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INVESTIGATION OF THE EFFECT OF DISINFECTANT AND ANTIOXIDANT INTRAUTERINE "ANIONIC OXYGEN" APPLICATION ON FERTILITY AFTER ARTIFICIAL INSEMINATION IN DAIRY COWS

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Abstract: In the study, a total of 30 clinically healthy fertile Holstein cows (aged 3-8 years old; with 500-700 kg average live weight and 28 litres average daily milk) were used in a private dairy farm in Milas district of Muğla province. Randomly selected cows were divided into two groups (control and treatment) (n=15 cows/group) with almost similar body condition scores (BCS) (as all having approximately BCS of 2.75±0.25 units, 1-5 scale). Cows were monitored for natural oestrus, especially during the 2 months postpartum. Control cows (n=15) showing oestrus were inseminated (throughout the 3 consecutive occasions at maximum) by routine AI via recto-vaginal intrauterine route using frozen bull semen (0.25 ml dose; 7.5 x 10^6 total motile sperm) and thawing at 37 °C for 30 seconds. Inseminations were carried out for 7 months (from December 2021 to June 2022). For the treatment group, the cows were given the antimicrobial agent (UB20 O8®) intrauterine 30 minutes after each AI. The disinfectant dose, given to the corpus uteri region after the AI, was used as a total of 50 ml mixture (40 ml warm saline and 10 ml disinfectant). Throughout the study, animals were routinely given a farm-specific "dairy cow" diet (with 23% protein/concentrated feed). In addition, cows were provided regularly with clean drinking water, shade, and free movement in paddocks. Total pregnancy rates of cows and the 'pregnancy index' (the number of inseminations per pregnancy) of 35-60-day post-insemination as checked via ultrasound examination were analysed statistically by using Chi-Square and Wilcoxon tests. The possible difference between the groups based on pregnancy rate and pregnancy index data was considered significant at the P<0.05 level. According to the results obtained, numerical differences observed due to local treatment after all insemination periods were not statistically significant (P>0.05). Pregnancy rates following the 1st, 2nd, and 3rd inseminations in the treatment and control groups were 60.00% vs. 46.66%, 86.66% vs. 66.66%, and 93.33% vs. 80.00%, respectively. The total pregnancy indexes in the groups were 1.57 (14 pregnancies/22 inseminations; treatment) and 2.25 (12 pregnancies/27 inseminations; control). Briefly, it was observed that the pregnancy rate and pregnancy index in the treatment group were consistently but only numerically higher than those in the control group, indicating that anionic oxygen could be an effective alternative for intrauterine treatment leading to high fertility early postpartum.

Keywords: Cow, Uterus, Insemination, Treatment, Antimicrobial, Pregnancy

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1. Introduction

Cases of infertility (possible subclinical endometritis) after artificial insemination (AI) in high-yielding dairy cows are very common (around 50%) in the field (Gökçen, 2008; Uçar and Ağırbaş, 2022).

In fact, the use of antimicrobials intrauterine after the AI may increase the pregnancy rate even in clinically healthy cows (Uçar, 2006; Uçar, 2021). In addition, a new/local product so called "Active Anionic Oxygen" (BAYNOVA Catalogue, 2021), as reported in the current literature to be very effective (with -sidal effect) on the respiratory (anti-viral) (Bursalı et al., 2020) and digestive systems (anti-carcinogen) (Apaydın Yıldırım, 2021), was considered herein as a treatment modality in terms of fertility outcomes. Hence, we presumed that it would also be effective (as antimicrobial-antioxidant) on the reproductive system in farm animals. Simply, our hypothesis, that UB20 O8® might have a potential positive effect on the genital tract of dairy cows was proved herein, to be a "novel intrauterine antimicrobial agent" along with the resultant high fertility.

2. Materials and Methods

2.1. Materials

In the study, a total of 30 clinically healthy fertile Holstein cows (aged 3-8 years old; with 500-700 kg average live weight and 28 litres average daily milk) were used in a dairy farm of private sector in Milas district of Muğla province, TÜRKİYE. For early pregnancy checking, ultrasound device used was HASVET 838

Veterinary Ultrasound Device® (HASVET, Antalya-Türkiye), as equipped with L50/7.5 MHz linear rectal probe (250 mm, standard).

2.2. Methods

The cows were arbitrarily divided into two groups (control and treatment) (n=15 cows/group) with almost similar body condition scores (BCS) (as all having approximately BCS of 2.75±0.25 units, 1-5 scale) (Çolak and Uçar, 2007).

Cows were monitored for natural oestrus especially for the 2 months postpartum.

2.2.1. Artificial inseminations

Control cows showing oestrus were inseminated (throughout the 3 consecutive occasional cycles at maximum) by routine AI via recto-vaginal intrauterine route using frozen bull semen (0.25 ml dose; 7.5 x 10^6 total motile sperm) and thawing at 37 °C for 30 seconds. Inseminations were carried out over 7 months (from December 2021 to June 2022).

2.2.2. Active anionic oxygen (UB20 O8)

For the treatment group, the cows were given the antimicrobial agent (H_2O_8, so called as "UB20 O8®", BAYNOVA-Ankara, Türkiye) for treatment via intrauterine route 30 minutes after each AI.

The disinfectant dose, given to the corpus uteri region after the AI, was used as a total of 50 ml mixture (40 ml warm saline and 10 ml disinfectant).

2.2.3. Ration

Throughout the study, animals were routinely given a farm-specific "dairy cow" ration (with 23% protein/concentrated feed).

In addition, cows were provided regularly with clean drinking water *ad libitum*, shade and free movement in paddocks.

2.2.4. Statistical analysis

Total pregnancy rates of cows inseminated and the 'pregnancy index' (the number of inseminations per pregnancy) (İleri, 2002) of 35-60-day post-insemination as found via ultrasound examination were analysed statistically by using Chi-Square and Wilcoxon tests (Önder, 2018).

The possible difference between the groups based on the mean pregnancy rate and pregnancy index data was considered significant at the P<0.05 level.

3. Results

According to the results, there were only numerical differences due to local treatment by UB20 O8® after all insemination periods (P>0.05) (Figure 1 and Table 1).

Pregnancy rates following the 1st, 2nd, and 3rd inseminations in the treatment and control groups were 60.00% *vs.* 46.66%, 86.66% *vs.* 66.66% and 93.33% *vs.* 80%, respectively.

The total pregnancy indexes in the groups were 1.57 (14 pregnancies/22 inseminations; as treatment) and 2.25 (12 pregnancies/27 inseminations; as control).

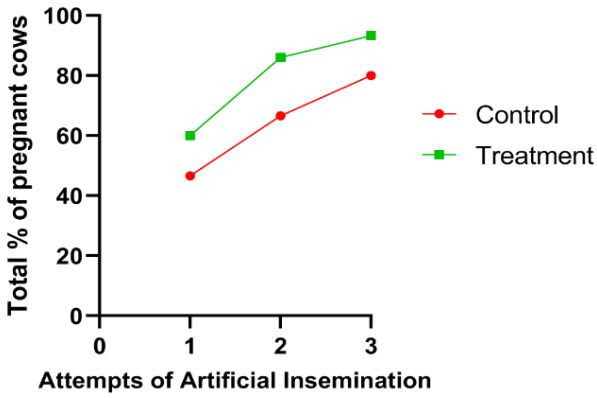

Figure 1. Comparing the intrauterine treatment (UB20 O8®) *vs.* control groups regarding the success rate of AI inducing pregnancy in Holstein dairy cows

Table 1. Results of the effect of intrauterine application of a new antimicrobial/antioxidant (Active Anionic Oxygen, UB20 O8®) on the pregnancy rates and pregnancy index in Holstein dairy cows

Groups	n	Pregnancy rates (%) after the inseminations (A.I.)			Pregnancy Index (PI)
		1st A.I.	2nd A.I.	3rd A.I.	
I-Treatment	15	60.00 (9/15)	86.66 (13/15)	93.33 (14/15)	1.57 (14/22)
II-Control	15	46.66 (7/15)	66.66 (10/15)	80.00 (12/15)	2.25 (12/27)
Significance (P value)		NS	NS	NS	NS

NS=not significant (P>0.05).

4. Discussion and Conclusion

Recently, we reviewed comprehensively the underlying mechanisms of bovine infertility and considered different approaches by using intrauterine antimicrobial therapy to increase pregnancy rate after artificial insemination dairy cows (Uçar and Ağırbaş, 2022). So, we briefly focused on the new results of our experiment conducted herein.

To sum up, when the AI is combined with intrauterine Active Anionic Oxygen (UB20 O8®) agent in Holstein dairy cows, relatively positive effects on the genital system (earlier conception and higher pregnancies, even by lower insemination numbers) (İleri, 2002; Gökçen,

2008; Uçar, 2021) were observed consistently, as in other body systems reported in the literature (Bursalı et al., 2020; Apaydın Yıldırım, 2021).

We believe that similar studies should be conducted in future in larger populations with different breeds and/or species in order to obtain more reliable and promising results under various feeding and management conditions (Dwyer, 2020; Uçar et al., 2020).

Author Contributions

The percentage of the author(s) contributions is presented below. All authors reviewed and approved the final version of the manuscript.

	Ö.U.	D.A.
C	75	25
D	75	25
S	100	
DCP	100	
DAI	100	
L	75	25
W	75	25
CR	75	25
SR	100	
PM	50	50
FA	50	50

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Approval/Informed Consent

Permissions were obtained from the Muğla Sıtkı Koçman University Animal Experiments Local Ethics Committee. All procedures performed involving animals were in accordance with the ethical standards approved by the Muğla Sıtkı Koçman University Animal Experiments Local Ethics Committee (protocol code: E-40051172-100-430002 and date: June 06, 2022).

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