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Use of vitamin e to augment fertility in cattle by optimizing endometrial thickness

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Abstract
Inadequate endometrial development is responsible for reduced fertility in both humans and cattle species. Vitamin E has been used in human patients with thin endometrium to attain adequate endometrial thickness. To test the hypothesis that oral feeding of vitamin E enhances fertility in cattle by increasing the endometrial thickness (ET) seventeen animals with history of repeat breeding having ET <8 mm on the day before ovulation, were fed with vitamin E for a period of one estrous cycle. All the animals were subject to ultrasound examination of the uterus and ovaries. Size of ovulatory follicle, ET, serum P4 and E2 were recorded and analyzed on the day prior to ovulation, before and after the treatment. 70 percent (12/17) of the animals treated showed an increase in the endometrial thickness (8.30±0.62) at the end of treatment of which nine animals (75%; 9/12) conceived. An overall conception rate of 58.8% (10/17) was obtained. Comparing the means of different parameters recorded before and after treatment with Vitamin E, using paired t test a statistically significant increase in the ET after treatment 4.416±0.139 vs 8.3018±0.622 was observed where as there was no statistically significant difference between the other parameters like follicular size, progesterone levels and estradiol levels (11.271±0.346 vs. 11.775±0.446, 0.490±0.537 vs 0,528±0.755 and 20.1±0.77 vs. 17.810±0.904 respectively). Further between the animals with ET > 8mm and < 8mm a higher percent of conception was recorded in the animals with ET > 8mm (75%), while other parameters did not differ significantly. In conclusion, feeding vitamin E enhanced the ET resulting in higher conception rate.

Keywords: Infertility, endometrial thickness, vitamin E

Introduction

The role of adequate endometrial development in influencing fertility is well documented in human patients [1, 2]. Endometrial thickness of <7 to 8 mm [3, 4] and > 14 mm [5] have resulted in lower fertility in humans. In cattle, Souza *et al.* [6] reported greater percentage of pregnancies per AI in animals with endometrial thickness >8mm while Singh *et al.* [7] have opined that ET at the time of estrus may be used as an indicator for timing of AI.

Although unlike in humans the endometrium does not shed following the follicular phase in cattle, it does undergo remodeling throughout the estrus cycle by cell proliferation and apoptosis mediated by estrogen and progesterone [8]. Coincident with the changes in the circulating levels of E2 & P4, the endometrial thickness varies through the estrus cycle and reaches a peak during the estrous phase [9, 10]. The increase in ET is caused by the release of endothelial growth factors (NO, VEGF,IGF- I, II) [11-13] mediated by the vasodilatory effect of estradiol. [14,15]. The knowledge that the vasodilatiory effect of estradiol enhances ET has led to the use of different therapeutic interventions aimed at increasing the uterine perfusion to treat thin endometria. For this purpose substances like estradiol [6], Vitamin E [13], potential nitric oxide donors such as l-argenine and seldinafil citrate [13] have been used to improve uterine perfusion in humans and cows. Vitamin E has been shown to improve capillary blood flow in a variety of organs not only by inhibiting the breakdown of lipids in red blood cell membranes and reducing the viscoelasticity of blood [16, 17] but also by protecting the endothelium from oxidative stress [18]. Compared to other substances like seldinafil and l-argenine, vitamin E is easy to administer in dietary form to cattle.

This experiment was carried out to test the hypothesis that oral feeding of vitamin E enhances ET leading to increased fertility in cows.

Materials and Methods

Animals

Pluriparous animals with history of repeat breeding maintained at the instructional livestock farm complex, Veterinary College, Hebbal, and MRS farm, GKVK, Hebbal, Bengaluru, Karnataka were subject to serial ultrasonographic examination of the ovaries and uterus from proestrus to one day after ovulation and concurrently blood sampling was done for P4 and E2 estimation. Seventeen animals with BCS 2.5-3 from the examined group with ET <8mm were selected for the trial. All the animals were maintained on uniform management conditions and nutritional plane.

Ultrasonography

Endometrial thickness was measured ultrasonographically (EASI-SCAN curve equipped with a 7.5 MHz Curved array transducer; IMV imaging®), with measurements done using electronic calipers in a 90-degree cross-sectional frozen image acquired 2 cm from the internal uterine body bifurcation. Minimum pressure was applied with the ultrasound transducer on top of the uterus, to avoid deformation of the uterine horns when performing these measurements. Endometrial thickness was defined as the distance between the edges of the endometrial lumen to the visualized interface between the endometrium and myometrium. Endometrial thickness was determined for each of the uterine horns in a separate frozen image, and then averaged to establish the final ET measurement for each cow. Serial ultrasonographic evaluations of the ovaries and uterus were performed from proestrus to one day after ovulation and concurrently blood sampling was done for P4 and E2 estimation. All the animals were fed 3200 mg of 50% pure vitamin E (as recommended by NRC) throughout one estrus cycle mixed in concentrate feed and similar serial ultrasonographic examination to measure the size of the ovulatory follicle and ET and blood sampling were performed in the next cycle. Size of ovulatory follicle, ET, serum P4 and E2 were recorded and analyzed on the day prior to ovulation before and after the treatment

Serum P4 and E2 concentrations were determined in extracted sera, using an antibody coated tube RIA kit

All the animals were inseminated with frozen semen 18 hours after observed heat at the end of treatment. Pregnancy diagnosis was performed 35 days post AI.

Statistical analysis

The results were expressed in mean values with standard error of means. Paired t-test was used to access the change in the recorded parameters before and after the treatment. Regression analysis was used to analyze the effect of the independent parameters like follicle size, estradiol and progesterone concentration on the endometrial thickness.

Results and Discussion

In the present study, an increase in endometrial thickness up to above 8mm following treatment with Vitamin E was seen in 70.58% of the animals (12/17) among which 75% (9/12) of the animals which were found pregnant. Similarly the effect of ET on fertility in cattle was recorded by Souza *et al.* [6] He observed an increase in pregnancies per AI from 20% to 40% when ET increased from 6 to 10 mm. Singh *et al.* [7] also have opined that ET at the time of estrus may be used as an indicator for timing of AI.

Comparison of means of the different parameters recorded using paired t test (Table I and Graphs I and II) before and after treatment showed statistically significant increase in the endometrial thickness after treatment as compared to before treatment (4.42±0.139 vs. 8.30±0.62) where as there was no statistically significant difference between the other parameters like follicular size, progesterone levels and estradiol levels (11.27±0.345 vs. 11.77±0.45, 0.49±0.54 vs. 0.53±0.75 and 20.10±0.77 vs. 17.81±0.90, respectively).

Although prior to the treatment, progesterone and estradiol levels were within the normal range [19, 20] the animals showed a thin endometrium (<8mm).

Changes in ET are effected by esradiol by increasing the blood flow to the endometrium through release of NO, stimulating VGEF production and enhancing endometrial growth by inducing mitotic changes [8, 12, 21]. Also decreasing progesterone along with increasing estradiol levels during the estrus phase may be a cue for increasing the ET [22]

Lowered ET in the previous cycle of animals with thin endometrium in this study may be, due to impedance of uterine radial arteries resulting in impairment of the growth of the glandular epithelium resulting in decrease in vascular endothelial growth factor (VEGF) expression a key factor for regulating angiogenesis in the endometrium [13]. Administration of vitamin E has been shown to improve capillary blood flow by inhibiting the breakdown of lipids in red blood cell membranes [17, 18] protecting the endothelium from oxidative stress [23] and by NO release from vascular endothelial cells which leads to the relaxation of vascular smooth muscle by activating cyclic guanosine monophosphate (cGMP) [24].

Following administration of vitamin E, in the group that responded to the treatment there was an increase in mean follicular size and progesterone level from 11.39±0.45 to 12.35±0.39 and 0.52±0.07 to 0.54±0.09 and decrease in estradiol level from 20.75±0.88 to 17.51±98 but these changes were not significant statistically. Whereas, an increase in ET from 4.43±0.16 to 9.57±0.24 was statistically significant. An increase in ET and decrease in RA-RI of patients in the next cycle was observed following administration of vitamin E [13] suggesting that vitamin E caused an increase in ET by increasing the blood flow to the uterus. Similar increase in ET was achieved in patients treated with a combination of vitamin E and pentoxyphylline [25] findings of this report suggest that Vitamin E treatment is effective in patients with endometrium which is unresponsive to estrogen.

A conception rate (75%) was observed in the animals with ET >8mm following treatment with vitamin E as compared to animals with ET <8mm (20%) (Table II). The animals that failed to achieve ET >8mm had a lower mean follicular size on the day of estrus as compared to those that showed an increase in *Et al* though this finding was not statistically significant.

Regression analysis was performed to evaluate the influence of the different independent parameters like follicular size, progesterone and estradiol levels on the endometrial thickness following treatment and it was observed that none of the parameters *viz;* follicular size (β=1.69), progesterone level (β =0.24) and estradiol level (Beta=0.04) had an influence on the endometrial thickness at an overall model fit of R^2 value of 0.19

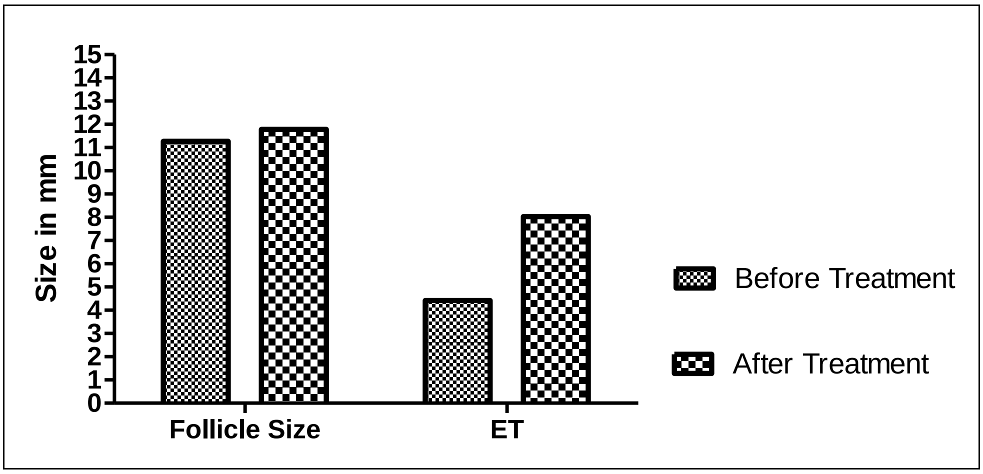
Table I: Mean ± SEM of Follicle Size, P4, E2, and ET before and after treatment with vitamin E for one estrous cycle. (n=17)

Parameters	Before treatment	After treatment
Follicle size (mm)	11.271±0.35[a]	11.775±0.45[a]
Progesterone (pg/ml)	0.49±0.54[a]	0.53±0.75[a]
Estradiol (ng/ml)	20.10±0.77[a]	17.810±0.90[a]
Endometrial thickness (mm)	4.42±0.14[a]	8.30±0.62[b]

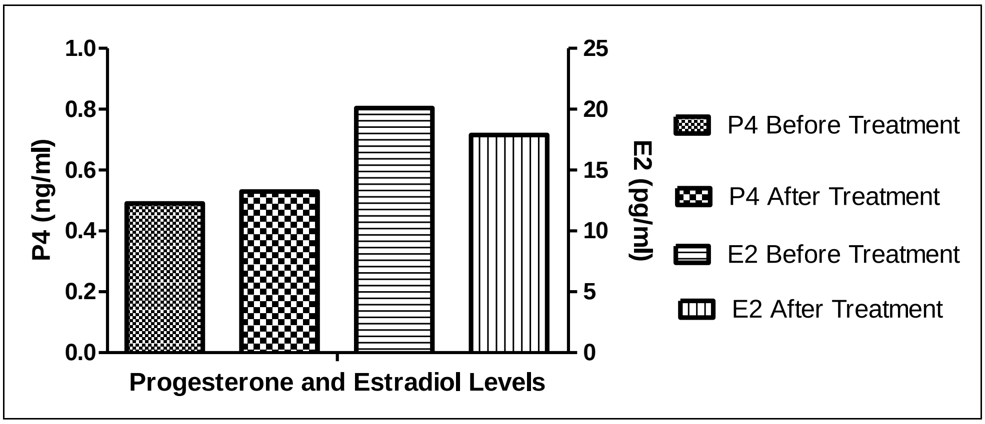
Different superscripts indicate statistically significant difference between the rows (P≤0.05).

Table II: Mean ± SEM of Follicle Size, P4, E2, and ET in animals with ET >8mm and <8mm, after vitamin E therapy.

Parameters	Endometrial thickness > 8mm (n=12)	Endometrial thickness < 8mm (n=5)
Follicle size (mm)	12.35±3.57	10.39±1.00
Progesterone (pg/ml)	0.541±0.16	0.50±0.12
Estradiol (ng/ml)	17.51±5.05	18.75±1.81
Pregnancy	75%	20%



Graph I: Comparative changes in Follicle Size and ET Before and After Treatment with vitamin E for one estrous cycle.



Graph II: Comparative changes in P4 and E2 Before and After Treatment with vitamin E for one estrous cycle.

Conclusion

Basing on the findings of this study we may conclude that ET plays a role in determining the fertility of cattle and oral feeding vitamin E for a period of one estrous cycle may enhance ET in animals with thin endometria.

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