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Optimising endometrial thickness to augment fertility in cattle using estradiol

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Abstract
The endometrium plays a vital role in determining the fertility of cattle. Failure to achieve optimal endometrial thickness (ET) at the time of estrus may lead to reduced fertility. Estradiol supplementation has been used to achieve adequate endometrial thickness in human patients with thin endometria. The present study was carried out to test the hypothesis that estradiol supplementation enhances fertility in cattle by increasing the endometrial thickness in cows with thin endometrium. For this purpose 15 parous HF cows with a history of repeat breeding having ET < 7mm were administered a single dose of estradiol 24 hrs prior to ovulation. 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 of ovulation before treatment and from three days prior to ovulation to a day after ovulation after treatment. A mean endometrial thickness of 4.70 ± 0.28 mm was recorded in all the cows on the day of estrus of the previous cycle which increased to a mean of 9.27 ± 0.40 mm in 60 % of the animals on the day of estrus in the cycle following treatment. Conception was seen in 6 of the 9 animals with ET above 7 mm. conception was not seen in any of the animals which did not attain endometrial thickness above 7 mm. The ET was positively correlated to serum estradiol concentration and negatively correlated to serum progesterone concentration. Findings of the study suggest that estradiol supplementation may enhance fertility in cows with thin endometria.

Keywords: Fertility, endometrial thickness, estradiol

Introduction
Reduced fertility of cows is related to poor oocyte quality and/or inadequate uterine environment to support early embryo growth before day 7 (Demetrio, 2007; Nascimento, 2013) [1, 2]. Estrogen and progesterone have long been described to provide adequate uterine environment for the establishment of pregnancy through their effect on the uterine tract particularly in the endometrium (Bridges., 2013) [3]. Estrogens increase blood flow to the uterus (Ford 1981; Shifren., 1996; Van Buren., 1992; Telfer., 1995; Chen., 2004) [4, 5, 6, 7, 8] and also increase cell proliferation and differentiation in the endometrium (Ohtani., 1996; Robinson., 2000) [9, 10]. Whereas, progesterone decreases blood flow to the uterus (Ford 1981) [4] and endometrial area (Wang., 2007) [11]. These effects of estradiol and progesterone alter the endometrial thickness throughout the estrous cycle (Sugiura., 2018) [12].
Adequate rise in estradiol following pre-exposure to progesterone is of major importance to prepare the uterus for embryo support and gestation (Bridges., 2013) [3]. Lowered fertility of cows may be partially explained by a suboptimal uterine environment as a result of lower circulating concentrations of both estradiol during pro-estrus and progesterone in the diestrus due to their greater steroid metabolism (Wiltbank., 2006) [13].
Ultrasonographic measurement of endometrial thickness (ET) near ovulation could be a good indicator of hormonal environment, and to assess exposure of uterus to adequate concentrations of hormones compatible with optimal fertility (Souza., 2011) [14].
Several studies in (Gonen., 1989; Kovacs., 2003) [15, 16] have associated endometrial thickness to fertility. A too thin or thick endometrium is associated with lowered fertility (Rashidi., 2005) [17]. Increased pregnancies are recorded in patients with ET between 9mm (Dickey., 1992) [18] to 14 mm (Weissman., 1999) [19]. In cattle higher pregnancies were recorded with ET > 8mm (Souza., 2011) [14].
Estradiol therapy has been used to treat human patients with thin endometria to achieve pregnancies with varying results (Dmowski., 1997; Fanchin., 2001; Chen., 2006; Liao., 2015) [20, 21, 22, 23].

Simillarly, Souza *et al.*, 2011 [14] reported increased pregnancies in cows with thin endometrium supplemented with estradiol.

The present study aims to test the hypothesis that estradiol supplementation increases pregnancies in cows with thin endometria.

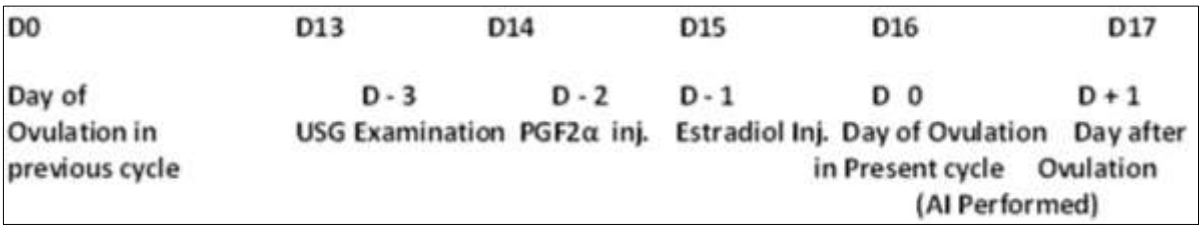
Materials and Methods

Animals

Thirty six parous HF cross cows with history of repeat breeding syndrome were examined on the day of estrus. Fifteen animals with ET < 7 mm were enrolled for the study. All the animals were maintained at the instructional livestock farm complex, Veterinary College, Hebbal, Bengaluru, Karnataka at latitude 13.1155° N, Longitude 77.6070° E.

All the 15 animals in this group were subjected to ultrasonographic examination of the ovaries on the 13th day following ovulation in the previous cycle for the presence of corpus luteum and follicular growth. On day 14 of the cycle the animals were administered 2 ml prostaglandin (PG) (Cloprostenol sodium; 250 mcg/ml, Estrumate, MSD Animal Health Intervet, India Pvt. Ltd) by intramuscular route. On day 15 of the cycle the animals were administered 2 mg of estradiol benzoate (EB) (Estradiol benzoate; 1 mg/ml, Pregheat, Virbac India Pvt. Ltd).

The animals were subject to serial ultrasonographic examination of the ovaries and uterus from day 13 to one day after ovulation to measure the size of the ovulatory follicle and ET. Concurrently blood sampling was done for P4 and E2 estimation.



Endometrial thickness was measured ultrasonographically (EASI-SCAN curve equipped with a 4.5 to 8.5 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. 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. Correlation analysis was used to analyse the effect of the independent parameters like follicle size, estradiol and progesterone concentration on the endometrial thickness. The statistical analysis was performed using SPSS software version 16.0.

Results and Discussion

In the present study all the animals ovulated the dominant follicle 48 hrs after $PGF2_{\alpha}$ administration and 24 hrs after estradiol administration. A mean endometrial thickness of 4.84 ± 0.28 mm, 5.64 ± 0.30 mm and 6.96 ± 0.49 mm were recorded on 3, 2 and 1 day prior to ovulation. Maximum mean endometrial thickness of 8.26 ± 0.43 mm was recorded on the day of ovulation following treatment which then decreased to a mean endometrial thickness of 8.18 ± 0.43 mm on the day following ovulation.

Twenty four hrs after $PGF_{2\alpha}$ administration, mean serum progesterone concentrations declined from 4.54 ± 0.23 ng/ml to 0.53 ± 0.43 ng/ml and estradiol concentrations increased from 18.33 ± 2.48 pg/ml to 20.93 ± 1.80 pg/ml. Twenty four hrs after estradiol administration, i.e. on the day of ovulation mean serum progesterone and estradiol concentrations of 0.57 ± 0.07 ng/ml and 30.50 ± 2.34 pg/ml were recorded. On the day after ovulation, mean serum progesterone concentration increased to 1.25 ± 0.56 ng/ml and estradiol concentrations of decreased to 23.03 ± 1.49 pg/ml (Table 1).

The mean endometrial thickness increased from 6.96 ± 0.49 mm to 8.26 ± 0.43 mm following estradiol treatment the increase in endometrial thickness was associated with an increase in the estradiol levels from 20.93 ± 1.80 pg/ml to 30.50 ± 2.34 pg/ml.

Table 1: Mean ± SE of follicle size, endometrial thickness, serum progesterone and estradiol concentrations during different days of estradiol treatment in cows (n=15)

Day of the cycle	Follicle Size (mm)	Endometrial thickness (mm)	Progesterone cocentration (ng/ml)	Estrogen cocentration (pg/ml)
Day Prior to Ovulation in Previous cycle	12.94 ± 0.52	4.70 ± 0.28	0.69 ± 0.06	19.49 ± 0.67
D -3	9.79 ± 0.43	4.84 ± 0.28	4.39 ± 0.25	12.44 ± 1.15
D -2	10.78 ± 0.52	5.64 ± 0.30	4.54 ± 0.23	18.33 ± 2.48
D -1	11.55 ± 0.51	6.96 ± 0.49	0.53 ± 0.43	20.93 ± 1.80
Day of Ovulation in Present cycle	12.32 ± 0.52	8.26 ± 0.43	0.57 ± 0.07	30.50 ± 2.34
Day +1		8.18 ± 0.43	1.25 ± 0.56	23.03 ± 1.49

Note: D -3 = day 13 of the cycle, D -2 = day of $PGF_{2\alpha}$ administration and D -1 = day of estradiol administration, D +1 = day after ovulation

The endometrial thickness was positively correlated to serum estradiol concentrations and negatively correlated with serum progesterone concentrations. Reports suggest that increasing estradiol levels combined with decreasing progesterone levels during the proestrus phase of the cycle is required to attain optimal ET on the day of ovulation(sugiura., 2018) [12]. In the present study a similar pattern of changes in the hormonal levels effecting change in the ET was recorded (Table 2 and Figure 1).

Table 2: Correlation coefficients among endometrial thickness, progesterone and estradiol concentrations during estradiol treatment (n=15)

Parameters	Endometrial thickness	Progesterone cocentration	Estradiol cocentration
ET	1.00	- 0.63***	0.45**
Progesterone		1.00	-
Estradiol			1.00

Note: Concentration of significance at $p < 0.05$** indicates moderately significant, *** indicates highly significant.

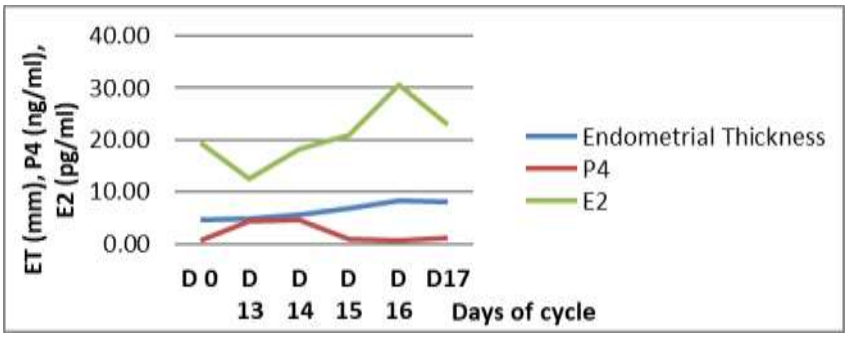


Fig 1: Changes in endometrial thickness in relation to serum progesterone and estradiol levels

A mean endometrial thickness of 4.70 ± 0.28 mm was recorded in all the cows on the day of estrus of the previous cycle which increased to a mean of 9.27 ± 0.40 mm in 60 % of the animals (9/15) on the day of estrus in the cycle following treatment. Conception was seen in 6 of the 9 animals with ET above 7 mm. Six animals which did not attain endometrial thickness above 7 mm and conception was not seen in any of these animals.

The increase in the endometrial thickness following estradiol treatment can be attributed to increase in vascularity and vascular permeability induced by estrogen in the endometrium (Shifren., 1996; Van Buren., 1992) [5, 6] and estradiol induced cell proliferation(Ohtani., 1996; Robinson., 2000) [9, 10]. Several authors have reported an increase in the endometrial thickness from 8.0 mm to 11.7 mm following treatment with estradiol by subcutaneous estradiol pillets, oral and vaginal methods routes of administration (Dmowski., 1997; Fanchin., 2001; Chen., 2006) [20, 21, 22].

Following administration of estradiol, in the group that responded to the treatment there was an increase in mean estradiol concentration from 19.49 ± 0.67 pg/ml to 30.50 ± 2.34 pg/ml and an increase in ET from 4.43 ± 0.16 mm to 9.57 ± 0.24 mm these changes were statistically significant (Table 3 and Figure 2).

Table 3: Mean ± SE of follicle size, endometrial thickness, serum progesterone and estradiol concentrations on the day of ovulation before and after estradiol treatment (n=15)

Parameters	Before treatment	After treatment
Follicle size (mm)	12.94 ± 0.52	12.32 ± 0.52
Progesterone cocentration (pg/ml)	0.69 ± 0.07	0.57 ± 0.07
Estradiol cocentration (ng/ml)	19.49 ± 0.67^{a}	30.50 ± 2.34^{b}
Endometrial thickness (mm)	4.70 ± 0.28^{a}	8.26 ± 0.43^{b}

Note: Vales with different superscripts inmdicate significant difference within the row ($p < 0.05$).

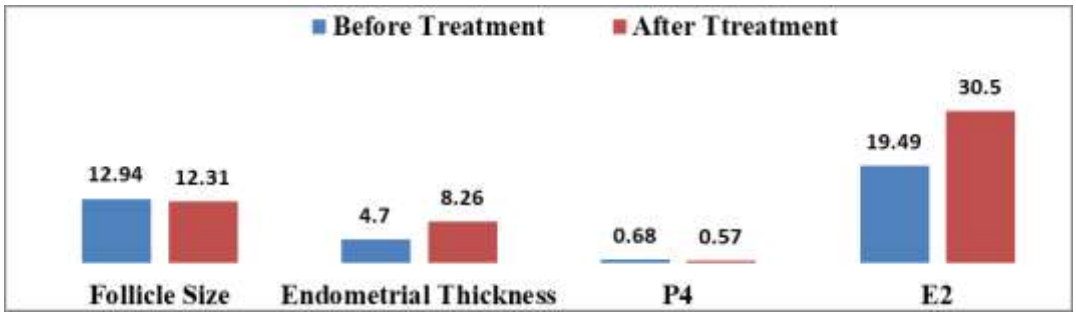


Fig 2: Changes in endometrial thickness, progesterone and estradiol concentrations on the days of ovulation before and after treatment

A conception rate (40.00 %) was observed in the animals with ET > 7 mm following treatment with estradiol as compared to animals with ET < 7 mm (0 %). The animals that failed to achieve ET > 7 mm had a lower mean follicular size of 11.33 ± 0.47 mm on the day of estrus as compared to mean follicular of 12.97 ± 0.75 mm in animals that showed an

increase in *Et al*though this finding was not statistically significant. higher conception rates are reported in cows that showed endometrial thickness above 8mm following treatment with estradiol (Souza *et al.*, 2011) [14] (Table 4 and Figure 3).

Increase in the conception rate were recorded in patients treated for thin endometrium, as observed in the present study by Liao, (2014) [23] who reported conceptions rate of 58.00 % and Dmowski, (1997) [21] reported conception in 27.00 % of patients treated.

In the present study, Increase in the endometrial thickness was seen in 60 % of the animals while 40 % of the animals did not respond to the treatment. The reason that some animals did not respond to the estradiol treatment may be attributed to a reduced expression of estrogen receptors, or even desensitization, which is not solved by increasing serum oestradiol concentrations(Garcia-Velasco., 2016) [24]. Liu (2015) [25] recommended longer duration of treatment with estrogen for patients who did not respond to estradiol treatment for thin endometria.

Table 4: Mean ± SE of follicle size, serum progesterone and estradiol concentrations in animals with ET > 7 mm and < 7 mm, in estradiol treatment (n=15)

Parameters	Endometrial thickness > 7 mm (n=9)	Endometrial thickness < 7 mm (n=6)
Follicle size (mm)	12.97 ± 0.75	11.33 ± 0.47
Progesterone cocentration (pg/ml)	0.60 ± 0.11	0.53 ± 0.12
Estradiol cocentration (ng/ml)	28.70 ± 3.0	33.20 ± 3.77
Pregnancy	40%	-

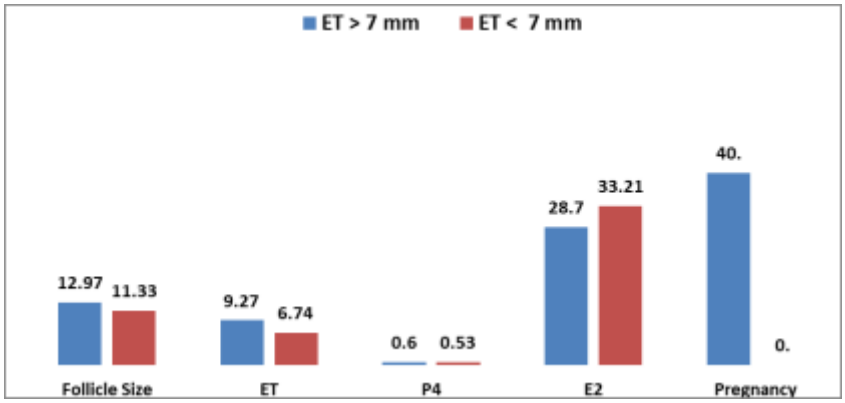


Fig 3: Changes in endometrial thickness, progesterone and estradiol concentrations in vitamin e treatment between cows with endometrial thickness > 7 mm and < 7

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