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NEW APPROACH FOR BIOLOGICAL TREATMENT OF ENDOMETRITIS IN DAIRY CATTLE

By

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ABSTRACT

Endometritis reduces fertility and is responsible for major economic losses in beef and dairy industries. The aim of this study was to evaluate an alternative therapy using platelet-rich plasma(PRP).PRP was tested invivo,after bovine intrauterine administration, and in vitro on endometrial cells Endometritis reduces fertility and is responsible for major economic losses in beef and dairy industries.The aim of this study was to evaluate an alternative therapy using platelet-rich plasma (PRP). PRP was tested in vivo, after bovine intrauterine administration, and in vitro on endometrial cells Endometritis reduces fertility and is responsible for major economic losses in beef and dairy industries.The aim of this study was to evaluate an alternative therapy using platelet-richplasma (PRP).PRP was tested in vivo,afterbovine intrauterine administration, and in vitro on endometrial cells Endometritis reduces fertility and is responsible for major economic losses in dairy breeders. The incidence of endometritis in cattle is variable, ranging from 3.4% to 40%. Platelet-rich plasma (PRP) has been recently applied in reproductive scenarios and is based on the knowledge that platelet growth factors can improve the endometrial environment, which is full of growth-factor receptors, adhesion molecules, cytokines, lipids,and other factors that enhance endometrial and embryonic development.

The aim of this study was to compare the using of platelet-rich plasma (PRP) and the traditional treatment (Gentamycin) and evaluation of each of the two treatments give good conception. Out of 220 dairy cows examined, 60 cows were affected with endometritis according to results of ultrasonographic examination and bacteriological isolation.

The affected group was subdivided into two equal groups. Group I (N=30) were treated with Gentamycin as a traditional treatment of endometritis. Group II (N=30) were treated by plasma rich platelet. Two blood samples were taken from each diseased animal (first sample

for serum separation and measurement of BUN, albumin, Total cholesterol, and glucose. Second blood sample was taken for preparation of PRP. Bacteriological swaps were taken from the affected animals before, after treatment for isolation, identification and sensitivity test of the isolated microorganisms.

INTRODUCTION

Endometritis impairs the function of not only the uterus **(Gilbert, 2011)** but also the ovary **(Williams *et al*., 2007).** The incidence of endometritis in cattle is variable, ranging from 3.4% to 40 % **(Mari *et al.,* 2012).** Endometritis causes economic losses which related to delay in the resumption of ovarian activity, increased number of services per conception, decreased milk yield, and costs of treatment of the disease **(Wagener *et al.,*2017).**

There is a relationship between uterine diseases and concentrations of albumin, blood urea nitrogen, and total cholesterol **(Ghanem *et al.,*2016).** Plasma proteins, including albumin, act as carriers for the transport of substances, such asfattyacids,during inflammation **(Mazzaferro *et al*.,2002),** and blood urea nitrogen (BUN) levels reflect protein metabolism in dairy cows **(Butler 1998).**Elevated serum beta-hydroxybutyric acid(BHBA) concentrations in the first 2 weeks postpartum indicate an increased risk of metritis **(Duffield *et al.,*2009),** which is a usual precursor of the development of clinical and subclinical endometritis **(Walsh *et al,* 2007).**

Uterine disease treatments aim at reverting inflammatory changes that impair fertility, whilst enhancing uterine defense and repair **(Shams *et al.,* 2004).** In cows, many therapeutic agents and procedures have been developed to treat endometritis, including systemic or intrauterine administration of antibiotics **(Runciman *et al.,* 2009).**

Platelet-rich plasma (PRP) is an emerging therapeutic application in tissue regeneration due to its enrichment in growth factors with mitogenic and anti-inflammatory potential **(Gentile, *et al.,* 2012,Nazari *et al*., 2016).** In particular, PRP is a concentration of platelets (3-5 fold the plasma baseline level) containing transforming growth factors, insulin-like growth factor1 (IGF-I),epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF) and hepatocyte growth factor (HGF) that are very important for regeneration processes **(Scully *et al*., 2018 and Aghajanova *et al*., 2018).** In addition**, Bos-Mikich *et al.*(2018)** recorded that, PRP is easy to obtain, low cost, and rich in growth factors, PRP is an autologous preparation, and therefore non-toxic and non-allergenic, it can

be used in various medical conditions as an adjuvant therapy to conventional treatment, with generally satisfactory results.

The aim of this study was to compare the using of platelet-rich plasma (PRP) and the traditional treatment (Gentamycin) and to evaluation each of other two treatments give good conception.

MATERIAL AND METHODS

Out of 220 dairy cows examined, 60 cows were affected with endometritis according to results of ultrasonographic examination and bacteriological isolation. The affected group was subdivided into two equal groups. Group I (N=30) were treated with Gentamycin intrauterine as a traditional treatment of endometritis. Group II(N=30) were treated by plasma rich platelet intrauterine.

All cows were housed in free stalls, fed twice daily with a total mixed ration meeting the requirements for milk production with *ad libitum* access to water.

All treated groups (I and II) were inseminated artificially. Conception rates were recorded.

Vaginal swabs collection and Bacteriological culture:

Vaginal swabs were taken (after gynecological examination per rectum) under complete aseptic conditions and then sent to the laboratory with no delay to avoid dryness of samples. Swabs were inoculated into nutrient broth and incubated over night at 37° C. Loopfuls were subcultured onto 5% sheep blood agar and MacConkey's agar. The plates incubated for 24 hrs at 37° C in aerobically condition**.** The growing colonies were described morphologically and microscopically. For further identification biochemical reactions were done after colonial purification according to **Ellen *et al.* (1994) and Quinn *et al.* (1994).**The bacterial species were identified based on colony morphology, Gram-stained smears, and biochemical characteristics **(Williams *et al.*,2005).**The bacterial species were classified based on their pathogenicity:**Actynomyces pyogenes** and*E.coli* (pathogenic bacteria)related to endometritis; 2, non-pathogenic bacteria **(Williams *et al.*, 2007).**

Antimicrobial susceptibility test:

The antimicrobial resistance patterns of isolates were estimated using the **Kirby Bauner disk diffusiontechnique(Brown *et al.*,1975).**Thedisks of the following antibiotics:Oxytetracyclin, Gentamycin,Ampicillin,Oxacillin,Sulphamethoxazole,Vancomycin and Enrofloxacin. Isolated colonies of same morphologic type were inoculated into 5 ml of nutrient broth at 37°C for

overnight until visible turbidity appeared. The turbidity was compared to 0.5 Mucfarland standards.

Mullar Hinton agar was used as medium. After inoculation of suspended broth on media antibiotic disks were applied to the surface of the inoculated plates with sterile forceps.

All disks were gently pressed down onto the agar with forceps to ensure complete contact with agar surface. The platelet was incubated inverted and then aerobically incubated for 24 hours at 37°C. The diameters of complete inhibition zones were measured to the nearest whole millimeter using zone inhibition scale.

Preparation of platelet-rich plasma

Blood was obtained from the selected cows suffering from endometritis. These animals were did not receive medication during the previous two months. The collection of blood and the preparation of PRP, with the method of double centrifugation, were performed as described by **Lange-Consiglio *et al.* (2014).** Blood was collected in tubes containing sodium citrate kept at (4°C) and sent to the laboratory.

Sampling:

Blood samples were collected from all animals before and after treatment. Blood samples were collected 3 h after feeding from the jugular vein in plain vacuum tubes and centrifuged at 3000rpm for 15 min. Serum was separated and stored at-20^{o}C till serum biochemical assays.

Serum biochemical parameters:

Serum blood urea nitrogen measured according to **Reiss *et al.*, (1965)**,albumin measured according to **Doumas, (1971),** serum total cholesterol level measured according to **Richmond (1973)** and glucose level measured according to **Trinder (1969).**

Statistical analysis:

Data were analyzed using SPSS Statistics® 16.0 (Version 16.0 released 2007, SPSS Inc., Chicago, IL, U.S.A.). One-way ANOVA was performed to compare the groups. The levels of significance was set at $P<0.05$.

RESULT

Table (1): Antibiogram of Actynomyces pyogenes and *E. coli* isolated from endometritis.

	Actynomyces pyogenes	***E. coli***
High sensitive (HS)	**Enroflaxcin Gentamycin**	**Enroflaxcin Gentamycin**
Moderate sensitive MS	**Oxytetracyclin Vancomycin Oxacillin**	**Oxytetracyclin Vancomycin**
Resistant R	**Ampicillin**	**Sulphamethoxazole**

Table (2): Concentration (mean ± SE) of some metabolic parameters before and after traditional and plasma rich platelet (PRP) treatment.

Some metabolic parameters	**Endometric cows before treatment (n=60)**	**Endometric cows after traditional treatment with Gentamycin(n=30)**	**Endometric cows after PRP treatment (n=30)**
Urea Nitrogen (mg/dl)	**20.85 ± 1.85^{a}**	**19.57 ± 1.25^{a}**	**15.57±2.25^{c}**
Albumin (mg/dl)	**1.16 ± 0.04^{c}**	**2.32 ± 0.02^{b}**	**3.91± 0.01 ^{a}**
Total cholesterol (mg/dl)	**51.72±5.95^{c}**	**69.12±9.38^{b}**	**81.42±8.05^{a}**
Glucose (mg/dl)	**53.28±2.59^{c}**	**62.84±1.12^{b}**	**76.25±3.15^{a}**

Means with different alphabetic subscripts in the same row were significantly differ at (*P< 0.05*).

Blood urea nitrogen showed significant increase (P< 0.05) in its level in endometric cows before treatment than in both traditional treated cows and PRP treated cows. Significant decrease (P< 0.05) in serum albumin, glucose and total cholesterol levels in endometric cows before treatment than traditional treated cows and PRP treated cows.

Table (3): Conception rates in traditional treatment and PRP of endometritis in dairy cattle.

	Group I (Traditional treatment)		**Group II (PRP) plasma rich platelet**	
	No	**Ratio %**	**No**	**Ratio%**
Conception rate	**30**	**17/30 (56.66)**	**30**	**22/30 (73.33)**

DISCUSSION

Endometritis is the localized inflammation of the endometrium persisting beyond normal uterine involution, and has been documented to be a major limitation on the optimal reproductive performance of dairy cows **(Agarwal *et al.*, 2013)**.

Serum albumin level showed significant decrease in endometric cows before treatment than treated ones. Albumin is known to be a negative reacting protein, and its level decreases during inflammation **(Bertoni *et al.*, 2008).** Albumin is known to be a negative reacting protein, and its level decreases during inflammation **(Mazzaferro *et al.*, 2002)** this may be a reason for the low albumin concentrations in the cows with endometrial inflammation **(Nazhat *et al.*, 2018).**

In the present study, found non-significant difference in BUN level in twcows with endometritis and treated groups. This result is similar to **Giuliodori *et al.* (2013) and Cui *et al.*, (2019)**, whereas **Senosy *et al.* (2011)** found an overall decrease of BUN.

In the present study, T- chol tended to be lower at endometritis group than treated groups. These findings are supported by those of **Patbandha *et al.*,(2016).** This is a clue that cows with endometritis suffer from disturbance in energy metabolism and delayed the physiological adaptation of cows for their energy needs **(Nazifi *et al*.2003).** Also, decreased total cholesterol levels during the first month of lactation caused longer intervals to first breeding after calving in Holstein dairy cows **(Kim and Suh,2003).**Treated cows showed high cholesterol levels which lead to cholesterol accumulation in macrophages and other immune cells, which

promote inflammatory responses. Cholesterol accumulation, through the promotion of inflammatory responses, probably has beneficial effects in the response to infections **(Tall and Yvan-Charvet, 2015).** Also, there were no significant differences in the T-cho concentrations among cows with or without uterine bacterial infection **(Ghanem *et al.,* 2016)**. In addition, Plasma glucose concentrations can be used to monitor the energy balance in cows, low glucose concentrations during the postpartum period reported to be associated with a significant decrease in reproductive efficiency **(Harrison *et al.,* 1990).** In the present study, the blood glucose concentration was markedly lower in cows with endometritis than those in the other cows. These findings are supported by those of **Nazifi *et al*.** (**2003),** who found that cows with uterine infection suffer disturbances in energy metabolism as well as delay in the physiological adaptations required to meet their energy needs. This delay might potentially be confirmed by decreased blood glucose concentrations in cows diagnosed with endometritis **(Sensoy *et al.,* 2011).**

Marini *et al.* (2016) found that, PRP should be considered a potential treatment for endometritis in vivo. Recently, its use of plasma rich platelet (PRP) therapy for endometritis showing promising results **(Molina *et al.,* 2018**, **Gonçalves *et al*., 2019),** since plasma rich platelet (PRP) is known to contain a number of growth factors and cytokines that may aid in accelerating cell proliferation, angiogenesis, and cell migration, resulting in rapid healing which enhance endometrial tissue regeneration **(Jang *et al*. 2017, Sills *et al.,* 2018).**

The conception rates at first AI tended to be higher in the PRP group than in the antibiotic group.

CONCLUSION

Collectively, the findings of the present study indicate that, the concentrations of urea nitrogen, albumin, total cholesterol, glucose, were altered in the serum of dairy cows affected with endometritis. Furthermore, PRP therapy shows its positive effects in promoting endometrial and follicular growth and gestation in assisted reproduction cycles than the traditional therapy. Moreover, Conception rate better in cattle treated with plasma rich platelet than in cattle with traditional treatment.

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