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Study of the cellular component of pig uterus after intra uterine administration of Lipopolysaccharide

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Abstract
A study was conducted on 8 sows to record the response of the uterus following intrauterine administration of lipopolysaccharide, a known immunomodulator by collecting uterine lavage from live animals, the immune response of the uterus was measured in terms of total leukocyte count (TLC) and differential leukocyte count (DLC), the mean TLC of uterine lavage collected from live animals was 1.57±0.21 × 10^6/ml before intrauterine infusion of LPS and 1.17±0.29 × 10^6/ml after infusion. In control group the mean values were 2.46±0.58 and 1.12±0.50 × 10^6/ml respectively. Mean percentage of neutrophils in the uterine lavage collected from live sows before and after intrauterine infusion of LPS was 99.88±0.10 and 99.82±0.05 respectively. For lymphocyte the corresponding mean percentages were 0.13±0.10 and 0.19±0.05. In the control live sows treated with PBS the mean percentage of neutrophils in the uterine lavage was 99.88±0.10 and 99.75±0.07 before and after infusion respectively, and that of lymphocyte was 0.13±0.10 and 0.25±0.07 respectively, the study revealed that intrauterine infusion of *E coli* lipopolysaccharide did not affect the total leukocyte count and differential leukocyte count of the uterus in pigs.

Keywords: sow- lipopolysaccharide-immune response-TLC-DLC

Introduction
The uterus in its normal state maintains a sterile environment but is subjected to microbial invasion during oestrus and post farrowing. In the uterus the polymorph nuclear neutrophilic cells are the first cells to arrive in response to exposure to any pathogen or foreign material (Lewis, 2004) [6]. The immune response of the uterus in pigs during oestrus and after mating is indicated by an influx of neutrophils into the endometrium and the uterine lumen (Lowell and Getty, 1968; Bischoff *et al.*, 1995; Engelhardt *et al.*, 1997; Rozeboom *et al.*, 1998, Kaeoket *et al.*, 2003) [7, 2, 4, 9, 5]. The inflammatory response of the uterus is usually measured by the infiltration of leukocytes in the uterine tissues and lumen within a given period of time.
Lipopolysaccharide is a component of the cell wall of Gram negative bacteria is a potent inducers of innate immunity. Neutrophils migrate to the uterine lumen in response to intrauterine infusion of lipopolysaccharide in guinea pig (Targowski, 1984) [13] and cattle (Singh *et al.*, 2000; Dhaliwal *et al.*, 2001; Prasad *et al.*, 2009) [12, 3, 8]. Commonly antibiotics have been used for the treatment of endometritis. However, the major disadvantages of antibiotics are development of bacterial resistance and reduction in uterine defense mechanism. *E. coli* lipopolysaccharide has been effectively used as an alternative to antibiotics for therapeutic management of endometritis in cattle (Singh *et al*., 2000; Prasad *et al.*, 2009) [8, 12]. The pigs, due to its less economic values and high cost of treatment, are usually culled when they develop reproductive problems. Hence, there appears little information on the treatment of reproductive problems in pigs. However there are encouraging reports that the intrauterine infusion of lipopolysaccharide increases the percentage of neutrophils in the uterine lavage. Reports are also available that *E. coli* lipopolysaccharide provokes an immune response involving serum protein and leukocyte. Protein level peaks within few hours of infusion and leukocyte peaks six hours later (Williamson *et al.*, 1987) [14].

Materials and Methods
Eight (8) cross bred sows (Ghungroo X Hampshire) maintained in the experimental pig farm

of National Research Centre on Pig, ICAR, Rani, Assam were selected for this study, the sows were 2 to $2\frac{1}{2}$ years of age and in their third to fourth parity. They were housed in naturally ventilated shed and fed as per the scientific feeding schedule followed in the NRC on. Sows were grouped under A1(treatment) and A2(control) to study the immune response of the uterus to intrauterine infusion of lipopolysaccharide (LPS) and Phosphate Buffer Saline (PBS) respectively by analyzing uterine lavage collected from live animals.

Sub group A1

i) In each sow, 100 ml of Phosphate buffer saline (PBS) was infused intrauterine with the help of catheter on 1st day of oestrus, kept for 10 minutes and the uterine lavage was collected with the help of Golden pig catheter and Menstrual Regulation Syringe Kit (Sigma) and the parameters studied.

ii) Immediately after, 100 µg of LPS in 100 ml of PBS was administered intrauterine with the help of A.I. catheter and kept in the uterus. The uterine lavage was collected on 3rd day of oestrus and studied for different parameter. For collection of uterine lavage, 100 ml of PBS was infused into the uterus and then withdrawn with the help of A.I. catheter and Menstrual Regulation Syringe Kit (Sigma).

Sub group A2

Same procedure as mentioned for sows under group A1 was followed, except that in step ii) 100 ml of PBS was used instead of LPS solution.

Uterine lavage collection from live animal

The uterine lavage was collected from the live animal with the help of a Golden pig catheter and menstrual regulation syringe kit. The Menstrual regulation syringe kit consisted of the following parts pinch valve button, pinch valve, valve lining, syringe barrel, O'ring, plunger stop clip and syringe plunger. The kit was assembled and the Pinch valve button depressed and locked. The syringe plunger was then pulled back until the locking tabs were engaged on both sides and kept ready. Sows in oestrus were detected by redness and swelling of vulva and stance reflex on applying back pressure. The perineum and vulva were cleaned and swabbed with povidone iodine solution and made ready for lavage collection. The golden pig catheter was also swabbed with iodine and the tip lubricated with a non- spermicidal gel. The catheter was then inserted through the vulva and locked in the cervix and 100 ml of PBS was infused into the uterine lumen using a semen cochette of 100 ml capacity. The PBS solution was then kept in the uterus for 10 minutes. The assembled syringe kit was then attached to the Golden pig catheter. Then the vacuum retaining pinch valve button was released slowly and the catheter was moved slightly forward and backward. This procedure was repeated twice or thrice till a considerable volume of lavage was collected. The volume of lavage collected ranged from 1 to 30 ml and varied from sow to sows. The uterine lavage was then transferred to a sterile conical flask and kept in ice for further laboratory test. The volume of the uterine lavage was measured and an aliquot of 1ml was kept for bacterial load count. The remaining lavage was centrifuged for 5 minutes at 1500 rpm. The supernatant was stored at -20 °C for estimation of total protein and immunoglobulin. The pellet was further processed for Total Leukocyte Count and Differential Leukocyte Count.

Preparation of lipopolysaccharide (LPS)

LPS stock solution was prepared by dissolving 10 mg of LPS (Sigma, *E. coli* LPS 026: B6≥10000 EU/mg) in 50 ml sterile PBS. An aliquot of 0.5 ml containing 100 µg of LPS was stored in appendorb at 2-8ºC in a refrigerator for a minimum period of three weeks.

Preparation of phosphate buffer saline (PBS)

Composition of Phosphate buffer saline (PBS) 1000 ml:

	Ingredients	Quantity
a.	Sodium chloride (NaCl)	8.0 gm
b.	Potassium dihydrogen phosphate (KH_2PO_4)	0.2 gm
c.	Di-sodium hydrogen orthophosphate (Na_2HPO_4)	1.16 gm
d.	Potassium chloride (KCl)	0.2 gm
e.	Distilled water upto 1000 ml	

The pH was adjusted to 7.3 and then autoclaved at 15 lb pressure for 15 minutes.

Artificial insemination (A.I.) technique

Oestrus in the sow was detected on the basis of physical signs and standing reflex. The vulva was cleaned and swabbed with povidone iodine solution (Betadiene). The golden pig catheter was swabbed with povidone iodine and the tip was lubricated with a non spermicidal gel. The catheter was inserted through the vulva in a forward and upward direction and locked in the cervix. Then the nozzle of the semen cochette was attached to the end of the catheter and the semen was allowed to flow down into the uterus by gravity. Little amount of pressure on the cochette may be necessary for complete infusion. Tactile stimulation was given in the flank region to enhance the uptake of semen.

Intrauterine infusion of LPS

The LPS solution was made ready for intrauterine infusion by the following procedure. The semen cochette was filled with 100 ml sterile PBS solution and sealed. The LPS stored in appendorb was thawed and then transferred to the PBS in semen cochette with the help of syringe and needle. The cochette was resealed at the site of puncture. The solution was mixed and then infused into the uterus.

Results and Discussion

Table 1: Total Leukocyte Count (TLC) of uterine lavage recovered before and after intrauterine infusion of LPS and PBS

I /U infusion Group	No. of observation	TLC Before infusion ($x10^6$/ml)		TLC After infusion ($x10^6$/ml)		t value
		Mean ± S.E	Range	Mean ± S.E	Range	
LPS	4	1.57 ± 0.21	1.09-2.22	1.17± 0.29	0.47-1.80	0.95 NS
PBS	4	2.46 ± 0.58	0.86-3.59	1.12±0.50	0.14-2.74	1.51 NS
t value		1.25 NS		0.22 NS		

NS Non-significant

Table 2: Mean differential leukocyte count (DLC) of uterine lavage recovered from sows before and after intrauterine infusion of LPS and PBS

Type of leukocyte		No. of observation	Before infusion %		After infusion %		t value
			Mean ± S. E	Range	Mean ± S. E	Range	
Neutrophils	LPS	4	99.88 ± 0.10 (87.97 ± 1.76)	98.00-100.00	99.82 ± 0.05 (87.55 ±1.26)	99.00-100.00	0.17 ^{NS}
	PBS	4	99.88 ± 0.10 (87.97 ± 1.76)	98.00-100.00	99.75 ± 0.07 (87.13 ±1.44)	99.00-100.00	0.32 ^{NS}
Lymphocyte	LPS	4	0.13± 0.10 (2.03 ± 1.76)	0.00-2.00	0.19 ± 0.05 (2.45 ±1.26)	0.00-1.00	0.05 ^{NS}
	PBS	4	0.13 ± 0.10 (2.03 ± 1.76)	0.00-2.00	0.25 ± 0.07 (2.87 ±1.44)	0.00-1.00	0.32 ^{NS}

^{NS} Non- significant Figure in parentheses indicate arcsin values

Results of the present study indicated that the intrauterine infusion of LPS did not affect TLC of the uterus in pig as estimated in the uterine lavage collected from the uterus of live animals as well as the excised uterus after slaughtering the animals. Similar was the result obtained by analyzing uterine lavage obtained from the control sows with intrauterine infusion of PBS. The mean TLC of uterine lavage collected before and after intrauterine infusion of LPS was 1.57±0.21 and 1.17±0.29 $\times 10^6$ /ml respectively and that of PBS 2.46±0.58 and 1.12±0.50$\times 10^6$ /ml respectively as shown in table no1, with no significant difference between the mean observed before and after intrauterine infusion in both cases of LPS and PBS. Singh *et al.* (2000) [12] and Sahadev *et al.* (2007) [11] reported that intrauterine infusion of LPS in cows led to a significant increase in TLC of uterine lavage collected after 6 to 24 hours of infusion. The reason for this difference in opinion regarding efficacy of LPS in increasing TLC of the uterus might be attributed to the difference in species of animal and time of recovery of the uterine lavage after treatment. In the present study intrauterine infusion of both LPS and PBS was carried out on the first day of oestrus and the uterine lavage was collected on the 3rd day. Singh *et al.* (2000) [12] observed that TLC of bovine uterus increased to a significantly higher level following intrauterine administration of LPS, reaching a peak level by 6 hours post treatment and gradually decreasing thereafter.

In the present study neutrophil was the major component of the total number of leukocytes found in the uterine lavage collected from live animals before and after intrauterine infusion of LPS as well as PBS. Mean percentage of neutrophil was 99.88±0.10 before infusion of LPS and 99.82±0.05 after infusion (Table no.2). Similar higher percentage of neutrophil was also observed in the uterine lavage collected from excised uterus and there was no significance difference between the mean values recorded before and after infusion of LPS as well as PBS. The other type of leukocytes found in the uterine lavage was only lymphocyte which varied from 0.13±0.10 to 0.19±0.05 per cent on an average (Table no.2). These results as obtained in the present study indicated that though intrauterine infusion of LPS had no significant effect on the TLC yet neutrophil appeared to be the major component of leukocytes. Predominance of neutrophil in the uterine lavage of gilts was also reported by Rozeboom *et al.* (1999) [10], mean values ranging from 92 to 99 per cent. Sahadev *et al.* (2007) [11] in cattle also recorded high influx of neutrophils following intrauterine infusion of LPS but not of PBS. Anderson *et al.* (1985) [1] working with intrauterine oyster glycogen reported that bovine uterine aspirate contained 10^6-10^9 number of polymorphonuclear neutroplils (PMN) after infusion, reaching a peak at 12 hours. In gilts, even artificial insemination was found to increase PMN percentage up to 95 per cent as reported by Rozeboom *et al.* (1998) [9].

Conclusion

In this study conducted to study the effect of Lipopolysaccharide in the uterus, It may be concluded that intrauterine infusion of 100µg of *E coli* lipopolysaccharide does not affect the total leukocyte count content of the uterus in the pig, yet significantly reduces bacterial load of the uterus thereby suggestive of reducing the risk of reproductive tract infection in pigs. Keeping in view larger size of the uterus in pigs, further study with a large number of animals and varying doses of lipopolysaccharide is suggested.

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