puerperal disorders (%) that developed after administration was determined; however, the fertility parameters of these animals were not evaluated.

STATISTICAL ANALYSIS

SPSS 14.0 for Windows® (Chicago, IL, USA) was used for statistical analysis. Descriptive statistics were used for the mean and standard deviation, while non-parametric tests were used for inter- and intra-group analyses. The One-Way ANOVA test was used for comparing values in the groups, and the chi-square test was used for comparing the OPR and FSPR values. P<0.05 was considered to be significant.

Results

COMPARISON OF PUERPERAL DISORDER RATES

When cows with dystocia (d) and normal parturition (np) were compared within the groups (Group I, II, III, and IV) according to the treatment options, it was observed that only GII demonstrated a significant difference between subgroups with regard to the percentage of cows that developed endometritis after treatment (P<0.05). In the other groups, no statistically significant difference was found between the subgroups-d and -np with regard to puerperal disorder rates after treatment (P>0.05). Group II presented the lowest endometritis rates of all the groups after treatment of subgroups-d and -np. No statistically significant difference was found between the groups with regard to cystic ovary rates after treatment (P>0.05; Table I).

![Table I: Puerperal disorder rates in the groups.](image-url)

np: Normal Parturition, d: Dystocia. #,*: Statistically significant difference between columns (P<0.05). a,b: Statistically significant difference between lines (P<0.01).
COMPARISON OF FERTILITY PARAMETERS

In all of the groups, it was found that the fertility parameters were not statistically different between cows with dystocia and normal parturition \((P>0.05;\) Table II). On the other hand, the FSPR values of subgroups GI and GIVd were lower than that of GIIId \((P<0.05; P<0.01)\). In cows with a normal parturition, FSPR was found to be significantly lower in the control group (GIVnp) than in GInp, GInp \((P<0.05, P<0.05;\) Table II).

The pregnancy index was found to be the highest in subgroup GIVnp and the lowest in subgroup GInp. However, there was no statistical difference between groups and subgroups with respect to PI values \((P>0.05;\) Table II).

The overall pregnancy rate was found to be the highest in subgroup GIId. Subgroups GInp, GInp, and GIVnp were found to have the highest OPR values in cows with normal parturition.

The days to first service and the D-PR values indicated that there were significant differences between the control and preventive treatment groups. The D-FS \((114\text{ days})\) in subgroup GIVd was lower than that observed in subgroups GIId, GIIId, and GIIId corresponding to \(57.0, 64.0,\) and \(54.3\) days, respectively \((P<0.001; P<0.01)\). No statistically significant difference was found between the subgroups of cows with dystocia and normal parturition \((P>0.05)\). D-PR was shorter in the subgroups of preventive treatment cows as compared to the control subgroup GIVd \((P<0.01)\). It was found to be longer in the control group \((P<0.001; P<0.01; P<0.05)\) when compared to the treatment groups of cows with normal parturition.

ULTRASONOGRAPHIC MEASUREMENTS OF UTERINE HORNS

There was a difference in the diameters of uterine horns in cows with puerperal disorders after the treatment. It was found that the diameters were greater in subgroups GIId and GIVd \((P<0.01; P<0.05)\). Ultrasonographic measurements indicated that the diameter of the uterine horn was smaller after treatment (Table III).

Discussion

In this study, an attempt was made to investigate the effect of combined preventive administration of CHR-OXT and PGF\(_{2\alpha}\) on fertility parameters after dystocia and normal parturition. The administration of CHR-OXT for three consecutive days combined with PGF\(_{2\alpha}\) might have a positive effect on fertility parameters and could be used for the prevention of uterus infection after cases of dystocia.

Pharmacokinetic studies have demonstrated that CHR can reach adequate therapeutic concentrations in infected tissues [42] and that ceftiofur residues in uterine tissue are higher in diseased animals than in normal pp cows, which might be used in the prevention or treatment of metritis [47]. Ceftiofur hydrochloride is used for therapeutic purpose, in both pp acute metritis [12, 17, 52] as well as RFM in cows [16, 18, 19, 46]. However, there is limited research available on pp prophylactic application.

The efficacy of systemic CHR treatment for preventing metritis clearly supports the findings of RISCO and HERNANDEZ [46].

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Groups</th>
<th>(P) value</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>GI</td>
<td>GII</td>
</tr>
<tr>
<td>FSPR (%)</td>
<td></td>
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</tr>
<tr>
<td>d</td>
<td>30.0(^a)</td>
<td>76.9(^b)</td>
</tr>
<tr>
<td>np</td>
<td>66.6(^a)</td>
<td>58.8(^{ab})</td>
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| OPR (%)        |            |                      |                      |                      |
| d              | 84.6\(^a\) | 100\(^b\)            | 75.0\(^{ac}\)        | 87.5\(^{abc}\)       |
| np             | 100\(^a\) | 97.0\(^{abc}\)       | 83.3\(^{b}\)         | 100\(^{ac}\)         |

| PI             |            |                      |                      |                      |
| d              | 1.6        | 1.3                  | 1.2                  | 1.6                  |
| np             | 1.3        | 1.5                  | 1.2                  | 2.1                  |

| D-FS (days)    |            |                      |                      |                      |
| d              | 57.0 ± 12.6\(^{a}\) | 64.0 ± 20.3\(^{ab}\) | 54.3 ± 17.7\(^{ab}\) | 114.0 ± 50.0\(^{c}\) |
| np             | 65.9 ± 23.4\(^{a}\) | 66.2 ± 18.8\(^{a}\) | 69.9 ± 33.3\(^{a}\) | 85.5 ± 27.9\(^{b}\) |

| D-PR (days)    |            |                      |                      |                      |
| np             | 72.3 ± 25.2\(^{a}\) | 75.8 ± 26.0\(^{ab}\) | 85.4 ± 39.6\(^{ac}\) | 108.0 ± 25.4\(^{d}\) |

\(\text{FSPR: First Service Pregnancy Rates, OPR: Overall Pregnancy Rates, PI: Pregnancy Index, D-FS: Days to First Service, D-PR: Days to Pregnancy, np: Normal Parturition, d: Dystocia. a,b,c: Statistically significant difference between lines (P<0.05; P<0.01; P<0.001). There is no statistically difference between columns (P > 0.05).}\)

**Table II: Fertility parameters in the study groups.**

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In contrast to the study in which it was concluded that no beneficial effect of a single injection of either OXT or carbetocin on the incidence of endometritis in cows with normal or abnormal calving [7], a decrease in endometritis rates was reported by administering OXT together with antibiotics for five days in cases of RFM [5, 8]. According to another study, the intrauterine infusion of ceftiofur hydrochloride reduced the prevalence of uterine infection in cows with clinical endometritis, and the prevalence of A. pyogenes, but did not affect the prevalence of subclinical endometritis or the fertility of dairy cows already receiving PGF2α [24]. In the present study, low endometritis rates in GII provide support for the preventive effect of CHR-OXT treatment. However, DUBUC et al. [15] reported that treatment with ceftiofur crystalline free acid in cows with high risk of uterine disease cows was not associated with the probability of metritis overall, but decreased the incidence of metritis in cows with high risk of uterine disease cows was not associated with the probability of metritis overall, but decreased the incidence of metritis in cows with normal or abnormal calving. On the contrary, the administration of CHR-OXT to cows with dystocia and normal parturition was found to be significantly higher (55.5%) in GIIId and normal parturition subgroups (GIInp) in the study groups for FSPR. The above results revealed that this type of combined treatment had a beneficial effect on the FSPR and PI parameters of both normal cows and those with calving problems. Further, the treatment was found to be especially effective on cows in subgroup GIId.

DRILLICH et al. [20] suggested that preventive ceftiofur administration in cows with RFM and cows with fever (≥39.5°C) results in the shortening of both D-FS and D-PR after combined treatment. In the present study, statistically significant reductions in the two parameters, D-FS and D-PR, were found (P<0.001 and P<0.01, respectively) in cows with dystocia as compared to non-treated cows. After the treatment was applied, it was observed that the D-FS and D-PR parameters of cows with calving problems approached those of normal animals (P>0.05). Significant differences between the control and preventive treatment groups indicated that higher values in these parameters (especially in D-FS) might relate to delayed uterine involution and ovarian activity, which agreed with the results of the mentioned studies.

Prostaglandin F2α has been used for stimulating uterine involution [40] and for enhancing fertility parameters [3] during the pp period. Furthermore, the administration of a long-acting analog of PGF2α, either 7 to 10 days or 14 to 28 days pp, has been determined to be effective for the treatment of delayed uterine involution and/or endometritis [41]. Some authors have found exogenous PGE2α treatment to be beneficial to the immune system during the absence of luteal function. It is debatable whether the effectiveness of PGE2α is owing to its luteolytic effect or because it directly affects the immune system [32]. Prostaglandin F2α has been recommended by some authors for the treatment of endometritis [41, 50], while others [39] have concluded that this treatment did not have a beneficial effect on endometritis. The administration of PGE2α [34] or OXT together with PGE2α [21] during the pp period has been reported to stimulate myometrium contractions and uterine motility.

RISCO et al. [45] reported that cows affected with dystocia, RFM, or both, and treated with PGE2α during early pp (day 12 and 26) had higher conception rates after first AI than untreated cows experiencing a normal or abnormal puerperium. Similarly, in the present study higher FSPR, D-FS and D-PR values were found in cows with dystocia and normal parturition in GIII (solely PGE2α administered 15 and 26 days after parturition) as compared with the non-treated control group (GIV). Thus, the authors concluded that preventive PGE2α.
administration did not alter the incidence of endometritis between 7 and 35 days after calving [27] and at 21 and 35 days pp [28]. Given the higher endometritis rates in the group that was only given PGF$_{2\alpha}$ (GII) and the control group (GIV), this research resulted in findings similar to the mentioned studies.

The uterine tone and the diameter of the uterine horns are related to each other in the early pp period in cows. Consequently, it was observed that cows with smaller uterine diameters had improved uterine tone [51]. In general, the uterine measurements proved that the preventive treatment applied to all animals in the study with or without calving problems gradually reduced the diameter of uterine horns as compared to the control group. RISCO and HERNANDEZ [46] reported that the administration of CHR or estradiol cypionate to cows with RFM did not alter the rate of uterine involution when compared to non-treated cows. Moreover, MELENDEZ et al. [40] found that cows affected by acute metritis had smaller gravid uterine horn diameter in comparison with the control group after two doses of PGF$_{2\alpha}$ at an eight-hour interval on day eight of CHR administration. Likewise, the use of combined CHR was found to be effective on uterine involution.

In the present study it was found that there was an increase in FSPR and OPR and a significant reduction in D-PR in cows with dystocia when treated with CHR-OXT for three consecutive days combined with PGF$_{2\alpha}$ at 15 and 26 days after calving (GIII). Furthermore, a positive effect was observed on involution of the uterus. The lower percentage of endometritis, improved fertility parameters, and smaller uterine diameter at day 45 pp after administrations suggest that the treatment with CHR-OXT for three consecutive days combined with PGF$_{2\alpha}$ could be useful for the prevention of uterus involution after cases of dystocia.

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