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Comparative efficacy of Vitamin E and estradiol in restoration of endometrial thickness in cattle

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Abstract

The present study was conducted to evaluate the therapeutic efficacy of vitamin E and estradiol in improving endometrial thickness in cattle. Forty-five parous HF crossbred cows with an endometrial thickness of less than 7 mm on the day of ovulation were selected and randomly allocated into three groups. Group I cows were treated with vitamin E at a dose of 1000 IU/10 kg DMI/day, cows in Group II received 2 mg estradiol benzoate from Day 15 of the estrus cycle until ovulation, and Group III served as the untreated control. All experimental animals were subjected to transrectal ultrasonography to measure endometrial thickness throughout the estrus cycle. The findings of the study revealed an increase in endometrial thickness from 4.45 ± 0.40 mm to 8.30 ± 0.61 mm in 73% of animals in Group I and from 4.42 ± 0.14 mm to 8.26 mm in 60% of animals in Group II, from three days before ovulation in the previous cycle to the day of ovulation in the present cycle. Findings of the study concluded that vitamin E treatment was more effective than estradiol in terms of the percentage of animals showing increased endometrial thickness.

Keywords: Endometrial thickness, Vitamin E, estradiol

1. Introduction

The endometrium is a dynamic tissue that undergoes cyclic changes involving cell proliferation and apoptosis throughout the estrus cycle under the influence of estradiol and progesterone (Miki *et al.*, 2013) ^[1]. It serves as the primary site of interaction between the maternal component and the developing embryo during the histotrophic phase of early pregnancy. Souza *et al.* (2011) ^[2] reported that a healthy endometrium is essential for fertility in dairy cows and demonstrated a higher probability of conception and pregnancy in animals with an endometrial thickness greater than 8 mm. Likewise, thin endometrium has been associated with reduced fertility in women undergoing assisted reproductive procedures (Rashidi *et al.*, 2005) ^[3].

Several therapeutic strategies have been investigated to improve endometrial thickness in human medicine. More recently, Gunaranjan *et al.* (2021a, 2021b) ^[4, 5] reported the successful use of vitamin E and estradiol for optimizing endometrial thickness in cattle. Takasaki *et al.* (2010) ^[6] demonstrated that vitamin E enhances endometrial growth by promoting glandular epithelial development and angiogenesis through increased expression of vascular endothelial growth factor (VEGF). Estradiol has also been shown to increase endometrial thickness by enhancing uterine perfusion (Ford and Chenault, 1981) ^[7] and stimulating the release of nitric oxide, a potent vasodilator (Van Buren *et al.*, 1992) ^[8]. Perusal of literature revealed scarce reports were available on the effects of Vitamin E and estradiol on endometrial thickness. In this context, the present study was undertaken to compare the efficacy of vitamin E and estradiol in restoring endometrial thickness in cattle.

2. Materials and Methods

2.1. Experimental Animals

The present study was conducted to evaluate the comparative efficacy of vitamin E and estradiol in increasing endometrial thickness in cows. A total of 45 apparently healthy parous

HF crossbred cows with an endometrial thickness of less than 7 mm, as determined by transrectal ultrasonography on the day of estrus, were selected for the study. The investigation was carried out in dairy farms located around Bengaluru, Karnataka. The selected cows were randomly allocated into three experimental groups as follows.

Group I: Animals were treated with vitamin E @ 1000 IU/10 kg DMI/day (50% pure oral powder; Agrimin India Pvt. Ltd.) from the day of estrus for one complete estrus cycle.

Group II: Animals were subjected to ultrasonographic examination of the ovaries on Day 13 following estrus of the previous cycle to confirm the presence of a corpus luteum and monitor follicular growth. Subsequently, the animals received 2 mg estradiol benzoate (Estradiol Benzoate 1 mg/mL; Pregheat®, Virbac India Pvt. Ltd.) from Day 15 of the estrus cycle until ovulation.

Group III: Untreated cows served as control group.

2.2. Ultrasonographic Examination

All animals were subjected to ultrasonographic examination of the uterus and ovaries from Day 13 of the estrous cycle until the day of ovulation using a portable ultrasonographic machine (EASI SCAN, BCF Technology Ltd., UK) equipped with a 4.5–8.5 MHz linear-array transducer. Frozen cross-sectional images were analysed using the electronic calipers provided in the Fiji open-source biological image analysis software (Schindelin *et al.*, 2012).

To minimize deformation of the uterine horns during examination, minimal pressure was applied with the transducer over the uterus. Endometrial thickness was measured separately in each uterine horn, and the mean value for both horns was considered as the endometrial thickness for each animal. The data were expressed as the mean endometrial thickness for each treatment group from three days before ovulation until the day of ovulation. Statistical analysis was performed using repeated-measures ANOVA, and differences were considered significant at $p < 0.05$.

3. Results and Discussion

3.1. Estrus Response Rate

In the present study, estrus response rates of 86.6% (13/15), 93.3% (14/15), and 80.0% (12/15) were observed in Groups I, II, and III, respectively, within 18–21 days of the estrus cycle. The estrus response was numerically higher in both treatment

groups than in the control group, indicating that administration of vitamin E and estradiol did not adversely affect cyclic ovarian activity.

3.2. Endometrial Thickness

Endometrial thickness increases progressively during the follicular phase of the estrus cycle, primarily due to enhanced uterine perfusion (Ford and Chenault, 1981)^[8], increased angiogenesis mediated by vascular endothelial growth factor (VEGF) (Shifren *et al.*, 1996)^[9], and proliferation of stromal and epithelial cells under the influence of estradiol (Ohtani *et al.*, 1996; Miki *et al.*, 2013)^[10,11]. Consequently, estradiol administration has been widely employed to improve endometrial thickness in subjects with thin endometrium. Furthermore, increased impedance to uterine arterial blood flow has been associated with reduced endometrial thickness, and therapies that enhance uterine blood perfusion have been shown to improve endometrial development (Takasaki *et al.*, 2010)^[6]. Vitamin E has also been reported to reduce blood viscosity and improve capillary blood flow (Chung *et al.*, 1995)^[11], thereby enhancing uterine perfusion and creating favourable conditions for endometrial growth.

In the present investigation, 73% of animals treated with vitamin E and 60% of animals treated with estradiol exhibited an increase in endometrial thickness to more than 7 mm, whereas none of the animals in the control group demonstrated a similar improvement between the day of ovulation in the previous cycle and the day of ovulation in the present cycle.

Animals treated with estradiol showed a significant increase in mean endometrial thickness from 4.42 ± 0.14 mm on the day of ovulation in the previous cycle to 8.26 ± 0.43 mm on the day of ovulation in the present cycle (Table I). These findings are consistent with those reported by Liao *et al.*^[2014]^[12] and Ranisavljevic *et al.* (2019)^[13], who demonstrated improved endometrial thickness following estradiol therapy in women with thin endometrium. The findings of the present study also corroborate with the earlier reports discussed in cattle (Souza *et al.* (2011)^[2] and Gunaranjan *et al.* (2021a)^[2,4]). The observed improvement in cows following estradiol administration may be attributed to enhanced uterine blood flow through nitric oxide-mediated vasodilation (Van Buren *et al.*, 1992)^[8], stimulation of angiogenesis via VEGF (Shifren *et al.*, 1996)^[9], and increased proliferation of endometrial stromal and epithelial cells (Ohtani *et al.*, 1996; Fanchin *et al.*, 2001; Miki *et al.*, 2013)^[10, 14, 1].

Table 1: Mean $\pm$ SE comparative rate of change in endometrial thickness between control (n=15), vitamin E (n=15), and estradiol treatment (n=15) in cows

Day of the cycle	Endometrial thickness (mm)		
	Control	Vitamin E Treatment	Estradiol Treatment
Day of Estrus (Previous Heat)	a	a	a
Three days Prior to Estrus	4.73 ± 0.36	6.26 ± 0.31	4.84 ± 0.28
Two days Prior to estrus	4.68 ± 0.36	6.96 ± 0.35	5.64 ± 0.30
One Day Prior to Estrus	5.34 ± 0.48	7.82 ± 0.49	6.96 ± 0.49
Day of Estrus	$4.67 \pm 0.28a$	$8.30 \pm 0.61b$	$8.26 \pm 0.43b$

*Values with different superscripts indicate significant difference within the columns ($p < 0.05$)

Similarly, animals treated with vitamin E exhibited a significant increase in mean endometrial thickness from 4.45 ± 0.40 mm on the day of ovulation in the previous cycle to 8.30 ± 0.61 mm on the day of ovulation in the present cycle (Table I). These observations agreed with the findings of Takasaki *et al.* (2010)^[6] and Krief *et al.* (2019)^[15], who also

reported improved endometrial thickness following vitamin E supplementation in women with thin endometrium. Comparable findings in cattle were reported by Gunaranjan *et al.* (2021b)^[5]. The beneficial effect of vitamin E may be attributed to reduced impedance to uterine arterial blood flow (Takasaki *et al.*, 2010)^[6] together with reduced blood

viscosity and improved microcirculation (Chung *et al.*, 1995) [11], resulting in enhanced uterine perfusion and improved endometrial development.

Compared with the treatment groups, animals in the control group did not exhibit any significant improvement in endometrial thickness during the study period, indicating that spontaneous restoration of thin endometrium was uncommon under the prevailing management conditions.

3.3. Conception Rate

Previous studies have demonstrated a positive association

between endometrial thickness and fertility. Noyes *et al.* [2001] [16] and Rashidi *et al.* [2005] [3] reported higher pregnancy rates in women with an endometrial thickness greater than 9 mm, whereas Souza *et al.* [2011] [2] reported improved fertility in cattle with an endometrial thickness exceeding 8 mm. These findings suggest that attainment of an optimal endometrial thickness is essential for successful conception. Consistent with these reports, the present study recorded overall conception rates of 58.80% (9/15), 40.00% (6/15), and 12.5% (1/15) in Groups I, II, and III, respectively.

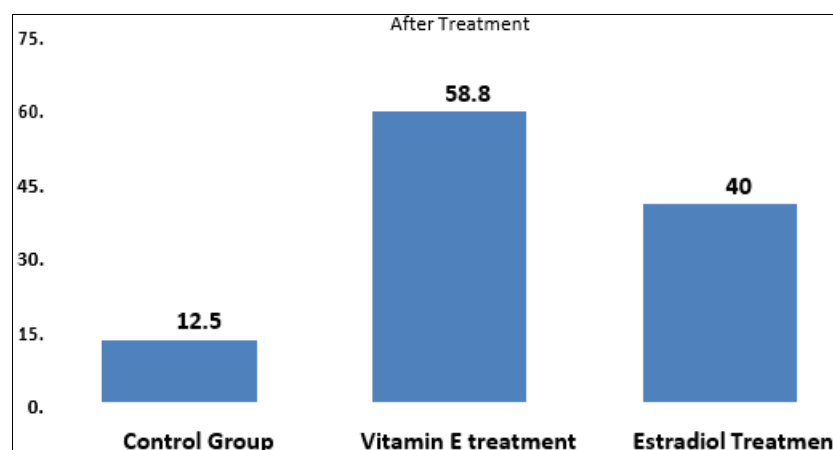


Fig 1: Graphical representation of Conception rate in different groups

Conclusion

Based on the findings of the present study, both vitamin E and estradiol can be used effectively for the treatment of thin endometria and to improve conception rate in animals with thin endometria, thereby providing a more favourable uterine environment for embryo implantation and establishment of pregnancy.

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Conflict of Interest

None of the authors has any conflict of interest.

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