STUDIES ON ULTRASONOGRAPHIC CHARACTERIZATION OF ENDOMETRIAL THICKNESS IN ESTROUS CYCLE OF COWS

SHARANABASAVA SAJJAN

DEPARTMENT OF VETERINARY GYNAECOLOGY AND OBSTETRICS
VETERINARY COLLEGE, BANGALORE
KARNATAKA VETERINARY, ANIMAL AND FISHERIES SCIENCES UNIVERSITY, BIDAR

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By

SHARANABASAVA SAJJAN

DEPARTMENT OF VETERINARY GYNAECOLOGY AND OBSTETRICS

VETERINARY COLLEGE, BANGALORE

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CERTIFICATE

This is to certify that the thesis entitled *"**STUDIES ON ULTRASONOGRAPHIC CHARACTERIZATION OF ENDOMETRIAL THICKNESS IN ESTROUS CYCLE OF COWS**"* submitted by **Mr. SHARANABASAVA SAJJAN., I.D. No. MVHK 1229** in partial fulfillment of the requirements for the award of **MASTER OF VETERINARY SCIENCE in VETERINARY GYNAECOLOGY AND OBSTETRICS** of the Karnataka Veterinary, Animal and Fisheries Sciences University, Bidar is a record of bonafide research work carried out by him during the period of his study in this University under my guidance and supervision, and the thesis has not previously formed the basis for the award of any degree, diploma, association ship, fellowship or other similar titles.

Bangalore,

July, 2014

Dr. G. SUDHA
Major Advisor

Approved by:

Chairman : _____
Dr. G. SUDHA

Members : _____
Dr. A. KRISHNASWAMY

: _____
Dr. T. G. HONNAPPA

: _____
Dr. R. BHASKARAN

Dedicated to my
Beloved Parents,
Sisters and Friends

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LIST OF ABBREVATIONS

ET	Endometrial thickness
P_4	Progesterone
E_2	Estrogen
Fig.	Figure
i.e	That is
±	Plus or minus
≤	Less than or equal
≥	More than or equal
®	Registered
α	Alpha
β	Beta
ng/ml	Nano gram per milliliter
Pg/ml	Pico gram per milliliter
mm	millimeter

Introduction

1. INTRODUCTION

Ideal uterine environment is essential for embryonic implantation and growth in mammalian species (Gray *et al*., 2001). Many factors, including hormonal milieu, nutritional status, clinical or subclinical uterine infections, alter the uterine environment and decrease fertility.

Dairy cow fertility has declined not only in association with increased milk production, but also partly due to increased embryonic mortality (Lucy., 2001; Diskin and Morris., 2008). Low systemic progesterone (P_4) concentrations, during early pregnancy (Lamming *et al*., 1989), and also in the estrous cycle preceding insemination were associated with an increased incidence of embryonic loss. Asynchrony between progesterone, luteinizing hormone and estrogen levels is another factor of cow infertility. In non-fertile cows, low progesterone levels after ovulation, elevated estrogen: progesterone ratio (approx. day 3 and 6), and the low LH surge prior to ovulation are reported when compared to normal fertile cows (Maurer and Echternkamp., 1982). There is a strong correlation between reduced fertility in dairy cows with lower progesterone concentration in systemic circulation preceding estrus than those animals which conceive for a single insemination (Meisterling and Dailey., 1987).

The effects of estrogen and progesterone on growth and regression of the nonpregnant uterus are reflected in cyclic patterns of cellular proliferation, vascular growth and blood flow that occur in the endometrium during estrous cycle (Reynolds *et al*., 1992; Johnson *et al*., 1997). During early pregnancy, proliferation of endometrial tissues is also supported by dramatic increases in cellular proliferation, vascularization

and blood flow (Reynolds *et al*., 1992; Zheng *et al*., 1993). The periods of endometrial growth and regression are synchronized with ovarian function through changes in circulating and or local levels of estrogen and progesterone, thereby ensuring the uterine environment will be conducive to embryonic development and placentation (Hericks *et al.*, 1973).

Although cows do not lose endometrial tissue due to menses, the thickness of the endometrium (Pierson and Ginther, 1987) and certain histological aspects (Dhaliwal *et al*., 2002; Wang *et al*., 2007) varied considerably during the estrous cycle. An early study using Holstein heifers (Pierson and Ginther, 1987), described remarkable ultrasonographic changes in the endometrium near ovulation. In that study, endometrial thickness increased at the time of expected luteolysis, with maximal endometrial thickness on the day before ovulation. Several studies have also described the decrease in endometrial thickness and changes in echotexture after ovulation, probably due to increasing circulating P4 concentrations (Pierson and Ginther, 1987; Bonafos *et al*., 1995; Jimenez-Krassel *et al*., 2009).

Methods to accurately evaluate uterine status prior to fertilization could be useful for predicting fertility, as well as for accurately determining optimal treatments for individual cows. Several techniques, including uterine cytology, biopsy, and ultrasonographic examinations, have been used to assess fertility in many species, including humans (Baerwald and Pierson., 2004). Ultrasonographic evaluation of the reproductive tract, provides a rapid and noninvasive diagnosis of uterine status. In women, several studies have reported the use of ultrasonography as a tool for predicting

fertility. Ultrasonographic evaluation of endometrial thickness (ET) has been used for over 20 years to evaluate endometrial receptivity during assisted reproduction techniques for humans (Baerwald and Pierson., 2004).

A increase in uterine weights near estrus was due to estrogen-induced endometrial tissue hypertrophy rather than hyperplasia is been reported by Johnson *et al* (1997). Ultrasonographic measurement of ET near ovulation might be a good indicator of hormonal environment, and could be used to assess whether the uterus has been exposed to adequate concentrations of hormones compatible with optimal fertility (Souza *et al*., 2011).

Information concerning morphologic changes of the bovine endometrium during the oestrous cycle is scanty and none have been reported from the Indian sub-continent therefore, endometrial thickness was measured in Holstein Friesean cows with the objective to

1. Characterize changes in endometrial thickness in cycling cows
2. Estimate progesterone concentration around estrus and ovulation

Review of Literature

II. REVIEW OF LITERATURE

2.1 Oestrous cycle and Hormone Profiles

Cattle are polyestrous and display estrus behaviour approximately every 21 days. The oestrous cycle is regulated by the hormones of the hypothalamus (gonadotrophin-releasing hormone; GnRH), the anterior pituitary (follicle-stimulating hormone; FSH and luteinising hormone; LH), the ovaries (progesterone (P_4), oestradiol(17β) and inhibins) and the uterus (prostaglandin F2α; PGF). These hormones function through a system of positive and negative feedback to govern the oestrous cycle of cattle (Roche, 1996). During the follicular phase of the oestrous cycle the hormone environment of basal progesterone due to the regression of the corpus luteum (CL). The increased E_2 concentrations, derived from the rapid proliferation of the pre-ovulatory dominant follicle (DF), with concomitant decrease in circulating concentrations of progesterone, induces GnRH surge which allows the display of behavioural estrus during which heifer/cow are sexually receptive and will stand to be mounted (Frandson *et al*., 2003). This pre-ovulatory GnRH surge induces a coincidental LH and FSH surge (Sunderland *et al*., 1994). Only when serum progesterone concentrations are basal and LH pulses occur every 40–70 min for 2–3 days does the dominant follicle ovulate (Roche, 1996). Ovulation occurs 10–14 hrs after estrus and is followed by the luteal phase of the oestrous cycle. Following ovulation, progesterone concentrations begin to increase due to the formation of the corpus luteum, for the establishment and maintenance of pregnancy and/or resumption of the oestrous cycle (Niswender, 1981).

2.1.1 Progesterone

Progesterone concentrations during the oestrous cycle of *Bos taurus* cattle have been reported by many workers. Plasma concentrations at estrus and diestrus have been reported to range from 0.8 ng/ml at estrus to peak of 11.7 ng/ml in diestrus (Peters, 1986) and from 0.5 to 5.1 ng/ml respectively (Diaz *et al*., 1986). Lower progesterone concentrations in *Bos indicus* than in *Bos taurus* cattle have been reported by many workers. Circulating progesterone concentrations of 4.5 ng/ml (Adeyemo and Heath, 1980) and 3.1 ng/ml (Vaca *et al*., 1985) in the luteal phase have been reported in *Bos indicus* compared to 5.2 ng/ml (Adeyemo and Heath, 1980) in *Bos taurus* cattle. Stahringer *et al*. (1990) reported that Brahman heifers with abnormal oestrous cycles had lower serum progesterone concentrations during the luteal phase than those that had regular oestrous cycles (1.35 ng/ml vs 2.22 ng/ml respectively: $P< 0.005$). Randel (1984) reported that Brahman cows had smaller corpora lutea, with lower progesterone concentration, than Hereford cows (2.7 vs 3.8 ng and 4.8 ng/ml vs 7.0 ng/ml respectively). The occurrence of low circulating progesterone might be due to lower preovulatory LH surge or lowered ovarian response to gonadotropin during CL formation (Randel, 1984) or smaller size corpus luteum (Adeyemo and Heath, 1980).

2.1.2 Estrogen

During the bovine oestrous cycle, plasma estradiol-17β concentrations increase markedly (45 pg/ml) during proestrous and estrus and again during the early luteal phase (mean 16 pg/ml) (Dieleman *et al*., 1986). The estradiol-17β concentration of *Bos taurus* cows has been reported to be 8.05 pg/ml during estrus and 2.88 pg/ml during the mid

luteal phase (Peters, 1986) and 7.1 pg/ml during oestrus and 3.9 pg/ml in diestrus (Schallenberger *et al*., 1988). Sergerson *et al*. (1984) reported concentrations of 7.3 pg/ml in Angus cows vs 5.9 pg/ml, ($P < 0.05$) in Brahman cows during estrus. The endogenous rise in serum estradiol-17β was recorded 24 h before estrus in Brahman cows and 8 h before estrus ($P<0.05$) in Hereford cows (Randel,1984). It is evident that serum estradiol-17β concentrations are lower in *Bos indicus* than in *Bos taurus* and estrus occurs sooner after the proestrous peak in *Bos taurus* than in *Bos indicus*.

2.1.3 Luteinizing hormone

LH values during estrus and diestrus have been reported to be 2.46 ng/ml and 1.8 ng/ml (Peters, 1986) and 1.1 ng/ml and 0.6 ng/ml respectively (Schallenberger and Walters, 1985). The preovulatory LH surge occurs earlier in relation to the onset of estrus and the preovulatory LH surge is shorter in Brahman cows (18.5 h) than in Herford Cows (22.9 h) (Randel and Moseley, 1977). and the preovulatory LH surge following estradiol-17β peak occurs later in Brahman cows (27.8 hrs) than in Hereford cows (22.1 hrs) (Randel, 1984).

2.2 Estrus Synchonization

Ovsynch a fixed-time A.I. synchronization protocol has been developed, tested and is being extensively used in lactating dairy cows (Pursley *et al*., 1998; Stevenson *et al*., 1999). The protocol builds on the basic GnRH-PGF format by adding a second GnRH injection 48 hours after the PGF injection. At the time of GnRH injection , the cow has dominant follicle that ovulates in response to the GnRH induced LH release furthermore, the increase in FSH induced by GnRH induces recruitment of a new pool of follicles in

approximately 2 days (day 7) and one of the follicles is selected to become the dominant follicle (Moreira *et al*., 2000). On day 12 of the cycle (7 days after the injection of GnRH), PG is injected to regress both the original CL present at day 5 of the cycle and a newly formed CL that was induced by the injection of GnRH on day 5 of the cycle. The decrease in progesterone associated with regression of the CL accelerates the growth of newly recruited dominant follicle and a second injection of GnRH 2 days after the injection of PG. Induces ovulation 24 to 32 hours later (Pursley *et al*., 1995). Predicting that ovulation will occur around this time, the timed insemination is performed approximately 16 hours after GnRH injection. This permits sufficient time for sperm accquire the capacity to fertilize the ova and, a fertile population of sperm are present to carry out fertilization.

If an interval of less then 7 days is used between GnRH and PG injection, the ability to effectively regress a newly developed CL is reduced. If the second injection of GnRH is delayed longer than 48 hours or 2 days, then more cows are detected in estrus prior to GnRH, cows become asynchronized, and the timing of insemination is not correct.

Success of the program dependents on whether lactating dairy cows are cycling as well as stage of the oestrous cycle at the time the Ovsynch protocol is initiated in cycling cows Pursley *et al*. (1998), if the group of cows are not cycling then the pregnancy rate is somewhat less even though the ovsynch protocol may induce some cows to begin to cycle and may have chance to conceive (Pursley *et al*., 1995).

Follicles that have periods of dominance longer than 5 days (Austin *et al*., 1999) or in those cows initiated ovsynch program is in the early stages of the oestrous cycle are less fertile (Vas concelos *et al*., 1999; Moreira *et al*., 2000). Though follicles may ovulate but oocytes are less fertile, or some cows may fail to ovulate in response to the second injection of GnRH.

2.3 The Uterus and Endometrium

2.3.1 Uterus

In mammals, the uterus is the organ of pregnancy (Senger, 2011). To establish pregnancy, endometrial cells change morphologically and functionally throughout the oestrous cycle (Weitlauf, 1994). and estradiol-17 and progesterone play important roles in regulating the function of the endometrium (Jabbour *et al*., 2006).

The wall of the uterus is composed of outer serous coat, the perimetrium; middle muscular coat, the myometrium and the inner mucous coat, the endometrium.

The perimetrium is derived from peritoneum and covers the entire organ except the lateral border. The myometrium is thick and composed of three layers of smooth muscles. Although poorly differentiated, these are outer longitudinal, middle circular, and inner longitudinal in orientation, the growth of myometrium is stimulated by estrogen (Mossman, 1987). Further increase in length and number of muscle fibers take place during pregnancy by the stimulation of placental estrogen (Gosh, 2006).

2.3.2 The Endometrium

The endometrium consists of surface epithelium and lamina propria. The endometrium or the mucous membrane is continuos with the peritoneum through the fimbriated ends of fallopian tubes and with the mucosa of vagina through the external os. The epithelium is lined by ciliated columnar cells at the horn, columnar in the body and neck and stratified at the external os. The cells lining the surface epithelium of the endometrium are tall at the time of estrus and they become more columnar or cuboidal at the post estrus phase. The lamina propria consists of stroma cells, tubular uterine glands, lymphatics, blood vessels and nerves. The endometrial glands are coiled tubular and branched structures, which open on the endometrial surface but not on the caruncular area. These glands extend upto the inner surface of the myometrium and are lined by columnar cells with occasional cilia. In the endometrium of the horn and body are found a large number (about one hundred) of caruncles or cotyledons.(Gosh, 2006).

All mammalian uteri contain endometrial glands that synthesize and secrete or transport a complex array of proteins and related substances termed histotroph. Uterine glands secretions are important for fetal well being in ruminants. Evidence has accumulated from during the last century supports an unequivocal role for secretions of endometrial glands as primary regulators of conceptus survival, development, onset of pregnancy recognition signals, and implantation/placentation (Bell., 1988). In primate and subprimate species, changes in endometrial secretory activity are proposed to regulate delayed implantation (Given and Enders, 1989; Renfree and Diapause, 1993). In marsupials, carnivores, and roe deer.

The proliferation and cell death of endometrial cells throughout the menstrual cycle is important for maintenance of endometrial function in women (Gargett *et al*., 2008). Where as in non primates since menstruation do not occur, the morphological change in the endometrium seem to be less important. However, cyclic cell proliferation and apoptosis have been observed in the uterus of murine (wood *et al*., 2007), rat (Sato *et al*., 1997), canine (Van cruchten *et al*., 2003), equine (Roberto da Costa *et al*., 2007) , porcine (Okano *et al*., 2007) and bovine (Miki *et al*., 2013).

Estradiol -17β induces expression of growth factors throughout the oestrous cycle in bovine endometrium (Robinson *et al*., 2000; Tasaki *et al*., 2010) and promotes the proliferation of endometrial epithelial cells by stimulating the production a variety of growth factors in stromal cells (Xiao and Goff, 1998; Miki *et al.*, 2013). In early luteal and follicular stages, cell proliferation is observed as a result of growth factors stimulated by estradiol 17β. (Miki *et al*., 2013*)*. The removal of unwanted cells by apoptosis plays an important role in the maintenance of homeostasis in the endometrium (Jin and El Diery, 2005). Endometrial apoptosis is promoted at the periovulatory periods or at luteolysis in several species (Wood *et al*., 2007). When pregnancy does not occur , endometrium gets renewed for the next chance to be pregnant. Endometrial withdrawl of progesterone induced endometrial apoptosis in rabbits (Rotello *et al*., 1992), withdrawl of esrtadiol 17β in mouse (Jo *et al*., 1993) or by withdrawl of both progesterone and estaradiol 17β in women (Song *et al*., 2002) while withdrawl of progesterone or estradiol 17β or both induces apoptosis of the endometrium in bovine (Miki *et al.*, 2013).

2.3.3 Endometrium and Conceptus

In pregnant cattle, endometrial glands undergo extensive hyperplasia and hypertrophy, presumably in response to increasing demands of the developing conceptus for uterine histotroph (Stewart *et al*., 2000.).

The growth and development of the conceptus (embryo/fetus and associated extraembrionic membranes) in mammals unequivocally requires progesterone and placental hormone actions on the uterus that regulate endometrial differentiation and function, pregnancy recognition signalling, uterine receptivity for blastocyst implantation, and conceptus-uterine interactions (Carson *et al*., 2000. Paria *et al*., 2000. Gray *et al*., 2001). Hormones from the conceptus act on the uterus in a paracrine manner to establish and maintain pregnancy.

The uterine endometrium plays a pivotal role for the establishment of early conceptus-maternal communication and maintenance of pregnancy. This involves dynamic changes in the uterine epithelia that are tightly regulated by changes in steroid hormones, cytokines, and growth factors and their receptors (SUNDER, S. and LENTON, E.A., 2000). These factors help to establish receptivity of the uterine luminar epithelium to the developing conceptus and play a key role in regulating differentiated functions of the uterine glandular epithelium, which is required for histotroph secretion and stromal cells, which are required for protection of the developing semiallograph conceptus from the maternal immune system (Spencer *et al*., 2008).

Available evidence supports the idea that hormones from the placenta act directly on the uterine endometrium to regulate cell differentiation and function (Xiao and Goff,

1998; Miki *et al*., 2013). In domestic animals, the endometrial glands undergo a program of hyperplasia followed by hypertrophy that appears to be dependent on temporal and spatial actions of hormones from the placenta (Miki *et al*., 2013). Endometrial gland morphogenesis during pregnancy allows for the endometrium to increase output of secretory proteins that are transported to the fetus. Histotrophic nutrition from the endometrium is the first available nutrition for the developing conceptus and appears to be essential for the survival and growth of conceptus throughout pregnancy in domestic animals (Fuller *et al*., 1998).

2.4 Endometrium and fertility

2.4.1 In human patients

Adequate/optimum uterine blood supply is required for steroid hormones for various growth factors and cytokines to reach the endometrium, especially to its functional layer (Coulam *et al*., 1994). The endometrium has to reach a certain thickness for successful pregnancy to occur (Gonen and Casper, 1990). They have found that endometrial thickness was significantly associated with in vitro fertilization outcome (Noyes *et al*., 1995). Pregnancy fails to occurr when the endometrium thickness was less than 7mm (Dickey *et al*., 1992). Two measures of uterine receptivity that are commonly used are the thickness and pattern of the endometrium as measured by ultrasound during the preovulatory period. Uterine receptivity is an important factor that may affect embryo implantation.

Several studies have suggested a poor pregnancy rates when the endometrium exceeded a certain thickness. One study showed that there were no pregnancies with an

endometrial thickness over 15 mm (Kupesic *et al*., 2001). Weissman *et al.* (1997) showed that pregnancy rate was significantly lower when the endometrial thickness exceeded of 14 mm, and they also suggested a possible increase in spontaneous abortion rates. Rashidi *et al* (2005) reported no pregnancies with an endometrial thickness >12 mm (n = 9). However, Richter *et al* (2007) and Ai-Ghamdi *et al.* (2008) demonstrated a significant increase in the pregnancy rates as endometrial thickness increased, which was independent of the number and quality of the embryos transferred.

Many studies have found a thin endometrium to be associated with a lower implantation rate, but no absolute cutoff for endometrial thickness exists (Gonen *et al*., 1989) optimum pregnancy rates have been reported in cycles with endometrium <6 mm, and a successful pregnancy has been reported with endometrial thickness of only 4 mm. Noyes *et al.* (2001) found that pregnancy rate and live birth rate were significantly lower when endometrial thickness was less than 8 mm than when endometrial thickness was ≥9 mm. Casper (2011). speculated that it may be related to oxygen tension. When the thickness measured by ultrasound is < 7 mm, the functional layer is thin or absent, and the implanting embryo would be much closer to the spiral arteries and the higher vascularity and oxygen concentrations of the basal endometrium. The high oxygen concentrations near the basal layer could be detrimental compared with the usual low oxygen tension of the surface endometrium.

In women, a thin endometrium, is caused by impairment of the normal process of endometrial growth. Low pregnancy rates are noted in patients with thin endometrium. (Richter *et al*., 2007; EI-Toukhy *et al*., 2008; Miwa *et al*., 2008). Little information is

available regarding the factors responsible for impaired endometrial growth in patients with a thin endometrium. It is suggested that a thin endometrium may be due to high blood flow impedance of uterine radial arteries, which are in the lower extremity of uterine arteries (Miwa *et al*., 2008). Uterine blood flow is an important factor for endometrial growth (Rogers *et al*., 1998; Ng EH *et al*., 2007).

It is found that high blood flow impedance of uterine radial arteries, could impair the growth of the glandular epithelium and results in a decrease in expression of vascular endothelial growth factor (VEGF), which is a key factor for regulating angiogenesis in the human endometrium (Sugino et al., 2002). Low VEGF levels cause poor vascular development, which in turn decreases blood flow to the endometrium. Vitamin E and potential nitric oxide (NO) donors such as L-Arginine and sildenafil citrate are reported to increase uterine radial artery blood flow (Akihisa *et al*., 2010).

Apart from blood flow, adequate uterine estrogenization is also a necessary condition for uterine receptivity (Li *et al*., 1992). Estrogens takes part in key mechanisms that regulate uterine preparation to embryo implantation, such as stimulation of endometrial proliferation and improvement of uterine (De Ziegler *et al*., 1991) and endometrial (Yang *et al*., 1999) perfusion. In addition, estrogens are also likely to stimulate myometrial contractile activity during the follicular phase of the menstrual cycle (Lyons *et al*., 1991). Subsequently, uterine contractility decrease during the luteal phase as a result of progesterone (Lyons *et al*., 1991), presumably to assist in embryo implantation (Fanchin *et al*., 1998).

Some patients fail to achieve proper uterine estrogenization and lead to defective endometrial thickness (Gonen *et al*., 1989; Hassan and Saleh, 1996), uterine perfusion (De Ziegler *et al*., 1991; Yang *et al*., 1999), and pregnancy rates (Gonen *et al*., 1989; De Ziegler *et al*., 1991; Hassan and Saleh, 1996; Yang *et al*., 1999). In these cases, vaginal E_2 administration may be an interesting alternative. A recent study by Tourgeman *et al.* (1999) showed that vaginal E_2 administration induces serum and endometrial tissue E_2 concentrations, respectively, 10-fold and 70-fold higher than the oral route at similar doses (Tourgeman *et al*., 1999).

2.4.2 In animals

In mammals uterus is the organ of pregnancy (Senger, 2011). To establish pregnancy, endometrial cells change morphologically and functionally throughout the oestrous cycle (Weitlauf, 1994). Ovarian steroids oestadiol- 17β and Progesterone play important roles in regulating the function of endometrium (Jabbour *et al*., 2006). The proliferation and cell death of endometrial cells throughout the menstrual cycle is important for maintenance of endometrial function in women (Gargett *et al*., 2008). Where as in non primate species donot menstruate, the morphological change in the endometrium seems to be less important. However, cyclic cell proliferation and cell death by apoptosis have been observed in the uterus of murine (Wood *et al*., 2007), rat (Sato *et al*., 1997), canine (Van cruchten *et al*., 2004), equine (Roberto da Costa *et al*., 2007), porcine (Okano *et al*., 2007) and bovine (Miki *et al*., 2013).

Estradiol -17β induces expression of growth factors throughout the oestrous cycle in bovine endometrium (Robinson *et al*., 2000; Tasaki *et al*., 2010) and promotes the

proliferation of endometrial epithelial cells by stimulating the production a variety of growth factors in stromal cells (Xiao and Goff, 1998; Miki *et al*., 2013). Cell proliferation in early luteal and follicular stages was observed as a result of growth factors stimulated by estradiol 17β (Miki *et al*., 2013). The removal of unwanted cells by apoptosis plays an important role in the maintenance of homeostasis in the endometrium (Jin and El Diery , 2005). Endometrial apoptosis is promoted at the periods of luteolysis or periovulatory period in several species (Wood *et al*., 2007). When pregnancy fails to occur, endometrium gets renewed for the ensuing pregnancy. Endometrial apoptosis is induced by progesterone withdrawl of in rabbits (Rotello *et al*., 1992), by 17β esrtadiol withdrawl in mouse (Jo et al., 1993) or by with drawl of both progesterone and estaradiol 17β in women (Song *et al*., 2002), where as in bovine endometrium withdrawl of progesterone or estradiol β or both induces apoptosis of the endometrium (Miki *et al*., 2013).

Although loss of endometrial tissue fail to occur in cows, but the thickness of the endometrium (Pierson and Ginther, 1987) and certain histological aspects (Dhaliwal *et al*., 2002; Wang *et al*., 2007) vary considerably during the oestrous cycle. An early study using Holstein heifers (Pierson and Ginther, 1987), described remarkable ultrasonographic changes in the uterus and endometrium near ovulation. In their study, endometrial thickness increased at the time of expected luteolysis, and maximum endometrial thickness was appreciable on the day prior ovulation (Pierson and Ginther, 1987). Nevertheless, also several studies have also reported decrease in endometrial thickness and associated changes in endometrial echotexture after ovulation, which

occurs probably due to increasing circulating P4 concentrations (Pierson and Ginther, 1987; Bonafos *et al*., 1995; Jimenez-Krassel *et al*., 2009).

Souza *et al.* (2011) studied the ultrasonography evaluation of endometrial thickness as a predictor of fertility in high producing dairy cows. They reported, that when the endometrial thickness of the cows were less than 7mm on the day of ovulation, pregnancy failed to occur. They established a strong relationship between endometrial thickness and fertility in cattle. and concluded that the endometrial thickness could be a predictor of ovulation and failure of pregnancy.

2.5 Ultrasounography

The real-time, B-mode diagnostic ultrasound has been used increasingly as an imaging modality in bovine reproduction, as it can provide some answers to questions dealing with the bovine reproduction cycle and its disorders.

Ultrasonography can be used to study the normal anatomy of reproductive tract, pregnancy diagnosis, time of ovulation and abnormal changes in uterus and ovaries. Depending on the physiological state of the organ, a variety of structures can be imaged within the stroma. In a small, inactive ovary the outer layers (cortex) can contain small, anechoic follicles while the innerzone (medulla) appears free of follicular activity. In a larger, active ovary the differentiation into z ones is less distinct, and the stroma is imaged as narrow, echogenic bands displayed around the more obvious features of CLs and pre-ovulatory follicles.

2.5.1 Ultrasonography in oestrous cycle

The uterine horns are an excellent location to obtain valid measurements of endometrial thickness, since the internal uterine bifurcation could be used as a guide to provide a consistent location for obtaining measurements from both horns in a cow (Souza *et al*., 2011).

The diestrus sonogram of cows revealed homogeneously gray with no evidence of interstitial edema. Anestrous uterus had prominent edematous development and heterogeneous utrasonographic texture. In the intermediate uterus, edema and heterogeneous texture were only moderately discernible (Pierson and Ginther, 1987).

Ultrasonographic monitoring of the uterus during the impending time of estrus revealed an increase in endometrial folding accompanied by a disruption in the imaging of distinct layers of the uterine wall due to oedema. This swelling is at maximum on the day of standing estrus, while the endometrial folding starts to recede two days post-ovulation. There is an increase in the anechoic fluid centre to the lumen, which continues up to the day of standing estrus, when the intrauterine fluid is released per vagina, a reduction in the diameter of the anechoic lumen are observed. The changes occurring in the ovary and resultant CL formation are described in the section on normal ultrasonographic anatomy (Pierson and Ginther, 1987; Boyd and Omran, 1991).

2.5.2 Ultrasonography of Uterine horns

As the uterine horn distal to the interarcuate ligament is in spiral form, the images produced when the transducer passes over the uterine coils will vary. The cross sectional

image is of an external hypoechoic layer, outlined by a dark ring, comprising the vascular coat and the longitudinal, circular and oblique layers of the myometrium. This muscular layer is imagined in differing planes and thus produces a signal of varying echogenicity. It is separated by a further dark ring from the hyperechoic endometrium, which forms a wide concentric layer around the inner anechoic uterine lumen. The lumen is seen as an anechoic central line running between the strongly echogenic endometrial bands. Variations in the ultrasonographic appearance of uterine horns will occur depending on the state of the reproductive cycle, as the tissue interfaces alter with the oedematous nature of the uterine wall seen at impending estrus.

2.5.3 Ultrasonography of Endometrium

In the follicular phase, the growing follicles produce increasing amounts of E_2 that induce proliferative endometrial changes. After ovulation, the corpus luteum produces progesterone, which leads to secretory changes. Since serum hormonal estrogen and progesterone levels cannot always accurately predict the development of the endometrium and other methods such as histological studies are too invasive, ultrasound has been used as a non-invasive technique to monitor. The measurements are easy to perform, easily reproducible, and have been shown to have a good interobserver correlation. Endometrial thickness and pattern have been implicated in the successful outcome of assisted reproductive technologies.

Characteristic changes of the tubular genitalia visible by ultrasonography involve thickness of the uterine body, evidence of increased vascularity and edema, and accumulations of mucus (Pierson and Ginther, 1987). The period of proestrous and estrus

Days -4 to -1 (Day 0 = ovulation) is characterized by 1) increasing thickness of the uterine body, 2) accumulation of luminal fluid first in the uterus followed in succession by fluid in the cervix and vagina, and 3) minimal curl of the uterine horns. Conversely, diestrus, (Days 3-16) is characterized by minimal thickness, minimal luminal fluid, and maximal curl to the uterine horns.

Ultrasonographic evaluation of endometrial thickness (ET) has been used for over 20 years to evaluate endometrial receptivity during assisted reproduction programs for humans (Baerwald and Pierson, 2004). Ultrasound measurement of endometrial thickness is a simple and reproducible method to evaluate endometrial proliferation (Delisle *et al*., 1998). Although cows do not lose endometrial tissue due to menses, the thickness of the endometrium (Pierson and Ginther, 1987) and certain histological aspects (Dhaliwal *et al*., 2002; Wang *et al*., 2007) varied considerably during the oestrous cycle.

Pierson and Ginther (1987), reported remarkable ultrasonographic changes in the uterus and endometrium near ovulation in Holstein heifers. In that study, endometrial thickness increased at the time of expected luteolysis, with maximal endometrial thickness on the day before ovulation. Several studies have described the decrease in endometrial thickness and changes in endometrial echotexture after ovulation, probably due to increasing circulating progesterone concentrations (Pierson and Ginther, 1987; Jimenez-Krassel *et al*., 2009: Bonafos *et al*., 1995).

2.5.4 Ultrasonography of uterine Pathology

Intrauterine pathology (e.g. fetal death and abortion, macerated fetus and pyometra) can be discerned sonographically. Pyometra usually appears as a distention of

the uterine lumen with an image of mixed echogenicity, containing hypoechoic material intermingled with some hyperechoic signals, often producing a swirling effect in real-time. With mummified or macerated fetus, irregular hyperechoic images are randomly detected within what may be either an anechoic or hypoechoic background, although usually these strong signals are without a regular anatomical outline. Fetal death is initially detected by an absence of heart-beat and this is accompanied by detachment of the fetal membranes from the endometrial layer. The membranes lose their tense appearance and begin to image as if floating within the anechoic uterine lumen. The conceptus, with membrane, will traverse through the uterine body, cervix ans vagina, and may be imaged in any of these areas accompanied by anechoic fluid. Even after the conceptus is expelled, the CL will maintain good size and echogenicity for a number of days post-abortion, and thus it is of importance when giving a positive pregnancy diagnosis from 24 days onwards that the operator should seek out the fetus and confirm viability with a heart-beat, and not just rely on imaging a distended anechoic uterine lumen and large CL (Taverne and Willemse, 1989).

A local inflammatory response within the endometrium would result in some degree of tissue thickening. Barlund *et al*. (2008) reported that endometrial thickness measurements >8 mm were less useful than endometrial cytobrush cytology with a sensitivity of 3.9% and a specificity of 89.2% compared to cytobrush cytology using a PMN threshold of >8% to diagnose subclinical endometritis. Measurement of endometrial thickness could be easily influenced by the location on the uterine horn where the measurement was taken and the positioning of the ultrasound probe during the measurements (Barlund *et al*., 2008).

Materials & Methods

III. MATERIALS AND METHODS

3.1. Location

The present study was conducted at the Instructional Livestock Farm, Veterinary College, Hebbal, Bangalore, between Nov. 2013 and Dec 2013. The Instructional livestock farm is located at latitude 12^0 58^I 34^{II} N, Longitude 77^0 36^I 11^{II}.

3.2. Animals

Ten Holstein Friesean cross bred cows aged 4 to 5 yr old in their second to third parity were selected. The experimental animals were maintained in good body condition under uniform managemental condition. They were free from Brucellosis and Tuberculosis and had been vaccinated regularly against foot and mouth disease, hemorrhagic septicaemia, black quarter and anthrax. They were treated regularly for flies and ticks whenever they appeared.

3.3. Reproductive Status

At the beginning of the experiment, the cows were examined per rectum. Uterus and ovaries were palpated to confirm that each animal had a palpably normal reproductive tract and was not pregnant and had no apparent uterine disorder prior to ovsynch protocol.

3.4. Ultrasonography

Ultrasonographic examination was conducted using a portable ultrasonography machine (Honda Electronics, Japan), equipped with 5-10 MHz linear probe. The animals were restrained in trevis and the perineum was cleaned with soap water. 5- 10MHz linear

rectal probe was guided into the rectum by carrying it in palm. The uterus was identified and probe was placed directly over the uterine horns for evaluation of endometrial thickness.

The endometrial measurements were carried out by using electronic callipers in cross sectional frozen image. 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 edge of the endometrial lumen to the visualized interface between the endometrium and myometrium. Endometrial thickness was determined for each of the uterine horns separately, and the average value was established for each cow.

Cows were examined for uterine health before the initiation of the ovsynch protocol. They were examined for endometrial thickness on the first GnRH administration of ovsynch protocol and for five days continuously from the day of PGF2α (for 2 day after the second GnRH treatment of the ovsynch protocol).

3.5. Estrus synchronization

To synchronize estrus the cows were treated with ovsynch protocol. Where in, on day 0 GnRH 20µg (Buserilin-Acetate; 0.004mg/ml; Receptal, MSD Animal Health Intervet, India Pvt. Ltd) given intramuscularly. The Prostaglandin (PG) (Prostaglandin $F_2\alpha$ 253mg/ml; Cloprostenol , MSD Animal Health Intervet, India Pvt. Ltd) was injected intramuscularly on seventh day after GnRH, after confirming the presence of CL by per-rectal examination. After 72hrs of PGF2α treatment animals were injected with second GnRH injection intramuscularly at a dose rate of 20µg. All animals were inseminated

16hrs after the second GnRH treatment. Synchronization protocol was used to reduce the work load by handling of animals as a group.

3.6. Estrus detection

Cows were observed for estrus after 24 – 48hrs of PGF2α injection. Females standing to be mounted by other females and displaying secondary signs of estrus were considered to be in estrus. The secondary signs of estrus considered were mucous discharge from the vulva, oedema, and reddening of the vulva, sniffing and bellowing. In the absence of behavioural signs of estrus, at the time of observation. Cows with no signs of estrus were considered to be not in estrus.

3.7. Artificial Insemination

Artificial Insemination were performed by using Frozen semen of Holstein Friesian from state semen collection centre, Hessarghatta, Bangalore. The Holstein Friesian semen, on thawing, had a motility of 40-50 percent. The frozen semen in 0.25 ml mini straws was thawed at 37^0C for 30 sec before insemination.The experimental animals were inseminated 16hrs after the second GnRH injection irrespective of whether or not they were detected in estrus.

3.8. Blood collection

The blood samples were collected by Jugular venipunture, into 10 ml in sterilized vacutainer tube without anticoagulant (BD Vacutainer®, USA). Blood samples were kept at room temperature as a slant for 4-8hrs for separation of blood serum. The expressed Serum was separated by centrifugation at 3000 rpm for 15 minutes. Serum was

transferred into sterilized serum vials, and stored at -20 ^{o}C until required for hormone assay.

3.9. Progesterone estimation

The concentration of serum progesterone were determined by eProcheck ® using progesterone test kit for Bovine serum, Germany. Progesterone concentration estimated was based on ELISA as per the manufacturer instructions.

3.10. Statistical Analysis

The mean and standard error for the blood serum progesterone and endometrial thickness of ovsynch group of animals were tabulated for different days. The relationship between the independent variable and progesterone concentration for different days were established by pearson correlation coefficient test as the methods described by Steel and Torrie, (1981). A value of $P \leq 0.05$ was considered significant.

Results

IV. RESULTS

All cows showed luteal regression following PGF2α treatment, with serum progesterone concentration decreasing to less than 1ng/ml. In 4 out of 10 cows progesterone concentration was less than 1ng/ml in 24 hrs post PGF2α while in the remaining six, progesterone values reduced to 1ng/ml at 48 hrs.

In the present study, based on initial evaluations, most cows responded adequately to the Ovsynch protocol, as reflected by low serum P4 concentrations (<1 ng/mL) 24 h after PGF2α treatment, except in one (cow no 10) which had progesterone concentration value of 0.2ng/ml at PGF2α injection and the progesterone concentrations ranged from 0.2 – 1.4ng/ml throughout the experimental period respond to ovsynch protocol. This animal did not show ovulation at the expected time after the first GnRH as evaluated by ultrasonography and indicated by progesterone concentration of 0.2ng/ml.

On the day of PGF2α injection uterine tone in the experimental animals were either flabby or subtonic. Except for One animal all the others had Corpus Luteum on ultrasonography and palpation.

Mean endometrial thickness and serum progesterone concentration from PGF2α to second GnRH treatment during the Ovsynch protocol in cycling cows is shown in table 1 and figure 1. Endometrial thickness and serum progesterone concentration from PGF2α to second GnRH treatment during the Ovsynch protocol in individual cycling cows is represented in graph as figure 2. Echogenicity and texture of endometrium with its measurement is shown in figure 3.

4.1 Progesterone concentration in Ovsynch protocol

On day 0, before the first GnRH injection of Ovsynch protocol, Progesterone values in six of the ten animals was > 1ng/ml and in the other four < 1ng/ml. On seventh day of protocol the day of PGF2α administration, the progesterone concentrations were 6.17 ± 1.2ng/ml ranging from 0.2 – 12.4 ng/ml. After the administration of PGF2 alpha, on the day of expected luteolysis (24hrs after PGF2α) there was drastic reduction of progesterone values in all animals from 6.17 ± 1.2ng/ml to 1.19 ± 0.17ng/ml. and the values remained at less than <1ng/ml on day 2,3 and 4 after PGF2α administration (Table 1).

4.2 Endometrial Thickness in Ovsynch protocol

The endometrial thickness which was 4.89 ± 0.21, on the day of PGF2α administration, showed an increase to 5.02 ± 0.35 at the time of expected luteolysis, approximately 24hrs after PGF2α administration. Expecting ovulation to happen around day 3 or 4 after PGF2α, it was observed that the endometrial thickness attained a maximum thickness during this time. On an average it was 5.92 ± 0.19mm on day three and 5.42 ± 0.19mm on day four after PGF2α. After ovulation endometrial thickness showed a decrease trend from 5.47 ± 0.41mm to 5.06 ± 0.18mm.

Endometrial thickness increased after PGF2α treatment from 4.89 ± 0.21mm to 5.02 ± 0.35mm in 24 hr, after PGF2α. It remained similar at day two (4.54±0.37mm) and day three (5.92±0.19mm) after PGF2α and after second GnRH treatment endometrial thickness decreased to 5.06 ± 0.18mm (Table 1).

4.3 Endometrial thickness and progesterone

On day three after PGF2α, ovulation occurred in 6 of 10 animals. These six animals showed maximum endometrial thickness ranging from 5.2 – 6.7mm and averaging at 6.18 ± 0.2mm and progesterone values at that time were 0.7 ± 0.2ng/ml. In the remaining 4 animals ovulation occurred on day 4 during which time the endometrial thickness ranged from 4.6 - 8.5mm, averaging at 6.47 ± 0.75mm and progesterone values ranging 0.3 – 0.8ng/ml with mean of 0.62 ± 0.11ng/ml.

After ovulation mean endometrial thickness of ten animals showed a decreasing trend from 5.47 ± 0.41mm to 5.06 – 0.18mm where as progesterone values showed an increasing trend from 0.76 ± 0.14ng/ml to 1.08 ± 0.28ng/ml. Despite decreasing concentration of progesterone near ovulation to <1ng/ml, there was considerable variation in endometrial thickness (range of 4.6 – 8.3mm) in the animals (Table 1, Figure 1).

Based on data from each cow, each day there was no correlation between serum progesterone concentration and Endometrial Thickness ($r = 0.430$, $p > 0.05$)

4.4 Endometrial thickness and uterine tone

On the day of PGF2α injection uterine tone in the experiment animals were either flabby or subtonic. After day 2 of PGF2α administration the endometrial thickness ranged between 2.9 to 6.9mm with the uterine tone ranging from tonic to subtonic. Next day as the endometrial thickness ranged from (4.6- 6.7mm), the tonicity of uterus changed from sub-tonicity to tonicity. There was gradual increase in uterine tone with increase in

endometrial thickness, despite lower or high progesterone values for example cow with endometrial thickness 8.3mm had a better tone than with 5.3mm of endometrial thickness.

In summary endometrial thickness increased after PGF2α treatment from 4.8 to 5.02 mm after PGF2α administration, then remained similar at second day (4.54 mm) and third day (5.9mm) after PGF2α administration. However after second GnRH treatment, endometrial thickness decreased to 5.06mm. Individually also 8 of 10 animals displayed similar trend of increasing endometrial thickness, where as 2 of them showed reducing endometrial thickness after PGF2α administration(Figure 2).

Table 1: Mean ± SEM profile of endometrial thickness, and serum P_4 concentrations from PGF2α to second GnRH treatment during the Ovsynch protocol in individual cows

Day	Endometrial thickness (mm)	Progesterone (ng/ml)	r value
0	4.47±0.24	3.78±1.14	0.577 (P > 0.05)
7	4.89±0.21	6.17±1.12	0.315 (P > 0.05)
8	5.02±0.35	1.19±0.17	0.330 (P > 0.05)
9	4.54±0.37	0.85±0.18	0.256 (P > 0.05)
10	5.92±0.19	0.71±0.12	0.066 (P > 0.05)
11	5.47±0.41	0.76±0.14	0.234 (P > 0.05)
12	5.06±0.18	1.08±0.28	0.225 (P > 0.05)

Figure 1: Mean ± SEM profile of endometrial thickness, and serum P_4 concentrations from PGF2α to second GnRH treatment during the Ovsynch protocol in individual cows.

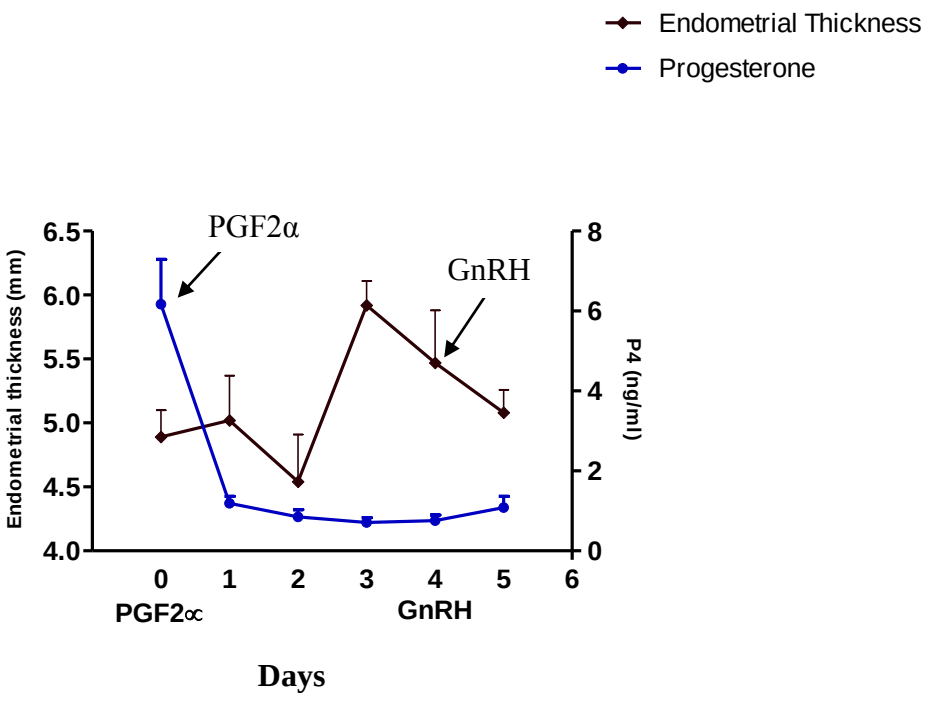
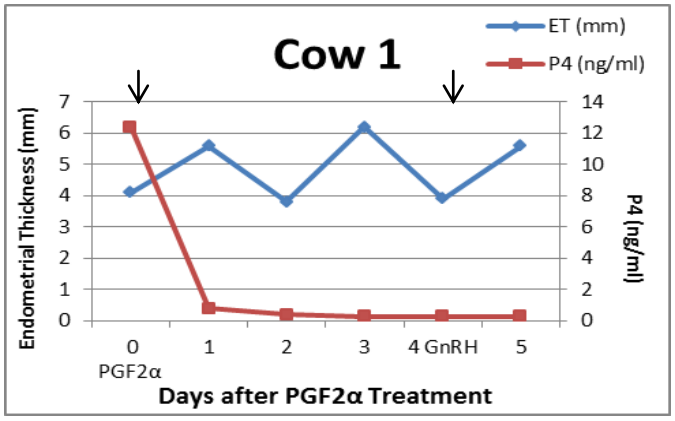
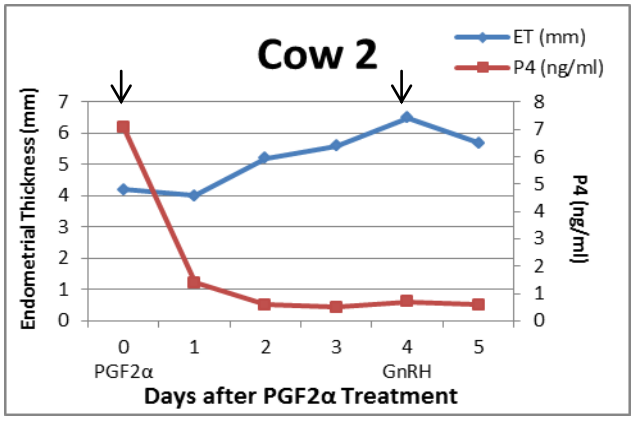
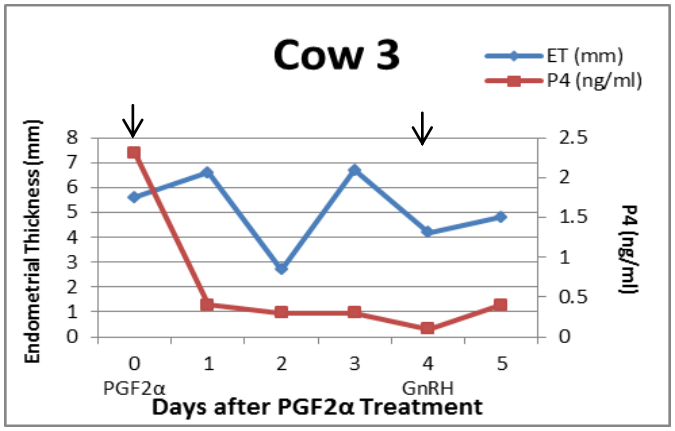
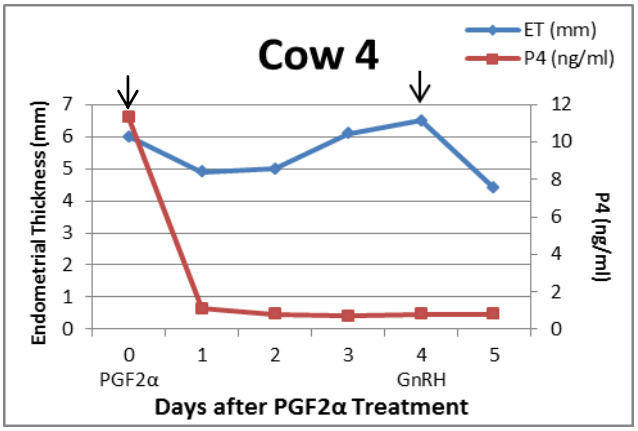


Figure 2: Profile of endometrial thickness, and serum P_4 concentrations from PGF2α to second GnRH treatment during the Ovsynch protocol in individual cows.









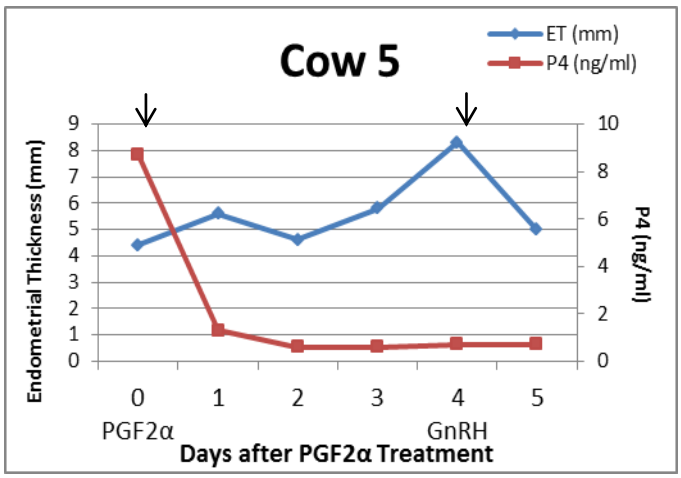
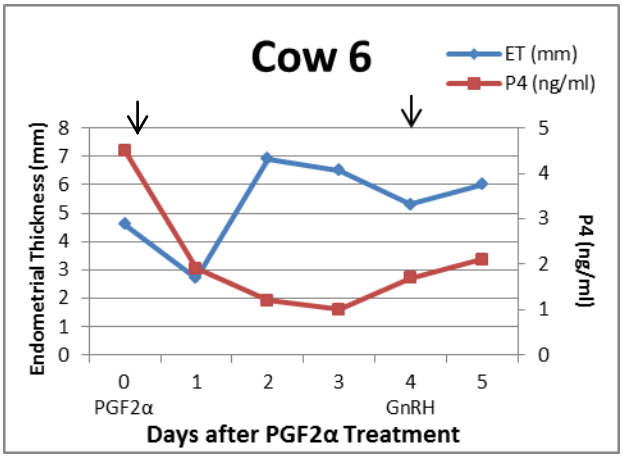
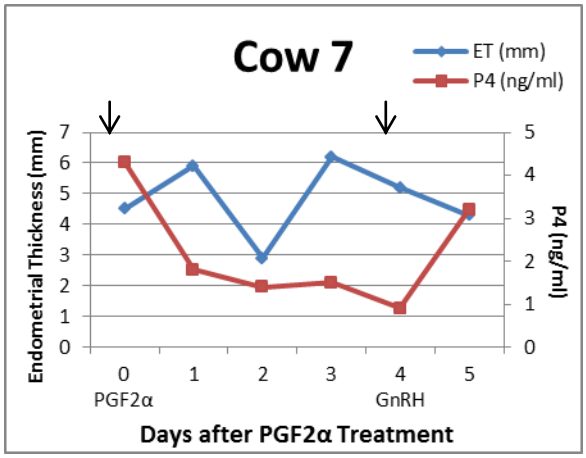
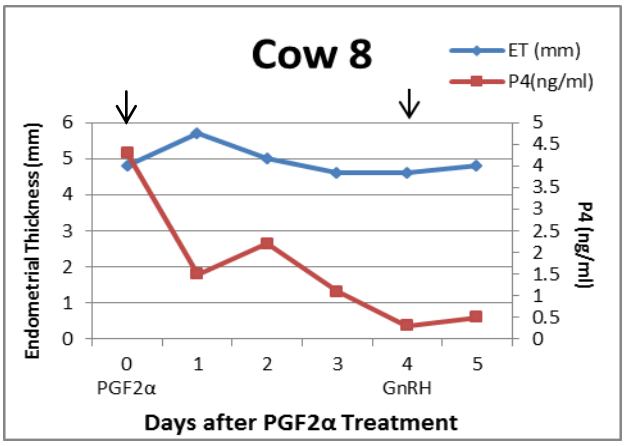
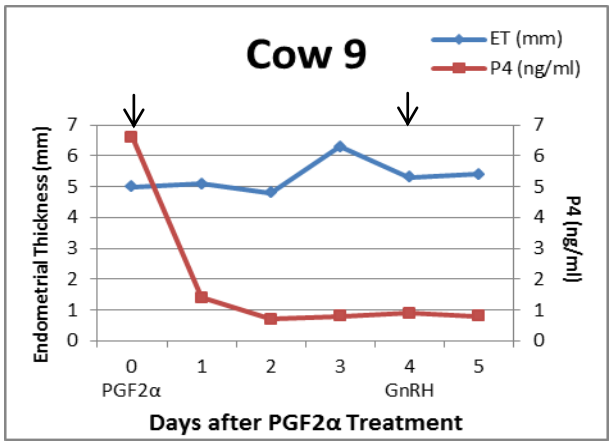


Figure 2: Profile of endometrial thickness, and serum P_4 concentrations from PGF2α to second GnRH treatment during the Ovsynch protocol in individual cows.









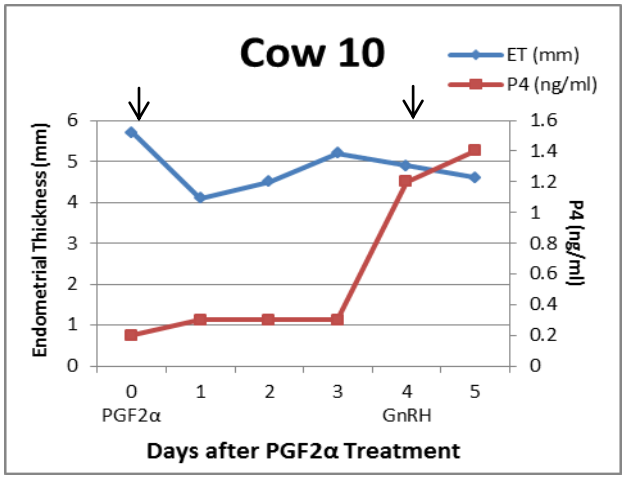
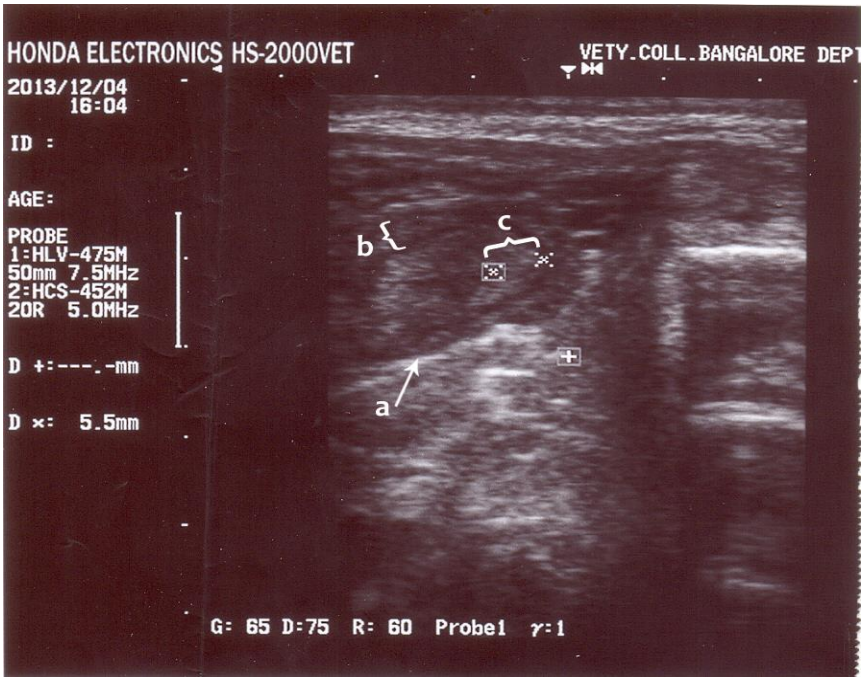
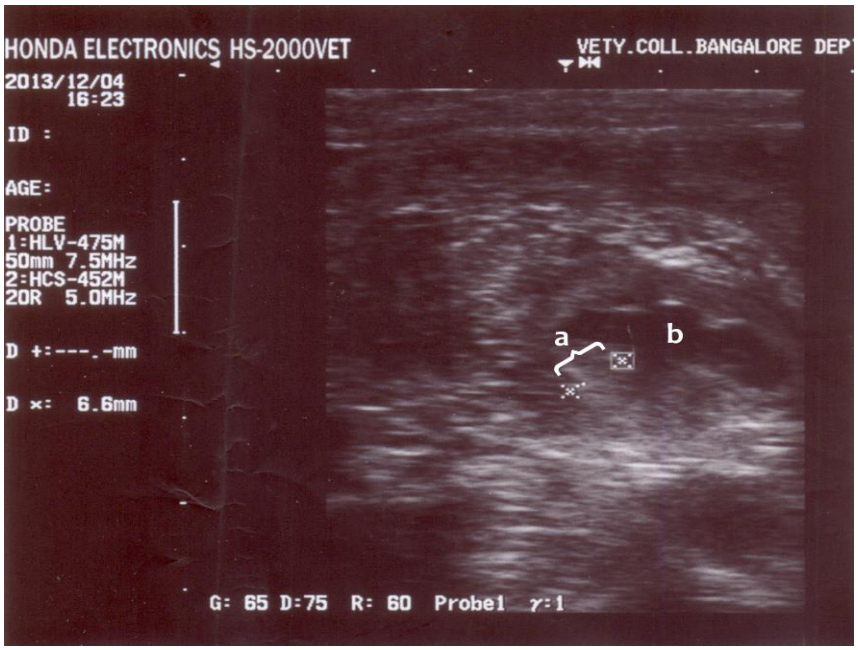


PLATE-1: Ultrasonographic image showing Cross section of uterus demonstrating different layers.

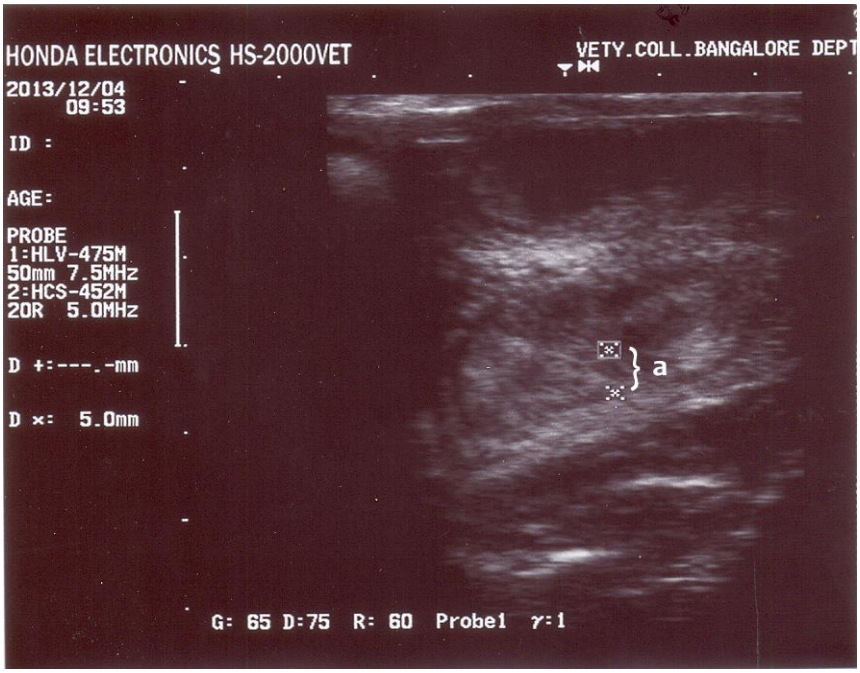


a = PERIMETRIUM ; b = MYOMETRIUM ; c = ENDOMETRIUM

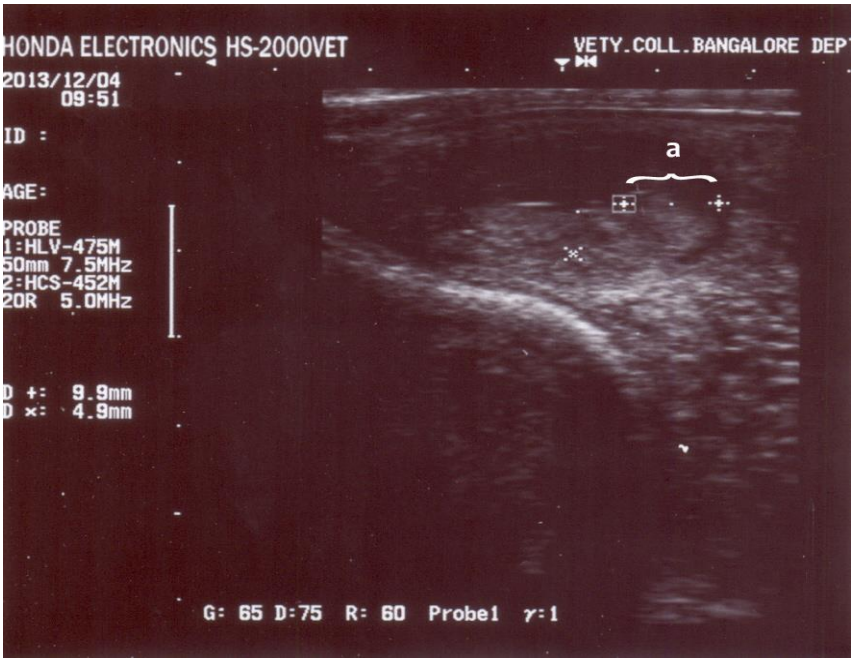


a = ENDOMETRIUM ; b = LUMEN

PLATE-2: Cross section of uterus showing endometrial thickness on ultrasonography.

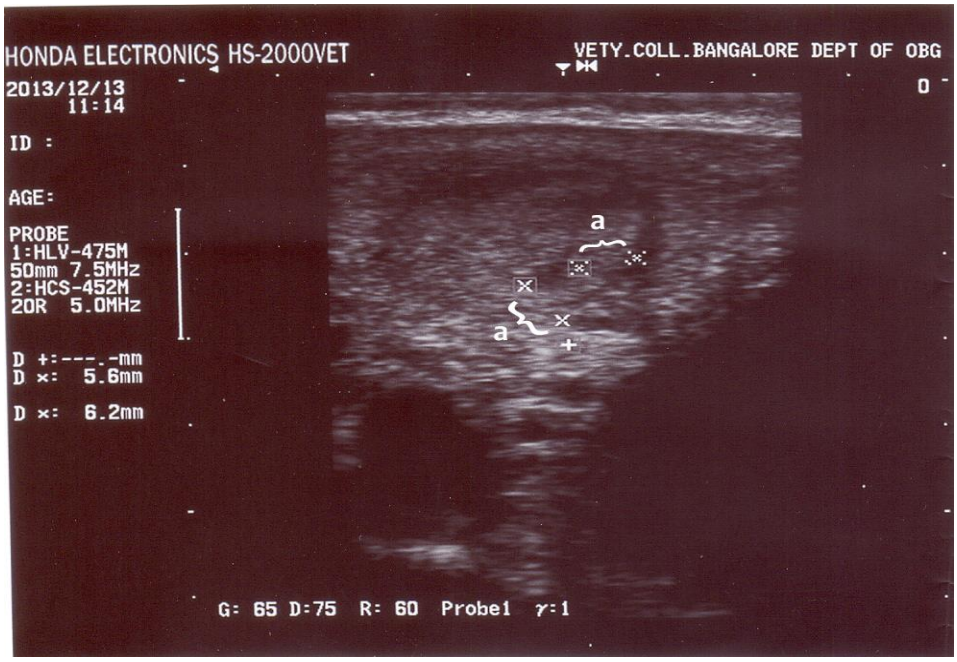


a = ENDOMETRIUM

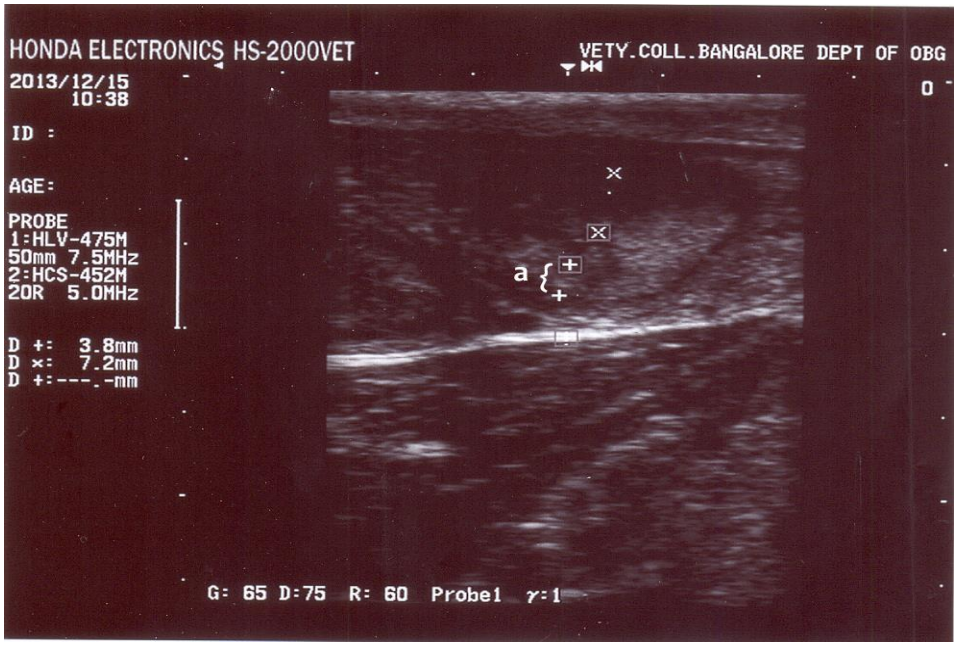


a = ENDOMETRIUM

PLATE-3: Cross section of uterus showing endometrial thickness on ultrasound scanning.

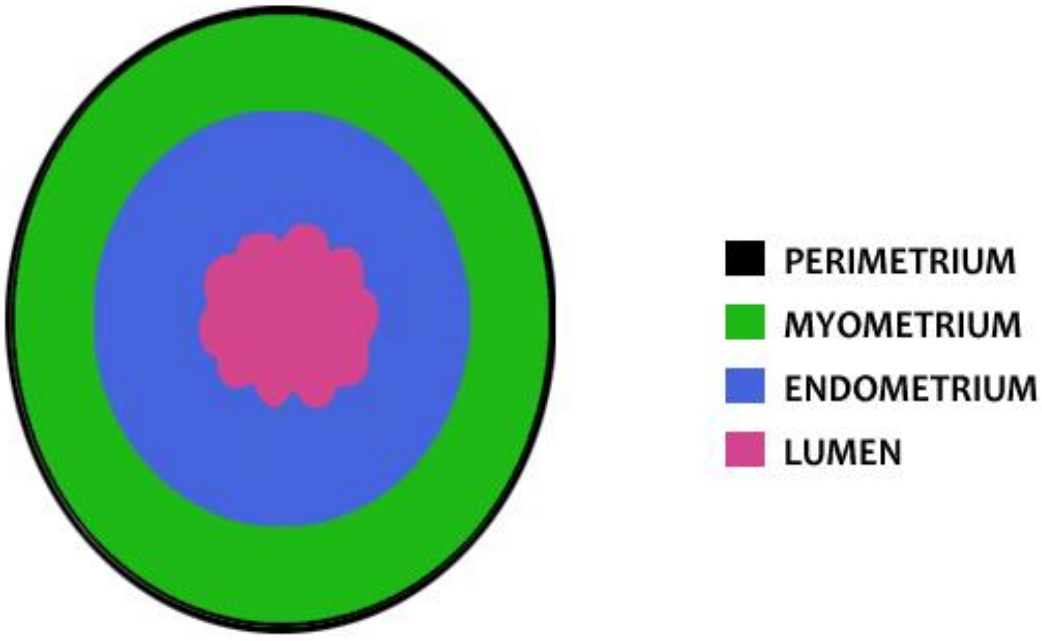


a = ENDOMETRIUM



a = ENDOMETRIUM

PLATE-4: Schematic representation of various layers of uterus.



Discussion

V. DISCUSSION

In the current study artificial control of oestrous cycle was done by using ovsynch protocol as a preparation for study of natural oestrous cycle and to reduce the workload by handling of animals as a group. All ultrasonic measurements were done on the uterine horns in this study similar to Souza *et al.* (2011), whereas Pierson and Ginther,(1987) performed ET measurements at the body of the uterus. The uterine horns were used to obtain valid measurements of endometrial thickness, since the internal uterine bifurcation could be used as a guide to provide a consistent location for obtaining measurements from both horns for each cow.

In the present study based on initial evaluations, most cows responded adequately to the Ovsynch protocol, as indicated by low serum P4 concentrations (<1 ng/ml) 24 h after PGF2α treatment, except for cow no 10 which had progesterone values of 0.2ng/ml concentration at PGF2α injection. She showed basal levels of progesterone concentrations ranging from 0.2 – 1.4ng/ml throughout the experimental period. This showed that the cow no 10, did not respond to ovsynch protocol. This animal did not show ovulation at the expected time after the first GnRH as evaluated by ultrasonography and indicated by progesterone concentration of 0.2ng/ml. This animal failed to respond to ovsynch protocol as it is found that ovulation do not consistently occur in response to first GnRH treatment in synchronized protocol (Macmillan and Thacther 1991) and ovulation rate in dairy cattle treated at random stages of oestrous cycle is observed to be as low as 54% (Colazo *et al*., 2005). This could be the probable reason why this particular animal did not respond to Ovsynch protocol.

5.1 Progesterone Concentration in Ovsynch protocol

In this study on the day of PGF2α administration that is the day with Corpus luteum progesterone values were 6.17 ± 1.2ng/ml ranging between 2.2 – 12.4 ng/ml . These values are consistent with Peter, (1986), and Diaz *et al*. (1986). Who reported progesterone values of 0.5-0.8ng/ml at estrus and 5.1-11.7ng/ml at diestrus in a normal oestrous cycle of *BosTaurus* cows. In this study on the day of expected luteolysis (24hrs after PGF2α) there was drastic reduction of progesterone values in all animals from 6.17 ± 1.2ng/ml to 1.19 ± 0.17ng/ml. and the values remained at less for <1ng/ml on day 2, 3 and 4 after PGF2α administration. This is in accordance with Ginther *et al*., (2007); Marcos *et al*., (2008) and Souza *et al*., (2011). They reported initiation of luteolysis within one hour of PGF2α administration and completion within 24hr in heifers.

5.2 Endometrial Thickness in Ovsynch protocol

Endometrial Thickness increased after PGF2 α treatment from 4.89 ± 0.21mm to 5.02 ± 0.35mm in 24 h. It remained similar on day two (4.54±0.37mm) and day three (5.92±0.19mm) after PGF2α administration. After second GnRH treatment endometrial thickness decreased to 5.06 ± 0.18mm. These findings are similar to that of Souza *et al*, (2011) where the trend in the change of endometrial thickness remained the same but reported a higher endometrial thickness values at each of these points. They reported endometrial thickness of 7mm at PGF2α administration which increased to 9.5mm 24hrs after PGF2α., remained at 9.2 and 9.1mm on day 2 and 3 after PGF2α and then decreased to 8.0mm and 7.4mm on days 1 and 2 day after GnRH.

The variation in the pattern of change in endometrial thickness where endometrial thickness increased from 4.89 ± 0.21mm to 5.02 ± 0.35mm at the expected luteolysis with maximum endometrial thickness achieved on day of ovulation in the present study and the objective are similar to the pattern of change of endometrial thickness as reported by Pierson and Ginther (1987) and Sauza *et al.,* (2011). Then an increase in endometrial thickness on day three to day four (Pierson and Ginther, 1987) to ovulation and decrease in endometrial thickness day one to day three after GnRH (Jimenez-Krassel *et al*.,2009; Souza *et al*., 2011) are similar to present study.

Throughout the study although the pattern change in endometrial thickness is similar to studies of Souza *et al.* (2011) and that of Pierson and Ginther (1987) endometrial thickness values show a variation of approximately 3mm from that of Souza *et al*. (2011) that is the value of endometrial thickness measured are not same as that of Souza *et al* (2011). This may be attributed to difference in the management practices and nutrition of the cows practised in this study and that of other studies.

5.3 Relationship of Endometrial thickness and progesterone

After ovulation the endometrial thickness showed a decrease trend from 5.47 ± 0.41mm to 5.06 ± 0.18mm, where as progesterone concentration increased from 0.76 ± 0.14ng/ml to 1.08 ± 0.28ng/ml. Despite decreasing concentration of progesterone near ovulation to <1ng/ml, there was considerable variation in endometrial thickness (range of 4.6 – 8.3mm) in the animals. The above observation made in this study is in concurrence with Souza *et al*, (2011) who also reported a decreasing endometrial thickness and increasing progesterone concentration after ovulation.

In the present study the correlation between serum progesterone concentration and endometrial thickness did not reveal any consistant association. (r = 0.430, P >0.05), However, Souza *et al*. (2011) reported a negative correlation between serum P4 concentrations and endometrial thickness (r = -0.28, P=0.05).

5.4 Endometrial thickness and uterine tone

Two days after PGF2α the endometrial thickness ranged between 2.9 to 6.9mm and the uterine tone varied from subtonic to tonic. The following day, the endometrial thickness varied from (4.6 to 6.7mm), the tonicity of uterus changed from subtonic to tonic. A gradual increase in uterine tone was associated with increase in endometrial thickness, independent progesterone concentration was observed in the present study However, cows with endometrial thickness of 8.3mm had better uterine tone than cow with 5.3mm of endometrial thickness. Some authors have reported highly coiled and tortuous uterus during the period associated with maximal serum progesterone concentrations (Pierson and Ginther 1987; Bonafos et al., 1995), However, in the present study, irrespective of progesterone concentration an increase in tone of uterus was observed with the increase in endometrial thickness. The observations made on the ET and uterine tone as observed in the present study are in accordance with Souza *et al.* (2011). Although the relationship between endometrial thickness and tonicity was seen, neverthe less underlying hormonal and molecular changes associated with uterine properties were not evaluated in the present study. It is reported that progesterone priming prior to periovulatory period and a sudden decrease in circulating progesterone and concomitant increase in circulating estrogen were important for changes in endometrial thickness during periovulatory period (Souza *et al.*, 2011).

The accuracy and better interpretation of the endometrial thickness in the oestrous cycle of a cow, along with the endometrial thickness measurement and progesterone concentration estimation of estrogen concentration seems to be essential since serum estrogen concentrations are positively correlated with endometrial thickness (Souza *et al*., 2011) and estrogen being a critical hormone responsible for uterine changes during the oestrous cycle.

Further, it is suggested that systematic studies on characterization of endometrial thickness during spontaneous and induced oestrous cycle, involving cows and heifers, of different breeds in different seasons are required.

Summary

VI. SUMMARY

This study was designed to characterize the endometrial thickness in the synchronized oestrous cycle of Holstein Friesean cows. Ten Holstein Friesian cross breed, cows aged 4-5 yr old in their second to third parity, with an apparently normal uterus and ovaries were selected for the study. The cows were synchronized with ovsynch protocol (GnRh -7PG-72 h GnRh-16 h AI). On the day 7 of the protocol, after the injection of PGF2α, using ultrasonography endometrial thickness was measured for five consecutive days. Blood samples were also drawn at the same time for serum progesterone estimation.

In the current study based on data from each cow, each day there was no correlation between serum progesterone concentration and endometrial thickness ($r = 0.430$, $P > 0.05$). Endometrial thickness increased after PGF2α treatment from 4.8 to 5.02 mm, then remained similar at second day (4.54 mm) and third day (5.9mm) after PGF2α administration. However after second GnRH treatment, endometrial thickness decreased to 5.06mm. Individually also 8 of 10 animals displayed similar trend of increasing endometrial thickness. After ovulation mean endometrial thickness of ten animals showed a decreasing trend from 5.47 ± 0.41mm to 5.06 ± 0.18mm where as progesterone values showed an increasing trend from 0.76 ± 0.14ng/ml to 1.08 ± 0.28ng/ml. Despite decreasing concentration of progesterone near ovulation to <1ng/ml, there was considerable variation in endometrial thickness (range of 4.6 – 8.3mm) in the experimental animals. After day 2 of PGF2α administration the endometrial thickness ranged between 2.9 to 6.9mm with the uterine tone ranging from tonic to subtonic. Next

day as the endometrial thickness ranged from (4.6 - 6.7mm), the tonicity of uterus changed from sub-tonicity to tonicity. There was gradual increase in uterine tone with increase in endometrial thickness, despite lower or high progesterone values for example cow with endometrial thickness 8.3mm had a better tone than with 5.3mm of endometrial thickness.

The variation in the pattern of change in endometrial thickness where endometrial thickness increased from 4.89 ± 0.21mm to 5.02 ± 0.35mm at the expected luteolysis with maximum endometrial thickness achieved on day of ovulation are similar to the pattern of change of endometrial thickness reported by Pierson and Ginther (1987) and Sauza *et al* (2011). Then an increase in endometrial thickness on day three to day four (Pierson and Ginther, 1987) to ovulation and decrease in endometrial thickness day one to day three after GnRH (Jimenez-Krassel *et al*., 2009; Souza *et al*., 2011) are similar to our study.

Throughout the study although the pattern change in endometrial thickness is similar to studies of Souza *et al.* (2011), and that of Pierson and Ginther (1987) endometrial thickness values show a variation of approximately 3mm from that of Souza *et al.* (2011), that is the value of endometrial thickness measured are not same as that of Souza *et al.*, (2011). This may be attributed to difference in the management practices and nutrition of the cows practiced in this study and that of other studies.

After ovulation Endometrial Thickness showed a decrease trend from 5.47 ± 0.41mm to 5.06 ± 0.18mm, where as progesterone showed an increasing trend from 0.76 ± 0.14 ng/ml to 1.08 ± 0.28ng/ml. Despite decreasing concentration of progesterone near

ovulation to <1ng/ml, there was considerable variation in endometrial thickness (range of 4.6 – 8.3mm) in the animals. The above observation made in this study is in concurrence with Souza *et al*. 2011, who also reported a decreasing endometrial thickness and increasing progesterone concentration after ovulation.

In the current study based on data from each cow, each day there was no correlation between serum progesterone concentration and endometrial thickness (r = 0.430, P >0.05), where as Souza *et al.* (2011), reported a negative correlation between serum P4 concentrations and endometrial thickness (r=-0.28, P=0.05). That is with increasing progesterone concentration there is decreasing endometrial thickness.

A gradual increase in uterine tone with increase in endometrial thickness, despite lower or high progesterone values is observed in this study for example cow with endometrial thickness of 8.3mm had better uterine tone than cow with 5.3mm of endometrial thickness. Some authors have reported highly coiled and tortuous uterus during the period associated with maximal serum progesterone concentrations (Pierson and Ginther 1987; Bonafos *et al*., 1995) where as in the present study irrespective of progesterone concentration an increase in tone of uterus was observed with the increase in endometrial thickness. Further the observations made in this study in relation to the ET and uterine tonicity are in accordance with Souza *et al.* (2011), It is suggested that studies on characterization of endometrial thickness that can compare natural to induced oestrous cycle, difference between cows versus heifers, indigenous breeds versus exotic breeds and also on changes in different seasons are required.

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Abstract

VIII. ABSTRACT

The effects of the, estrogen and progesterone, on growth and regression of the nonpregnant uterus are reflected in the cyclic patterns of cellular proliferation, vascular growth, and blood flow that occur in the endometrium during the estrous cycle. The objective of the present study was to evaluate changes in endometrial thickness (ET) near the time of a synchronized ovulation and to assess the relationship of ET and progesterone concentration in Holstein cows. In the present study ten cows were subjected to an Ovsynch protocol examined with transrectal ultrasonography, once daily for 5 days, starting concurrent with PGF2α (PGF) treatment. The endometrial thickness increased rapidly after PGF (5.92 ± 0.19mm), remained high for the next 2 days, then decreased to 5.06 – 0.18mm, on day 1 and 2 respectively, after the second GnRH.. The progesterone concentration on day after PGF2α was 0.7 ± 0.2ng/ml, after second GnRH the progesterone values were 1.08 ± 0.28ng/ml. Based on data from each cow, no significant correlation between serum progesterone concentration and endometrial thickness ($r = 0.430$, $p > 0.05$), could be established. The uterine tone was either flabby or subtonic, on the day of PGF2α there was gradual increase in uterine tone with increase in Endometrial Thickness, despite eg: Cow with endometrial thickness 8.3 had better tone than with 5.3mm of endometrial thickness. In conclusion, a single ultrasonographic evaluation of ET 48 hrs after PGF treatment in an Ovsynch program in HF cows was a good predictor of ovulation failure.