



Effect of intra-uterine administration of *Lactobacillus* bacteria on the steroid hormone profile in sub-clinical endometritis affected cows

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Sub-clinical endometritis is prevalent in high-producing dairy cows which leads to decreased pregnancy rates and extended inter-calving period (Kasimanickam *et al.* 2004, Gilbert *et al.* 2005). Sub-clinical endometritis is the inflammation of the endometrium without any visible clinical signs and often without symptoms of infection (Madoz *et al.* 2014 and Marino *et al.* 2017). In addition to reproductive failure, clinical as well as sub-clinical endometritis may negatively affect milk production (Bell *et al.* 2007). Current treatments of sub-clinical endometritis mostly include antimicrobials and prostaglandin (PGF₂ α). However, the reproductive performance was neither improved by antibiotics nor PGF₂ α (Lima *et al.* 2013). Therefore, an alternative strategy for the effective management of sub-clinical endometritis for improving fertility rates without the unwanted side effects of antimicrobials would be of great advantage. In humans, bacterial vaginosis is being treated with lactobacilli as an alternative to antibiotics (Vicariotto *et al.* 2014). Gram-positive lactobacilli has the capacity to produce acetic and lactic acid, hydrogen peroxide and bacteriocins, which are considered to be useful in managing the pathogenic bacteria (Aroutcheva *et al.* 2001). Considering these documented facts, objective of this study was to determine the role of steroid hormone and its concentration in sub-clinical endometritis affected cows before and after intra-uterine treatment with lactobacillus bacteria.

Fourteen postpartum cross-bred cows affected with sub-clinical endometritis and ten healthy cows brought to Large Animal Gynaecology Unit were utilised for the study. The signalment, anamnesis, clinical parameters were recorded for all the cows diagnosed with sub-clinical endometritis. All these pluriparous cows were tested for sub-clinical endometritis by white-side test and endometrial cytology. All the 14 cows were assigned into two groups as

Group I and II. The group I cows were treated with 20 ml of 10¹⁰ CFU intra-uterine on the day of estrus. The group II cows were treated with liq. Lenovo 60 ml intra-uterine for three consecutive days. Artificial insemination was done in the following estrus. Ten healthy cows were selected and vaginal secretions were collected with sterile cotton swabs during estrus.

The swabs were inoculated into MRS broth and incubated for 24 h, then the cultures was streaked over MRS agar (Cat.No. M641, Himedia) (specific for lactobacillus) and incubated anaerobically for 24 h at 37°C. The microorganism was phenotypically identified as gram positive bacilli by gram staining. To identify the organism PCR amplification targeting the 16s rRNA gene with forward primer 5'-CTTGTACACACCGCCCGTCA-3' and reverse primer 5'-CTCAA-CTAAACAAAGTTTC 3' was done. The PCR confirmed the presence of *Lactobacillus* spp. 10 ml of lactobacillus culture with each ml containing 1.5 \times 10⁹ CFU/ml was prepared and made into a 20 ml solution with normal saline. The 20 ml of prepared solution contains 1.5 \times 10¹⁰ CFU. This freshly prepared lactobacillus suspension was used for intra uterine treatment.

Blood collection was done during estrus before and after the treatment in both the groups. Blood samples were collected in sterile, heparinized vacutainers through jugular vein puncture from all these cows as per the standard protocol. The samples were brought to laboratory in ice box and the serum was separated by centrifugation at 3000 rpm for 15 min and then stored at -20°C until estimation. The stored serum samples were used for estradiol 17- β and progesterone estimation by Radio Immuno Assay kit using the Calbiotech, Inc (CBI) and Progesterone Radio Immuno Assay kit, respectively as per the kits protocol.

The mean serum estradiol 17 β -values are given in Table 1 and Fig. 1 for both the groups. The mean estradiol-17 β concentration (pg/ml) in Group I *Lactobacillus* treated cows before and after treatment was 17.13 \pm 0.67 and 26.44 \pm 1.17, respectively whereas it was 25.22 \pm 2.55 and 29.24 \pm 2.75 in Group II Lenovo treated cows, respectively.

The mean serum concentration of estradiol-17 β in group

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Table 1. Mean (\pm SE) Serum estradiol-17 β concentration in sub-clinical endometritis cows before and after treatment with *Lactobacillus* and Lenovo intra-uterine

GROUP	Estradiol-17 β (pg/ml)		p-value
	Before treatment	After treatment	
Group I (n=7)	17.13 \pm 0.67 ^A	26.44 \pm 1.17 ^B	6.16**
Group II (n=7)	25.22 \pm 2.55	29.24 \pm 2.75	0.87 ^{NS}

Means bearing different superscript (a,b) differ significantly between groups (between rows); otherwise non-significant. Means bearing different superscript (A,B) differs significantly within the group (between columns); otherwise non-significant. ^{NS} Non-significant ($p > 0.05$). *, Significant at $p < 0.05$ level; **, Significant at $p < 0.01$ level; X^{A,B}, Comparison within the groups before and after treatment.

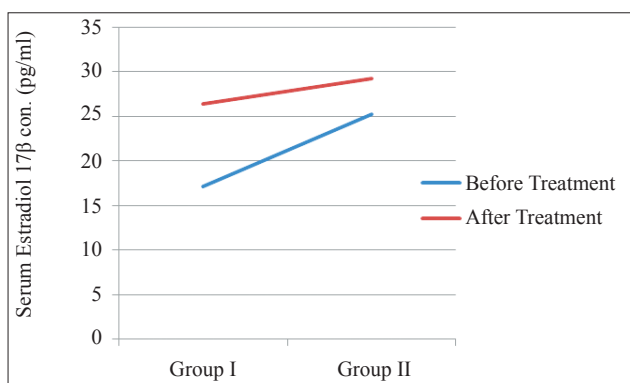


Fig. 1. Mean (\pm SE) Serum estradiol-17 β concentration in sub-clinical endometritis cows before and after treatment with *Lactobacillus* and Lenovo intra-uterine.

I cows got increased significantly ($p < 0.01$) after intra-uterine therapy. In the Lenovo group II cows there was no significant difference found in the estradiol concentration before and after treatment.

Estrogens are hormones produced by the ovary, transported by carrier proteins and act on the central nervous system causing the estrus behaviour in females. Estrogen plays an important role in the regulation of the endocrine and behavioural functions associated with estrous cycle. Peripheral plasma estradiol levels are similar in cattle and buffaloes, with maximum concentrations observed before and during the preovulatory surge of gonadotropins which reaches a basal value in the next few days with minor fluctuations throughout the estrous cycle (Chaudhary *et al.* 2015). Estrogen induces the preovulatory LH surge and estrus behavior (Hafez and Hafez 2000). After a certain threshold of estradiol-17 β is attained, LH surge occurs which leads to ovulation (Lyimo *et al.* 2000). It is well documented that the micro-organisms in uterine pathogens may reduce reproduction either by direct endometrial damage or by their toxins (Williams *et al.* 2007). Bacterial infections may affect estradiol and progesterone synthesis, disrupt follicular growth and corpus luteum (Shimizu *et al.* 2012), interfere with LH production and cause ovulatory defects (Lavon *et al.* 2010), may upregulate PGE₂ synthesis and prolongs corpus luteum life (Herath *et al.* 2009) and may lead to early embryonic death (Soto *et al.* 2003).

In the present study a low value of estradiol was reported in sub-clinical endometritis when compared with other researcher's findings in normal infection free cows. Suresh A *et al.* (2021) reported that the serum estradiol level was significantly ($p < 0.05$) lower (41.85 \pm 3.01 pg/ml) in repeat breeders cows (which could be major cause for sub-clinical endometritis) than the normal cycling animals (50.49 \pm 1.14 pg/ml). Though they reported a lower estradiol value in normal cyclical cows, it was higher than our values in subclinical endometritis. A similar value (16.38 \pm 0.18) of estradiol was reported by Suresh Kumar *et al.* (2021) in normal cyclical buffaloes during estrus. Since buffaloes have silent estrus, normally the estradiol values are lesser than in cows during estrous cycle.

Cows with postpartum uterine infection had slower growth of the first postpartum dominant follicle and lower peripheral plasma estradiol concentrations around the time of maximal follicle diameter, and in those animals that did ovulate, peripheral plasma progesterone concentrations were lower 5–7 days after ovulation (Sheldon *et al.* 2002 and Williams *et al.* 2007). These effects of uterine microbes on ovarian function could be caused by PAMPs or inflammatory mediators acting on the hypothalamus, pituitary, or ovary. This could be the possible reason for reduced levels of estradiol in sub-clinical endometritis affected cows

The mean serum progesterone values are given in Table 2 and Fig. 2 for both the groups. The mean serum

Table 2. Mean (\pm SE) Serum progesterone concentration in sub-clinical endometritis cows before and after treatment with *Lactobacillus* and Lenovo intra-uterine

Group	Progesterone (ng/ml)		p-value
	Before treatment	After treatment	
I	0.75 \pm 0.08	0.65 \pm 0.04	1.12 ^{NS}
II	0.84 \pm 0.06	0.68 \pm 0.04	2.35 ^{NS}

Means bearing different superscript (a,b) differ significantly between groups (between rows); otherwise non-significant. Means bearing different superscript (A, B) differs significantly within the group (between columns); otherwise non-significant. ^{NS}, Non-significant ($p > 0.05$). *, Significant at $p < 0.05$ level; **, Significant at $p < 0.01$ level; X^{a,b}, Comparison between groups; X^{A,B}, Comparison within the groups before and after treatment.

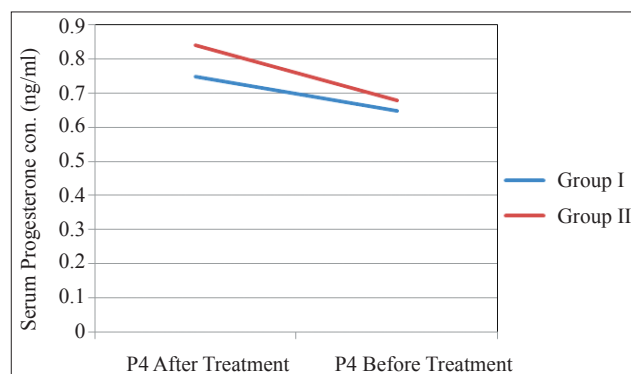


Fig. 2. Mean (\pm SE) Serum progesterone concentration in sub-clinical endometritis cows before and after treatment with *Lactobacillus* and Lenovo intra-uterine.

progesterone concentration (ng/ml) was 0.75 ± 0.08 and 0.65 ± 0.04 in group I and 0.84 ± 0.06 and 0.68 ± 0.04 in group II cows respectively. This progesterone concentration did not differ significantly in both the groups before and after intra-uterine therapy. Suresh A *et al.* (2021) found a similar non significant progesterone values of 2.41 ± 0.12 pg/ml in normal cyclical and 2.19 ± 0.29 pg/ml in repeat breeder cows. A similar progesterone value (0.72 ± 0.14 ng/ml) was reported by Suresh Kumar R *et al.* (2021) in cyclical buffaloes during estrus.

Progesterone is produced by the corpus luteum, formed after ovulation. If the animal doesn't become pregnant, degeneration of the corpus luteum occurs leading to a decline in progesterone levels by around day 18-19 and return to estrus by day 20-22 (Ahammed *et al.* 2018). Higher plasma progesterone concentrations around estrus had been associated with accelerated transport of the embryo, which may cause premature entry of the zygote into the uterus (Newcomb and Rowson 1975). Luteal insufficiency (with low progesterone) has also been reported as a major reason of early embryonic mortality. The present study revealed a lower (although non-significant) level of progesterone during estrus before and after intrauterine therapy in sub-clinical endometritis cows which concurs with Barui *et al.* (2015) who have shown significant differences between progesterone levels of normally cyclic (5.61 ± 0.74 pg/ml) and repeat breeder cows (3.36 ± 0.49 pg/ml).

The effect of uterine disease on follicular function may be further enhanced by cytokines released by the endometrial cells because granulosa cell steroidogenesis is also impaired by proinflammatory cytokines (Spicer L J and Alpizar E 1994). If animals ovulate, the cytokines secreted by the infected endometrium may also partly explain the reduced progesterone secretion from the corpus luteum because bovine luteal cells are highly responsive to a range of cytokines and cytokines are also important in luteolysis (Petroff *et al.* 2001, Okuda and Sakumoto 2003).

SUMMARY

Infertility in bovines is a major cause of serious economic loss to the dairy farmers. A major reason for infertility is sub-clinical, retrograde uterine infections. Sub-clinical endometritis is endometrial inflammation without apparent clinical manifestation and usually without signs of infection. This condition may be due to uterine infections or an extended inflammatory period that exists after elimination of bacteria. At present, infertility treatment is mainly done with intrauterine antibacterials, antiseptics and less commonly by hormones. These days, the major problem faced by animal reproductive health workers is the multiple drug resistance pathogens because of the indiscriminate use of antibiotics. Therefore, a different approach for more effective treatment of subclinical endometritis with *Lactobacilli* and its role on the steroid hormone profile was studied. In the present study, estradiol-17 β values were lower in the sub-clinical endometritis affected cows. Whereas, intra-uterine

administration of *Lactobacillus* bacteria caused significant increase in estradiol level in a cycle after treatment in cross-bred cows. Similarly, the circulating serum progesterone concentration was also found to be low in subclinical endometritis affected cows. These findings could be used for clinical and experimental interpretations. Further, the information on pulsatile release of gonadotropins and steroid hormones status, receptors and its control, the close relationship among hormones could be necessary for better understanding of the role of steroid hormones in sub-clinical uterine infections.

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