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Research Article

The Effect of Intrauterine Infusion of Carvacrol After Insemination on Conception Rate in Repeat Breeder Cows Subjected to Progesteron Based Estrus Synchronization Protocol

Necdet Cankat LEHİMCİOĞLU [1,a] Yavuz ÖZTÜRKLER [1,b] Savaş YILDIZ [1,c] Umut Çağın ARI [1,d]

[1] Department of Reproduction and Artificial Insemination, Faculty of Veterinary Medicine, University of Kafkas, TR36100 Kars - TURKEY

[a] ORCID: 0000-0001-8780-616X; [b] ORCID: 0000-0002-7089-6522; [c] ORCID: 0000-0001-6459-6841; [d] ORCID: 0000-0002-7089-6522

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Abstract

Repeat breeder (RB) is one of the crucial causes of economic loss in dairy cows. The aim of this study was to determine the effects of intrauterine carvacrol administration after timed artificial insemination (TAI) on the conception rate (CR) in RB cows. The study involved 155 RB cows returned to service for more than three times and without any significant pathologic defects in genital tract. All cows were subjected to following estrus synchronization method: An intra-vaginal apparatus (IVA) containing 1.55 g progesteron were inserted for 9 days and administered one dose of $PGF_{2\alpha}$ one day before removing IVA, then injected one dose of GnRH 6 h before TAI. All the cows were inseminated in 56th h after removing IVA. All RB cows were randomly divided to three groups. Group I (GI; n=52) and Group II (GII; n=51), treatments groups were infused with 0.1% diluted Carvacrol and 0.1% Lugol's (GII) solution. Group III (GIII; n=51) the control group, was received normal saline only. All infusions were administered one dose of 30-50 mL (according to uterine size) into uterus half hour after insemination. In the observations performed in 21 to 30 days after insemination, non-return rates (NRR) were 71.15%, 69.23% and 68.62% in groups of GI, GII and GIII, respectively. CR detected by rectal palpation at 60 days following artificial insemination were 67.30%, 63.46% and 46.15% in GI, GII and GIII, respectively. However, the differences between groups were not statistically significant. As a result: In the treatment of RB cows, it may be suggested that carvacrol infusion to the uterus may be preferred as it has improved pregnancy rates to some extent. Nevertheless, further investigations are needed to confirm these results.

Keywords: *Cow, Carvacrol, Artificial insemination, Repeat Breeder, Estrus synchronisation, Progestron, PRID, $PGF_{2\alpha}$, GnRH*

Progesteron Temelli Östrus Senkronizasyonu Protokolüne Tabi Tutulan Repeat Breeder İneklerde Suni Tohumlama Sonrası İntrauterin Carvacrol İnfüzyonunun Gebelik Oranları Üzerine Etkisi

Öz

Repeat Breeder (RB), süt ineklerinde ekonomik kaybın önemli nedenlerinden biridir. Bu çalışmanın amacı, RB ineklerinde zamanlanmış suni tohumlama sonrası intrauterin carvacrol uygulamasının gebelik oranlarına (GO) etkilerini belirlemektir. Çalışma, 3 defadan fazla geri dönen, genital sisteminde önemli patolojik bozukluk saptanmayan 155 adet RB inekte yürütüldü. Tüm inekler aşağıdaki östrus senkronizasyonu yöntemine tabi tutuldu: 1.55 g progesteron içeren bir intra-vajinal aparat (IVA), 9 gün boyunca yerleştirildi ve IVA çıkarılmasından bir gün önce bir doz $PGF_{2\alpha}$ uygulandı ve suni tohumlamadan 6 saat önce bir doz GnRH enjekte edildi. Tüm inekler, IVA çıkarıldıktan 56 saat sonra tohumlandı. Tüm hayvanlar rastgele üç gruba ayrıldı. Uygulama grupları olan grup I (GI; n=52) ve grup II (GII; n=51)'ye sırasıyla %0.1 seyreltilmiş Carvacrol ve %0.1 Lugol (GII) çözeltileri verildi. Kontrol grubu grup III'e (GIII; n=51) sadece normal serum fizyolojik infüze edildi. Tüm infüzyonlar, tohumlamadan yarım saat sonra uterus içine bir doz 30-50 mL (uterus boyutuna göre) olacak şekilde uygulandı. Tohumlamadan 21 ila 30 gün sonra yapılan gözlemlerde, geri dönmeyenlerin oranı (NRR), GI, GII ve GIII gruplarında sırasıyla %71.15, %69.23 ve %68.62 olarak belirlendi. Suni tohumlamayı takiben 60. günde yapılan rektal palpasyonla teşhis edilen GO ise GI, GII ve GIII'de sırasıyla %67.30, %63.46 ve %46.15 olarak saptandı. Ancak, gruplar arasındaki farklar istatistiksel olarak anlamlı değildi. Sonuç olarak: RB ineklerin tedavisinde, uterusa carvacrol infüzyonunun gebelik oranlarını bir dereceye kadar arttırdığı için tercih edilebileceği kanaatine varıldı. Fakat, bu sonuçları pekiştirmek için başka araştırmalara ihtiyaç olduğu düşünülmektedir.

Anahtar sözcükler: *İnek, Carvacrol, Suni tohumlama, Repeat breeder, Östrus senkronizasyonu, Progesteron, PRID, $PGF_{2\alpha}$, GnRH*

İletişim (Correspondence)
+90 474 2426807 GSM: +90 533 6836847 Fax: +90 474 2426853
nclehimcioglu@hotmail.com

INTRODUCTION

Reproductive performance plays a crticial role in productivity of the dairy cattle industry. An ideal reproductive health can be described as "interval of calving-repregnancy in a cow herd is kept in an optimum range of economic scale". The main goal in herd management in dairy cows is considered to be as "getting a calf per year [1]. Repeat breeder (RB) is one of the main problems in cows that leads to reproductive waste and significant decline in profitability in dairy industry due to decreasing of reproductive performance [2]. According to results of one study on RB syndrome which were carried out in Michigan, RB was observed in 24% of 3.309 lactations in twenty-two dairy herds, the cost of delayed conception, extra inseminations, extra veterinary service, and losses due to wastage of livestock were calculated as $385 [3]. In Indian, the incidence of clinical endometritis and sub-clinical endometritis were estimated as 54.15% and 1.40%, respectively in RB cows [4]. Besides, it has been also reported that RB is seen in 5-32% frequency in cattle [5] and alone contributed to reproductive problems in the rate of 2.29 to 42.7% [6-8].

According to the most common description, the cows that are cyclic and nearly showing normal heat, have no clinically detectable pathological lesion of their genital tract, failure of conceiving and returning back to estrus after three mating with a fertil bull has been definited as "RB" [9,10]. RB is caused by an abnormal uterine media established by harmful bacteria, histological lesions and pathological defects such as endometritis, subclinical endometritis, nutritional deficiency, thereby embriyo survival is terminated. RB syndrome depend on many multifactorial situations and causative factors such as uterine inflamation, improper oestrus detection and also endocrine imbalance and uterine infection [11,12]. Although many creative factors are existed under the RB, among the various etiological factors, subclinical endometritis is regarded as the most effective etiological factor which decreases reproductive performance and increases the insidence of RB (52.7%) [13]. Subclinical endometritis is defined as endometrium inflammation without systemic findings and is correlated with delayed uterine involution which depends on various factors such as age, race, nutrition [11].

Many methods have been implemented in the treatment of RB syndrome in cows. For therapeutic process of RB, the effects of GnRH injections [14] intrauterine infusions of various antiseptic and antibiotic solutions [15] had been investigated. However, cattle with metritis are commonly treated by using various antibiotics (gentamicin, oxytetracycline, ampicillin, cloxacillin, penicillin, ceptiofur), antiseptics (2% Lugol's solution, povidine iodine) and hormones [16-18]. Whereas, undesirable resistance effects of antibiotics in animal and humans are well known. Widely use of antibiotic resistant strains of microorganisms requires the discovery of alternative therapeutic compounds. For this purpose, recently, instead of antibiotics, some alternative therapy options are sought and new methods are tried to develop in order to treat the RB [19,20]. At his point, nowadays, the use of medicinal herbs obtained from natural sources has become an option and alternative for therapeutic purposes [21].

From past to present, uterine effective herbal drugs has been used for treatment of endometritis in animals. Thus, it has been reported that polyherbal intrauterine infusion has yielded succesful conception results (70%) compared with control group (40%) in treating in repeat breeder cattle, and it showed that polyherbal drugs have worked safety and also not seen any any irritation effect to mucous membrane of uterus [20]. From this point on, some *in vitro* studies have been performed on carvacrol which has wide spectrum antimicrobial power and is safe in use of animal and human conditions [22]. Carvacrol has also a strong inhibition effect on growing of bacterial isolates except *Pseudomonas aeroginosa* [23]. Moreover, it has been demonstrated that the carvacrol has a inhibitive effect against antibiotic resistant bacteria in vitro and in food, and use of herbal preparations with organic origin in the treatment of cows with toxic puerperal metritis have provided significant benefits in terms of food safety [21,24]. Additionally, it has been demonstrated that the residual of carvacrol or its derivatives in urine was found in very small amounts after one day only in rats [25]. On the other hand, it has been declared that herbal derived oils such as carvacrol and thymol have also been accepted as generally recognized as safe (GRAS) natural antimicrobial and non-antibiotic effective that are traditionally employed to conserve food and enrich flavor [26,27]. Further, it is known that the oils obtained from thyme contain thymol, carvacrol, p-cymene and α-pinene. Many investigators have declared that carvacrol has antioxidant, antibacterial, antiviral, anti-obesity, hepatoprotective, antifungal, antiseptic, anticarcinogenic, anti-inflammatory, spasmolytic, vasoconstrictive, immuno-modulatory effect and biological and pharmacological effects [21,28-30]. In this regard, it has been suggested that Optimum UterFlush, containing carvacrol (Van Beek® Natural Science, Orange City, IA) is an organic certified product used in the treatment of toxic puerperal metritis cows, and it can be used is in the treatment by transvaginally and IU infusion [30]. Although this product has been applied to cure the metritis, but up to now, not tested just after insemination in RB cows in order to treat the probable subclinical endometritis. However, some researchers have showed that application of various drugs as IU after artificial or natural insemination to the RB cows in the field conditions increases the pregnancy rates [18,31-34]. In the past, a similar method had applied by Riedel and Astrom was realised by using dilute iodine tincture and Lugol's solution for the first time in 1935. Since then, this method has been considered as an option for treatment after insemination in the cows carrying light subclinic chronic endometritis and this application has

been called as "Astrom Theraphy after that time" [32]. This intrauterine treatment has been applied either shortly after insemination or 15th minute as well as after 12th or 24th h [18,24,31,33]. However, in the literature search, we did not find any study which has investigated the effect of intrauterine infusion of carvacrol (uteroflash) after artificial insemination combined with oestrus synchronisation protocol. Therefore, under the light of above knowledges, in the present study, it was aimed to investigate the effects of intrauterin carvacrol infusion following artificial insemination in cows exposed to estrus synchronization protocol including PRID plus $PGF_{2\alpha}$ and GnRH.

MATERIAL and METHODS

This study was received approval by the Animal Experiments Local Ethics Committee of Kafkas University (KAU HADYEK; 2017-102).

Upon farmers' demand, a total of 300 cows were examined in accordance with the anamnesis and reviewed records of the owners in 6 intensive barns in three villages, near to Kars Province (40° 25′ 0″ Nort and 43° 4′ 59″ East), Turkey. Among them, repeat Breeder cows were selected as described by Levine [10] and Taşal [35].

In the selection of RB cows the following criteria were applied:

a- Not to be pregnant at least three times breeding

b- Being cyclic

c- Clinically not show any detectable disease

d- No detect any pathological findings in rectal palpation

e- No pathological discharge from the vagina

One hundred fifty five of the cows at various ages (3-7), races (Simental, Swiss Brown and crossbred) and 90-120 days range of postpartum period were considered as "RB cows" based on the above criteria, then included in the study. The remaining cows were disqualified because they were pregnant or have given birth. For feeding of animals, appropriate ration (coarse/concentrate) was given as twice daily with ad-libitum water.

All the cows were subjected to following synchronization method: An intra-vaginal apparatus (IVA; PRID DELTA® containing 1.55 g progesteron, CEVA-DIF) was inserted for 9 days and administered one dose of $PGF_{2\alpha}$ (5 mL/IM Enzaprost®-T, CEVA-DIF) one day before removing IVA, injected one dose (2 mL of GnRH (Ovarelin®, CEVA-DIF, containing 0.1 mg Gonadorelin diasetat/mL 6 h before TAI and then inseminated in 56th h after removing IVA. All animals were randomly divided to three groups. Cows in Group I (GI; n=52) and Group II (GII; n=51) were administered intrauterine with 0.1% diluted Carvacrol (GI) (Optimum UterFlush, Van Beek® Natural Science, Orange City, IA; containing 1 oz fl 13.9 g carvacrol, cinnamaldehydes, tymol, 440 mg yucca extract, stock containing 2.13 mL+ 997.7 mL distilled water) and 0.1% (w/v) Lugol's (GII) solution (1g iodine (I), 2 g Potassium iodide (KI) 1000 mL distilled water), respectively. Cows in Group III (GIII; n=51), control were received intrauterine normal saline only. All infusions were performed as one dose of 30-50 mL (according to uterine size) into uterus half hour after insemination.

Days 21 to 30 following inseminations, the percentage of cows returning to service were determined according to the estrus observations, but, pregnancy rates were confirmed by rectal palpation on the 60th day following insemination. All data were statistically analysed with SPSS (20.0) chi-square test program.

RESULTS

The rates of non-returned cows (NRR) at 21-30 days after the insemination were 71.1% (37/52), 69.23% (36/52) and 68.62% (35/51), in groups GI, GII and GIII, respectively *(Table 1)*. There was no statistically significant difference among groups in view of non-return rates (P=0.958).

Table 1. *The proportion of non-returned cows 21 to 30 days after insemination (NRR%)*

Groups	n	Number of Non-Returned Cows	NRR (%)
Carvacrol (GI)	52	37	71.15
Lugol's (GII)	52	36	69.23
Control (GIII)	51	35	68.62
There was no significant difference among groups (P=0.958)			

Table 2. *Pregnancy rates at the day 60th after insemination*

Groups	n	Number of Pregnant Cows	Pregnancy Rate (%)
Carvacrol (GI)	52	35	67.30
Lugol's (GII)	52	33	63.46
Control (GIII)	51	24	46.15
There was no statistically significant difference among groups (P=0.085)			

Pregnancy rates determined by rectal palpation on day 60 following AI were 67.30% (35/52) 63.46% (33/52) and 46.15% (24/51) in GI, GII and GIII groups, respecrtively *(Table 2)*. There was no statistically significant difference among groups (P=0.085).

DISCUSSION

Although Intrauterine infusions with a variety of antiseptic and antibiotic solutions have provided successful results for therapy in repeat breeder cows for a long time [15] we encountered a limited number of articles directly related to intrauterine infusion of carvacrol or uteroflash after insemination with estrus synchronisation in the literature review documented here.

In the present study, despite no statistical difference was found between percentages of NNR (71.15%, 69.23% and 68.62%) in GI, GII and GIII groups (P=0.490), it was noticed that carvacrol and Lugol's solution groups were numerically higher than the control group.

Pinedeo et al.[30] were treated with Uterflush (Stock Uterflush 3.75 mL/117 distilled water) and povidin iodine (200 mL povidin iodine/2lt distilled water) in natural estrus in the treatment of toxic puerperal metritic cows. They achieved the preganacy rates in 61.7% and 56.6%, respectively. In our study, the pregnancy rates obtained from carvacrol and Lugol's treatment groups (67.30%, 63.46%) were found higher than those of the study mentioned. In the present study, the results of Lugol's treament were also near to those of the values of Öztürkler et al.[31] (71.43%), Çolak and Öztürkler [33] (70%) and Vandeplasche [36] (80%). However, Öztürkler et al.[31] (60%)'s Lugol's results were found to be lower than the control group (69.23%) in those of our study. Additionally, Ahmed et al.[37] provided passable recovery rates (63.64, 61.54 and 60.00%) in the treatments with mineral mixture, GnRH and Lugol's solution in repeat breeder buffalo-cows. This study demonstrates that also the mineral support and special care to animals are positively effected on management of RB animals. Despite any supplementation and specical care did not enforce to animals in the current study, the conception rates were successful. Moreover, Sharma and Singh [38] found that 0.1% Lugol's iodine was successful for the management of suspected fungal endometritis, also they considered it as inexpensive therapeutic choose. At the same time, they have declared that the administration of irritant solutions to healthy uterus may not have an negative effect on conception but infusion of them in to sick uterus may adversely affect fertility in cows [38]. In this point, it is understood that all these research results mentioned above including our study's findindgs show that Lugol infusion also give successful results for treatment of endometritis. Several investigators claimed that it is not possible to determine the subclinical endometritis during routine examinations in RB cows. So, in RB cases, also, it is not exactly possible to diagnose metritis with the rectal and vaginoscopic examination of cows [1,39]. Subclinical endometritis can only be diagnosed by cytological examination, biopsy and the other laboratory methods [40,41]. In our study, any laboratory diagnostic method was not performed in cows except for rectal palpation and clinical inspection in the cows in order to determine only RB cows. Because, as known well, RB cows are mostly suffered from subclinical endometritis [42-45]. Actually, It is necessary to point here that, when the present study was designated, it was speculated that potential slight subclinic endometritis might be major cause of the RB. As it is known, RB might be caused by mainly subclinical endometritis and delayed ovulation or hormonal imbalance. Since the subclinical endometritis has been a major causative factor (in incidence of 52.7%) of RB in cows, the present study was basically established to elimination by assuming presence of such a problem [13]. However, it has been commented that despite both treatments (Uteroflash and Lugol's solution) did not show statistically significant positive effect, but also it can be said that any negative effect was not seen. Nonetheless, it was noteworthy that results of group I (Uterflash) had numerically higher compared to other groups (Lugol's and control group). In this context, in our study, the numerical increase in pregnancy rates of treatment groups compared to the control group can be interpreted that Carvacrol and Lugol's therapies may be effective on subclinical endometritis, so, these results confirm our speculation mentioned above.

On the other hand, it is seen that there are many studies related to intrauterine antibiotic infusion for treatment of endometritis. Such that, Shams-Esfandabadi et al.[45] reported that intrauterin infusion of oxytetracycline and procain penicillin G sodium gave 49.2 and 47.7% pregnancy rates, respectively following first service in dairy cows. Also they did not see any advantage of antibiotic treatment comparing with control group. Besides, Gümen et al.[46] and Mosaferi et al.[47] obtained the lower pregnancy rates (40-44% with cephapirin; 32 and 22% with cephapirin and oxytetracycline) than those of our study's results. It is understood that the results obtained from the present study are superior than those of several studies mentioned above which used the intrauterine antibiotic infusion twenty-four hours after inseminaton. Nevertheless, it is seen that the pregnancy rates of the present study are lower than those of some intrauterine antibiotic infusion's studies such as with cephapirin (70%) and with combination ciprofloxacin and tinidazole (70 to 78%) [48,49] and cephalexin and enrofloxacin (83 and 75%) or similar to an gentamicin treatment (67%) [50]. These studies indicate that impact of uteroflash may be considered as competitive comparing those of other studies that focused on effects of intrauterine antibiotic infusion for treatment of RB. In another study, Oral et al.[19] found that pregnancy rate was 66.6% following the intrauterine application of 5% oregano oil (contains carvacrol) in cows

with chronic endometritis. In the present study, the pregnancy rate obtained from carvacrol group was similar (67.30%) to those of them. Also, Carvacrol group in the present study (67%) was found to be numerically higher than other groups (63.4%, 47%), although there was no statistically significant difference among the all groups (P=0.252). With reference to theese results, it can be commented that carvacrol may have a improving effect on pregnancy rate in RB cows.

In the present study, it was observed that pregnancy rates obtained by rectal palpation in all groups were lower than NRR%. This may be due to the incidence of early and late embryonic deaths during the gestation or fertilisation failure [51]. On the other hand, Inskeep and Dailey [52] reported that conception failure in cows were due to various causes such as embriyonic mortality (57%), late placentation (10%), early placentation (4%), fetal (3%), lethal gene (5%), re-bred (1%) and ovum transport (8%). Differences between the our work and others may be depended on research protocols, care and nutrition conditions, age, breed, environmental factors, different yield characteristics and many unknown attributed factors [53-55]. Thus, in RB Cows, repeat breeding is not only depend on endometrial inflamations and infections, but also accociated with hormonal, mineral and antioxidant imbalance [37].

In accordance with the above litaretures and discussions, it can be argued that carvacrol (uteroflash) increased preganacy rates compared to Lugol's and control group, but it was not also seen negative effect on pregnancy rates. In this respect, it is said that carvacrol has also some advantages such as inhibition effect on antibiotic resistant bacteria and no side effect on uterus as well as improving the fertility.

In conclusion, for treatment of RB cows, it can be suggested that carvacrol infusion to the uterus may be preferred as it has improved pregnancy rates to some extent. But, to confirm these results, further are needed.

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