

RESPONSE OF INTRAUTERINE INFUSION OF LUGOL'S IODINE IN INFECTIOUS REPEAT BREEDING CATTLE

LAKHWINDER SINGH¹, V.K GANDOTRA², JAGIR SINGH³ AND A.K ARORA⁴

Department of Veterinary Gynaecology and Obstetrics

Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana-141 004.

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ABSTRACT

Repeat breeding cows (n=24) with endometritis were divided into four groups of six animals each. In group I (control), single infusion of 30 ml sterile distilled water whereas, in groups II, III and IV 30 ml of lugol's iodine 0.5 (single infusion), 0.25 (single infusion) and 0.1 per cent (double infusion at 24 h interval), respectively, were administered intrauterine on the day of estrus. A total of 27 bacterial isolates yielded from the uterine swabs of these cows before treatment were *E. coli* (29.6%), *Bacillus* spp. (25.9%), *Klebsiella* spp. (22.2%), *Streptococcus* spp. (14.8%) and *Staphylococcus aureus* (7.4%). The polymorphonuclear leucocytes (PMNLs) were 6-11 per cent in the cellular contents of uterine secretions before treatment in all the groups. The PMNLs increased to 70.6±6.48 (group II), 58.58±4.75 (group III) and 51.00±7.90 per cent (group IV) by 24 h of treatment as compared to 12.00±4.77 per cent in group I and remained significantly higher in all the treatment groups upto 72 h. One cow in group I, five in group II, two in group III and none in group IV yielded microbial growth on next estrus post treatment; four cows in group II and one from group III conceived. It is evident from the study that lugol's iodine increased the PMNL influx in the uterine secretions to combat infection. The best response in terms of clearing infection and conception was achieved with 0.5 percent lugol's iodine which can be used effectively to treat bacterial endometritis.

Key words: Lugol's iodine, Repeat breeder, Cattle

Repeat breeding in cattle is a main cause of infertility and economic loss to the dairy farming community. Endometritis due to bacterial infection is the major etiological component of repeat breeding (Saini *et al.*, 1995). Inconsistent success, high cost of treatment, milk disposal after treatment and emergence of microbial resistance are the constraints of antibiotic therapy of the disease condition. Alternate therapies involving bacterial endotoxin (*E. coli* lipopolysaccharide; LPS) and oysteryglycogen that stimulate uterine defense mechanism through increased influx of neutrophils have been reported (Hussain and Daniel 1992, Saini *et al.*, 1995 and Singh *et al.*, 2000) but non-availability and cost of treatment renders their use limited. Lugol's iodine, besides its antiseptic properties, stimulates uterine tone and motility, mobilizes neutrophils (Roberts 1971) and is easily available and economical. The present study was conducted to assess the ability of lugol's iodine in different concentrations to optimize the uterine defense and its efficacy to cure endometritis in cattle.

The investigation was conducted in 24 repeat breeder cows having normal estrous cycle and apparently normal palpable genitalia but abnormal genital discharge at estrus. The animals under investigation were divided randomly into four groups: On day of estrus, the cows were infused intra uterine with 30 ml sterile distilled water- Group I (control), 30 ml of 0.5 per cent lugol's iodine- Group II, 30 ml of 0.25 per cent lugol's iodine- Group III and 30 ml of 0.1 per cent lugol's iodine twice at 24 hrs interval- Group IV.

Uterine swabs were taken for bacterial culture with Neilson's sterilized uterine swab catheter on the day of estrus and at subsequent estrus, transported to laboratory and processed as per standard procedures on to sheep blood agar and Maconkey's lactose agar plates. The plates were incubated aerobically at 37°C for 24 hrs and observed for growth of bacterial colonies. The organisms were characterized on the basis of different cultural, morphological and biochemical characteristics (Carter, 1973).

Uterine secretions were collected from each animal on the day of estrus before treatment, 24 and 72 hrs after the treatment by a sterilized two way Rucsh

1. Veterinary Officer, Distt. Gurdaspur, 2. Professor and Head and corresponding author 3. Senior Gynaecologist, 4. Associate Professor, Department of Veterinary Microbiology

catheter. Thirty ml flushing solution of sterile phosphate buffer saline (PBS, pH 7.4) was infused, left in the uterus for five min, allowed to flush out after gentle massage of uterus per rectum, collected in a sterilized vial and transferred to laboratory on ice. The flushings were centrifuged in refrigerated centrifuge at 3000 rpm for 10 min. The sediment was used to prepare cytospin slide smear at 1000 rpm for 5 min. The smear was air dried, fixed in methanol for two min and stained with Giemsa stain diluted 1:8 with phosphate buffer (pH 6.8) for 60 min. The smears were examined for polymorphonuclear leucocytes (PMNLs) count, lymphocyte, epithelial cells and other cells under oil emersion at 100X magnification. A total of 200 cells were counted per slide and number of PMNLs was recoded as per cent. Artificial insemination was performed in all the cows showing no growth on subsequent estrus and pregnancy was confirmed per rectum 60 days post insemination. The data was analyzed statistically by two ways ANOVA and means were compared using Duncan's multiple range test.

Pre-treatment uterine swabs in all the cows showed bacterial growth. 27 bacterial isolates obtained from the uterine swabs of these cows before treatment were *E. coli* (29.6%), *Bacillus* spp. (25.9%), *Klebsiella* spp. (22.2%), *Streptococcus* spp. (14.8%) and *Staphylococcus aureus* (7.4%). Twenty one cows yielded one type of bacterial isolate and three others yielded mixed bacterial infection. Most of the bacteria isolated were same in all the groups. No growth was observed in one control, five treated with 0.5 per cent (group II) and two cows treated with 0.25 per cent lugol's iodine (group III) where as infection was persistent in the rest of cows as similar micro organisms were isolated at subsequent estrus post treatment. The uterine swab from one cow each in groups II and IV, following

treatment could not be processed as the swabs were dried off.

For normal function of the reproduction, the female genital tract should be free from pathogenic bacteria (Fitch and Bishop, 1932). The bacterial organisms were present in 77 to 100 per cent of repeat breeding cows (Dholakia *et al.*, 1987, Shukla, 1988 and Singla *et al.*, 1991). In the present study also, pre-treatment uterine swabs at estrus in all the endometritis cows indicated presence of bacterial infection. *Staphylococcus aureus*, *Actinomyces pyogenes*, *Streptococcus* and *Bacillus* spp. have been reported to be predominant in repeat breeding bovines (Singh *et al.*, 1989, Sharda *et al.*, 1991 and Saini *et al.*, 1999).

The differences in the pre-treatment PMNLs count per cent in all the four groups was non-significant (Table). The PMNLs increased significantly ($P < 0.05$) at 24 hrs post-treatment from pre-treatment values in all treatment groups and the values remained higher ($P < 0.05$) even at 72 hrs post-treatment. However, there was non significant decline in number of PMNLs at 72 hrs. The PMNLs influx was the maximum in the cows infused with 0.5 per cent lugol's iodine, though the values were non-significantly different at various intervals except at 24 and 72 hrs ($P < 0.01$) between group II and IV, respectively.

Lugol's iodine stimulates uterine tone and mobilizes neutrophils (Roberts 1971). The PMNLs influx was the maximum at 24 hrs and that too in cows treated with 0.5 per cent lugol's iodine. Nikahara *et al.*, (1977) and Singh *et al.*, (1988) reported direct relationship between concentration of lugol's iodine and its inflammatory response. Phagocytic action of neutrophils thereby killing the invading the micro-organisms (Tizard, 1987) could have resulted in

Table: Polymorphonuclear leucocytes (PMNLs; percent mean \pm S.E.) in cellular contents of uterine secretions of endometritis cows

Treatment group	—		
	Pre- treatment	Post- treatment	
	0 h	24 h	72 h
Group I; n=6 (Control; sterile distilled water)	10.48 \pm 4.11 ^a	12.00 \pm 4.77 ^a	14.58 \pm 5.67 ^a
Group II; n=6 (0.5% lugol's iodine)	7.33 \pm 3.84 ^a	70.6 \pm 6.48 ^b	56.00 \pm 5.38 ^b
Group III; n=6 (0.25% lugol's iodine)	8.58 \pm 4.27 ^a	58.58 \pm 4.75 ^b	54.00 \pm 3.63 ^b
Group IV; n=6 (0.1% lugol's iodine)	6.66 \pm 1.48 ^a	51.00 \pm 7.90 ^b	43.91 \pm 7.88 ^b

Means bearing different superscripts differ significantly ($P < 0.01$; Duncan's Multiple Range Test)

clearance of bacterial infection. The clearance of bacterial infection was more effective following treatment with 0.5 per cent lugol's iodine as microorganisms were not isolated in five of the six cows as compared to 0.25 and 0.1 per cent lugol's iodine treated and control cows. Persistence of infection in group III and IV cows could be due to lack of optimum inflammatory response and hence less number of PMNLs influx into the uterus as compared to group II cows. Four out of five (group II) and one out of two (group III) recovered cows conceived following insemination.

Clearance of different types of bacterial infection in endometritis cows and subsequent conception shows a very good therapeutic response of intra uterine infusion of 0.5 per cent lugol's iodine in repeat breeder cows. The constituents of the preparation are cheap, boost natural uterine defense and there is no risk of development of drug resistance as in other antibiotics.

REFERENCES

- Carter, G.R. (1973). Diagnostic procedures in Veterinary Bacteriology and Mycology. Charles C. Thomas Publishers, Springfield, Illinois.
- Dholakia, P.M., Shah, N.M., Purohit, J.H. and Kher, N.N. (1987). Bacteriological study on non-specific genital infection and its antibiotic spectra in repeat breeders. *Indian. Vet. J.*, **64**: 637-640.
- Fitch, C.P. and Bishop, L.M. (1932). Bacteriological study of the gravid and non-gravid bovine uterus. *Cornell Vet.*, **22**: 225-238.
- Hussain, A.M. and Daniel, R.C.W. (1992). Effects of intrauterine infusion of Escherichia coli endotoxin in normal cows and in cows with endometritis induced by experimental infection with Streptococcus agalactiae. *Theriogenology*, **37**: 791-810.
- Nikahara, T., Domeki, I. and Yamauchi, M. (1977). Local effect of intrauterine injection of iodine solution on the life span of the corpus luteum of the cow. *J. Reprod. Fertil.*, **86**: 425-435.
- Roberts S.J. (1971). Veterinary Obstetrics and Genital Diseases. 3rd Edn., Woodstock, Vermont.
- Saini P.S., Grewal, A.S., Nanda, A.S. and Arora, A.K. (1995). Intrauterine immunotherapy with immunomodulator lipopolysaccharide (LPS) for bacterial endometritis associated clinical cases of repeat breeders in dairy cattle (*Bos taurus* x *Bos indicus*) and buffaloes (*Bubalis bubalis*). *Bull. Soc. Fr. Jpn. Sci. Vet.*, **6**: 128-134.
- Saini, P.S., Nanda, A.S., Grewal, A.S. and Singh, J. (1999). Uterine defense modulation for the treatment of repeat breeding due to infectious endometritis in bovines. *Indian. J. Anim. Sci.*, **69**: 309-309.
- Sharda, R., Moghe, M.N. and Tanwani S.K. (1991). Antibiotic sensitivity pattern of bacteria isolated from repeat breeding animals. *Indian. Vet. J.*, **68**: 179-200.
- Shukla, S.P. (1988). Studies on biochemical changes in the uterine fluid in relation to bacteriology and histopathology of the uterus in repeat breeding cattle. Ph.D. Thesis submitted to Punjab Agricultural University, Ludhiana.
- Singh, J., Sidhu, S.S., Dhaliwal, G.S., Pangaonkar, G.R., Nanda, A.S. and Grewal, A.S. (2000). Effectiveness of lipopolysaccharide as an intrauterine immunomodulator in curing bacterial endometritis in repeat breeding crossbred cows. *Anim. Reprod. Sci.*, **59**: 159-166.
- Singh, K.C.P., Pandey, J.N., Singha, M.N., Prasad, C.B., Prasad, C.R. and Singh S.S. (1989). Studies on genital microflora of repeat breeder cows. *Indian. Vet. Med. J.*, **13**: 61-63.
- Singh, U., Gupta, R.C., Sharma, D.N. and Sinha, A.K. 1988. Effect of various concentrations of lugol's iodine and oxytetracycline in the cytomorphology of the endometrium of repeat breeding cows. *Indian. J. Anim. Res.*, **9**: 87-89.
- Singla, V.K., Verma, H.K., Dwivedi, P.N. and Gandotra, V.K. (1991). Bacteriological isolates in repeat breeder cows. *Indian. J. Anim. Sci.*, **61**: 181-182.
- Tizard, I. (1987). Veterinary Immunology. 3rd Edn., E.B. Saunders Co., Philadelphia.