



Therapeutic Evaluation of Levofloxacin and Lugol's Iodine for Subclinical Endometritis

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ABSTRACT

Study was conducted in cross-bred cows (>60 days-in-milk) to evaluate various therapeutic regimens for Subclinical Endometritis (SCE). A total of 60 animals tested positive for SCE by Endometrial Cytology (EC) were divided into five groups with equal number. Therapeutic regimens viz. 0.3% Lugol's iodine I/U (G-I), Lenovo-AP I/U (G-II), Meriflox I/M (G-III), Meriflox I/M + AI (G-IV) and No treatment + AI as control (G-V), each treatment opted for 3 days. In G-I, II and III, Whiteside test (WST) of Estrual Mucus and Artificial Insemination (AI) was done on subsequent estrus to evaluate recovery and conception rates, respectively. However, in G-IV and G-V, AI was performed on concurrent estrus to evaluate conception rate. Treatment efficacy was analyzed by recovery rate based on negative WST at next estrus (G-I, II, III) and conception rate evaluated based on pregnancy diagnosis after two months of AI in all groups. Group-I, II and III revealed recovery rate of 83.3%, 50% and 100% as well as conception rate of 50%, 50% and 66.7%, respectively. However, no difference was found in conception rate of G-IV (33.3%) and G-V i.e. control (33.3%). Further, treatment cost calculated per animal was ₹-9 for Lugol's iodine, ₹-135 for Lenovo-AP and ₹-230 for Meriflox. In conclusion, Meriflox (Levofloxacin) by intramuscular route and AI on subsequent estrus can be recommended for treatment of SCE, and to improve conception rate. Apart from being economical, Lugol's iodine laden with enormous benefits can be advocated as alternative treatment option via intrauterine administration for SCE.

HIGHLIGHTS

- Efficacy of Lugol's iodine compared with antibiotic as it has numerous advantages.
- Effective treatment option was found to be Levofloxacin.
- Lugol's iodine was slightly less effective but the most economical treatment option.

Keywords: Subclinical endometritis, Endometrial cytology, AI, conception, Levofloxacin, Lugol's iodine

Subclinical endometritis (SCE) is inflammation of the internal lining of uterus (endometrium) in the dearth of quantifiable signs of endometritis, which causes considerable decline of reproductive performance (Sheldon *et al.*, 2009). It is also called cytological endometritis and is associated with an amplified percentage (proportion) of

polymorphonuclear cells (PMN) in samples of endometrial cytology (Kasimanickam *et al.*, 2004).

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Regarding the treatment of SCE, there is no evidence-based, valid single recommendation for therapy of SCE (Wagener *et al.*, 2017) as described by few studies on treatment strategies. Currently, in the literature quite a few controversial reports are available regarding the effectiveness of treatment protocols for SCE, i.e. Cephalosporin (Kasimanickam *et al.*, 2005; Denis-Robichaud and Dubuc, 2015), PGF2 α (Lima *et al.*, 2013), Ceftiofur (Dubuc *et al.*, 2011), combination of Levofloxacin-Ornidazole and α -Tocopherol (Markandeya *et al.*, 2010), Lugol's iodine (Sood *et al.*, 2012; Ahmed and Elsheikh, 2014), Moxifloxacin (Purohit *et al.*, 2013), NSAIDs (Priest *et al.*, 2013), Oxytetracycline (Singh *et al.*, 2014), Levofloxacin (Kumar *et al.*, 2017) and many more.

Uterine bacterial culture sensitivity test has shown Fluoroquinolone group as highly sensitive antibiotic group (Malinowski *et al.*, 2011; Purohit *et al.*, 2013; Sharma *et al.*, 2017). However, the preferred treatment should not be limited to clinical or microbiological cure but should improve the fertility along with economical consideration. Use of antibiotics to combat uterine infections results in variable success and often requires milk disposal due to fear of residual antibiotics in milk. Apart from high cost of treatment, there are also chances of development of antibiotic resistance. Conversely, Lugol's iodine has no withdrawal period which is truly advantageous and except in excess administration it does not penetrate into the milk (Carleton *et al.*, 2008) besides having antifungal, antiprotozoal and even antiviral properties in addition to its antibacterial properties (Sekhon, 2012) and intra-uterine infusion of diluted Lugol's iodine significantly reduces the rate of services per conception and mean length of days open (Ahmed and Elsheikh, 2014).

MATERIALS AND METHODS

This investigation was carried out at Veterinary Clinical Complex (VCC), FVSc & AH, Shuhama, Srinagar and Mountain Livestock Research Institute (MLRI), Manasbal, Ganderbal. Screening was done in crossbred postpartum cows of >60 DIM that were presented for AI at VCC and MLRI. The main criteria's for screening were no apparent clinical signs of any disease, clear estrual mucus discharge and without any gross reproductive tract pathology as examined per-rectally. All the cows were in age group of 3-12 years with 73.3% cows falling in age group of 3-5

years. Similarly, parity was ranging from 1-7 parities with 63.3% cows falling in parity range of 1-2 parities.

Sixty (60) animals were diagnosed positive for SCE by EC using Cytobrush with $\geq 4\%$ PMNs as cut-off value as per Singh *et al.* (2016). EM from study animals was collected at 6 to 12 hr after the onset of oestrus as described by Bhat *et al.*, 2014 with slight modification. All the positive animals were divided into five treatment groups of 12 animals each (Table 1).

Table 1: Different treatment regimens followed in the study

Group (N=60)	Treatment regimen followed
I (n=12)	0.3% Lugol's Iodine @ 30ml I/U \times 3 Days + WST and AI on next heat
II (n=12)	Lenovo AP @ 30ml I/U \times 3 Days + WST and AI on next heat
III (n=12)	Meriflox @ 4-5 mg/kg body weight I/M \times 3 Days + WST and AI on next heat
IV (n=12)	Meriflox @ 4-5 mg/kg body weight I/M \times 3 Days + AI at the same heat
V (n=12)	Control Group, AI + NO Treatment

N = total number of animals; n = number of animals in one group.

Group-I (n=12)

Intra-uterine administration of 30 ml of 0.3% lugol's iodine once a day for three days was carried out in this group. Lugol's iodine (0.3%) solution was prepared fresh from 5% stock solution of lugol's iodine (Sisco Research Laboratories Pvt. Ltd., India) and final volume was made with distilled water. On the next estrus, EM was again collected from the treated cows, subjected to WST and then artificially inseminated with proven good quality frozen semen deposited in body of uterus. AI was performed after 12 hrs of onset of estrus in all the study animals by the same veterinarian.

Group-II (n=12)

In this group, 30 ml of Lenovo AP (Intas Pharmaceuticals Ltd, India) which contains levofloxacin (20 mg/ml), ornidazole (40 mg/ml) and alpha-tocopherol (5 mg/ml) was administered by intra-uterine route once a day for three days. On the next estrus, EM was collected and

subjected to WST and the cows were then artificially inseminated as in group-I.

Group-III (n=12)

Meriflox (Vetoquinol India Animal Health Pvt. Ltd., India) which contains levofloxacin (100 mg/ml) was given at a dose rate of 4-5 mg per kg body weight by intra-muscular route once a day for 3 days. On the next estrus EM was collected and subjected to WST and the cows were artificially inseminated as in group-I.

Group-IV (n=12)

Along the treatment with Meriflox (4-5 mg/kg body weight) by intra-muscular route once a day for three days, the cows were also artificially inseminated as in group-I at the same estrus.

Group-V (n=12)

The animals in this group were kept as control without treatment but were artificially inseminated as in group-I at the same estrus.

Evaluation of efficacy of treatment regimen of Group I, II and III was evaluated based on WST at next estrus and conception rate while for Group IV, was evaluated only on conception rate in comparison to Group V (Control). Assessment of conception rate in all the groups was made by pregnancy diagnosis (PD) after 50-60 days of AI by rectal palpation.

RESULTS AND DISCUSSION

In group-I, 83.3% animals showed recovery based on negative WST and conception rate of 50% was found based on pregnancy diagnosis after 2 months of AI. Similarly, in group-II, both the recovery and conception rates were found to be 50%. However, in group-III, in which Meriflox was given intramuscularly for three days, 100% animals showed recovery and conception rate of 66.7% was detected.

In group-IV, the conception rate was found to be 33.3%. In group-V i.e control, in which only insemination was done without any treatment, the conception rate was also found to be 33.3% (Table 2).

Table 2: Recovery rate and conception rate in different treatment groups (N=60)

Group No	Treatment	Recovery rate (-ve WST)	Conception rate
I (n=12)	Lugol's Iodine (0.3% × 30ml × I/U × OD × 3 days)	83.3%	50.0%
II (n=12)	Lenovo AP (30 ml × I/U × OD × 3 days)	50.0%	50.0%
III (n=12)	Meriflox (4-5mg/kg bw × I/M × OD × 3 days)	100%	66.7%
IV (n=12)	AI + Meriflox (4-5mg/kg bw × I/M × OD × 3 days)	*	33.3%
V (n=12)	AI + No treatment (Control)	*	33.3%

N = total number of animals; n = number of animals in one group.

Economy of treatment

When comparing the treatment cost per animal, Lugol's iodine was found to be cost effective with only nine rupees approx (₹ 09) treatment cost per animal while Meriflox treatment came out to be much expensive at ₹ 230 approx per animal. For the third treatment regime opted in which Lenovo AP was used, cost of treatment per animal remained in between the former treatments at ₹ 135 approx (Table 3).

Table 3: Economics of treatment per animal and milk withdrawal period comparison

Treatment option	Amount used/ animal	Cost /unit	Approx Cost / animal (₹)	Milk Withdrawal period
Lugol's iodine	90 ml of 0.3%	₹ 182 / 125 ml (5%)	09	0 days
Lenovo AP	90ml	₹ 45 / 30 ml	135	7 days
Meriflox	30ml Approx	₹ 115 / 15 ml	230	7 days

Fluoroquinolones (intramuscular as well as intrauterine) and Lugol's iodine (intrauterine) have been previously used with variable efficacy. The present study revealed that Group-III treated with Meriflox (Levofloxacin) administered I/M for three days with AI at next estrus showed best results in terms of recovery rate which was 100% (negative WST) and conception rate (66.7%)

in comparison to other treatment regimens. However, Groups-I & II treated with 0.3% Lugol's iodine, I/U for three days and Lenovo AP (Levofloxacin, Ornidazole and alpha-tocopherol) I/U for three days respectively, showed comparable results in terms of conception rate although more number of animals (83.3%) recovered (negative WST) when treated with Lugol's iodine as compared to Lenovo AP (50%). Further, no difference was found in terms of conception rate between Group IV treated with Meriflox (Levofloxacin), I/M for three days after AI and Group-V (Control) where it was 33.3% in both cases which is far less than other groups.

G-I, in which Lugol's iodine (I/U) was given, showed recovery rate of 83.3% and conception rate of 50% which was similar to the results reported by Singh *et al.* (2018) with recovery rate of 87.50% and conception rate of 42.86% but poor results were reported by Sood *et al.* (2012) and, Sharma and Singh, (2012) in comparison to present study. Sood *et al.* (2012) reported recovery rate of 56.2% which could be due to use of treatment for only one day and also diagnostic method used was CFU/ml of EM while Sharma and Singh, (2012) reported recovery rate of 58.3%, which could be due to use of low concentration of Lugol's iodine (0.1%) and post treatment presence of fungal growth in uterine discharge was used as diagnostic criteria.

G-II, in which Lenovo AP (I/U) was given, showed recovery rate of 50% and conception rate of 50%. Similar conception rate of 56.5% was reported by Bhattacharyya *et al.* (2011) along with a higher recovery rate of 78.3% which could be due to treatment regimen followed for five days. Markandeya *et al.* (2010) reported recovery rate of 77.78% and conception rate of 66.67%, both higher than the present investigation and could be due to use of double dose of 60ml of Lenovo AP (I/U). Likewise, elevated recovery rate and conception rate (86.7% and 80% respectively) was reported by Purohit *et al.* (2013), which could be due to use of high dose rate of 2000 mg Moxifloxacin in comparison to 600 mg of Levofloxacin in the present study. Singh *et al.* (2018) reported higher recovery rate of 87.9% but conception rate of 48.3% coinciding with the present study.

G-III, in which Meriflox I/M was administered, showed recovery rate of 100% and conception rate of 66.7% which was in close concurrence with the results reported

by Pillai, (2012) and Singh *et al.* (2018). In another study, Pillai, (2012), reported 100% recovery rate and 57.9 % conception rate whereas Singh *et al.* (2018), reported recovery rate of 95.20% with slightly lower conception rate (50%).

G-IV, in which Meriflox I/M was administered in addition to AI at the same estrus revealed conception rate of 33.3% which was similar to the control group. To the best of our knowledge there is no published research available in relation to this treatment regimen.

G-V, i.e. control in which AI was performed without any treatment, exhibited conception rate of 33.3% which was mediocre as compared to other treatment groups. Poorer conception rates of 25% and 20% were reported by Singh *et al.* (2014) and Singh *et al.* (2018), respectively in untreated control group.

The comparison of results showed Levofloxacin as best treatment for improving recovery as well as conception rate but use of Lugol's iodine also came up with comparable results and it has got other benefits like being lot more economical, has zero withdrawal period, has anti-bacterial, anti-viral, anti-fungal and anti-protozoal properties (Carleton *et al.*, 2008; Sekhon, 2012).

CONCLUSION

Administration of Meriflox (Levofloxacin) parentally with AI on subsequent estrus can be recommended for treatment of SCE and improving conception rate. Apart from being economical, Lugol's iodine laden with enormous benefits can be advocated as an alternative treatment option via intrauterine administration for SCE.

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