



The Effectiveness of Intrauterine Treatment with *Momordica charantia* L. on Some Hormone Parameters in Repeat Breeder Cattle

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ABSTRACT

Background: Repeated breeding is a substantial problem in dairy cattle affecting reproductive efficiency. The goal of the current study was to see how intrauterine treatment of phytotherapeutic plant extract affected some hormonal indicators and conception rates in dairy cattle with fertility issues.

Methods: In the study, 40 repeat breeder (RB) cattle were randomly divided into two groups. Forty ml (0.25 g/mL) *Momordica charantia* L. (MC) extract was administered intrauterine to cattle in group I (n=20) and 40 ml of pure olive oil was administered intrauterine to cattle in group II (n=20) at a time. The control group, group III (n=20), was composed of healthy heifers. Endometrial smear samples were taken from all RB cattle for cytological examination before the treatment and all were stained with Giemsa. Estradiol (E2), progesterone (P4) and insulin-like growth factor (IGF-1) levels were assessed by collecting blood samples starting from intrauterine treatment on days 0, 7 and 14. After the last blood sample was collected, the estrus cycles of all cattle were synchronized through a double dose of PGF_{2α} administration at 14-day intervals. GnRH was administered with single insemination at the 60th hour following the second PGF_{2α} treatment.

Result: Subclinical endometritis was determined in 82.5% (33/40) of RB cattle. Pregnancy rates in group I, group II and group III were determined as 55%, 35% and 35%. respectively (P>0.05). There was no significant difference in E2 values according to time and between groups. For P4, there were also no significant differences between three time-dependent measurements, but there was a difference in the 7th and 14th days of the groups (P<0.01). For IGF-1, significant differences were found between the three time-dependent measurements of the groups (P<0.001). In conclusion, it was observed that the MC extract treatment, especially in RB with SCE, can improve the pregnancy rate by positively affecting the increase in IGF-1 which is a key element of the extremely complex endocrine system.

Key words: Cow, Endometritis, *Momordica Charantia* L., Phytotherapy, Repeat Breeder.

INTRODUCTION

Low fertility is one of the key issues that cause economic losses in dairy cattle herds. The goal of fertility is to be able to get a calf every year. For this to happen, the cattle that give birth must have a healthy puerperium period (Arthur *et al.*, 1989). Fertility is disrupted by traumatic, pathogenic, metabolic, breeding and nutrition abnormalities or insufficiencies that negatively affect the postpartum (pp) period (McEntee, 1990). The situation of animals that do not become pregnant despite being inseminated three or more times in practice is defined as repeat breeder, which is one of the key etiological determinants of infertility (Noakes, 1988).

Depend on the nature of infection, systemic antibiotics, hormones, enzymes and phytotherapeutic substances are used to treat uterine infections in cattle (Busch and Grüssel, 1998; Risco *et al.*, 2007). Because of the toxicity and side effects of allopathic therapy, herbal medicine is becoming more popular. Some herbal products have been used as therapeutic agents and have recently been the subject of scientific research. *Momordica charantia* L. (MC), which is a plant of the family Cucurbitaceae, growing mainly in

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tropical and subtropical regions. It has been determined to have an antihyperglycemic (Rao *et al.*, 2001), antifungal (Wang *et al.*, 2016), antimicrobial, immunotoxic, antifertility (Krawinkel and Keding, 2006; Nerurkar and Ray, 2010) and antioxidant effects (Talukdar *et al.*, 2014).

Estrogens have a key role in many physiological processes, especially in the development and maintenance of normal sexual and reproductive activities in females (Alonso and Rosenfield, 2002). Estrogen, along with progesterone and gonadotropins, is involved in the regulation

of the estrus cycle (Gray *et al.*, 2001). Estradiol-17 β (E2) stimulates the development of the uterus by increasing DNA synthesis and cell proliferation in the uterus and regulating the activity of growth hormone and insulin-like growth factor-1 (IGF-1) (Leung *et al.*, 2004). An increase in IGF-1, which is a key element of the endocrine system, causes beneficial effects on fertility, immunity and lactation (Lucy, 2000). Stress factors such as infection, heat stress, or NEB reduce the number of growth hormone receptors (GHRs) in the blood and, consequently, the level of IGF-1 decreases (Ferry, 1999).

The aim of the present study is to investigate the effectiveness of intrauterine infusion of *MCL* extract on some hormonal parameters and to determine the conception rate in lactating RB dairy cattle.

MATERIALS AND METHODS

The study was carried out in a private enterprise in Şanlıurfa province in 2019-2021 and laboratory studies were carried out in Harran University laboratories. This research was conducted with 20 healthy heifers and 3-8-year-old 40 Holstein-Friesian dairy cattle which had given at least one birth and had regular sexual cycles and no clinical disorders and abnormal discharges in their genital organs, but they could not conceive even though artificial insemination was done at least three times, demonstrating no other clinical complaints other than infertility. The body condition score of infertile cattle ranged between 2.75-4.5, the milk yield at the last lactation (305 days) was 7540 kg on average (5265-8650 kg) and the postpartum period was recorded as 182 days on average (108-349 days).

During selection of cattle, rectal palpation, ultrasonography and vaginoscopy methods were used and endometrial smears were taken from all cows with RB using an endocervical brush. The smears were stained according to Giemsa method and the presence of inflammatory cells was assessed under a light microscope. The neutrophil density of 5% and more in postpartum cattle was defined as a criterion for SCE (Melcher *et al.*, 2014).

Repeat breeder cows are split into two main groups disregarding the results of cytological examination. 40 ml (0.25 g/mL) MC extract was administered intrauterine to cattle in group I (n=20) and pure olive oil (40 ml) was administered intrauterine to cattle in group II (n=20). The control group (n=20), group III was composed of healthy heifers and no intrauterine administration was exerted.

Derivation of *Momordica charantia* L. plant extract

The mature fruits of the plant were collected from the vicinity of Mersin, Tarsus and Adana. The mixture was prepared as indicated in our previous study (Emre *et al.*, 2017) and its density was calculated as 0.25 g/mL.

Collecting blood samples

To determine estradiol-17 β (E2), progesterone (P4) and insulin-like growth factor (IGF-1) levels in all groups, blood

samples were collected into the tubes without heparin from the coccygeal vein on the days 0, 7 and 14 of the treatment, respectively. The blood samples were centrifuged at 3000 rpm and serum were stored in a freezer (-20°C) until analyzed.

Estrus synchronization

After 14 days from the last bloodletting, PG. (25.mg, IM; Dinolytic®, Pfizer, Turkey) was used to cattle in double doses at intervals of 14 days, so estruses were synchronized. After the second PG administration, GnRH (10 μ g; IM; Receptal®, Intervet, Turkey) injection was executed at 60th hours along with insemination. The pregnancy examination was done 45 days after artificial insemination by using ultrasonography (100 Falco Vet, Pie Medical),.

Determination of hormonal parameter levels

The levels of hormonal parameters were determined in the in the Biochemistry Department, Medicine Faculty by the spectrophotometric method using ELISA kits. Serum E2 levels (Cayman® Estradiol ELISA Kit, 582251, Gaziantep, Turkey), serum P4 levels (Rel Assay® Bovine Progesterone ELISA Kit, 201711, Gaziantep, Turkey) and serum IGF-1 levels (Rel Assay® Bovine Insulin-like Growth Factors-1 ELISA Kit, 21711, Gaziantep, Turkey) were performed based on the manufacturer's protocols of commercially available diagnostic kits.

Statistical analysis

The obtained data were evaluated by using SPSS 22.0 (SPSS Inc. Chicago, IL, USA) software. The Shapiro-Wilk test was used to see if the data provided normal distribution assumptions. In addition, the homogeneity of variance was controlled by Levene's test. Logarithmic or square root transformation was applied to data that did not show normal distribution. To detect time-dependent variation within the group, the data were evaluated primarily by two-way variance analysis in the repetitive measurements. In the case where the time factor or time*group interaction is significant, posthoc analysis of the time-dependent changes of each group in itself was carried out by additional coding to the syntax menu in the General Linear Model (GLM) procedure. Pregnancy rates were evaluated by chi-square analysis. $P < 0.05$ was considered significant. The data in the table, figure and results section were expressed as average $\pm$ standard error $\bar{X} \pm \bar{S\bar{X}}$.

RESULTS AND DISCUSSION

Repeat breeder is one of the most important problems in addition to many etiological factors affecting reproductive performance in dairy cattle enterprises (Gustafsson and Emanuelson, 2002; Yusuf *et al.*, 2010). The important etiologies include ovarian dysfunction, failure of fertilization and subclinical endometritis (Pothmann *et al.*, 2015; Yusuf *et al.*, 2010). Hormones, antibiotics and immunomodulators are widely used in the treatment of uterine infections in cattle (Purohit *et al.*, 2015) and it is difficult to suggest a specific treatment. Accordingly, in this study, it was aimed to

determine the efficiency of treatment with *Momordica charantia* L. in RB cattle on the level of E2, P4, IGF-1 and pregnancy rate, supposing that it can provide activation of natural defense mechanism in the uterus and may be an alternative treatment with immunomodulatory properties.

Endometrial cytology

The incidence rate of SCE was determined as 82.5% (33/40) as a result of cytological examinations in RB cattle at the beginning of the study. According to the results of the cytological examination of animals considered as healthy, prismatic uterine epitheliums were observed intensively. In the animals diagnosed with SCE, lymphocytes and neutrophil cells were intensively determined along with prismatic epithelium.

It is stated that clinical and subclinical endometritis (SCE) is one of the most important reasons for delaying the time of resumption of postpartum ovarian activity (Sheldon *et al.*, 2009). In previous studies, it was determined that the incidence of SCE in RB cattle ranged from 12.7 to 94% (Agaoglu *et al.*, 2020; Pothmann *et al.*, 2015; Salasel *et al.*, 2010; Yazlik *et al.*, 2021). In the presented study, similar to the results of previous studies (Agaoglu *et al.*, 2020; Salasel *et al.*, 2010), one of the most important causes of RB was found to be SCE (82.5%). Pothmann *et al.* (2015) determined this ratio as 12.7% and reported that SCE is not an important factor in the etiology of RB. The reason for such a high rate of SCE in our study may be explained by the fact that the pp day and neutrophil threshold values are different from other studies. However, as another reason, it was thought that the farm in which we were conducting the study was not a professional enterprise and therefore it might have been caused by management errors.

Pregnancy findings

In group I, group II and group III pregnancy rates were determined as 55%, 35% and 35%, respectively ($P>0.05$).

The highest pregnancy rate at the first insemination was determined as 55% in the phytotherapy group. Pregnancy rates had variety in studies where intrauterine plant extract was applied in RB cattle with SCE. Bhardwaz *et al.* (2019) reported that the pregnancy rate at the first insemination was 50% in the group where neem extract was applied every 24 hours for 7 days and the same researcher determined this rate was 60% when garlic was applied in another study (Bhardwaz *et al.*, 2018). Brahmanand *et al.* (2019) determined the pregnancy rate at first insemination was 66.67% in the methanol fractioned neem oil (2 days at 24 hrs interval) group and 33.33% when it was applied as 2 days at 48 hrs intervals. They found 16.67% in the Neem bark extract group (3 days at 24 hrs intervals) and showed a similar pregnancy rate (16.67%) when applied for 3 days at 48 hrs intervals. Brahmanand *et al.* (2019) found that the pregnancy rate increased (total pregnancy rate 83.33, 66.67, 50 and 33.33%, respectively) as the number of inseminations increased in comparison with the first insemination rate in RB cattle with SCE. It was thought that the basis of the the variety in the pragnency rate might be depend on the severity or intensity of the infection, the concentration of the therapeutic agent, the frequency of administration and the duration of treatment. For higher pregnancy rates, the number of intrauterine applications may be increased in further studies. It should be considered that there may have been an increase in the pregnancy rate in all groups depending on the double-dose PG protocol (Galvão *et al.*, 2009; Hendricks *et al.*, 2006) used as a treatment option for uterine infections from the 14th day of the study.

Hormonal measurement findings

The mean values of the hormonal parameters belonging to all three groups and the time-dependent change curves are presented in the relevant Table 1, 2, 3.

Table 1: Mean E2 values between groups (pg/ml).

Group	n	Day 0	7 th day	14 th day	P _{ANOVA}
		$\bar{X} \pm \bar{S}\bar{X}$	$\bar{X} \pm \bar{S}\bar{X}$	$\bar{X} \pm \bar{S}\bar{X}$	
Group I	20	42.28±3.73	43.78±4.25	46.39±3.89	***
Group II	20	44.95±4.23	47.01±3.70	49.39±4.23	***
Group III	20	40.57±2.87	38.16±2.05	38.04±2.76	***
P _{two-way ANOVA}	***	***	***		

***p<0.001

Table 2: Mean P4 values between groups (ng/ml).

Group	n	Day 0	7 th day	14 th day	P _{ANOVA}
		$\bar{X} \pm \bar{S}\bar{X}$	$\bar{X} \pm \bar{S}\bar{X}$	$\bar{X} \pm \bar{S}\bar{X}$	
Group I	20	8.09±1.13	7.96±1.43 ^a	8.75±0.94 ^a	***
Group II	20	6.77±1.17	3.38±0.62 ^b	6.87±1.14 ^{ab}	***
Group III	20	7.78±0.84	6.97±0.78 ^a	4.14±0.53 ^b	***
P _{two-way ANOVA}	***	**	**		

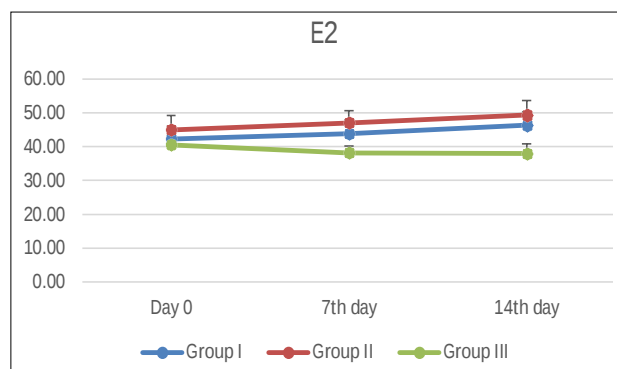
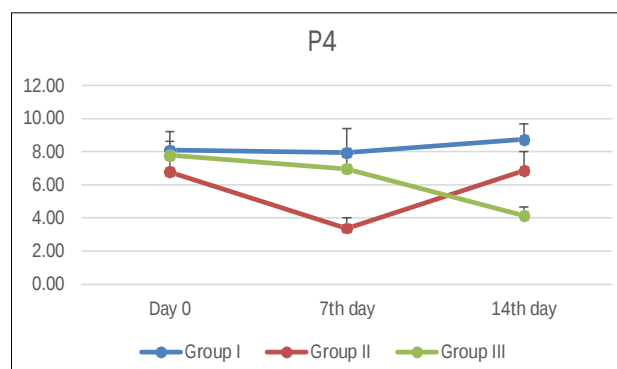
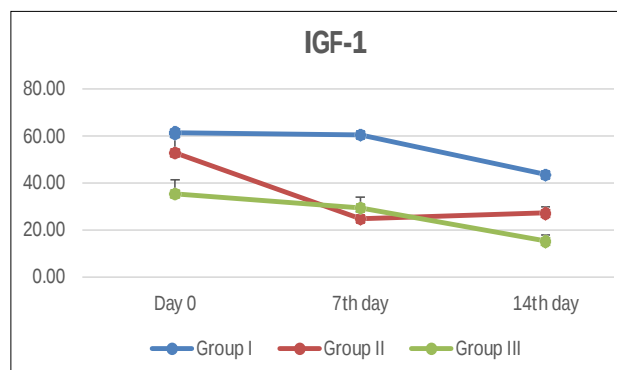
***p<0.01, ***p<0.001

Table 3: Mean IGF-1 values between groups (ng/ml).

Group	n	Day 0	7 th day	14 th day	P _{ANOVA}
		$\bar{X} \pm \text{SX}$	$\bar{X} \pm \text{SX}$	$\bar{X} \pm \text{SX}$	
Group I	20	61.5 $\pm$ 7.7 ^a	60.4 $\pm$ 6.1 ^a	43.6 $\pm$ 5.9 ^a	***
Group II	20	52.8 $\pm$ 6.6 ^{ab}	24.9 $\pm$ 3.0 ^b	27.3 $\pm$ 4.6 ^b	***
Group III	20	35.5 $\pm$ 2.7 ^b	29.5 $\pm$ 2.6 ^b	15.4 $\pm$ 2.4 ^b	***

P_{two-way ANOVA}

*p<0.05, ***p<0.001

**Fig 1:** Weekly average E2 concentration (pg/ml) in control and treatments groups.**Fig 2:** Weekly average P4 concentration (ng/ml) in control and treatments groups.**Fig 3:** Weekly average IGF-1 concentration (ng/ml) in control and treatments groups.

The intra-group time-dependent variation in E2 value was not significant ($p>0.05$) and there was no interaction between the groups and E2 variation ($p>0.05$) (Fig 1).

The intra-group time-dependent variation in the P4 value was not significant ($p>0.05$), but an interaction between the P4 value and the groups was determined ($p<0.05$). On the seventh day, the differences between group I and II ($p<0.05$) and between group II and III were significant ($p<0.05$), while there was no significant difference between group I and III ($p>0.05$) (Fig 2).

The time-dependent change in IGF-1 value within the group was significant ($P<0.05$) and there was an interaction between the groups and the IGF-1 variation ($p<0.001$). While there was a difference between group I and III on day 0 ($p<0.05$), there was no difference between group I and II ($p>0.05$) and group II and III ($p>0.05$). On day 7, there was a significant difference between group I and II ($p<0.05$) and group I and III ($p<0.05$), while no differences were found between group II and III ($p>0.05$). On the 14th day, there was a significant difference between group I and II ($p<0.05$) and group I and III ($p<0.05$), while no differences were found between group II and III ($p>0.05$) (Fig 3).

The number of neutrophil leukocytes, immunoglobulins, cytokines and pregnancy rates was evaluated in intrauterine medicinal herbal treatment studies (Bhardwaz *et al.*, 2019; Brahmanand *et al.*, 2019), however, evaluations on ovarian activity were not encountered. In this study, serum concentrations of P4, E2 and IGF-1 were measured to evaluate the ovarian activity of intrauterine MC extract administration. For estradiol, no interaction was recorded between the three time-dependent measurements and the groups. However, the absence of any changes in the E2 concentration after phytotherapy administration suggested that the effectiveness of a single application on the ovary was not sufficient and that better results could be obtained from the longer-term application. For P4; it was observed that there was no significant difference in the change between the three time-dependent measurements and the P4 value was always high in group I which underwent phytotherapy. It was thought that intrauterine MC extract with PG administration in group I, where the level of progesterone is still high on the 14th day, increased the pregnancy rate due to both luteolysis and immunomodulatory effects (Lewis, 2004). However, a significant decrease in the average P4 level was observed on the 7th day in the pure olive oil group. Here it can be considered that pure

olive oil exerts a luteolytic effect. In the study, intrauterine application of MC extract did not affect estradiol and progesterone concentrations depending on time. Unfortunately, the effect of phytotherapy on three time-dependent measurements was not fully scrutinized in this study since the application was not started in the same period of the sexual cycle. It was shown that many studies should be conducted on the administration of MC. However, the values obtained as a result of the hormone measurement on the day 0 and the high SCE incidence in the study reveal that ovarium is active and RB did not occur by ovarium dysfunction (Agaoglu *et al.*, 2020; Pothmann *et al.*, 2015). Contrary to our findings, it was also reported that besides an affected endometrium, it may also be associated with impaired ovarian function as a possible cause of failure to conceive in RB (López-Gatius *et al.*, 2004; Kendall *et al.*, 2009). However, Pothmann *et al.* (2015) reported that SCE, uterine infections, or inactive ovaries were not strongly associated with RB.

Insulin-like growth factor-1 (IGF-1) is known to be highly associated with energy balance, follicular growth, ovarian cycle resumption and prediction of reproductive performance in cattle (Velazquez *et al.*, 2009; Zulu *et al.*, 2002). For IGF-1, significant differences was observed depending on time between three measurements of the groups in which we were able to monitor the effect of the treatment. In the 7th and 14th days, IGF-1 levels were observed to maintain concentration and remain high compared to other groups in the phytotherapy group. The results here indicate that IGF-1 has an important role in the fertility of dairy cattle and MC administration may affect the treatment. However, there are many studies in which there is a low concentration of IGF-1 in uterine infections (Kasimanickam *et al.*, 2013; Pascottini and LeBlanc, 2020). It is noted that estradiol-17 β stimulates the development of the uterus by increasing DNA synthesis and cell proliferation in the uterus and regulating the activity of growth hormone and IGF-1 (Leung *et al.*, 2004; De Catanzaro, 2015). Although there was no significant difference in E2 concentration in this study, it should be considered that it supports IGF levels. Because IGF-1 levels remained high in the group undergoing phytotherapy and in parallel, the pregnancy rate was also observed to be the highest. Considering that the endometrium of heifers was not affected by any pathological changes, they were selected as a healthy/control group. However, another point that drew attention was that the IGF-1 values were low because these heifers in the control group did not reach sufficient body weight due to environmental and management conditions, despite being of an appropriate age. Because it was determined that the level of IGF-1 in the systemic circulation is closely related to the feeding of the animals and ovarian functions (Zulu *et al.*, 2002).

CONCLUSION

In conclusion, it was determined that subclinical endometritis is one of the most substantial factors in the etiology of RB. It was re-established that uterine cytology

can be used to elucidate the state of the endometrium in RB cattle. The high rate of SCE in the enterprise indicates that the problem occurs based on herd rather than individual sensitivity. At the stage of deciding on treatment in RB cattle, the ovarian hormone profile should be conceived. It was observed that the intrauterine application of the oily form of the MC as a phytotherapeutic plant has positively affect the treatment and can be used as an alternative treatment option. It was observed that this application can also improve the pregnancy rate in RB cattle in parallel with the level of IGF-1, which is a key element of the endocrine system. It is believed that many studies should be conducted on the MC in animal welfare. In future studies on RB with SCE, post-treatment cytological examination should also be included in the study. However, ovarian activity should be monitored ultrasonographically as well as measuring hormones.

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Conflict of interest

The author have stated that there is no conflict of interest at the end of the article.

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