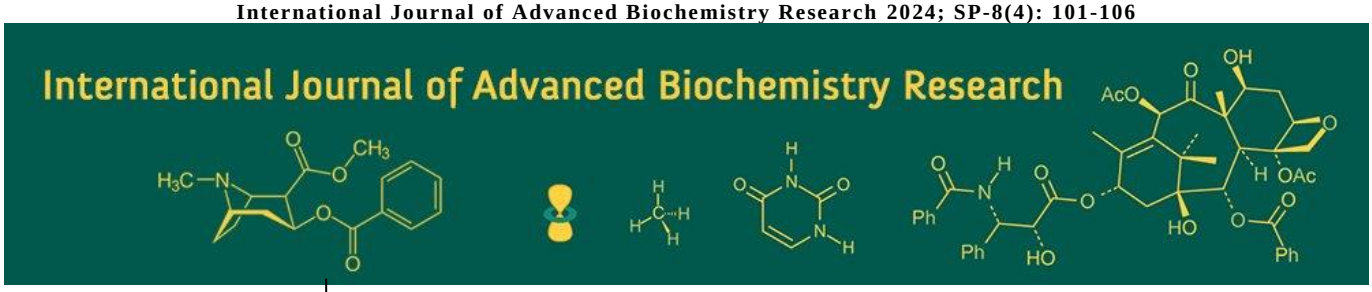

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Study on efficacy of different intrauterine treatments of endometritis in buffaloes

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Abstract
A study was carried out in dairy farms in and around Hyderabad. The buffaloes with the history of abnormal uterine discharge and repeat breeders that did not conceive in earlier services were selected through clinico-gynaecological examinations, estimation of pH of cervico-vaginal mucus and white side test of cervico vaginal mucus to diagnose the endometritis. A total of Thirty-two buffaloes with endometritis were selected and randomly divided into four groups of eight animals in each group. The buffaloes of group-I not given any treatment and were inseminated at observed estrus, Group II were administered intrauterine Lenovo I.U. 60ml intrauterine for 3 consecutive days from day of estrus, Group III were administered intrauterine *E. coli* lipopolysaccharide 100µg in 20ml of sterile phosphate buffer saline single dose on the day of estrus and Group IV were administered intrauterine Oyster glycogen 500mg in 30ml phosphate buffer saline single dose on the day of estrus and artificial insemination was done during subsequent observed estrus when white side test was found negative. According to the results it was concluded that the buffaloes with abnormal cervico-vaginal discharges and with conception failure could be effectively treated with the intrauterine infusion of *E.coli* LPS and Oyster glycogen to improve the fertility and conception rate. The *E.coli* LPS and Oyster glycogen can also be used effectively as immunomodulator with highest safety to the endometrium and without causing any systemic effects.

Keywords: Treatment of endometritis, *E. coli* LPS, oyster glycogen, Lenovo IU

Introduction
Reproductive efficiency is high priority in all systems yet is considered higher in seasonal calving systems as the opportunity to calve and become pregnant is time limited to ensure a calf per year (Dillon *et al.*, 2006) [12]. Infertility among dairy animals remains a major bottleneck in exploiting fullest potential of our animal wealth leading to loss of rupees 1500 crores each year (Mehra, 2023) [20] Though, other factors like poor nutrition, management and diseases etc. are also important for low productivity. The mean reduction in net revenue from one day increase in adjusted calving interval was estimated at $4.7 (Canadian) per cow (Plaizier *et al.*, 1997) [25]. Fertility is a multifactorial trait and its deterioration is caused by interplay of genetic, environmental, managerial factors, and its complex interactions make it difficult to determine the precise reason for its decline (Walsh *et al.*, 2011) [36]. Reproduction may be affected by various factors but the pathological changes in the reproductive tract caused by microorganisms beside the presence of specific venereal infection or low grade non-specific genital infections which are being introduced following dystocia and surgical interventions employed to aid in cases of dystocia, retention of foetal membrane, stillbirth, prolapse of uteri, unhygienic artificial insemination, Natural service with infected bulls and the use of intrauterine irritants. These infections reduced the fertility by affecting uterine environment resulting into impairment of sperm transport, sperm death and environment hostile to subsequent development and maintenance of conceptus (Sheldon *et al.*, 2009 and Brahmanand *et al.*, 2018) [30, 9]. The major cause of repeat breeding in cattle is endometritis. The occurrence rate of endometritis in buffaloes is much more common than cows which might be due to improper closure of vulval lips, poor hygienic environment, vaginal stimulation for milk letdown and wallowing habit (Moghaddam and Mamoei, 2004) [21]. In the present study, *E. coli* lipopolysaccharide, Oyster glycogen and Lenovo AP were infused intrauterine one in each group of buffaloes with the objective to evaluate the efficacy of

different intrauterine treatment protocols for the management of endometritis in buffaloes, to compare the efficacy of different intrauterine immunomodulators and antibiotics and to study the rate of recovery from endometritis and conception.

2. Materials and Methods

The present study was carried out on Thirty-two buffaloes free from anatomical abnormalities of reproductive tract aged between 4 - 7 years with body condition score of 2.5 to 3.0 on scale of 5.0, with a history of more than two months of postpartum period, return to estrus within 20-24 days after insemination with good quality semen (repeat breeder) and with positive result for white side test of cervico-vaginal mucus were selected in and around Hyderabad. The study was carried out over a period of seven months (from June 2023 to December 2023). The different intrauterine drugs used are Lenovo I.U. marketed by INTAS Pharmaceuticals, Matoda, Ahmedabad, India., L4391 Lipopolysaccharides from *Escherichia coli* O111:B4, γ-irradiated, BioXtra, marketed by SIGMA ALDRICH, 3050 Spruce Street, Saint Louis, MO 63103, USA. Made in Israel and 49740 Glycogen ex. Oyster for molecular biology, 85%, marketed by Sisco research laboratories pvt. ltd plant site 2:H-4, MIDC, Taloja, Maharashtra, India.

2.1 Experimental design

Group 1: (control) (n=8) Animals in this group on observed estrus artificial insemination was done and monitored for pregnancy diagnosis after 60 days post artificial insemination.

Group 2 (n=8): Animals in this group were administered intrauterine Lenovo IU (each 5ml contains Levofloxacin 100mg + Ornidazole 200mg + Tocopherol (vit E) 25mg) 60ml intrauterine for 3 consecutive days from day of estrus and artificial insemination was done during subsequent observed estrus when white side test was found negative.

Group 3 (n=8): Animals in this group were administered intrauterine *Escherichia coli* lipopolysaccharide 100µg in 20ml of sterile phosphate buffer saline single dose on the day of estrus and artificial insemination was done on subsequent observed estrus when white side test was found negative.

Group 4 (n=8): Animals in this group were administered intrauterine Oyster glycogen 500mg in 30ml phosphate buffer saline single dose on the day of estrus and artificial insemination was done on subsequent observed estrus when white side test is found negative.

The efficacy of the treatment was determined based on the following parameters i.e., pH of cervico-vaginal mucus before and after treatment at subsequent estrus (.using pH meter), result of White side test of cervico-vaginal mucus before and after treatment at subsequent estrus, polymorphonuclear leucocytes percentage before treatment, 24 hrs after treatment and at subsequent estrus and Bacterial load of cervico-vaginal mucus before treatment and after treatment at subsequent estrus. The CVM and uterine flushings are collected aseptically to prevent contamination from outside environment and to assess the above mentioned parameters appropriately. The pregnancy diagnosis was done on 30th day post AI and conception rate was determined.

3. Results

3.1 White side test

The white side test was performed on all the buffaloes under study on their cervico-vaginal mucus before and after treatment at subsequent estrus in therapeutic groups and at observed estrus in control group. The colour reaction to white side test before treatment was recorded as light yellow colour in 12 (37.50%), yellow in 16 (50.00%) and dark yellow in 4 (12.50%) buffaloes. The White side test results after 1st and 2nd treatment are shown in (Table 1& 2).

Table 1: White side test on cervico–vaginal mucus in endometritic buffaloes of different groups before and after treatment (subsequent estrus)

S. No.	Group wst result after 1st treatment at subsequent estrus	No. of animals	Positive for wst result before treatment/ at observed estrus	Wst result after 1st treatment at subsequent estrus	
				Positive	Negative
1	G-I	8	100%	-	-
2	G-II	8	100%	37.50%	62.50%
3	G-III	8	100%	12.50%	87.50%
4	G-IV	8	100%	12.50%	87.50%

Table 2: White side test on Cervico–vaginal mucus in endometritic buffaloes of different groups after 2nd treatment at Subsequent estrus

S.no	Group	No. of animals	Positive for wst after 1st treatment	Wst after 2nd treatment at subsequent estrus	
				Positive	Negative
1	G-II	3	37.50%	0	100%
2	G-III	1	12.50%	0	100%
3	G-IV	2	12.50%	0	100%

3.2 pH of cervical mucus

The mean pH values of cervico-vaginal mucus collected from endometritic buffaloes in this study pre-treatment and post-treatment in all the 3 therapeutic groups and at observed estrus in control group are presented in (Table 3, Fig.1)

Table 3: pH of Cervico-vaginal mucus in endometritic buffaloes before and after treatment at subsequent estrus (Mean ± SE)

S. no	Groups	No. of animals	pH of cervico-vaginal mucus	
			Before treatment / At Observed estrus	After treatment
1	G-I	8	8.29 ± 0.05^{a}	-
2	G-II	8	$8.19\pm0.05^{a,y}$	$7.39\pm0.04^{a,x}$
3	G-III	8	$8.18\pm0.06^{a,y}$	$7.46\pm0.02^{ab,x}$
4	G-IV	8	$8.16\pm0.05^{a,y}$	$7.51\pm0.02^{b,x}$

[a,b] Means sharing different superscripts in the same row differ significantly ($p<0.05$).
[x,y] Means sharing different superscripts in the same column differ significantly ($p<0.05$).

3.3 Bacterial load (X 10^6 per ml)

The mean bacterial load in cervico-vaginal mucus samples collected from all therapeutic groups, before treatment and at subsequent estrus after treatment and in control group at subsequent estrus were presented in (Table 4, Fig 2)

Table 4: Bacterial load (X 10^6 /ml) in cervico–vaginal mucus of endometritic buffaloes before and after treatment (at subsequent estrus) (Mean ± SE)

S. No	Groups	No. of animals	Bacterial load (X 10^6 /ml)	
			Before treatment/ At Observed Estrus	After treatment
1	G-I	8	72.88 ± 0.61^{ab}	-
2	G-II	8	$72.25\pm0.37^{a,y}$	$6.5\pm0.50^{a,x}$
3	G-III	8	$73.25\pm0.56^{ab,y}$	$5.5\pm0.42^{a,x}$
4	G-IV	8	$74.25\pm0.61^{b,y}$	$5.88\pm0.52^{a,x}$

[a,b] Means sharing different superscripts in the same row differ significantly ($p<0.05$).
[x,y] Means sharing different superscripts in the same column differ significantly ($p<0.05$).

3.4 Polymorphonuclear cells (pmn cells) percentage

The mean percent of polymorphonuclear cells in uterine flushing of all therapeutic group of animals at day 0, 24 hrs after treatment and subsequent estrus after treatment and in control group animals at subsequent estrus were presented in (Table 5, Fig 3).

Table 5: Percentage of polymorphonuclear cells in uterine flushing of endometritic buffaloes in different groups at day 0, 24 hrs post treatment and subsequent estrus after treatment (Mean ± SE)

S. No	Groups	No. of animals	Percentage of PMNL cells		
			Day 0 / At observed estrus	24 hrs post treatment	At subsequent estrus
1	G-I	8	24.125 ± 0.64^{a}	-	-
2	G-II	8	$23.88\pm0.77^{a,y}$	$42.75\pm0.88^{a,z}$	$5.5\pm0.50^{a,x}$
3	G-III	8	$25.50\pm0.96^{a,y}$	$72.38\pm0.80^{c,z}$	$5.38\pm0.32^{a,x}$
4	G-IV	8	$23.88\pm0.30^{a,y}$	$69.88\pm0.58^{b,z}$	$4.5\pm0.42^{a,x}$

[a,b,c] Means sharing different superscripts in the same row differ significantly ($p<0.05$).
[x,y,z] Means sharing different superscripts in the same column differ significantly ($p<0.05$).

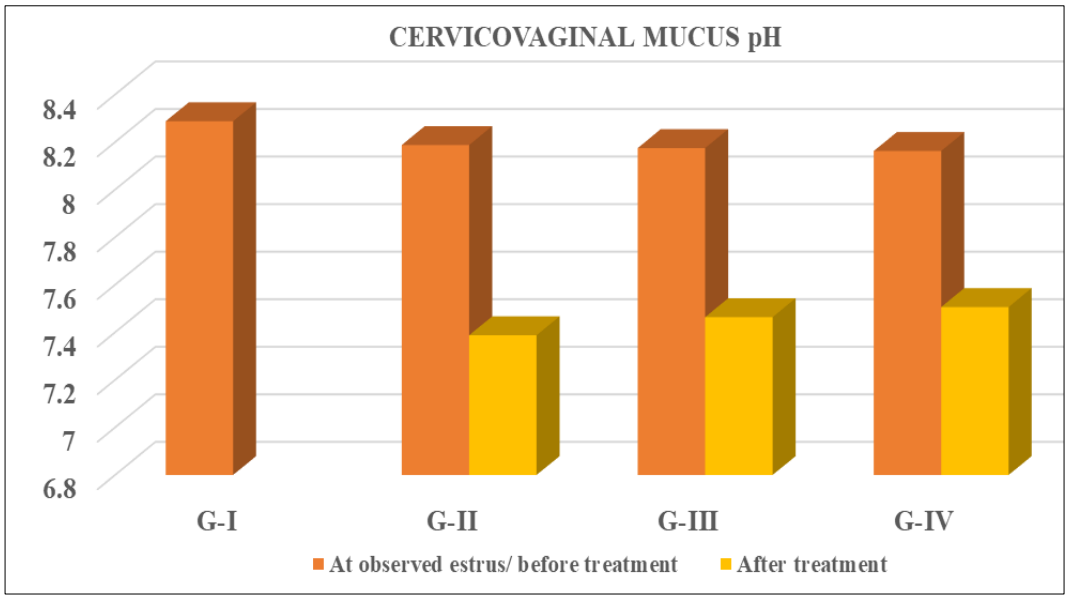

Fig 1: pH of cervical mucus before and after treatment in different groups

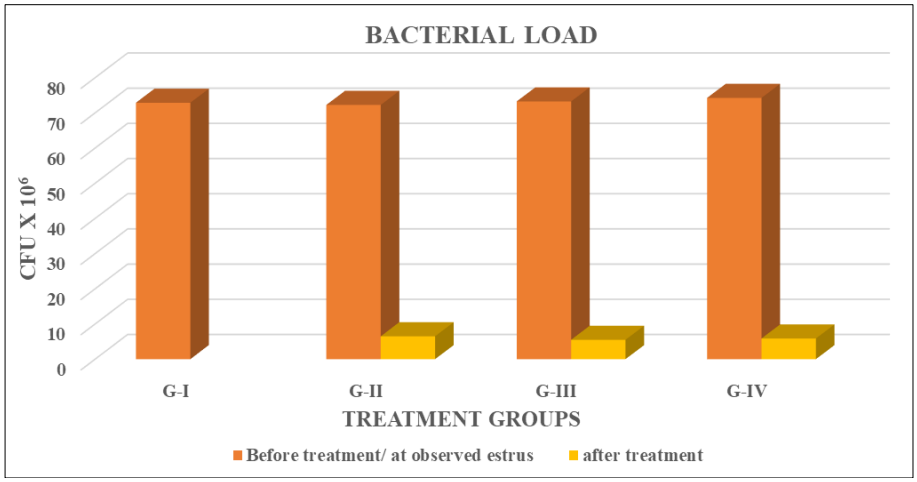

Fig 2: Bacterial load (10^6/ml) of cervical mucus before and after treatment in different groups

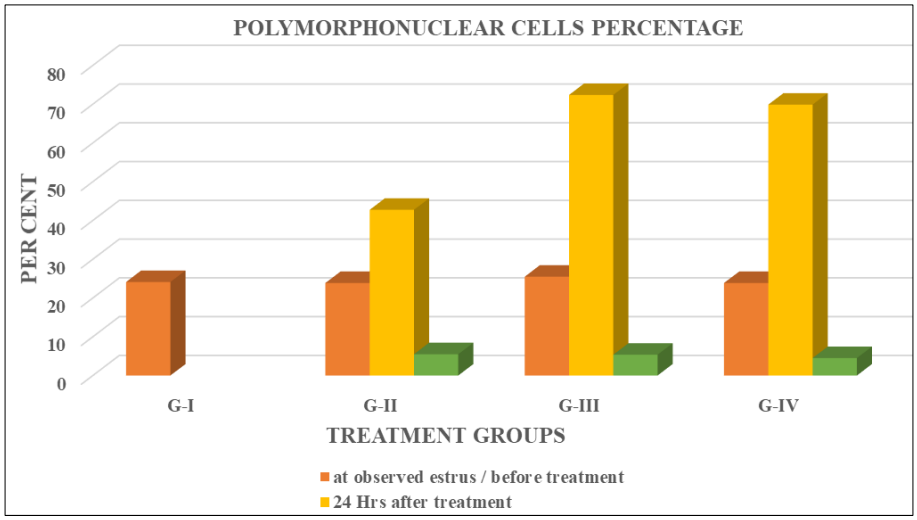

Fig 3: Percentage of PMNL cells at day 0, day 1 and subsequent estrus in different groups

3.5 Conception Rate: The conception rate in recovered experimental buffaloes was recorded (Table 6).

Table 6: Recovery and Conception rate after treatment of endometritic buffaloes

Groups	No. of Animals	No. of animals recovered / Inseminated	Conception rate		Overall conception rate (%)
			1st estrus	2nd estrus	
I	8	8	0.00 (0/8)	12.50% (1/8)	12.50% (1/8)
II	8	8	50.00%(4/8)	25.00% (2/8)	75.00% (6/8)
III	8	8	62.50% (5/8)	25.00% (2/8)	87.50% (7/8)
IV	8	8	62.50% (5/8)	25.00% (2/8)	87.50% (7/8)

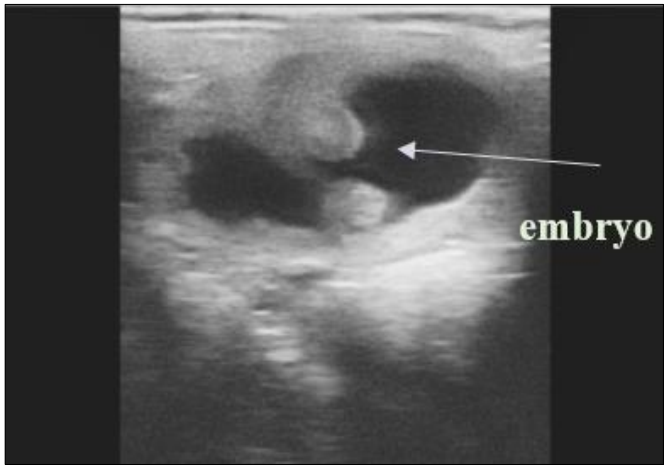

Fig 4: Picture showing ultrasonographic image of gravid uterus of buffalo on 30th day post insemination

4. Discussion

High reproductive performance is required for successful running of dairy. Buffaloes are polyoestrous species but a distinct seasonal pattern has been reported in India (Gangawar, 1980 and Barile, 2005) [13, 3]. Pregnancy rate after insemination measures the economy of dairy farm. But, one of the major constraints of profitable dairy farming is low conception rate. Reproduction is affected by many factors, but the pathological alteration caused by microbes in the genital tract of the animal appears to be the main factor for infertility (Prajapati *et al.*, 2005) [24]. Postpartum endometritis has a negative effect on reproductive performance, causing an increase in the number of services per pregnancy and in the length of service period (Bell and Roberts, 2007) [5].

In the present study all the buffaloes are positive for white side test before treatment. Kumar *et al.* (2015) [19] Bajaj *et*

al. (2016) [4], Kumar *et al.* (2017) [17] and Singh *et al.* (2017) [32] observed similar colour reaction to white side test with dark yellow, yellow, light yellow and no colour changes.

The mean pH values of all the groups before the treatment is in range of 8.1 to 8.6 and with overall mean pH of all the experimental groups before treatment is 8.21±0.05. These findings are in agreement with Afreen (2021) [1] who reported that pH value of cervico- vaginal mucus from buffaloes affected with subclinical endometritis and clinical endometritis ranged between 7.5 to 8.0 and 8.5 to 9.0, respectively. Hussaini (2021) [14] who reported that the buffaloes with pH of cervico-vaginal mucus ranging from 7.5 to 8.5 is considered to be affected with subclinical endometritis. The mean pH value revealed a significant decline towards normal in all the therapeutic groups from pre-treatment to post-treatment. This similar decline in the pH of cervico-vaginal mucus is seen in studies conducted by Palanisamy *et al.* (2014) [22] and Puro *et al.* (2018) [26].

The mean bacterial load in cervico-vaginal mucus samples of all groups before treatment is 73.16±0.54 and after treatment, the mean bacterial load recorded of all the therapeutic groups is 5.96±0.48, these findings are in agreement with work of Solmonraju *et al.* (2006) [34] and Venkatesh (2021) [35] who recorded mean bacterial colony count in endometritic buffaloes 79.04 ± 8.10 x 10^6/ml and 77.12± 0.05 x 10^6/ml respectively. There is significant (p<0.05) decline in bacterial load after the treatment, this finding is also seen in the research conducted by Kumar *et al.* (2004) [18], Solmonraju *et al.* (2006) [34], Raju *et al.* (2009) [27], Jena *et al.* (2018) [15] and Venkatesh (2021) [35]. The reduction in bacterial load after treatment might be due to an infiltration of neutrophils into uterine lumen after infusion of immunomodulators, which are responsible for the clearance of bacteria from the uterus in case of *E. coli* LPS and Oyster glycogen Group, whereas the decline in the bacterial load in Lenovo IU Group is due to antibiotic effect of Levofloxacin and Ornidazole.

The mean of PMN cell percentages of all groups recorded before treatment is 24.35±0.67 which is in alignment with study of Raval *et al.* (2018) [29] who recorded the mean percent PMN cells in endometrial cytology of repeat breeding cows having clear stringy, turbid viscous, and watery white flaky cervico-vaginal mucus as 21.80±8.39, 32.18±3.26 and 29.25±6.63, respectively and Azawi *et al.* (2008b) [2] who recorded the mean PMN cells from the endometritic buffalo cows as 24.5±1.32 percent. Whereas, Brodzki *et al.* (2014) [10] and Dar *et al.* (2015) [11] recorded the mean PMNs to be 13.86±4.05 and 11.57, respectively which are lesser than the present finding. Cytological examination of buffaloes with endometritis revealed that, presence of higher percent of PMNL cells than the healthy buffaloes. These findings are in agreement with the findings of Azawi *et al.* (2008b) [2] and Raval *et al.* (2018) [29]. There is a significant (p<0.05) increase in the percent of PMNL cells in the uterine flushing at 24hrs after treatment and the percent of PMNL cells declined significantly (p<0.05) at subsequent estrus in all therapeutic groups.

The overall conception rate is 75% in Lenovo IU Group in this study this finding is in agreement with Bhattacharyya *et al.* (2011) [7] who recorded overall conception rate of 72.22%. Resume and Singh (2016) [28] recorded lower conception rate of 60.00% using Lenovo IU. Whereas, Singh *et al.* (2014) [31] and Bhat *et al.* (2013) [6] recorded higher conception rate of 89.28% and 93.33% respectively using Lenovo IU. The overall conception rate is 87.50 percent in *E. coli* LPS group in this study and These findings were in close agreement with findings of Bhuyan *et al.* (2015) [8], Puro *et al.* (2018) [26] and Venkatesh *et al.* (2021) [35] who used *E. coli* LPS in treatment of endometritis in buffaloes achieved conception rate of 88.88, 83.33, 83.33 percent, respectively. The overall conception rate is 87.50 percent in Oyster glycogen group these findings are in close agreement with findings of Krishnamurthy *et al.* (2021) [16] who used Oyster glycogen 500mg to treat endometritis and achieved conception rate of 87.50%. Whereas, Solanki *et al.* (2019) [33] recorded a lower conception rate of 70.00%. and Parikh *et al.* (2022) [23] recorded lower conception rate of 37.50% at subsequent estrus after treatment with Oyster glycogen. The animals administered with *E. coli* LPS and Oyster glycogen have higher conception rates than the animals treated with Lenovo IU.

5. Conclusion

The present study concluded that, the buffaloes with endometritis can be effectively treated with the intrauterine infusion of *E. coli* LPS and Oyster glycogen and there by improving conception rate. The *E. coli* LPS and Oyster glycogen after intrauterine administration effectively increased the infiltration of PMNs into the uterine lumen and there by significantly decreased the bacterial load when evaluated at subsequent estrus after treatment so they could be used effectively as immunomodulator and as an alternative to the traditional use of antibiotics and other antimicrobial agents with better efficacy to control the infection by stimulating the uterine immune response and safer as intrauterine infusion without getting absorbed into systemic blood flow, and without causing any damage to the uterine walls.

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