

## THE EFFECT OF hCG AND GENTAMICIN ADMINISTRATION RELATED TO ARTIFICIAL INSEMINATION FOLLOWING OESTRUS SYNCHRONISATION UPON THE CALVING RATES IN REPEAT BREEDER COWS

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**Summary:** The aim of this study was to increase the calving rates (CRs) by administration of hCG and Gentamicin related to artificial insemination (AI) following oestrus synchronisation (OS) in repeat breeder (RB) cows. In this study, 56 RB cows of various ages and breeds were used. The cows were divided into two groups: the first group was assigned as treatment group while the other was as control. In Group 1 (n=36), following OS by i.m. 25 mg Dinoprost Tromethamine, a PGF<sub>2</sub>α analogue (Dinolytic-ECZACIBAŞI) injection (twice, 11 days apart), intrauterine AIs were performed at 72 and 96 h. In addition, i.v. 1,500 IU hCG (Pregnyl-ORGANON) injection at 72 h and intrauterine infusion of 250 mg Gentamicin sulphate (Gentadol-ECZACIBAŞI) in 50 cc 0.9% NaCl solution at 30 min after 96 h were applied. In Group 2 (n=20), the AIs were performed at 72 and 96 h following OS (as above) only. The CRs after 280 (± 10) days of AIs were recorded. The results showed that treatment methods used had a significant effect upon the CRs (P<0.001, F=51.46). The CR in Group 1 (38.89% ± 1.37) was significantly higher than those of Group 2 (15.00% ± 1.83) (P<0.05). The findings suggest that oestrus synchronisation (by PGF<sub>2</sub>α) alongside the applications of hCG and Gentamicin related to artificial insemination can increase the calving rates in repeat breeder cows.

**Key Words:** Cow, repeat breeder, artificial insemination, PGF<sub>2</sub>α, hCG, Gentamicin, calving

### Repeat Breeder İneklerde Östrus Sinkronizasyonu Sonrası Sun'i Tohumlamaya Bağlı hCG ve Gentamisin Uygulamasının Doğum Oranları Üzerine Etkisi

**Özet:** Bu çalışmada, repeat breeder (RB) ineklerde östrus sinkronizasyonu (ÖS) sonrası sun'i tohumlamaya (ST) bağlı hCG ve Gentamisin kullanılarak doğum oranlarının (DO) artırılması amaçlanmıştır. Çalışmada, değişik ırk ve yaşlardaki toplam 56 adet RB inek kullanıldı. İnekler, ilk grubu tedavi, diğeri ise kontrol olmak üzere iki gruba ayrıldı. Grup 1'e (n=36), i.m. 25 mg Dinoprost Tromethamine, bir PGF<sub>2</sub>α analogu (Dinolytic-ECZACIBAŞI) enjeksiyonu (2 kez, 11 gün ara) ile ÖS sonrası 72. ve 96. saatlerde intrauterin yolla ST yapıldı. Ayrıca, 72. saatte i.v. 1.500 IU hCG (Pregnyl-ORGANON) enjeksiyonu ve 96. saatten 30 dak. sonra 50 cc %0,9 NaCl çözeltisi içinde 250 mg Gentamisin sulphate (Gentadol-ECZACIBAŞI) intrauterin yolla uygulandı. Grup 2'ye (n=20), ÖS (yukarıdaki gibi) sonrası sadece 72. ve 96. saatlerde ST yapıldı. ST'lerden 280 (± 10) gün sonraki DO'ları kaydedildi. Kullanılan tedavi yöntemlerinin DO üzerine önemli bir etkisi gözlemlendi (P<0.001, F=51.46). Grup 1'deki DO (%38,89 ± 1,37) Grup 2'dekinden (%15,00 ± 1,83) önemli oranda daha yüksekti (P<0.05). Sonuç olarak, repeat breeder ineklerde östrus sinkronizasyonu (PGF<sub>2</sub>α ile birlikte sun'i tohumlamaya bağlı hCG ve Gentamisin uygulanarak doğum oranlarının artırılacağı kanısına varıldı.

**Anahtar Sözcükler:** İnek, repeat breeder, sun'i tohumlama, PGF<sub>2</sub>α, hCG, Gentamisin, doğum

## INTRODUCTION

The cows; (i) younger than 10-years-old, (ii) delivered at least once, (iii) showing regular sexual cycles, (iv) with no apparent clinical signs of reproductive organ defects or abnormal discharge but not conceiving (v) in spite of AI at least 2-3 times (or more), are so-called as *repeat breeder*<sup>1,2</sup>. The aetiology of repeat breeding can be classified as follows: (i) fertilisation failures, (ii) early embryonic death, (iii) infectious agents, (iv) genetic factors, (v) hormonal imbalance, (vi) congenital or acquired morphological (anatomical) factors, (vii) intrauterine milieu, (viii) nutrition, (ix) management and environmental factors and (x)

age<sup>5,7</sup>. There has been only a very low incidence of pathological or inflammatory changes in the genital tracts of repeat breeder cows<sup>3,4</sup>. Aköz<sup>5</sup>, reviewing the literature on repeat breeding cows concluded that the incidence of cows not conceiving following 3 AIs (or more) were 8.5 to 18.0% of the herd. Öztürkler *et al.*<sup>6</sup> further notified that the incidence of repeat breeder cows (particularly in Kars district) cannot be underestimated.

Various compounds (i.e. hormones<sup>7</sup>, antibiotics<sup>5</sup>, antiseptics<sup>6</sup> and immunomodulators<sup>8</sup>) or acupuncture technique<sup>9</sup>

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are used to overcome repeat breeding problem in the cow. Hormonal therapy includes; administrations of Prostaglandin ( $\text{PGF}_2\alpha$ -analogues), Oestrogen, Gestagens, GnRH, PMSG, hCG, FSH and corticosteroids<sup>7</sup>. Aköz<sup>5</sup> noted that  $\text{PGF}_2\alpha$  applications could be used for both synchronisation and treatment of endometritis. hCG can be used for induction of ovulation in postpartum cows<sup>10</sup>. The applications of 1,500-2,000 IU hCG related to AI were reported to be adequate for increasing the conception rates in repeat breeder cows. Additionally, the conception rates might be increased 6-17% following GnRH applications related to AI, as compared to non-treated controls. It was also reported that there was marked (13-60%) improvement in conception rates by GnRH applications in cows not conceiving following repeated inseminations in spite of having no signs of reproductive infection but with delayed ovulation or unovulation<sup>12</sup>. However, most AI strategies adopt multidisciplinary protocols using hormones plus antibiotics/antiseptics<sup>5,11</sup>. Antibiotics (e.g. Rifaximin<sup>6</sup> and Gentamicin<sup>13,14</sup>) or antiseptics (e.g. Lugol sol.<sup>6</sup>) are successfully used to combat microbial infections of the uterus. Furthermore, our preliminary observations in the field showed that Gentamicin was very successful antibiotic for treatment of endometritis in cows.

Therefore, the objective of this study was to increase the calving rates by administrations of hCG and Gentamicin related to AI following oestrus synchronisation (by a  $\text{PGF}_2\alpha$  analogue) in repeat breeder cows.

## MATERIALS and METHODS

In our study, 56 repeat breeder cows (in Kars district) of various ages and breeds were used. The cows were divided into two groups: the first group was assigned as treatment group while the other was as control. In Group 1 (n=36), following oestrus synchronisation by i.m. 25 mg Dinoprost Tromethamine, a  $\text{PGF}_2\alpha$  analogue (Dinolytic-ECZACIBAŞI) injection (twice, 11 days apart), intrauterine AIs were performed using  $25 \times 10^6$  bull sperm/0.25 ml frozen-thawed straws at 72 and 96 h. In addition, i.v. 1,500 IU human Chorionic Gonadotrophine, hCG (Pregnyl-ORGANON) injection at 72 h and intrauterine infusion of 250 mg Gentamicin sulphate (Gentasol-ECZACIBAŞI) in 50 cc 0.9% NaCl solution at 30 min after 96 h were applied. In Group 2 (n=20), as control, the inseminations were performed at 72 and 96 h following oestrus synchronisation (as above) only. The calving rates after 280 ( $\pm 10$ ) days of inseminations were recorded.

Data presented as means  $\pm$  SEM were analysed by Analysis of Means and Regression Analysis using Minitab<sup>15</sup>. Differences between the means were significant when  $P < 0.05$ <sup>16</sup>.

## RESULTS

The results showed that treatment methods used had a significant effect upon the calving rates in repeat breeder cows ( $P < 0.001$ ,  $F = 51.46$ ) (Table 1). The calving rates ( $38.89\% \pm 1.37$ ) in Group 1 ( $\text{PGF}_2\alpha$  + hCG + Gentamicin) was significantly ( $P < 0.05$ ) higher than those ( $15.00\% \pm 1.83$ ) of Group 2 ( $\text{PGF}_2\alpha$  only).

**Table 1.** The effect of hCG and Gentamicin administrations related to AI following oestrus synchronisation (by a  $\text{PGF}_2\alpha$  analogue) upon the calving rates in repeat breeder cows ( $P < 0.001$ )

**Tablo 1.** Repeat breeder ineklerde östrus sinkronizasyonu (bir  $\text{PGF}_2\alpha$  analogu ile) sonrası sun'i tohumlamaya bağlı hCG ve Gentamisin uygulamalarının doğum oranları üzerine etkisi ( $P < 0.001$ )

Groups	No. of Cows (n)	Treatments	% Calving Rates (Mean $\pm$ SEM)
I	36	- $\text{PGF}_2\alpha$ (twice, 11 days apart), - AI at 72 and 96 h, - i.v. 1,500 IU hCG at 72 h, - intrauterine 250 mg Gentamicin sulphate (in 50 cc 0.9% NaCl sol.) 30 min after 96 h	38.89 <sup>b</sup> $\pm$ 1.37 (14/36)
II	20	- $\text{PGF}_2\alpha$ (twice, 11 days apart), - AI at 72 and 96 h	15.00 <sup>a</sup> $\pm$ 1.83 (3/20)

<sup>a-b</sup>The values in rows with different superscripts are significantly different from each other ( $P < 0.05$ ).

<sup>a-b</sup>Farklı harf taşıyan satırlardaki değerler arasındaki fark önemlidir ( $P < 0.05$ ).

## DISCUSSION

The results of this study showed that the use of treatment methods related to AI had a marked effect upon the calving rates in repeat breeder cows ( $P < 0.001$ ).

A number of previous studies showed that PGF<sub>2</sub>α applications prior to AI could be used not only for synchronisation but also for treatment of endometritis<sup>5,17-21</sup>. However, various conception rates (33 to 60%) were reported following PGF<sub>2</sub>α application in repeat breeder cows<sup>5,21</sup>. In our study, the calving rate achieved was 15% with PGF<sub>2</sub>α only. This indicates that the application of PGF<sub>2</sub>α only related to AI may not result in satisfactory conception (or delivery) rates because of many other factors (e.g. early embryonic death, infectious agents and nutrition) playing a crucial role in the aetiology of repeat breeding<sup>5,7</sup>.

Antibiotics such as Gentamicin<sup>13,14</sup> were also successfully used to combat microbial infections of the uterus in repeat breeder cows. Awasthi and Kharche<sup>13</sup>, studying the effect of intrauterine Gentamicin sulphate application following AI in repeat breeding cows achieved 50% conception rates as compared to 20% in control group following the first service. Likewise, Singla *et al.*<sup>14</sup> achieved 89% conception rate following Gentamicin application for treatment of endometritis in repeat breeders. In our study, the delivery rate achieved was 39% with PGF<sub>2</sub>α + hCG + Gentamicin while it was 15% in control group (with PGF<sub>2</sub>α only). The results of our study seem to be lower than those of the previous studies. This might be due to small number of animals used and the criteria of success (conception rate vs delivery rate) in their studies. Undoubtedly, the conception rates are expected to be lower than the delivery rates due mainly to the embryonic losses (25-40%)<sup>22</sup>. Roberts<sup>1</sup>, reviewing the literature on repeat breeding concluded that antibiotics and other infusions to correct possible infection in repeat breeding cows may or may not, usually the latter, reveal significant results over non-treated controls. However, our findings indicate that

Gentamicin application may increase the fertility rates in repeat breeding cows. Indeed, the vast majority of researchers<sup>14,23-25</sup> studying the antibiogram tests of bacteria isolated from the cultures of genital tract of repeat breeder animals showed that isolated bacteria have been the most sensitive against Gentamicin. However, differences between the antibacterial sensitivities against Gentamicin infusion<sup>5,26</sup> might be due likely to (i) bacteriological milieu of the uterus, (ii) dose (or the course) of antibiotics treatment and (iii) type of endometritis, etc.

There are controversial reports on the effect of hCG application for treatment of repeat breeding cows<sup>27,28</sup>. Of course, hCG can be used for induction of ovulation for treatment of delayed ovulation and thus increasing the fertility rates following AI<sup>7-10</sup>. However, delayed ovulation and unovulation are uncommon causes for repeat breeding<sup>1</sup>. Furthermore, Çoyan and Tekeli<sup>7</sup> have reported that the incidence of delayed ovulation causing repeat breeding has been less than 2% in the cattle. By contrast, infectious agents are one of the major factors causing repeat breeding<sup>5,7</sup>. Indeed, Dholakia *et al.*<sup>29</sup> reported that one third of repeat breeding cows had had uterine infections. Hence, it might be postulated that marked improvement in delivery rate in treatment group (PGF<sub>2</sub>α + hCG + Gentamicin) was mainly caused by Gentamicin in the present study. However, further studies are warranted to elucidate the actual effect of hCG application in repeat breeder cows.

In conclusion, the overall results suggest that oestrus synchronisation (by a PGF<sub>2</sub>α analogue) combined with the administration of hCG and Gentamicin related to artificial insemination can increase the calving rates in repeat breeder cows.

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