

**STUDY ON THE OVARIAN FOLLICULAR DYNAMICS DURING
OESTROUS CYCLE IN PUNGANUR CATTLE**

By

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This is to certify that the thesis entitled "**Study on the ovarian follicular dynamics oestrous cycle in Punganur cattle**" submitted in partial fulfilment of the requirements for the degree of Master of Veterinary Science of the Sri Venkateswara Veterinary University, Tirupati, is a record of the bonafide research work carried out by **Mr. Ravikiran B R**, ID. No. TVM/2015-47 under my guidance and supervision. The subject of the thesis has been approved by the Student's Advisory Committee.

No part of the thesis has been submitted by the student for any other degree or diploma. The published part has been fully acknowledged. All assistance and help received during the course of the investigations have been duly acknowledged by the author of the thesis.

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LIST OF SYMBOLS AND ABBREVIATIONS

AI	Artificial insemination
BCS	Body condition score
CL	Corpus Luteum
° C	Degrees Celsius
D	Day
DF	Dominant follicle
et al	And other associates
ELISA	Enzyme linked immuno sorbent assay
Fig	Figure
FSH	Follicle stimulating hormone
GnRH	Gonodotropin releasing hormone
HF	Holstein Friesian
h/hrs	Hours
IOI	Interovulatory length
LH	Lutenizing hormone
MHZ	Mega hertz
Mg	Micro gram
mm	Milli meter
ml	Milliliter
ng	Nano gram
OCL	Oestrous cycle length
pg	Pico gram
POF	Preovulatory follicle
P4	Progesterone
PGF2 α	Prostaglandin2alpha
SPSS	Statistical package for social science
SE	Standard error
vs	Versus
%	Percentage
<	Less than

>	Greater than
±	Plus or minus
<	Less than or equal to
≥	Greater than or equal to
®	Registered sign
©	Copyright sign

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DECLARATION

I, **Mr. RAVIKIRAN B R**, ID. No. TVM / 2015 – 47 hereby declare that the thesis entitled “**Study on the ovarian follicular dynamics during oestrous cycle in Punganur cattle**” submitted to Sri Venkateswara Veterinary University, Tirupati for the degree of “**Master of Veterinary Science in Veterinary Gynaecology & Obstetrics**” is the result of original research work done by me. I also declare that the materials contained in this thesis have not been published earlier.

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ABSTRACT

The follicular dynamics during two consecutive oestrous cycles of eight Punganur cows stationed at Livestock Research Station, Palamaner, Chittoor district was monitored on every alternative day by using an ultrasound device. The Punganur cows indicated the presence of 2 follicular wave (25.00 %) and 3 wave (75.00 %) estrous cycle. The number of waves did not show any significant difference in the exhibition of estrus intensity with overall mean estrus duration of 16.02 ± 0.74 h.

The mean values on day of onset of follicular wave, day of follicular wave ending, duration of follicular wave, maximum diameter of dominant follicle and day of maximum diameter of dominant follicle in cows showing 2 wave cycle during 1st and 2nd waves were 0.8 ± 0.37 and 13.00 ± 1.08 ; 12.00 ± 0.68 and 21.00 ± 0.63 ; 11.8 ± 0.49 and 9.25 ± 2.98 ; 7.3 ± 0.63 and 9.4 ± 0.47 mm; 6.6 ± 1.21 and 21.6 ± 1.63 and 0.64 ± 0.10 and 0.73 ± 0.24 mm, respectively while the same during 1st, 2nd

and 3rd waves in cows showing 3 wave cycle were 1.83 ± 0.27 , 7.6 ± 0.5 and 13.66 ± 0.87 ; 9.59 ± 1.03 , 15.73 ± 0.76 and 21.7 ± 0.51 day; 8.16 ± 0.95 , 7.93 ± 0.98 and 7.6 ± 0.25 days; 6.75 ± 0.45 , 6.4 ± 0.29 and 8.33 ± 0.38 mm and 4.75 ± 0.63 , 11.4 ± 0.49 and 21.4 ± 0.56 day and 0.71 ± 0.10 , 0.68 ± 0.10 and 0.66 ± 0.10 mm per day, respectively. While, the mean day of onset of atresia of follicular wave in cows showing 2 waves oestrous cycle was 8.2 ± 1.24 for 1st wave and the same in 3 waves cycle was 7.16 ± 0.61 and 13.33 ± 0.49 day for 1st and 2nd, respectively.

The difference in the diameter of corpus luteum within 2 wave and 3 wave oestrous cycle cows was significant ($P < 0.05$). While the difference in the period of wave duration, mean maximum diameter of DF and growth rate of follicle within 2 wave and 3 wave oestrous cycle cows was insignificant ($P > 0.05$).

The diameter of the preovulatory follicle was higher ($P < 0.05$) than the diameter of the other dominant follicles of previous waves in oestrous cycles in cows with either two or three waves. The mean preovulatory follicular diameter was correlated positively and significantly ($P < 0.05$) with the corresponding day of oestrous cycle estrogen concentration ($r = 0.94$).

The mean overall diameter of CL during 4, 6, 8, 10, 14, 16, 18 and 20 days of oestrous cycle in Punganur cows was 8.49 ± 0.49 , 11.17 ± 0.50 , 12.90 ± 0.45 , 14.46 ± 0.34 , 14.80 ± 0.29 , 15.09 ± 0.46 , 13.34 ± 0.51 , 11.51 ± 0.63 and 10.38 ± 0.43 , respectively. The mean maximum diameter of corpus luteum was 15.09 ± 0.46 on 14th day and regression of corpus luteum was initiated on 16th day of oestrous cycle. The increase in the diameter of the CL was significant throughout its growth from the day 4 of oestrous cycle to the day of regression with a maximum diameter of 15.09 ± 0.46 on 14th day. The difference in the diameter between and within 2 wave and 3 wave oestrous cycle cows was significant ($P < 0.05$) and was positively and insignificantly ($P < 0.05$) correlated with the progesterone concentration ($r = 0.66$) but negatively and significantly ($P < 0.05$) correlated with the estrogen concentration ($r = -0.92$) on day 14 of oestrous cycle.

The mean overall progesterone concentration on day 0, 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 of oestrous cycle was 1.06 ± 0.29 , 3.14 ± 0.91 , 2.38 ± 0.48 , 4.29 ± 0.50 , 4.49 ± 0.97 , 5.44 ± 0.74 , 5.47 ± 0.74 , 6.30 ± 1.20 , 7.04 ± 0.62 , 5.10 ± 0.87 and $2.47 \pm$

0.87 ng/ml respectively and the overall estrogen concentration was 44.82 ± 1.07 , 38.75 ± 1.68 , 41.65 ± 1.45 , 40.72 ± 1.43 , 36.77 ± 4.77 , 42.09 ± 1.91 , 42.89 ± 1.81 , 42.78 ± 1.34 , 42.07 ± 0.65 , 42.40 ± 0.85 and 44.82 ± 0.70 pg/ml, respectively. The difference in the serum progesterone and estrogen mean concentrations between 2 wave and 3 wave oestrous cycle cows was not significant ($P>0.05$) but difference between days of oestrous cycle progesterone was significant ($P<0.05$) and estrogen was not significant ($P>0.05$).

The study concluded that follicular dynamics in Punganur cattle is characterized by a higher incidence of 3 wave oestrous cycles with slightly insignificantly longer oestrous cycles and the trend of estrogen and progesterone hormones during oestrous cycle indicates the normal cyclicity like any other Indian cattle breed.

CHAPTER I

1. INTRODUCTION

India has a large population of humped cattle (*Bos indicus*) estimated to be 150 million (Livestock census, 2012). Most of the indigenous breeds are undergoing genetic degradation due to indiscriminate cross breeding and irregular mating among the breeds situated in each other's vicinity. As a result several indigenous breeds with many desirable traits are threatened with extinction, while others are in the process of getting replaced completely by certain high producing cross breed animals.

Punganur is one such breed of Indigenous cattle which is under threat of extinction (Ramesha *et al.*, 2001). Punganur, the world's one of the shortest, humped cattle with long tail and switch touching the ground. It's native tract is situated in Chittoor district south-eastern tip of the Deccan Plateau (Reddy *et al.*, 2004). Animals are white and light grey in colour with a broad forehead and short horns with the average height of 60-100 cm having a body weight of 115-200 kg. These animals are efficient milkers with an average milk yield of 3-5 liter / day on a daily feed intake of 5 kg (Reddy *et al.*, 2004). The bulls are more docile than females with grey color on the neck and hump and are tight sheathed (Rao *et al.*, 1999). Disease resistance of these animals is remarkable and the productive and reproductive efficiencies are believed to be on par with the other dual purpose Indian breeds (Veerabramhaiah *et al.*, 2012). Punganur cattle can thrive well on less quantities of feed and fodder with reasonable returns to the farmers. The Punganur breed of cattle is under the threat of extinction as the population is alarmingly dwindled to 733 animals only (Livestock Census, 2012). As per the FAO norms (Bodo, 1989) this small number indicates that the breed is in an alarming state of extinction. Therefore up-liftment and propagation

of this population can be undertaken by using available reproductive technologies, but there is a lacuna in the information on reproductive physiology of this breed wherein, the follicular dynamics is the one of the important subjects of reproductive physiology which is important in understanding the cyclicity of the breed and also for optimization and successful utilization of assisted reproductive technologies to control and / or to improve fertility in among Punganur cattle.

Follicular dynamics is characterised by the wave of follicular growth and regression during estrus and different number of waves exists among different breeds of cows (Ginther *et al.*, 1989). Each follicular wave is comprised of emergence, selection, deviation, dominance, atresia and/or ovulation. Subsequently, corpus luteum (CL) will develop at the site of the ovulated graffian follicle and regulate the oestrous cycle. It has been well established in *Bos taurus* cattle that more than 95 % of all oestrous cycles consist of two or three follicular waves (Adams, 1999 and Evans, 2003). Similarly, few studies on follicular characterization have been done in some Zebu breeds of cattle like Nellore (Mollo *et al.*, 2007) and Gir (Gambini *et al.*, 1998) with variation in the number of follicular waves even up to four-wave cycles (Bo *et al.*, 2003) and occasionally five-wave cycles (Viana *et al.*, 2000). The follicular wave developmental patterns are being influenced by wave order (Ginther *et al.*, 1989), breed (Figueiredo *et al.*, 1997), reproductive stage (Roche and Boland, 1991) etc. There are certain variations in the follicular dynamics especially in the steroid profile (Purohit *et al.*, 2000), dominant follicle diameter (Figueiredo *et al.*, 1997), number of follicular waves (Viana *et al.*, 2000) between Zebu cows and European breeds of cows. Knowledge on these aspects is important for understanding the correct ovulation parameters, its managerial procedures (Viana *et al.*, 2000)

and application of reproductive technologies like synchronisation of ovulation, superovulation etc, so as to augment the fertility.

Since the information on the peculiarities of follicular dynamics and luteal development are not available for Punganur cattle, this study was conceived to fill in these existing information gaps by studying the follicular dynamics and luteal developmental trends during the oestrous cycle in the Punganur cattle with the following objectives:

- 1) To study the follicular dynamics during oestrous cycle of Punganur cows.
- 2) To estimate steroid concentrations during oestrous cycle of Punganur cows.

CHAPTER II

2. LITERATURE REVIEW

2.1 Estrus detection

2.1.1 Intensity of estrus behaviour

Intensity of estrus is the subjective measurement of sex drive of the animal (Hafez, 1987). Rottenston and Touchberry, (1957) classified the intensity of estrus depending on the degree of expression of estrus symptoms as weak, medium (normal) and Intense.

Anderson, (1971) found that the strength of estrus symptoms increased with an increase in the time elapsed from calving in Danish cows. He has also reported that Danish Red, Danish Black Pied and Danish Jersey breeds 64, 56 and 63% of cows, respectively with strong intensity were conceived to first insemination as against 45, 54 and 47% of cows, respectively with intermediate intensity and 45, 48 and 36% of cows, respectively with weak intensity symptoms.

Kuhlamann *et al.* (1998) monitored Angus cows and heifers with heat watch® and found that animals which became pregnant had more mounts during estrous (40 vs. 23).

Rorie *et al.* (2002) reported that the intensity of estrus (number of mounts) was related to fertility and also stated that cows that became pregnant had significantly more mounts than those that did not.

It is evident, from the literatures reviewed, that the duration of estrus and the expression of intensity of the different behaviours vary with different breeds. It also varies with the parity, milk yield and the environment of the cow. The duration and

intensity of the estrus is somewhat low in case of Zebu cattle in comparison to its European counterparts (Layek, 2010).

Intensity of estrus in different cattle breeds

Breed / Species of the animals	Intensity of estrus (%)			Reference
	Weak	Medium	Strong	
Brown Swiss cross	10.00	62.50	27.50	Gupta, 1975
Buffaloes	39.43	45.19	15.38	Agarwal and Purbey, 1983
HF x TP Crossbred	26.39	52.78	20.83	Singh and Kharche, 1985
Ongole cows	19.08	63.30	17.55	Naidu and Rao, 2006
Buffaloes	73.24	25.35	1.41	Gunasekaran <i>et al.</i> , 2007
Crossbred	49.26	44.85	5.88	Gunasekaran <i>et al.</i> , 2008
Sahiwal cows	52.00	34.00	14.00	Layek, 2010

2.1.2 Duration of estrus

Duration of estrus in Zebu and their crosses was shorter than that of the European breeds. In both the cattle and in all location the estrus of heifers reported shorter than that of the cows (Hafez, 1968; Warnick and Duarte, 1979; Mukasa-Mugerwa *et al.*, 1989 and Galina and Arthur, 1990).

In a study with radio telemetry showed the average duration of 7 h estrus duration with the range of 33 min to 36 h (Dransfield *et al.*, 1998).

Koppel *et al.* (1984) observed that the average estrus duration was 13 h in Zebus, Holsteins and their crosses. Further, several authors have indicated that the

duration of the estrus is dependent on the season, number of cows in estrus, parity of the animals, milk production of the animals etc.

Duration of estrus in different breeds of bovine

Breed / Species of the animals	Duration of estrus (h)	Reference
Brahman cows	6.70 ± 0.78	Plasse <i>et al.</i> , 1970
Buffaloes	17.65 ± 0.45	Gill <i>et al.</i> , 1973
Friesian cows	15.10 ± 4.40	Esselmont and Bryant, 1976
Sahiwal cows	15.52 ± 0.21	Dash, 1980
Haryana cows	14.53 ± 0.43	Dash, 1980
Zebu cows	13.60 ± 1.00	Galina <i>et al.</i> , 1987
Zebu cows	7.66 ± 4.68	Mattoni <i>et al.</i> , 1988
Baoule cows	10.70 ± 5.10	Chicoteau <i>et al.</i> , 1989
Nellore cows	10.50 ± 1.00	Pinheiro <i>et al.</i> , 1998
Crossbred cows	34.34 ± 1.99	Gunasekaran, 1998
Beef cows	16.50 ± 11.90	Floyd <i>et al.</i> , 2001
Fogera cows	10.60 ± 4.50	Negussie <i>et al.</i> , 2002
HF cows	11.80 ± 4.40	Roelofs <i>et al.</i> , 2005
Ongole cows	15.54 ± 0.28	Naidu and Rao, 2006
Mithun cows	12.60 ± 2.30	Mondal <i>et al.</i> , 2006
Sahiwal cows	14.53 ± 0.80	Layek, 2010

2.1.3 Oestrous cycle length

Ginther *et al.* (1989) recorded that the mean length of 2-wave oestrous cycle length was significantly shorter (20.40 ± 0.30 days) than for 3-wave intervals (22.80 ± 0.60 d).

Figueiredo *et al.* (1997) observed that the mean length of 2-wave oestrous cycle length was significantly shorter (20.60 ± 0.50 d) than that of 3-wave interval (22.0 ± 0.4 d) in Nellore cattle.

Taylor *et al.* (1993) recorded that the estrus cycle length of two wave cycle was significantly shorter (20.8 ± 1.7 d) compared to three wave cycles (29.7 ± 4.9 d) in dairy cattle.

Ruiz *et al.* (1999) recorded that oestrous cycle length was 21.0 ± 3 days in Zebu cows.

Viana *et al.* (2000) observed a non-significant ($P > 0.05$) difference in oestrous cycle length (21.11 ± 1.76 vs 22.25 ± 1.71 days) between cycles with three and four waves and they have also recorded a period of 19 and 32 days cycle length in two and five wave cycle, respectively in Gir breed.

The average inter-estrous interval was 21 days for both *Bos taurus* and *Bos indicus* cattle (Bó *et al.*, 2003).

Nosier *et al.* (2003) observed that mean length of the 2-wave oestrous cycle was significantly shorter (19.8 ± 0.6 days) than that of 3- wave interval (22.5 ± 0.8 days) in native breed of Egypt.

Sartori *et al.* (2004) reported that two-wave (19 days) cycle length was significantly shorter ($P < 0.05$) than three-wave (23 days) cycles and they have also

reported that high-producing lactating Holstein cows have exhibited longer oestrous cycle length (23 days) due to a prolonged time between luteolysis and ovulation.

Patel *et al.* (2006) recorded significantly longer oestrous cycle for 3-wave than 2-wave cycles in HF cows. They have also significantly longer oestrous cycle for 3 wave than 2 wave cycle in HF cows.

Akter *et al.* (2010) reported that oestrous cycle length was 21.8 ± 0.8 days in zebu cattle having two waves cycle.

Satheshkumar *et al.* (2011) observed that the oestrous cycle length was insignificantly greater in crossbred cows (22.67 ± 1.20 days) than in buffaloes (21.80 ± 0.54 days).

Sakhong *et al.* (2011) reported that in Thai native cows (*Bos indicus*) having 1, 2, 3 and 4 follicular waves the estrus cycle length was 10.50 ± 0.96 , 15.50 ± 1.46 , 21.63 ± 0.51 , and 25.00 ± 1.36 days, respectively. While, in cows having 3 wave pattern the oestrous cycle length was significantly longer than in heifers.

Muraya, (2013) observed a positive correlation ($P > 0.05$) between the number of follicle waves per cycle and oestrous cycle length. They have stated that mean oestrous cycle length was 25.0 ± 4.0 days for 4 wave cycles, 23.6 ± 1.05 days for 3 wave cycles and 18.6 ± 1.9 days for two-wave cycles, in Kenyan Boran cows.

Degefa *et al.* (2016) compared the follicular dynamics of purebred and crossbred Boran cows in Ethiopia and stated that the mean oestrous cycle length was similar in Boran (19.4 ± 0.2 days) and B×H cows (20.1 ± 0.4 days).

Hadiya *et al.* (2016) recorded an inter estrus interval of 21.50 ± 0.29 and 22.00 ± 0.00 days in two and three wave cycle, respectively in crossbred cattle and also

concluded that oestrous cycle length (22-23 days) and duration of oestrous cycle (21-22 days) were statistically not different between 2 and 3 wave cycles.

Siregar *et al.* (2016) observed that the average oestrous cycle length for Aceh cattle was 17.8 ± 0.4 days in cow having three wave cycle.

Imron *et al.* (2016) recorded that the inter estrus interval was 22.0 ± 1.6 and 22.8 ± 2.9 days in three and four wave cycle, respectively in Peranakan Ongole Cattle.

2.2 Follicular dynamics

2.2.1 Follicular Growth

Rajakoski, (1960) was the first to propose the two waves concept of follicular turn over based on gross and histological studies of ovaries during bovine oestrous cycle and he has stated that follicles having ≥ 5 mm diameter showed two wave follicular growth pattern wherein the 1st follicular growth was observed between third and fourth day and the other wave between 12th and 14th day of oestrous cycle. The large follicle of the 1st wave undergoes atresia between 12th day to 17th day and large follicle of the 2nd wave ovulates after its final maturation during the estrus cycle.

Follicular growth in cattle is not continuous (Matton *et al.*, 1981) but cyclic and is dependent on the stage of oestrous cycle (Fortune, 1994). Ovarian follicular dynamics is characterized by waves of follicular growth and regression during the oestrous cycle. Each wave is comprised of successive phases referred as recruitment, selection, deviation, dominance and atresia (Moore and Thatcher, 2006). During each wave there will be a synchronous development of a group of follicles and subsequent regression of subordinate follicles (Ginther *et al.*, 1989 and Taylor and Rajamahendran, 1991).

Growth of follicular wave begins with increase in the concentration of serum FSH followed by recruitment and growth of 8-41 small follicles in 2-3 days (Ginther *et al.*, 1997). However, circulating concentrations of inhibin increase concurrently with emergence of waves and suppresses the synthesis and secretion of FSH (Kulick *et al.*, 2001). Plasma FSH concentration decreases within 2-3 days after the emergence of wave leading to the follicular deviation (Ginther *et al.*, 1999 and Kulick *et al.*, 2001) and selection of a dominant follicle (Adams *et al.*, 1993). The growth of the selected DF continues and in turn causes the smaller follicles to become subordinate follicle (SF) that would undergo the process of atresia.

Estradiol (E2) concentration in the follicular fluid of dominant follicle and factors such as Insulin Growth Factors, Inhibin and Follistatin produced by growing follicles regulate FSH release and availability (Singh *et al.*, 1999). The release of Luteinizing Hormone (LH) is enhanced by higher concentrations of E2 and lower concentrations of Progesterone (P4) (Kaneko *et al.*, 1991). The increased levels of LH may lead to initiation of luteolysis and final maturation during the growing phase of DF leading to the ovulation of DF. If there is no luteolysis during the growing period of DF and it may undergo a process of atresia (Vasenna *et al.*, 2003; Jaiswal *et al.*, 2004 and Adams *et al.*, 2008).

Nosier, (2003) reported that the follicular growth wave consists of a simultaneous growth of a group of follicles, in which one of them will become the dominant follicle and reach the largest size and suppress the growth of other smaller follicles.

Azawi *et al.* (2009) defined follicular dynamics as “A process of continual growth and regression of antral follicles” and stated that one to four waves of

follicular growth and development occur during a single estrus cycle in cattle, but most commonly two to three waves appear during each oestrus cycle.

The dominant follicle of each wave grows continuously at an accelerated rate and if the development coincides with corpus luteum lysis that will leads to decreased concentration of progesterone which in turn results in to ovulation of DF (Torres *et al.*, 2012).

2.3 Transrectal Ultrasonography

Ultrasound examination of follicle appear as dark, sometimes delineated, anechogenic structures, surrounded by a fine wall and with a diameter usually < 25 mm (Pierson and Ginther, 1984).

Edmondson *et al.* (1986) reported that follicles with a diameter 6 mm can be detected with a 3.5 MHZ transducer and follicles with a diameter 2 mm can be detected by 5 MHZ transducer.

Ginther *et al.* (1996) recorded that transrectal ultrasonic imaging provides a means for repeated, direct, non invasive monitoring and measuring of follicles regardless of their depth within the ovary.

Zeitoun *et al.* (1996) reported that the diameter of each follicle and CL was determined by taking the mean of the length and width of the respective structures.

Estimating the stage of oestrous cycle, status and number of pre-ovulatory follicles, ovulation, monitoring the development and morphology of corpus luteum are the potential applications of ultrasonographic examination of the ovaries (Evans, 2003).

Lemma *et al.* (2006) reported that follicles as small as 2 – 3 mm can be visualized and the corpus luteum can usually be identified throughout its functional life using ultrasound.

2.3.1 Follicular population:

New born calf ovaries contain approximately > 1 lakh primordial follicles which are not renewable and gradually depleted until it reaches near zero when the cow attains the age of 15 to 20 years (Hafeez, 2008).

It was noticed that the number of follicles recruited always depends on the individual animal and these were lower in buffalo than in cattle (Adams *et al.*, 1993).

Baruselli *et al.* (1996) reported that there was a non-significant correlation between the number of follicles recruited during each follicular waves and the number of follicular waves in each oestrous cycle (2-wave cycle, $r = 0.752$, $P < 0.01$ and 3-wave cycle, $r = 0.536$, $P < 0.01$). However, they have also recorded that the larger number of follicles in the first wave were more than in the subsequent waves.

Morita and Tilly, (1999) postulated that the ultimate fate of primordial follicles (more than 99.9%) endowed in the ovaries at birth leads to death through apoptosis.

Filho *et al.* (2001) recorded that the waves of follicular growth were characterized by the recruitment of a group of four to six follicles having 3.00 ± 0.50 mm diameter in Girolando cattle.

Muraya, (2013) reported higher incidence of ovulatory DFs in the right ovary and it was attributed to fact that the right ovary receives more blood supply compared to the left one.

Degefa *et al.* (2016) compared the follicular dynamics of purebred and crossbred Boran cows and stated that Boran cows possessed a greater number of ovarian follicles than B×H crossbred cows and they have also observed that both the genotypes had displayed more activity on their right ovary than left.

2.3.2 Number of follicular waves per oestrous cycle

Ovarian follicular development in cattle occurs in waves (Sirois and Fortune, 1988). A wave of follicular growth involves the synchronous development of a group of follicles, one of which become dominant and achieves the greatest diameter suppressing the growth of the subordinate smaller follicles (Pierson and Ginther, 1987b).

In cattle, the wave-like pattern of follicle development has been studied by various authors (Ginther *et al.* 1989a; Roche *et al.*, 1998; Adams 1999; Ireland *et al.*, 2000 and Fortune *et al.*, 2001). Taylor and Rajamahendran, (1991) and Figueiredo *et al.* (1997) have reported that the luteal phase length was the main determinant of the number of follicular waves since luteal phase keeps high concentrations of progesterone, blocks ovulation of functional dominant follicles, induces the atresia and the onset of a new follicular wave.

While, Knopf *et al.* (1989) recorded prevalent two wave follicular activity during an oestrous cycle in cattle.

Similarly, a higher prevalence of cycles with three follicular waves was observed by many authors in European cows (Savio *et al.*, 1988 and Sirois and Fortune, 1988) and in Zebu cows (Viana *et al.*, 2000).

Studies in *Bos taurus* cattle have indicated that the occurrence of two to four follicular waves during the oestrous cycle, with predominance of two waves and very

rarely four waves (Sirois and Fortune 1988; Townson *et al.*, 2002 and Sartori *et al.*, 2004).

It was reported in various studies that Zebu cows viz., Nelore heifers (Mollo *et al.*, 2007), Gir cows (Gambini *et al.*, 1998 and Viana *et al.*, 2000) and Brahman cows (Zeitounet *et al.*, 1996) showed predominance of three waves oestrus cycle and also some observation of two, four and even a few five waves per cycle.

Savio *et al.* (1990) recorded one follicular wave in cows during post-partum period after first ovulation.

Adams *et al.* (1992) reported that emergence of these waves coincides temporally with a surge of FSH secretion during which one or two dominant follicles and several subordinate follicles developed.

Evans *et al.* (1994) observed short oestrous cycles with one follicular wave around the time of puberty.

Rhodes *et al.* (1995) reported that Brahman heifers have exhibited 1 to 4 follicular waves with predominance of 3 follicular waves.

Wolfenson *et al.* (1995) stated that heat stress and low plane of nutrition have been reported to cause increase in occurrences of three-wave patterns in Taurine cows. However, wave patterns in Zebu cows are shown to be influenced by parity (Figueiredo *et al.*, 1997).

Manik *et al.* (1998) recorded that there was two-wave pattern (5/6 animals) and three-wave pattern (1/6 animals) in buffaloes.

Adams, (1999) observed that more than 95% of oestrous cycles in cattle showed either two or three follicular waves.

Higher prevalence of cycles with three follicular waves was observed in European cows (Savio *et al.*, 1988 and Sirois and Fortune, 1988) and Zebu cows (Viana *et al.*, 2000).

Filho *et al.* (2001) recorded that out of 24 monitored cycles in five-eighths Girolando cattle, 15 (62.5%) cycles presented two waves of follicular development and nine (37.5%) cycles presented three waves.

Townson *et al.* (2002) reported that the cows showing two follicular waves per cycle tend to have shorter ($P < 0.01$) cycles, ovulate larger ($P < 0.05$) and older ($P < 0.01$) follicles but the cows were less fertile than cows with three waves per cycle.

Bó *et al.* (2003) reported that there was 3-wave pattern in cows with poor nutrition and heat-stress.

Martínez *et al.* (2003) recorded that the mean length of the oestrous cycle was positively correlated to the number of waves.

Nosier *et al.* (2003) observed that 14 inter-ovulatory intervals 5 out of 7 cows (71.4%) had 3 waves of follicular development and remaining cows (28.6%) had 2 wave cycle in native breed of Egypt.

Gaur *et al.* (2007) recorded that the higher proportion of cycles with two follicular waves (78.57%) compared to three follicular waves (21.42%) in *Bos indicus* cattle.

Adam *et al.* (2008) recorded that the most heifers (65%) of Nellor cattle (*B. indicus*) showed 3-wave, while most of cows (83%) showed 2-wave pattern.

Akter *et al.* (2010) reported that all cows (N=10) taken under study showed two follicular waves with oestrous cycle length 21.8 ± 0.8 days.

Sakhong *et al.* (2011) compared follicular dynamics in Thai heifers and cows and observed that majority of Thai native cows (82.76%) exhibited a 3-follicular wave pattern, whereas the heifers exhibited both 2 (38.10%) and 3 (47.62%) follicular waves pattern.

Satheshkumar *et al.* (2011) reported that 3-wave pattern emerged more frequently in the winter than the 2-wave pattern and the same was reversed in the summer.

Muraya, (2013) recorded that the percentage of three wave, two wave and four wave cycles were 66.67%, 25.6% and 6.67%, respectively in Kenyan boran cows.

Hadiya *et al.* (2016) observed that there were either 2-waves (66.66%, n=4) or 3-waves (33.33%, n=2) follicular waves in postpartum cyclic crossbred cows.

Imron *et al.* (2016) recorded 3 (66%) and 4-waves (34%) follicular pattern in Peranakan ongole cattle.

Siregar *et al.* (2016) observed that follicular wave happened 3 times in one oestrus cycle in Aceh cattle.

2.3.3 Day of onset of follicular wave

Savio *et al.* (1988) observed that the onset of first and second (ovulatory) waves in a two-wave cycle was commenced on day 2- 4 and day 10 -11, respectively.

Ginther *et al.* (1989) recorded that the follicular waves were first detectable with 4 -5 mm follicular diameter on day 0 and 10 for two-wave cycles and on day 0, 9 and 16 for three-wave cycles and they have also stated that the mean day of emergence of the 1st and 2nd waves were non significantly ($P<0.05$) different between 2 wave and 3 wave interval.

Knopf *et al.* (1989) observed that follicular waves were emerged on day 0.6 ± 0.4 and 9.2 ± 0.7 in animals with two waves and on day 0.7 ± 0.4 , 10 ± 0.6 and 16.1 ± 0.1 in cycles with three waves in Holstein cows.

Adams *et al.* (1992) reported that FSH plays a significant role in regulating the follicular wave emergence and deviation in cattle and after each FSH surge, a group of small follicles appear in the ultrasonographic examination.

Figueiredo *et al.* (1997) observed that in the Nelore cattle the detection of the first and second follicular waves in 2-wave cycle was on day 1.50 ± 0.15 and 12.0 ± 0.91 , respectively while for 3-wave cycle, detection of the first, second and third wave was on day 1.57 ± 0.20 , 9.14 ± 0.47 and 15.14 ± 0.48 , respectively.

Manik *et al.* (1998) recorded that the first wave was detected on day 0.20 ± 0.19 (day 0 = day of ovulation) and second wave was on sets on day 9.20 ± 1.06 in buffaloes.

Santos *et al.* (1998) recorded that emergences of wave occurred on day 0 and 11 in cycles with two waves and on day 0, 9 and 15 in cycles of three waves cycle in Gir cattle.

Kulick and Kot, (1999) observed that the circulating concentrations of inhibin has increased concurrently with emergence of waves and in turn suppressed the synthesis and secretion of FSH.

The day of onset of follicular wave was defined as “The day on which the dominant follicle was retrospectively traced to be 4 to 5 mm in diameter” (Burke *et al.*, 2000).

Viana *et al.* (2000) reported that on set of first, second and third wave was on day 0.78 ± 0.44 , 7.11 ± 1.05 and 13.22 ± 2.44 , respectively in case of three wave cycle and the first, second, third and fourth wave was on day 1.25 ± 0.50 , 7.25 ± 0.96 , 11.25 ± 0.96 and 15.00 ± 1.41 , respectively in four wave cycle of Gir breed.

Filho *et al.* (2001) recorded that on set of first and second wave in two wave cycle was on day 0.6 ± 0.4 and 9.2 ± 0.7 , respectively whereas the nine cows with three follicular waves showed emergences of wave on day 0.7 ± 0.4 , 10.0 ± 0.6 and 16.0 ± 0.1 in five-eighths Girolando cattle. They have also stated that there was a small difference in the onset of waves among cattle and attributed it to the genetic, environmental, nutritional factors, age and number of calvings, which may exert an influence over ovarian dynamics.

Nosier *et al.* (2003) observed that the first wave was detected on day 0 and the 2nd wave was detected on day 8.7 in 3-wave IOI. While, the 3rd wave was detected on day 15 of IOI.

Gaur *et al.* (2007) recorded that the first wave of a two-wave cycle emerged on day 2.10 ± 0.36 and the second wave (ovulatory) emerged on day 10.5 ± 0.6 while in cows with three-wave cycle the first, second and third (ovulatory) waves emerged on day 0.7 ± 0.5 , 7.2 ± 1.0 and 13.2 ± 3.4 of estrus, respectively.

Akter *et al.* (2010) reported that the first wave was emerged on day 3.3 ± 1.0 and the second wave emerged on day 12.3 ± 1.0 in two way cycle of the zebu cattle.

Satheshkumar *et al.* (2011) compared the follicular dynamics in cross breed cattle and buffalo and observed that the day of emergence of the first follicular wave was significantly ($P < 0.05$) earlier (day 0.89 ± 0.31) and the emergence of second

wave was significantly ($P < 0.05$) later (day 9.44 ± 0.34) in crossbred cows when compared with buffaloes (day 1.80 ± 0.26 and 8.60 ± 0.20 , respectively)

Sakhong *et al.* (2011) compared the follicular dynamics in Thai heifers and cows and observed that there was a non-significant difference among the patterns in the linear growth rate of dominant preovulatory follicle. However, the first and second wave in both heifers and cows of all patterns were not different. But the third wave of 3-wave pattern appeared earlier in heifers than in cows (12.57 ± 0.56 , 14.29 ± 0.36 d; $P < 0.05$).

Muraya, (2013) observed that first wave in two-wave cycles was emerged on day 0.61 ± 0.06 while the second wave was emerged on day 10.40 ± 1.17 . In three-wave cycle the waves were emerged on day 0.42 ± 0.16 for wave one and the second and third waves on day 8.15 ± 0.56 and 15.00 ± 0.98 , respectively. Four wave cycle animals had their waves emergence on day 0.5 ± 0.5 , 7.0 ± 2.0 , 14.5 ± 3.5 and 17.0 ± 3.0 for waves one, two, three and four, respectively.

Hadiya *et al.* (2016) recorded that there was a non-significant difference for the period of emergence of first as well as second dominant follicle between 2 and 3 wave cycles. However, preovulatory follicle of 2-wave cycle appeared significantly ($P < 0.01$) earlier than 3-wave cycle (day 9.00 ± 0.71 vs 14.50 ± 0.50) in postpartum cyclic crossbred cows.

Imron *et al.* (2016) recorded that the first wave of 3 and 4-waves pattern was emerged on day 0.4 ± 0.9 and 1.4 ± 1.1 , respectively. The second wave of 3 and 4-wave pattern was emerged on day 9.8 ± 1.5 and 7.4 ± 1.9 , respectively in Peranakan ongole cattle.

Siregar *et al.* (2016) recorded that wave initiation of first, second and third waves in Aceh cattle was on day 0, 8th and 14th of oestrus cycle, respectively.

2.3.4 Duration of follicular wave

Figueiredo *et al.* (1997) observed that in the Nelore cattle ovulatory wave was always the shortest regardless of 2 or 3 wave pattern. However, the ovulatory wave was longer ($P < 0.05$) in cows with 2 waves than for those with 3 waves ($P < 0.05$). In the 2-wave cows the first follicular wave was longer ($P < 0.10$) than the second ($P < 0.05$) compared with that of the 3 wave animals and in cow with 2 wave cycle 1st and 2nd waves lasted for 14.75 ± 0.70 and 9.05 ± 0.69 days, respectively. Similarly 1st, 2nd and 3rd wave of 3 wave cycle lasted for 13.00 ± 0.65 , 11.50 ± 0.42 and 6.86 ± 0.51 days, respectively.

Baruselli *et al.* (1996) reported that the ovulatory wave was longer ($P < 0.05$) in buffaloes with 2 waves than for those with 3 waves (20.67 ± 1.18 vs 17.90 ± 3.47 days; $P < 0.05$). They have also recorded that the wave duration was 7.39 ± 1.55 and 11.44 ± 0.91 days in 1st and 2nd of 2 wave cycle, respectively and 5.50 ± 1.22 , 5.20 ± 0.61 and 7.70 ± 0.52 days in 1st, 2nd and 3rd wave of 3 wave cycle, respectively.

Viana *et al.* (2000) reported that the wave duration was 13.00 ± 1.58 , 11.44 ± 2.19 and 7.67 ± 1.80 days in 1st, 2nd and 3rd wave of 3 wave, respectively and 11.50 ± 2.38 , 9.50 ± 1.92 , 9.25 ± 1.71 and 7.25 ± 1.89 days in 4 wave cycle, respectively in Zebu cattle.

Nosier *et al.* (2003) observed that there was a significant ($P < 0.05$) difference in wave duration of preovulatory follicle of 2 wave and 3 wave cycles.

Guar *et al.* (2007) observed that the wave duration was 13.35 ± 1.72 and 10.45 ± 0.98 days in 1st and 2nd wave of two wave cycle, respectively. Similarly, wave

duration in 1st, 2nd and 3rd wave of 3 wave cycle was 13.00 ± 1.58 , 11.44 ± 2.19 and 7.67 ± 1.80 days, respectively in Rathi cattle.

Hadiya *et al.* (2016) recorded that in cyclic crossbred heifers, the duration of preovulatory follicles differed significantly ($P < 0.01$) between 2 and 3 wave cycles (12.00 ± 0.70 vs 7.00 ± 0.00 days) wherein they have recorded that the wave duration in 1st and 2nd wave of 2 wave cycle was 10.00 ± 0.40 and 12.00 ± 0.70 days, respectively and 10.00 ± 0.00 , 6.00 ± 0.00 and 7.00 ± 0.00 days, respectively in 3 wave cycle. They have also reported that duration of waves in 2 and 3 wave cycle in crossbred cows was almost similar with reference to the wave duration (7.75 ± 0.48 and 12.00 ± 0.70 days in 1st and 2nd wave of 2 wave cycle, respectively and 8.50 ± 0.50 , 7.00 ± 0.00 and 7.50 ± 0.50 days in 1st, 2nd and 3rd of 3 wave cycle, respectively).

Siregar *et al.* (2016) recorded that the wave duration was 8, 6 and 4 days in 1st, 2nd and 3rd wave of 3 wave cycle, respectively in Ache cattle.

2.3.5 Maximum diameter of Dominant follicle and its day of appearance

Ginther *et al.* (1989) recorded that in 2-wave cows, the maximum diameter attained by the dominant an-ovulatory follicle was not different from the diameter of the preovulatory follicle on the day before ovulation but in 3-wave cows the mean maximum diameter of the 1st an-ovulatory follicle was greater ($P < 0.01$) than 2nd an-ovulatory follicle and pre-ovulatory follicle.

Ginther *et al.* (1996) reported that FSH release inhibition was proportional to the maximum size of the DF and that larger DFs were related to higher intervals between follicular waves.

Fortune, (1994) reported that the dominant follicle is functional only during growth or early plateau phases and the period in which it can induce atresia of subordinate follicles thereby block follicular recruitment and emerging of a new wave.

Figueiredo *et al.* (1997) observed that maximum diameter (10 to 12 mm) of dominant follicle in Nelore cattle was smaller than the *Bos taurus* heifer and cow (14 to 20 mm). They have also stated that maximum diameter of dominant follicle of 2nd wave was smaller than the 1st and 3rd wave of 3 wave cycle and that might be due to low progesterone levels since the corpus luteum was still maturing. This would not have prevented LH release leading to enhanced follicular growth because of failure to have strong negative feedback of progesterone at the hypothalamus and pituitary. Same authors have stated that during second follicular wave mature CL would secrete high concentrations of progesterone that inhibit LH release. In the third wave, the CL demise causes a sharp decrease in progesterone levels, which leads to increase in LH concentration, followed by fast follicular growth and ovulation.

Alvarez *et al.* (2000) recorded that maximum diameter (11.4 ± 0.6 mm) of the dominant follicle of the first wave of follicular development was lesser ($P < 0.01$) in Angus cows than in Brahman ($15.3 \pm .6$ mm) or Senepol (13.9 ± 0.5 mm) cows. While the maximum diameter (15.6 ± 0.5) of the preovulatory follicle was greater ($P < 0.01$) in Brahman than in Angus (12.8 ± 0.4 mm) or Senepol cows (13.6 ± 0.4 mm).

Viana *et al.* (2000) reported that the max DF diameter was 11.78 ± 1.20 mm, 10.44 ± 2.13 mm and 12.44 ± 1.59 by the day 6.45 ± 0.88 , 12.89 ± 3.06 and 20.44 ± 1.42 of first, second and third wave of the Gir cattle. Simultaneously, they have

observed that the DF maximum diameter (10.44 ± 2.1 mm) of the second wave was smaller ($P < 0.05$) than the diameter (12.44 ± 1.59 mm and 11.78 ± 1.2 mm) of the other DFs. Further, they have postulated that the differences might be due to the fact that the second wave emerges during the period of higher progesterone, whereas the first and third waves emerged during the luteogenic and luteolytic periods.

Filho *et al.* (2001) recorded that in two follicular waves, the first dominant follicles reached maximum diameter of 13.8 ± 1.4 mm on day 6.2 ± 0.9 . The second dominant follicles reached an average diameter of 13.8 ± 0.7 mm on day 15.6 ± 1.2 . In the cycles with three follicular waves, the maximum diameter of the dominant follicles of the first and second waves were 11.8 ± 0.8 mm and 12.0 ± 0.6 mm on days 6.7 ± 0.7 and 13.7 ± 0.3 , respectively.

Bó *et al.* (2003) reported that the number of *B. indicus* cows (16%) which had 4 follicular wave pattern was higher ($P < 0.01$) than *B. taurus* (0%) and was attributed to smaller diameter of dominant follicle (10–12 mm) and shorter dominant duration in *B. indicus* cows.

Nosier *et al.* (2003) observed that the maximum diameter preovulatory follicle was 10.9 ± 0.3 mm and 11 ± 0.9 mm in 2 and 3 wave cycle, respectively. Similarly maximum diameter of non ovulatory DF were 6.8 ± 0.3 mm and 7.3 ± 0.7 mm in 2 and 3 wave respectively and also observed a significant ($P < 0.05$) increase in the maximum diameter of DF in 3- wave inter ovulatory intervals.

Gaur *et al.* (2007) recorded that maximum DF diameter of first and second wave was appeared on day 5.00 ± 0.63 and 17.50 ± 0.50 , respectively with a maximum diameter of 11.75 ± 1.59 and 14.65 ± 1.24 mm, respectively in Rathi breed cattle.

Akter *et al.* (2010) reported that maximum DF diameter of first and second wave was appeared on day 9.3 ± 1.0 and 18.7 ± 1.0 , respectively with a maximum diameter of 12.1 ± 1.1 and 18.1 ± 0.6 mm, respectively in zebu cattle of Bangladesh.

Sakhong *et al.* (2011) compared follicular dynamics in Thai heifers and cows and observed that the maximum diameters of dominant preovulatory follicles in heifers was 12.13 ± 0.62 , 11.31 ± 0.38 , and 11.28 ± 0.34 mm on day 9.33 ± 0.79 , 15.13 ± 0.48 , 18.57 ± 0.51 in 1-wave, 2-wave and 3-wave heifers, respectively and in cows 12.65 ± 0.76 , 12.45 ± 0.76 , 12.82 ± 0.22 , and 12.80 ± 1.08 mm on day 10.50 ± 0.96 , 15.50 ± 1.46 , 21.63 ± 0.51 in 1-wave, 2-wave and 3-wave cow, respectively. They have concluded that maximum diameters in the 3-wave pattern for heifers was smaller and shorter than in cows ($p < 0.05$).

Satheshkumar *et al.* (2011) reported that the DF of the first-wave in buffaloes reached the maximum size significantly ($P < 0.01$) earlier (Day 5.80 ± 0.50) than in crossbred cattle.

Muraya, (2013) observed that the maximum diameters (10.95 ± 0.34 mm and 13.52 ± 0.50 mm) of both the non-ovulatory and preovulatory follicles of the Boran cow seem were smaller than those reported for taurine cows. They have also recorded that the maximum DF diameter of first and second waves was 13.6 ± 0.92 and 14.00 ± 0.85 mm on day 7.20 ± 0.37 and day 19.40 ± 2.01 , respectively in case of two wave cycle and in three wave cycle, maximum DF diameter of first, second and third wave of 10.95 ± 0.34 mm, 10.63 ± 0.58 mm and 13.52 ± 0.50 mm on day 6.31 ± 0.44 , day 14.54 ± 1.22 and day 22.31 ± 1.04 , respectively in Kenyan boran cows.

Siregar *et al.* (2016) recorded that the maximum DF diameter on day 5, 12 and 18 was 10.00 ± 1.83 , 8.75 ± 0.50 and 13.75 ± 1.71 mm, respectively in Aceh cattle having three wave cycle.

2.3.6 Diameter of preovulatory follicle

Ginther *et al.* (1989) reported that the dominant preovulatory follicle differed significantly between the 2-wave and 3-wave intervals in the diameter on the day before ovulation.

Baruselli *et al.* (1997) reported that the pre-ovulatory follicle size in 2 wave and 3 wave cycles was 15.5 ± 0.16 and 13.4 ± 0.13 mm in Murrah dairy buffaloes, respectively.

Curran, (2000) reported that the dominant pre-ovulatory follicle can be predicted one or three days prior to ovulation and the time of ovulation can be determined by disappearance or greatly reduced size of the dominant follicle.

Viana *et al.* (2000) reported that diameter of preovulatory was always more than the diameter of non ovulatory DF of other non ovulatory wave and length of ovulatory follicular wave was shorter than the first wave, but was similar to that observed for the intermediate waves ($P < 0.05$) reflecting the lower persistence of these waves by considering that the preovulatory wave has no atresia period. The diameter of preovulatory follicle in three and four wave cycles was 20.44 ± 1.42 and 21.25 ± 1.71 mm, respectively in Zebu cattle.

Sartori *et al.* (2001) reported that bovine follicles achieve preovulatory capacity at ≥ 10 mm in diameter. They have also stated that a larger dose of luteinizing hormone was required to induce ovulation of a 10 mm follicle than to induce ovulation of larger follicles.

The number and size of follicles on a given ovary will vary widely and are dependent on the time of year and the reproductive stage in different animals (Godoi *et al.*, 2002).

Celik *et al.* (2005) reported that size of the pre-ovulatory follicle was influenced by the number of follicular waves during the oestrous cycle.

Sartorelli *et al.* (2005) compared the follicular dynamics of both Nelore cow and Holstein and stated that the maximum diameter of the dominant follicle is about 11 mm in Nelore whereas 16 mm in Holsteins.

Gaur *et al.* (2007) observed that maximum diameter of preovulatory follicle was 20.44 ± 1.42 mm in Rathi cattle.

Satheshkumar *et al.* (2011) reported that the third-wave DF (preovulatory follicle) in buffaloes developed at a slow pace and reached a maximum diameter (10.9 ± 0.7 mm) which was significantly smaller than its counterpart in crossbred cattle (13.33 ± 0.72 mm) and also stated that smaller size and slower growth of DF was the single largest problem affecting the reproductive efficiency in water buffaloes thereby increasing the inter-calving period.

Pfeifer *et al.* (2012) have grouped follicles in to five classes viz., 10-12; 13-15; 16-18; 19-21 and > 22 mm and observed that a higher proportion of cows presented follicles between 13-15 mm rather than between 10-12 and > 22 mm of diameter.

Centurion-Castro *et al.* (2013) reported that the pre-ovulatory follicle diameter in BCS1-2, BCS3-4 and BCS5-6 was 9.8 ± 0.9 , 10.8 ± 1.3 and 12.9 ± 1.0 respectively indicating that the diameter of the pre-ovulatory follicle was positively correlated with the BCS.

Muraya, (2013) observed that the largest diameter (14.00 ± 0.85 mm) was attained only by the preovulatory DF ($P < 0.05$) in the Boran cows. The maximum diameter of preovulatory follicle of two and three wave cycle was 13.52 ± 0.50 and 14.00 ± 0.85 mm respectively.

Keskin *et al.* (2016) observed that the percentage of cows having medium sized preovulatory follicles (≥ 14 and < 18 mm) was 63.9, whereas the percentage of cows with small (≥ 10 and < 14 mm) and large follicles (≥ 18 mm) was 18.3 and 17.8, respectively.

Degefa *et al.* (2016) stated that mean diameter of the dominant follicle was similar ($P > 0.10$) in both genotypes (In Boran cows 15.8 ± 1.5 mm and in B×H cows 19.4 ± 2.9 mm).

Hadiya *et al.* (2016) recorded that the diameter of preovulatory follicle of 2-wave cycle was significantly ($p < 0.05$) larger than 3-wave cycle (17.22 ± 0.79 vs 14.65 ± 0.45 mm) in crossbreed heifers and also stated that the difference in the diameter of preovulatory follicle between 2-wave cycle and 3-wave cycles was insignificant ($P > 0.05$).

Siregar *et al.* (2016) recorded that the maximum DF diameter on day 18 was 13.75 ± 1.71 mm in Aceh cattle having three wave cycle.

2.4 Growth rate:

Spicer *et al.* (1986) noted that the rate of growth of small follicles (1 to 3 mm) into larger follicles was constantly increased as the oestrous cycle progress from day 1 to 18 (day 0 = estrus).

Savio *et al.* (1988) defined growth rate of ovarian follicle as “The maximum size of the follicle divided by duration of growth” and also reported that the growth rate was not linear each day but the mean growth rates per day were 5.5, 4.3 and 3.6 mm in 1st, 2nd and 3rd wave dominant follicles, respectively.

The main characteristics of follicular growth and atresia are affected by wave order (Ginther *et al.*, 1989a) due to many factors such as breed (Figueiredo *et al.*, 1997), reproductive stage (Roche and Boland, 1991), season (Badinga *et al.*, 1994), and energy balance (Rhodes *et al.*, 1995).

Grimard *et al.* (1995) reported that energy balance will affect IGF, insulin and GH systemic levels and alters LH pulse frequency leading to altered follicular growth.

Alvarez *et al.* (2000) compared growth rate of three breeds and commented that there was a non-significant difference ($P > .10$) in growth rate of the preovulatory follicle among Angus, Brahman, and Senepol cows. They have also reported that Angus and Brahman cows had predominantly two waves of follicular development during the oestrous cycle (72.7 and 55.6%, respectively) and Senepol cows had mostly (70%) three waves of follicular development during the oestrous cycle and these breed differences were non-significant ($P > .05$).

Viana *et al.* (2000) reported that growth and atresia rates did not differ among waves ($P > 0.05$) and noted that growth rate (mm/day) was 1.00 ± 0.50 , 1.03 ± 0.16 and 1.01 ± 0.33 in first, second and third wave, respectively in three wave cycle. Similarly animals with four wave cycle recorded the growth rate was 0.73 ± 0.25 , 0.98 ± 0.24 , 0.88 ± 0.25 and 0.92 ± 0.17 mm/day in first, second, third and fourth wave, respectively in Gir breed.

Bó *et al.* (2003) reported that the growth rate of follicle was season dependent and the growth rate of the dominant follicle in *B. indicus* cows was slower in the fall (1.1 mm per day) than in the spring (1.5 mm per day), whereas the dominant follicle in *Bos taurus* cows tended to grow faster during the fall (1.6mm per day) than in the spring (1.4mm per day).

Nosier *et al.* (2003) stated that development of dominant anovulatory follicles comprised of three phases i.e., growing, static, and regressing phases. A progressive increase in the size of dominant anovulatory follicle and estradiol concentration was observed during growth phase and progressive decrease in estradiol concentration during static phase with more or less constant size of the follicles.

Gaur *et al.* (2007) recorded that the growth rate (mm/day) of dominant follicle in Rathi cattle was 1.03 ± 0.24 , 1.07 ± 0.23 and 1.08 ± 0.26 during first, second and third waves of three wave cycle, respectively and growth rate (mm/day) was 1.81 ± 0.32 and 1.16 ± 0.22 during first and second wave of two wave cycle, respectively.

Adam *et al.* (2008) observed that group of follicle had the same growth rate at the beginning of growth until one follicle was selected for continuous growth to become a DF.

Sakhong *et al.* (2011) compared follicular dynamics in Thai heifers and cows and observed that there was non-significant difference among the patterns in the linear growth rate of dominant preovulatory follicle and DF growth rate (mm/day) of 1.16 ± 0.17 , 1.34 ± 0.17 , 1.16 ± 0.05 and 1.16 ± 0.04 in first, second, third and fourth wave, respectively in four wave cycle.

Satheshkumar *et al.* (2011) compared follicular dynamics of buffaloes and cross bred cattle and reported that in buffaloes the DF of the first-wave reached the

maximum size significantly ($P < 0.01$) earlier (day 5.80 ± 0.50) at a significantly ($P < 0.01$) faster growth rate (2.10 ± 0.14 mm / day) than in cattle (1.58 ± 0.09 mm / day). The growth rate of second (1.42 ± 0.10 mm / day) and third (1.95 ± 0.30 mm / day) wave cross breed cattle were non significantly ($P < 0.01$) higher than the second (1.50 ± 0.10 mm / day) and third (1.73 ± 0.10 mm / day) wave of the buffaloes.

Muraya, (2013) observed that growth rate of first and second wave DF of two wave cycle was 0.92 ± 0.04 and 0.78 ± 0.04 mm / day respectively. Similarly, growth rate of first, second and third wave was 0.87 ± 0.07 , 0.73 ± 0.52 and 0.87 ± 0.81 mm / day, respectively in case of three wave cycle in Boran cow.

Hadiya *et al.* (2016) recorded that in postpartum cyclic crossbred cows linear growth rate (mm/day) (1.21 ± 0.12 and 1.22 ± 0.17 mm/day) of first dominant follicle was almost similar between 2- and 3-wave cycles and the linear growth rate (mm/day) of preovulatory follicle was significantly lower ($P < 0.05$) in 2 wave than 3-wave cycle (0.90 ± 0.07 vs 1.31 ± 0.15).

Imron *et al.* (2016) reported that growth rate of dominant follicle was non-significantly different between 3 and 4 wave (0.87 ± 0.23 and 0.94 ± 0.25 mm/day, respectively) in Peranakan ongole cattle.

2.5 Rate of atresia:

Savio *et al.* (1988) defined atresia rate of ovarian follicle as “The maximum size of the follicle divided by duration of atresia”. They have reported that mean rates of atresia for the 1st and 2nd dominant follicles was 1.9 and 3.9 mm per day ($P < 0.05$) and the mean lengths of atresia was 10 and 4-6 days for the 1st and 2nd follicles ($P < 0.05$), respectively in Friesian× Hereford cyclic beef heifers.

Taylor and Rajamahendran, (1991) observed that there was a non-significant difference in the onset of regression (first dominant follicle) between 2- and 3-wave cycles.

Viana *et al.* (2000) reported that growth and atresia rates did not differ among waves ($P>0.05$) and recorded that the DF atresia rate (mm/day) was 1.02 ± 0.30 , 0.92 ± 0.29 and 0.76 ± 0.07 in first, second and third waves, respectively in four wave cycle. Similarly in case of three wave cycle the DF atresia rate (mm/day) was 0.97 ± 0.26 and 0.96 ± 0.33 in first and second wave, respectively in Gir breed.

Gaur *et al.* (2007) recorded that there was a non-significant ($P>0.05$) difference between the growth and atresia rates among waves and recorded that the DF atresia rate (mm/day) was 0.97 ± 0.26 and 0.96 ± 0.33 in first and second waves in three wave cycle and DF atresia rate was 0.55 ± 0.2 in first wave of two wave cycle in Rathi cattle.

Satheshkumar *et al.* (2011) compared follicular dynamics of buffaloes and cross bred cattle and reported that there was a non-significant difference in DF atresia rate of among the cross breed cattle and buffaloes. They have observed that the DF atresia rate (mm/day) was 1.50 ± 0.23 and 1.96 ± 0.10 in first and second wave of three wave cycle of buffaloes, respectively. Similarly in cross breed cattle the DF atresia rate (mm/day) was 1.47 ± 0.14 and 2.24 ± 0.25 in first and second wave of three wave cycle, respectively.

Throughout ovarian development and function in mammals, a highly orchestrated, periodic process known as “follicular atresia” occurs that destroys and eliminates follicles and oocytes from the ovary indicating that in the adult female, atresia ensures that only the healthiest follicles, containing oocytes of optimal quality

for fertilization and remain available throughout the reproductive period (Towson *et al.*, 2002).

Muraya, (2013) observed that atresia rate of first wave DF of two wave cycle was 1.01 ± 0.29 mm / day and the same for first and second wave DF of three wave cycle was 0.91 ± 0.10 mm / day and 01 ± 0.10 mm / day, respectively in Boran cow.

Hadiya *et al.* (2016) recorded that the mean linear regression rate of first wave in two and three wave cycle was 1.39 ± 0.09 and 1.33 ± 0.14 mm / day, respectively. Similarly atresia rate of 2nd wave in three wave cycle was 1.80 ± 0.29 mm / day in postpartum cyclic crossbred cows and they have concluded that there was a non-significant difference in the duration of the static and regression phase of the first dominant follicle between 2- and 3-wave cycles.

Imron *et al.* (2016) recorded that mean DF atresia rate of three and four wave cycle was 0.83 ± 0.21 and 0.91 ± 0.31 mm/day, respectively in Peranakan ongole cattle.

2.6 Effect of preovulatory follicle diameter on maximum diameter of CL

McNatty *et al.* (1979) suggested that development of a normal CL depends on preovulatory follicles having: (i) an adequate number of granulosa cells, (ii) an adequate number of luteinizing hormone receptors on granulosa and thecal cells, and (iii) granulosa cells capable of synthesizing adequate amounts of progesterone after luteinization.

Baruselli *et al.* (1996) reported that preovulatory follicle had no difference in the diameter when correlated to the position of the CL (same ovary: 1.55 ± 0.15 cm, n=10 vs opposite ovary: 1.55 ± 0.19 cm, n=9) for 2-wave cycles and (same ovary:

1.32 ± 0.13 cm, n=5 vs opposite ovary: 1.38 ± 0.14 cm, n=5) for 3-wave cycles in buffaloes.

Vasconcelos *et al.* (2001) reported that CL diameter on day 7 post AI for smaller preovulatory follicles (≤ 14 mm) and larger preovulatory follicles (≥ 15 mm) was 4,949 and 5,735 mm³, respectively with a significant positive correlation.

George *et al.* (2005) reported that ovulation of small follicles (11.5 ± 0.2 mm) results in the development of small CLs and ovulation induced larger follicles (14.47 ± 0.39 mm) results in the development of bigger CLs in cattle.

Pfeifer *et al.* (2009) reported that heifers with mean preovulatory follicles diameters of 11.49 ± 0.48 and 13.57 ± 0.35 mm at AI had mean CL diameter of 20.79 ± 1.06 and 24.17 ± 0.83 mm, respectively and in cows with mean preovulatory follicles diameters of 14.2 ± 0.34 and 16.5 ± 0.52 mm at AI have mean CL diameter of 22.2 ± 0.76 and 24.7 ± 0.69 mm, respectively.

Pandey *et al.* (2011) reported that mean of pre-ovulatory follicle diameters of 14.1 ± 0.3 and 12.2 ± 0.03 mm had post luteal diameters of 15.7 ± 0.7 and 13.3 ± 0.4 on day 5 post AI, respectively.

Pfeifer *et al.* (2012) reported that preovulatory follicles larger than 19 mm resulted in larger corpus luteus than preovulatory follicles with < 15 mm diameter.

Rahman *et al.* (2012) reported that on day 5 post ovulation CL size was positively correlated (CL: $r=.74$, $P<0.01$) with pre-ovulatory follicle diameter.

Pinaffi *et al.* (2015) reported that cows with mean preovulatory follicles diameter of 13.0 ± 0.2 and 12.7 ± 0.13 mm at AI have lead to the development of CL with a diameter of 17.5 ± 0.3 and 17.1 ± 0.3 mm, respectively.

2.7 Effect of preovulatory follicle diameter on progesterone profiles

Vasconcelos *et al.* (2001) reported that serum progesterone concentration on day 7 post AI for smaller preovulatory follicles (≤ 14 mm) and larger preovulatory follicles (≥ 15 mm) was 1.22 ± 0.15 and 1.61 ± 0.13 respectively with a significant correlation. Further, they have also stated that when the follicles of the dairy cows were aspirated to reduce their size (11.5 ± 0.2 vs 14.5 ± 0.4 mm for non aspirated control) the serum progesterone concentration at day 7 was affected correspondingly (1.16 ± 0.12 vs 1.42 ± 0.11 ng/ml, respectively).

George *et al.* (2005) reported that small follicles (11.5 ± 0.2 mm) results in to the development of small CLs and secretes less progesterone than that in cattle induced to ovulate larger follicles (14.47 ± 0.39 mm).

Busch *et al.* (2008) reported that ovulating follicles having a diameter of 10.0 to 12.5 mm had reduced serum concentration of progesterone compared with ovulating follicles having a diameter of ≥ 15 mm in cows.

2.8 Subordinate follicle

Figueiredo *et al.* (1997) stated that maximum diameter of subordinate follicle of first and second wave of two wave cycle was 7.53 ± 0.40 and 7.17 ± 0.36 mm respectively. Same with reference to first, second and third wave of three wave cycle was 6.14 ± 0.37 , 5.80 ± 0.45 and 6.21 ± 0.39 mm, respectively.

Viana *et al.* (2000) reported that the maximum diameter of subordinate follicle of first, second and third wave of three wave cycle was 7.25 ± 0.71 , 7.22 ± 1.39 and 7.00 ± 1.32 mm, respectively and the maximum diameter of subordinate follicle of first, second, third and four wave of four wave cycle was 7.50 ± 0.58 , 7.50 ± 1.29 , 6.75 ± 0.96 and 7.25 ± 2.50 mm, respectively.

Gaur *et al.* (2007) observed that the maximum diameter of subordinate follicle of first and second wave of two wave cycle was 7.42 ± 0.58 mm and 7.60 ± 0.90 mm, respectively, and same in first, second and third wave of three wave cycle was 7.25 ± 0.71 , 7.22 ± 1.39 and 7.00 ± 1.32 mm, respectively in Rathi cattle.

Muraya, (2013) observed that the maximum diameter of largest subordinate follicle of first, second and third wave of three wave cycle was 6.74 ± 0.42 , 6.89 ± 0.37 and 7.28 ± 0.40 mm with a growth rate of 0.60 ± 0.90 , 0.49 ± 0.07 and 0.57 ± 0.10 mm/day, respectively. While the same in first and second wave of two wave cycle was 7.40 ± 0.82 and 7.12 ± 0.42 mm with an atresia rate of 0.74 ± 0.19 and 0.43 ± 0.92 mm/day, respectively in Boran cows.

Imron *et al.* (2016) recorded that the growth rate of subordinate follicle of third and fourth wave was 0.83 ± 0.25 and 0.72 ± 0.33 mm/day, respectively in Peranakan ongole cattle.

2.9 Characteristics of corpus luteum

Figueiredo *et al.* (1997) characterized the luteal phase length as the main determinant of the number of follicular wave as it keeps high concentrations of progesterone and blocks the ovulation of functional dominant follicles inducing their atresia and the onset of a new follicular wave. Supporting this hypothesis, factors like the artificial lengthening of oestrous cycle by exogenous progesterone administration (Sirois and Fortune, 1988 and Taylor *et al.*, 1993) and the maintenance of corpus luteum during initial gestation (Roche and Boland, 1999 and Ginther, 1993) result in the continuous onset of new follicular waves.

Figueiredo *et al.* (1997) stated that average size of CL for 3-wave Nelore cattle (17.69 ± 0.40 mm) was smaller than those breeds of *Bos taurus* (20 to 25 mm).

Quintal-Franco *et al.* (1999) reported that LH pulses are necessary for both structural development (corpora luteum with typical diameter) and normal function (production of progesterone) of corpora luteum 48 h before the time of preovulatory surge of LH (d 0) to d 7 of the oestrous cycle in cattle.

Taponen *et al.* (2000) reported that the CL grew to a peak diameter of 22.7 ± 1.1 mm on the mean Day of 11 or 12 in Finnish Ayrshire breed cows and heifers.

Sartori *et al.* (2002) reported a significant ($P > 0.05$) positive correlation between size of the preovulatory follicle and size of the CL and that seems likely that increased preovulatory follicular size has led to increased numbers of granulosa cells which differentiate into large luteal cells following the LH surge and subsequent increased size of the CL.

Siqueira *et al.* (2009) reported that greater percentage of CL appeared in heterogeneous echotexture in the beginning of the oestrous cycle (metestrus) and near to the subsequent ovulation (proestrus) than during diestrus. Further, the corpus luteum morphological differentiation (development of luteal tissue) was highly correlated to CL function (progesterone production), however there was a temporal difference between physical regression and loss of function after the onset of luteolysis.

Satheshkumar *et al.* (2011) recorded that the mean maximum diameter was 21.58 ± 0.36 mm in crossbred cows but the size was attained on day 9.33 ± 0.51 of cycle and initiation of luteal regression was noticed from day 16.25 ± 0.76 onwards.

Muraya, (2013) observed that in Boran cows the day of onset of luteal regression was directly related to the number of waves per cycle and the duration of cycle for all cows. Similarly they have reported that the maximum diameter of CL in

two, three and four wave cycle was 17.0 ± 1.29 , 15.9 ± 0.64 and 15.1 ± 0.9 mm on day 11.2 ± 2.18 , 12.7 ± 0.61 and 15.5 ± 4.50 , respectively and the onset of regression was started on day 10.8 ± 1.74 , 14.4 ± 0.92 and 15.4 ± 0.87 , respectively.

2.9.1 Effect of CL size on progesterone concentration

Spencer *et al.* (1989) reported that luteal structure >11 mm usually correlates with a progesterone concentration and it was greater than 5 ng/ml.

Baruselli *et al.* (2001) reported that CL >20 mm in diameter had 2.44 ng/ml, CL of 15 mm in diameter had 1.75 ng/ml and CL less than 15 mm in diameter had 1.19 ng/ml plasma progesterone concentrations suggesting that CL diameter and plasma progesterone concentration were significantly correlated.

Veronesi *et al.* (2002) demonstrated a good correlation between the diameter of the CL and peripheral P4 concentrations.

Atanasov *et al.* (2015) observed that 24.19 % of the cows had lower than 1 ng/mL P4 concentrations even though the CL was larger than 25 mm in diameter.

2.10 STEROIDS CONCENTRATION:

Follicular development in cattle is thought to be regulated primarily by gonadotrophic hormones (Lucy *et al.*, 1992). The hormonal profiles of progesterone and oestradiol during the oestrous cycles were characterized by increasing serum concentrations of progesterone during different stages of the oestrous cycle.

2.10.1 Progesterone

Following ovulation, progesterone concentrations begin to increase due to the formation of the CL in which the granulosa and theca cells of the ovulated DF lutenise and produce progesterone in readiness for the establishment and maintenance

of pregnancy and/or resumption of the oestrous cycle (Niswender, 1981). During the di-oestrous phase, progesterone concentrations remain elevated and recurrent waves of follicle development continue to be initiated by release of FSH from the anterior pituitary. However, these DFs that grow during the luteal phase of the oestrous cycle do not ovulate (Hafeez, 1987).

The progesterone dominant luteal phase of the oestrous cycle, through negative feedback, only allows the secretion of greater amplitude but lesser frequency LH pulses that are inadequate for ovulation of the DF (Rahe *et al.*, 1980). Finally, during the pro-oestrous period, progesterone concentration decrease when the CL regresses in response to PGF secretion from the uterus (Hansel and Convey, 1983). Edgar (1953) reported that a rise of preovulatory progesterone was apparently absent in the cow, as indicated by the low, barely detectable progesterone values found at estrus. Progesterone levels increased from 0.25 ± 0.2 ng/ml plasma on day 4 to 4.36 ± 0.53 mg/ml on day 9 of the first cycle.

Sprague *et al.* (1971) reported that progesterone concentration declined at estrus and reached a low point 8 to 20 hr after LH peak. Further, they also stated that progesterone levels were lowest at 20 hr. after detection of estrus. The concentration rose gradually and to remain above 2.50 ng / ml between days 6 and 12 with peaks on days 9 and 12 and declined thereafter to the next estrus.

Edquist *et al.* (1973) reported a progesterone value of 0.7 ng/ml 2 days after estrus in bovines.

Echternkamp and Hansel (1973) reported in lactating Holstein cows the progesterone concentration on days 0, 3, 9, 12, 15, 18, 21 p4 pg/ml that were $0.24 \pm$

0.13; 1.65 ± 0.67 ; 4.36 ± 0.53 , 3.81 ± 0.78 , 3.45 ± 1.04 , 4.17 ± 0.53 and 0.24 ± 0.21 , respectively.

Smith *et al.* (1975) reported that the levels of progesterone reaches a peak of 6-7 ng/ml in mid-cycle, then began to fall 4 days before estrus to values of about 1 ng/ml at estrus and remained low for a further 4 days.

The progesterone concentrations (ng/ml) on 0, 2, 5, 9, 17 and 20 day of oestrous cycle in cows were 0.28 ± 0.03 , 0.17 ± 0.01 , 0.65 ± 0.02 , 1.5 ± 0.7 , 2.0 ± 0.5 and 0.40 ± 0.06 , respectively (Agarwal *et al.*, 1978).

Smith *et al.* (1975) reported that the levels of progesterone reaches a peak of 6-7 ng/ml in mid-cycle, then began to fall 4 days before estrus to values of about 1 ng/ml at estrus and remained low for a further 4 days.

Rawlings *et al.* (1980) reported that the undetectable circulating progesterone concentrations indicate absence of an ovulation and of a functional CL.

During various phases of reproduction in normal fertile cows the progesterone concentration (ng/ml) on day 1, 3, 10 and 18 of oestrous cycle were 0.23, 0.92, 1.7, and 2.07 respectively (Pargaonkar and Kaikini, 1989).

In non lactating normal cycling crossbreed cows which fed on maintenance ration and sub maintenance ration the plasma progesterone concentrations (ng/ml) during oestrous cycle of 20 days length on 0, 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 were (0.40 ± 0.03 , 0.47 ± 0.04), (0.45 ± 0.04 , 0.47 ± 0.05), (0.85 ± 0.09 , 0.67 ± 0.08), (1.46 ± 0.23 , 1.29 ± 0.21), (1.89 ± 0.31 , 1.75 ± 0.25), (1.60 ± 0.15 , 2.21 ± 0.40), (2.64 ± 0.30 , 2.45 ± 0.30), (2.46 ± 0.39 , 2.21 ± 0.32), (1.57 ± 0.29 , 1.52 ± 0.27), (0.71 ± 0.10 , 0.52 ± 0.05), (0.42 ± 0.03 , 0.40 ± 0.04), respectively in winter and (0.26 ± 0.03 , 0.39 ± 0.05), (0.41 ± 0.05 , 0.47 ± 0.10), (0.44 ± 0.08 , 0.80 ± 0.20), (1.96 ± 0.23 , $1.31 \pm$

0.51), (1.38 ± 0.24, 1.60 ± 0.39), (2.12 ± 0.41, 2.34 ± 0.82), (2.52 ± 0.56, 2.87 ± 0.71), (1.50 ± 0.51, 2.21 ± 0.58), (0.85 ± 0.25, 1.34 ± 0.29), (0.35 ± 0.07, 0.60 ± 0.08), (0.36 ± 0.02, 0.37 ± 0.03), respectively in summer (Juneja and Arora, 1990).

Savio *et al.* (1993) observed that from day 8 onwards, plasma concentrations of progesterone ranged from 5.5 to 8.5 ng/ml and reached peak values between days 15 and 18.

Peters *et al.* (1994) reported that stimulation by LH pulses is required for the normal progesterone production by corpora luteum from d 2 to 12, but not from d 12 to 17 of the oestrous cycle of cattle.

Baruah and Goswami (1997) recorded that the levels of serum progesterone (ng/ml) at various stages of oestrous cycle (days) 0, 3, 12, 16, 18 and 20 were 0.43 ± 0.06, 0.63 ± 0.03, 3.40 ± 0.23, 2.69 ± 0.19, 1.84 ± 0.23 and 0.45 ± 0.02, respectively in cross breed heifers (Jersey X Assam cows).

Figueiredo *et al.* (1997) reported that Nelore cows and heifers, progesterone concentration in the blood increased steadily from the second day post-ovulation and reached a plateau at approximately Days 8 to 16.

Palta *et al.* (1998) reported that progesterone concentrations in ovarian follicular fluid are much higher than those in peripheral circulation and progesterone concentrations (pmol/ml) were not related to follicular diameter and were not different among small (330.99 ± 27.32), medium (384.84 ± 26.20) and large follicles (253.25 ± 32.23) in buffaloes.

Roche *et al.* (1999) reported that only when serum progesterone concentrations were basal and LH pulses occur every 40–70 min for 2–3 days does the DF ovulate.

Shukla *et al.* (2000) reported that progesterone concentration in normal breeding cyclic animals on 0, 4, 11 and 16 of oestrous cycle were 0.40 ± 0.03 , 0.78 ± 0.05 , 2.25 ± 0.20 and 2.82 ± 0.26 , respectively.

Dhabale *et al.* (2000) reported that the serum mean progesterone concentrations (ng/ml) on 0, 10th, 20th and 30th days of oestrous cycle in crossbred cows were 1.98 ± 0.47 , 2.43 ± 0.75 , 1.97 ± 0.59 and 1.85 ± 0.78 , respectively.

Mondal *et al.* (2001) compared the cow with overt estrus and silent estrus, and recorded that in cows with overt estrus, plasma progesterone concentrations during periestrus, early luteal, midluteal and late luteal phase were 0.40 ± 0.02 , 0.74 ± 0.10 , 1.94 ± 0.22 and 0.63 ± 0.16 ng/ml, respectively and the corresponding values in cows with silent estrus being 0.47 ± 0.03 , 0.94 ± 0.08 , 1.39 ± 0.13 and 0.95 ± 0.19 ng/ml, respectively. The overall plasma progesterone levels in cows that exhibited overt estrus were 1.23 ± 0.99 ng/ml. It was concluded that progesterone levels were lower ($P > 0.05$) in cows that exhibited silent estrus compared to overt estrus.

Mondal and Prakash, (2003) reported that in Sahiwal cows peripheral progesterone levels (ng/ml) during periestrus, early luteal, mid luteal and late luteal phase were 0.40 ± 0.02 , 0.74 ± 0.10 , 1.94 ± 0.22 and 0.63 ± 0.16 ng/ml respectively.

Nosier *et al.* (2003) stated that the 2-wave and 3-wave period showed negative correlation with follicular size and progesterone concentration.

Mehrotra *et al.* (2005) reported that the progesterone concentration (ng/ml) during -8, -4, 0, 4, 8 and 12 days of estrus cycle were 0.682 ± 0.05 , 0.564 ± 0.09 , 0.718 ± 0.06 , 0.724 ± 0.04 , 0.682 ± 0.03 , 0.638 ± 0.07 and 0.712 ± 0.03 , respectively in crossbred (Holstein Friesian X Harijana) cows.

Venkatesan *et al.* (2005) observed that the serum concentrations of progesterone (ng/ml) on the day 0 and day 7 of estrus cycle were 0.1922 ± 0.077 and 3.151 ± 0.0092 , respectively in regular breeding cows.

Mutharao *et al.* (2005) reported that -4, -1, 0, 1, 2, 3, 4, 5, 6 and 7 days of oestrous cycle the progesterone concentrations (ng/ml) were 11.48 ± 0.38 , 0.47 ± 0.09 , 0.62 ± 0.27 , 0.98 ± 0.42 , 1.98 ± 0.43 , 7.23 ± 0.69 , 9.30 ± 1.08 , 11.70 ± 1.36 , 15.07 ± 1.48 and 16.25 ± 1.48 , respectively in Ongole cows.

In Sahiwal cows, Harpreet Sing *et al.* (2006) recorded that the plasma progesterone concentrations (ng/ml) on days 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 of oestrous cycle were 0.40 ± 0.04 , 13.94 ± 1.41 , 9.36 ± 0.68 , 8.38 ± 0.84 , 7.07 ± 0.72 , 5.88 ± 0.98 , 4.78 ± 1.09 , 3.35 ± 0.92 , 1.19 ± 0.53 , 0.24 ± 0.02 , and 0.13 ± 0.03 , respectively in cows and 0.49 ± 0.11 , 17.20 ± 3.84 , 19.15 ± 3.30 , 18.33 ± 3.03 , 14.50 ± 1.66 , 12.52 ± 0.97 , 9.28 ± 1.02 , 8.79 ± 1.71 , 1.27 ± 0.68 , 0.37 ± 0.22 , and 0.14 ± 0.02 in heifers, respectively.

Mondal *et al.* (2007) reported that blood progesterone raised to peak levels on day 13 to 15 of the oestrous cycle in buffaloes.

Siqueira *et al.* (2009) observed that the decline in plasma progesterone concentration usually precedes luteal tissue regression.

Sakhong *et al.* (2011) reported that the plasma progesterone concentrations increased slowly from day 3 to 5, reached the peak of approximate 3.74 ng/ml on day 9 and then declined to baseline by day 15 in Thai native heifers

Naik *et al.* (2013) recorded overall mean concentration of progesterone during oestrous cycle on the day of estrus and day 3, 6, 9, 12, 15, 18, 19, 20 of estrous cycle and subsequent estrus day 0 were 0.43 ± 0.89 , 1.89 ± 0.89 , 5.39 ± 0.89 ,

6.24 ± 0.89 , 9.72 ± 0.89 , 10.67 ± 0.89 , 8.64 ± 0.89 , 5.22 ± 0.89 , 1.67 ± 0.89 and 0.34 ± 0.89 mg per ml, respectively.

2.10.2 Estrogen

Increasing serum concentrations of 17β -estradiol by an increase in both frequency and amplitude of 17β -estradiol pulses from the ovary were observed during the early luteal phase of the oestrous cycle, corresponding to the greater frequency of LH pulses observed during this same period (Walters *et al.*, 1984). The increasing concentrations of circulating 17β -estradiol in cows during the early luteal phase of the oestrous cycle are accompanied by the concurrent appearance of the large estrogen active follicle in the ovary (Ireland and Roche, 1982).

Echternkamp and Hansel, (1973) noticed a rise in plasma estradiol concentration in lactating Holstein cows 2 to 3 days prior to estrus with maximal levels on day 0 of the first estrus and 24 hr. and the same on days 0, 3, 9, 12, 15, 18 and 21 (pg/ml) were 16.15 ± 5.17 ; 3.94 ± 2.79 ; 1.85 ± 0.75 ; 0.68 ± 0.06 ; 2.18 ± 0.58 , 1.93 ± 1.32 , 0.51 and 7.83 ± 0.94 , respectively.

Christensen *et al.* (1974) observed that estrogen levels were lowest at estrus (1.31 ± 0.84 ng/ml) and remained below 2 ng/ml until day four after estrus. Later it was increased to a peak of 6.15 ± 1.47 ng/ml on day 15 of the cycle.

Corah *et al.* (1974) found that estradiol was 4 to 8 pg/ml one day prior to estrus and reached 8.4 ± 1.7 pg / ml on the day of first postpartum estrus. While in subsequent oestrous cycle, estradiol values ranged between 4 and 6 pg/ml and reached a peak of 10.0 ± 1.3 pg/ml at the estrus.

Lemon *et al.* (1974) reported that the estradiol concentration at the time of estrus in cows was 20 to 25 pg/ml.

Smith *et al.* (1975) reported that peak levels of oestradiol (11.2 ± 1.3 pg/ml) occurred on the day before estrus and a second peak (7.9 ± 2.9 pg/ml) occurred on Days 6-8 of the cycle.

Agarwal *et al.* (1978) reported that the estradiol concentrations (pg/ml) in cows were 9.0 ± 1.5 , 6.00 ± 1.10 , 6.50 ± 1.60 , 5.5 ± 0.6 , 6.0 ± 1.2 and 4.70 ± 0.55 on 0, 2, 5, 9, 17 and 20 days of oestrous cycle, respectively.

In Holstein heifers, plasma oestradiol 17 concentration was lower at the start of luteal regression (2.2 ± 0.5 pg/ml) and increased to 3.8 ± 0.6 pg/ml on the next day and reached 6.6 ± 0.9 pg/ml (Glencross and Pope, 1981).

Agarwal *et al.* (1989) noticed comparatively lower levels of (9 pg/ml) of estradiol in blood serum on the day of estrus in cows which conceived and 5.7 pg/ml in those which were not conceived.

Jain and Pandey (1983) recorded higher blood serum estradiol levels (36.2 pg/ml).

While, Jimenez *et al.* (1988) observed that the estradiol concentration was 15.2 pg/ml on the day of estrus.

Savio *et al.* (1993) observed that oestradiol concentrations increased at the early stages of the oestrous cycle (days 4 to 6; 5 pg/ml) and returned to a basal concentration throughout the luteal phase.

Estradiol concentration in blood serum on day of estrus ranged from 11.6 to 18 pg/ml with on average value of 13.2 pg/ml, (Gupta *et al.*, 1998).

Alvarez *et al.* (2000) reported that the plasma concentration of estradiol on the day of estrus in Angus, Brahman and Senepol cows were 0.4, 0.4, and 0.3 pg/ml, respectively.

Estradiol concentrations (pg/ml) in cross bred cows on 0, 4, 11 and 16 days of cycle were 20.25 ± 0.70 , 11.20 ± 0.84 , 7.71 ± 1.49 and 6.56 ± 1.31 , respectively (Shukla *et al.*, 2000).

Purohit *et al.* (2000) reported that the estradiol concentration of Rathi cattle on the day of estrous, on 5th, 10th, 14th, 18th, and 22nd day of the oestrous cycle were 8.00 ± 1.88 , 10.15 ± 2.18 , 4.97 ± 0.43 , 6.75 ± 1.15 , 5.93 ± 1.50 , 6.09 ± 1.04 and 9.53 ± 2.11 ng/ml, respectively.

Shukla *et al.* (2000) studied hormonal levels during different stages of oestrous cycle from 0 day to 16th day and noticed higher basal estrogen levels (20.93 to 9.3 pg/ml) in repeat breeding than in normal cyclic cross bred cows (20.75 to 6.56 pg/ml).

Plasma estradiol (pg/mL) concentration on 8, 10, 12 and 0 day of oestrous cycle were 10.5 ± 0.1 , 12.0 ± 1.4 , 12.7 ± 1.3 and 15.5 ± 1.1 , respectively in crossbred cows (HF, Jersey and Brown Swiss) (Selvaraju *et al.*, 2002).

Nosier *et al.* (2003) stated that the 2-wave and 3-wave period showed positive correlation with follicular size and oestrodol concentration and also indicated that ovulation occurred when estradiol concentration attained its peak (5.95 ± 0.70 pg/ml in 2-wave periods and 5.23 ± 0.29 pg/ml in 3-wave periods).

Sartori *et al.* (2004) recorded that maximum serum estradiol concentration preceding ovulation was lower ($P \leq 0.0001$) in cows than in heifers (7.9 ± 0.8 vs. 11.3

± 0.6 pg/mL) even though preovulatory follicles were larger ($P \leq 0.0001$) in cows (16.8 ± 0.5 vs. 14.9 ± 0.2 mm).

Mehrotra *et al.* (2005) observed in Holstein Friesian X Hariana cattle that the serum estrogen concentration (pg/ml) on oestrous cycle -8, -4, 0, 4, 8, 12 and 16 days was 61.0 ± 4.30 , 73.0 ± 7.51 , 69.0 ± 5.78 , 60.0 ± 4.18 , 63.0 ± 6.63 , 59.0 ± 6.78 and 69.0 ± 9.27 , respectively.

Venkatesan *et al.* (2005) reported that in regular breeder cows estrogen concentration (pg/ml) on the day '0' and day 7 was 7.201 ± 1.29 and 2.99 ± 0.05 , respectively.

In Ongole cows, Mutharao *et al.* (2005) reported that estradiol concentrations (pg/ml) on different day of cycle (-4, -1, 0, 1, 2, 3, 4, 5, 6 and 7) were 37.83 ± 4.08 , $88, 28 \pm 3.71$, 49.48 ± 35.40 , 51.48 ± 15.82 , 47.48 ± 14.29 , 63.60 ± 21.84 , 33.55 ± 7.43 , 33.78 ± 14.87 , 34.90 ± 12.34 and 35.98 ± 9.74 , respectively.

Harpreet Sing *et al.* (2006) observed that estradiol concentrations (pg/ml) during 0,1,2,3,4,5,6,7,8,9 and 10 days of oestrous cycle were 13.00 ± 4.65 , 12.50 ± 3.40 , 21.00 ± 0.70 , 45.00 ± 10.26 , 34.25 ± 4.03 , 31.25 ± 4.64 , 53.50 ± 2.99 , 53.00 ± 6.72 and 32.00 ± 10.17 in Sahiwal cows and 11.50 ± 0.96 , 11.25 ± 39.81 , 74.75 ± 17.44 , 27.50 ± 9.03 , 18.50 ± 3.10 , 17.00 ± 1.73 , 17.25 ± 1.70 , 25.75 ± 1.55 and 24.50 ± 1.50 in Sahiwal heifers, respectively.

Opera *et al.* (2006) reported that the serum estradiol -17 β concentration (pg/mL) in non pregnant cyclic Bunaji cattle without corpus luteum and with large ovarian follicle (>3 mm) was 78.1 ± 0.2 , while in non pregnant cows having corpus luteum and large ovarian follicle (>3 mm) was 1591.6 ± 2.0 .

Naik *et al.* (2013) recorded overall mean concentration of estrogen in Punganur cows during estrous cycle on the day of estrus and the day 3, 6, 9, 12, 15, 18, 19, 20 of estrous cycle and subsequent estrus were 20.24 ± 1.17 , 11.26 ± 1.17 , 14.25 ± 1.17 , 10.53 ± 1.17 , 15.24 ± 1.17 , 12.36 ± 1.17 , 17.83 ± 1.17 , 20.28 ± 1.17 , 21.47 ± 1.17 and 22.25 ± 1.17 pg/ml, respectively.

Perry *et al.* (2014) stated that In all cows ($P < 0.01$) and those exhibiting estrus ($P = 0.02$), a significant positive relationship was there between follicle diameter and peak estradiol concentration, but there was no relationship ($P=0.52$) between follicle diameter and peak estradiol concentration in cows not exhibiting estrus and the above variants also correlate with pregnancy rate.

CHAPTER-III

3. MATERIALS AND METHODS

The study was performed at Livestock Research Station, Palamaner, Chittoor district, Andhra Pradesh. The institute is located at an altitude of 2550 feet above mean sea level and at 78° longitude and 13.15° Latitude. The present experiment was conducted during the period from March, 2017 to July, 2017. The mean environmental temperature, relative humidity and vapour pressure ranged between 12-40°C, 59-85 per cent and 7-17 mm Hg, respectively.

3.1 Experimental Animals

A total of eight Punganur cows (Fig.1) in estrus having a Body Condition Score of 2.5 to 3.5 in a scale of 1-5 (Westendorf *et al.*, 1988) were selected for the study.

3.1.1 Management

All the animals were maintained under uniform managerial and husbandry conditions in well-ventilated asbestos roofed sheds with cemented anti-slippery floor. The concentrate mixture consisted of maize 40 per cent, wheat bran 37 per cent, ground nut cake 20 per cent, mineral mixture 2 per cent and common salt 1 per cent on dry matter basis was fed to selected cows. Fresh green grass and clean drinking water were fed *ad libitum* during the entire experiment period. The cows were dewormed using Truemectin® Ivermectin against both external and internal parasites and no disease vaccination was carried out within the study period. After proper restraining the animal ovarian ultrasonographic scanning procedures were performed for understanding the reproductive status of the animal.



Fig. 1 : Photograph of Punganur cow



Fig. 2 : Photograph showing the ultrasonography of Punganur cow genital tract

3.2 Estrus detection

All the cows were constantly observed for the exhibition of estrus symptoms at six o'clock in the morning and six o'clock in the evening for a period of 30 minutes.

3.2.1 Estrus intensity

The intensity of estrus was measured by using a score card outlined by Rao and Rao (1981) and Callesen *et al.* (1993) with slight modifications mentioned in the below table. Further the intensity of estrus was classified as weak, intermediate and intense estrus based on the type of the symptoms exhibited by the animal.

Score card for assessment of intensity of estrus in crossbred cows

S.No	Symptoms exhibited	Points allotted
1	Bellowing and restlessness	15
2	Excitement	10
3	Estrual mucus discharge	20
4	Edema, moist vulva	15
5	Placid response to placement of palm or hand on the rump, raising of tail	10
6	Micturation	10
7	Cervical relaxation	5
8	Uterine tonicity	15
	TOTAL	100

These parameters were added and sum of these were quantified into weak estrus (0-40), intermediate estrus (41-70) and intense estrus (71-100) of that animal.

3.2.2 Duration of estrus

The duration of estrus was estimated in hours from the time of first appearance of behavioural symptoms to disappearance of symptoms of estrus.

3.2.3 Oestrous cycle length

The oestrous cycle length was estimated in days from the time of onset of estrus to the appearance of subsequent estrus.

3.3 Ultrasonography of Ovary

Ovarian ultrasonographic evaluation was carried out on all eight selected cows during the period of two oestrous cycles on every alternative day. Each oestrous cycle was taken as one unit while calculating the number of follicles, follicular diameter, number of waves, initiation of follicular wave, follicular wave length, dominant follicle diameter, day of initiation of corpus luteum development, duration of CL period, day of CL regression etc. Ultrasonography was performed by using (Medicon, MAN – 600SV – EP23300, Korea) ultrasound scanner with 7.5 MHz linear-array trans rectal transducer. Each cow was palpated per rectum and ovarian structures were evaluated with the help of probe and printed the images by the SONY® printer attached to the scanner.

Procedure:

- i. The animal was restrained in the trevis and rectum was evacuated completely for provision of better contact between the probe and the genital structures (Fig. 2).

- ii. The external genitalia including the perineum were cleaned thoroughly. At the same time linear probe set at 7.5 MHz was placed in a disposable sleeve filled with Ultrafroz® acoustic gel in order to cover the surface of the transducer and to prevent soiling the probe with dung.
- iii. Then the ovaries were palpated by hand to ascertain the location of ovary and for the presence of follicles and/or CL.
- iv. Thereafter, a linear probe set at 7.5 MHz with the guidance of the hand was inserted into the rectum and moved cranially towards to dorsal surface of the ovary.
- v. The transducer was then moved slowly back and forth across the ovary (either right or left) to scan the entire surface and after completion of one ovary examination the probe was moved to the opposite side to scan the other ovary.
- vi. After scanning the both ovaries the images of the ovarian structures were stored by printing with the printer attached to the scanner.
- vii. All palpated and scanned structures, together with their relative locations within the ovary were sketched on a note book and the sketches were used to trace the same structures within the same ovary on the following day scanning time.
- viii. Ovarian assessment and the ultrasonic identification of the ovarian structures were done as described previously (Ginther, 1995).
- ix. Briefly, the ovary was traced as a globular irregular hypo-echoic structural area with a surrounding hyper-echoic tunica. The clear and diffuse area in the inferior part was indicative of the ovarian stroma while the white area was the tunica albuginea.

- x. The medullar layer of the ovary was seen as hyper-echoic diffuse pattern, while the cortical layer was normo-echoic or moderately hypo-echoic. The hilus was traced as a hyper- echogenic line that penetrated the ovary.

During each ultrasonographic examination following activities were carried out as per the procedure described by Alvarez *et al.* (2000) and Burke *et al.* (2000):

- i. General body health assessment
- ii. Rectal palpation of the uterus and ovaries
- iii. Ultrasonographic scanning of the ovaries
- iv. Drawing of sketches of the largest follicles within the scanned ovary
- v. Measurement of follicle diameters
- vi. Counting and recording follicular populations within the scanned ovary
- vii. Recording of all ovarian structures observed
- viii. Measuring and recording the CL diameters
- ix. Getting printing and storing all static images obtained during scanning.

3.3.1 Follicles diameter

Both the ovaries were scanned for the appearance of follicles having more than three millimeters diameter in such a way that the upper and lower lines of the greater lengths of the follicular is visible as thin white lines within the image of the ovary (Fig. 3). Immediately the ultrasound image was freezed and the diameters were



Fig. 3 : Ultrasonographic image showing follicles recruited into follicular wave



Fig. 4 : Ultrasonographic image showing dominant follicle

then determined by using inbuilt electronic calipers of the scanner. All these activities were repeated on every alternative day for a period of two oestrous cycle lengths on both the ovaries.

3.3.2 Dominant follicle diameter

The follicle was described as dominant follicle (DF) of a wave when its diameter measured at least 7 mm and above, and clearly exceeding the diameter of all other follicles in the wave (Ginther *et al.*, 1996) (Fig. 4). A subordinate follicle (SF) was described as the one that originated from the same follicular pool as the dominant follicle but then terminated growth after the deviation period (Fig. 5). Maximum diameter and growth rate of the dominant follicle within all the follicular waves were also measured and estimated. Growth rate (mm/day) of follicles was calculated by subtracting the diameter on the day of detection from the maximum diameter and dividing this by the interval in days (Rhodes *et al.*, 1995).

3.3.3 Follicle atresia

Atresia onset was considered as the day from which follicles began reducing in size during the respective follicular wave period except during the period of last wave of oestrous cycle at which period of time the dominant follicle will undergo for ovulation.

3.3.4 Follicular wave

Description of number of waves was done as per the procedure described by Ginther *et al.* (1989a). Oestrous cycles were described as cycles having two, three or four waves of follicular development depending on number of waves that formed before ovulation of dominant follicle. For instance, a cow in which the DF of the first wave was regressed and that of the second wave follicle ovulated was noted to be

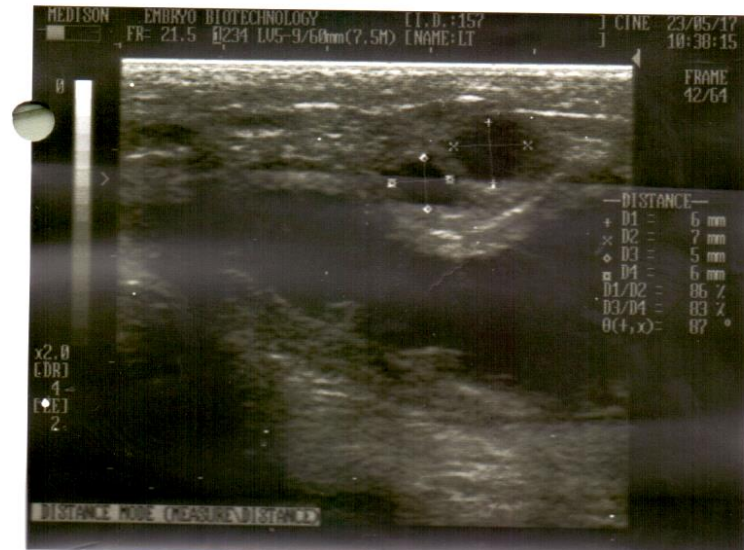


Fig. 5 : Ultrasonographic image showing subordinate follicle



Fig. 6 : Ultrasonographic image showing corpus luteum

having two follicular waves during that oestrous cycle. Similarly three and four wave oestrous cycles had their respective preovulatory DF in its third and fourth waves of follicle development, respectively.

3.3.5 Onset of follicular wave

The day of wave onset/wave emergence was defined as the day on which the follicle was retrospectively traced to be 1 to 3 mm in diameter (Burke *et al.*, 2000). Such definitions were made possible by obtaining the growth rate for each particular follicle so as to trace back the day of the cycle when that follicle emerged and appearance of follicle on the day of ovulation was treated as day 0.

3.3.6 Duration of follicular wave

Duration of follicular wave was calculated as the period between the day of initiation of follicle having more than three millimeter diameter to the day of disappearance of follicle either by due to atresia or ovulation.

3.3.7 Number of follicular waves

During oestrous cycle synchronous follicular development of a cohort of follicles in the ovary of the cow, which often referred to as a “follicular wave”. The emergence of these waves occurs either one or two or three or four times as first, second, third and fourth follicular waves, respectively. Usually during each follicular wave one or two dominant follicles and several subordinate follicles develops.

3.3.8 Corpus luteum diameter

Ovulation was described whenever disappearance of the dominant follicle was noted and subsequently a CL formed at the same location on the ovary. Its growth rate was assessed on every alternative day of the oestrous cycle by measuring the diameter in mm (Fig.6).

3.4 Estimation of steroid hormones

Blood samples were collected from jugular vein of the Punganur cows on every alternative day of second oestrous cycle. The serum was separated from the blood samples and transferred into sterile eppendorf tubes, labelled and stored in deep freeze (-20 °C) until hormonal assay for progesterone and estrogen was carried out. The procedures described below are as per the instructions of the kit supplied by the manufacturer.

3.4.1 Progesterone

Serum progesterone concentrations were determined by ELISA (enzyme linked immune sorbent assay) based commercial diagnostic kit (Progesterone ELISA, The Calbiotech Inc®, Spring Valley, CA). The test kit contained P4 standards of 0, 2.5, 5, 10, 20 and 40 ng/ml, The sensitivity of the assay for P4 was 0.22 ng/ml.

Assay Procedure

Prior to assay, bring all reagents to room temperature. Gently mix all reagents before use.

1. Place the desired number of coated strips into the holder
2. Pipet 10 µl of Progesterone standards, control and patient's serum samples.
3. Add 200µl of Progesterone Enzyme Conjugate to all wells.
4. Incubate for 60 minutes at room temperature (18-26° C).
5. Remove liquid from all wells. Wash wells three times with 300 ml of 1X wash buffer. Blot on absorbent paper towels.
6. Add 100 µl of TMB substrate to all wells.
7. Incubate for 15 minutes at room temperature.

8. Add 50 μ l of stop solution to all wells. Shake the plate gently to mix the solution.
9. Read absorbance on ELISA Reader at 450 nm within 15 minutes after adding the stop solution.
10. The progesterone concentration was measured in ng/ml.

3.4.2 Estrogen

Serum estrogen concentrations were determined by ELISA (enzyme linked immune sorbent assay) by using commercial diagnostic kit (Total Estrogens ELISA, The Calbiotech® Inc, El Cajon, CA) based on the principle of competitive binding between Estrogens in the test specimen and enzyme labeled estrogens for a constant amount of rabbit anti estrogens antibody. The test kit contained estrogen standards of 0, 0.10, 20, 100, 200, 500 and 1500 pg/ml. The sensitivity of the assay for estrogen was 2.69 pg/ml.

Assay Procedure

Prior to assay, allow reagents to stand at room temperature (20-26°C). Gently mix all reagents before use.

1. Place the desired number of coated strips into the holder.
2. Add 50 μ l of Total Estrogens standards, controls and patient's samples into designated microwells.
3. Add 100 μ l of the working solution of Total Estrogens enzyme reagent into each well.
4. Add 50 μ l of anti Total Estrogens antibody reagent into each well.

5. Cover the plate and incubate for 120 minutes at room temperature with shaking (600rpm).
6. Remove liquid from all wells. Wash wells three times with 350 μ l of 1X Wash Buffer.
7. Blot on absorbent paper towels.
8. Add 100 μ l of TMB Substrate into each well.
9. Cover the plate, and incubate for 30 minutes at room temperature with shaking (600rpm).
10. Uncover the plate and add 50 μ l of Stop Solution into each well. Mix, gently, for 10 seconds.
11. Immediately absorbance of all the wells was read against the blank (AI) at 450 nm with the help of an ELISA automatic reader.
12. The estrogen concentration was measured in pg/ml

3.5 Statistical Analysis

The data obtained were analyzed by analysis of variance from summary data (follicular parameters), analysis of variance - one way classification (CL diameter and steroid hormones) and correlation (preovulatory follicle and CL diameter and steroids hormones concentrations) to understand the differences among parameter recorded. SPSS 12.0 for windows was employed in the analysis. The significance of all the parameters was measured at $P < 0.05$ level of significance (Snedecor and Cochran, 1994).

CHAPTER -IV

4. RESULTS

The present study was undertaken by utilizing eight Punganur cows stationed at Livestock Research Station, Palamaner, Chittoor Dist. Experiment was designed to study the estrus response and follicular dynamics in Punganur cows during two consecutive oestrous cycles on every alternative day with the help of ultrasonography. All the animals with good health were examined to study the estrus response by recording the time of onset of estrus, end of estrus signs, duration of estrus and intensity of estrus.

4.1 Estrus detection

4.1.1 Estrus intensity

Estrus intensity in Punganur cattle was 64.8 ± 0.33 (range from 40 to 85) and 63.7 ± 0.40 (range from 40 to 80) in 2 wave and 3 wave cows, respectively. The estrus intensity was intense in 3 out of 16 estrus period observed (18.75 %); intermediate in 10 out of 16 estrus period (62.50 %) and weak in 3 out of 16 estrus period (18.75 %) (Table No. 1). The difference in the overall estrus intensity between 2 wave and 3 wave estrus was not significant ($P>0.05$).

4.1.2 Duration of estrus

The mean duration of estrus in Punganur cattle was 16.02 ± 0.74 h with a range of 11.5 to 34 h. The estrus duration was below 12 h in 14 %, between 12-18 h in 42 % and 18-24 h in 24 % (Table No. 1).

4.1.3 Oestrous cycle length

The mean oestrous cycle length in Punganur cattle was 20.25 ± 0.48 days (range from 19 to 21) in 2 wave cycle and 21.27 ± 0.50 days (range from 19 to 21) in

Table No 1: Mean (\pm SE) duration and intensity of estrus in Punganur cows

Characteristics	Mean values (\pmSE)
Duration of estrus (h)	16.02 \pm 0.74
Weak estrus (%)	18.75
Intermediate estrus (%)	62.50
Intense estrus (%)	18.75

Table No. 2: Characteristics of follicular dynamics in two wave oestrous cycle of Punganur cows

Follicular parameters	First wave N=4	Second wave N=4
Day of onset follicular of wave (day)	0.80 \pm 0.37	13.00 \pm 1.08
Day of wave ending (day)	12.00 \pm 0.68	21.00 \pm 0.63
Day of maximum diameter of the follicle (day)	6.60 \pm 1.21	21.60 \pm 1.63
Maximum diameter of the follicle (mm)	7.30 \pm 0.63 ^a	9.40 \pm 0.47 ^a
Duration of follicular wave (days)	11.80 \pm 0.49 ^a	9.25 \pm 2.98 ^a
Growth rate of follicle (mm/day)	0.64 \pm 0.10 ^a	0.73 \pm 0.24 ^a
Day of onset of follicular atresia (day)	8.20 \pm 1.24	
Atresia rate of follicle (mm/day)	0.65 \pm 0.85	

a, b ,c -Values in row with different super scripts differ significantly (P<0.05)

3 wave cycle. The difference in the overall oestrous cycle length between 2 wave and 3 wave estrus was not significant ($P>0.05$).

4.2 Follicular dynamics

4.2.1 Number of follicular waves per cycle

Follicular dynamics in Punganur cows during oestrous cycle exhibited characteristic pattern of follicular waves with the initial development of a group of follicles having 3 to 5 mm diameter, followed by selection, development and atresia of a dominant follicle. In the present study, among the eight Punganur cows during 2 consecutive oestrous cycles (n=16 cycles) two wave follicular waves (Fig.7) were exhibited in 4 cycles (25.00 %) and three wave follicular waves (Fig.8) were exhibited in 12 cycles (75.00 %).

4.2.2 Mean day of onset of follicular wave

The mean day of onset of follicular wave in cows showing 2 wave oestrous cycle was 0.80 ± 0.37 and 13.00 ± 1.08 during 1st and 2nd wave, respectively. While, the same in cows showing 3 follicular waves oestrous cycle was 1.83 ± 0.27 , 7.60 ± 0.50 and 13.66 ± 0.87 during 1st, 2nd and 3rd waves, respectively (Table No. 2 and 3) (Fig.7 and 8).

The day of onset of 1st follicular wave of 2 wave oestrous cycle was insignificantly ($P>0.05$) earlier than in cows showing 3 wave oestrous cycle. While, the same during 2nd wave in cows showing 2 wave oestrous cycle was significantly ($P<0.05$) earlier than in cows showing 3 wave oestrous cycle (Table No. 4).

4.2.3 Day of wave ending

The mean day of follicular wave ending in cows showing 2 wave oestrous cycle was 12.00 ± 0.68 and 21.00 ± 0.63 during 1st and 2nd wave, respectively. While

Table No. 3: Characteristics of follicular dynamics in three wave oestrous cycle of Punganur cows

Follicular parameters	First wave N=12	Second wave N=12	Third wave N=12
Day of onset of follicular wave (day)	1.83 ± 0.27	7.6 ± 0.5	13.66 ± 0.87
Day of wave ending (day)	9.59 ± 1.03	15.73 ± 0.76	21.70 ± 0.51
Day of maximum diameter of the follicle (day)	4.75 ± 0.63	11.40 ± 0.49	21.40 ± 0.56
Maximum diameter of the follicle (mm)	6.75 ± 0.45 ^a	6.40 ± 0.29 ^a	8.33 ± 0.38 ^a
Duration of follicular wave (days)	8.16 ± 0.95 ^a	7.93 ± 0.98 ^a	7.60 ± 0.25 ^a
Growth rate of follicle (mm/day)	0.71 ± 0.10 ^a	0.68 ± 0.10 ^a	0.66 ± 0.10 ^a
Day of onset of follicular atresia (day)	7.16 ± 0.61	13.33 ± 0.49	
Atresia rate of follicle (mm/day)	0.66 ± 0.10 ^a	0.65 ± 0.1 ^a	

a, b ,c -Values in row with different super scripts differ significantly (P<0.05)

Table No. 4: Day of onset of follicular wave analysis during oestrous cycle in Punganur cows

Number of waves	First wave	Second wave	Third wave
2 wave	0.80 ± 0.37 ¹	13.00 ± 1.08 ¹	
3 wave	1.83 ± 0.27 ¹	7.60 ± 0.50 ²	13.66 ± 0.87

1, 2 - Values in column with different super scripts differ significantly (P<0.05)

Fig No 7 : Follicular wave pattern in 2 wave oestrous cycle of Punganur cows.

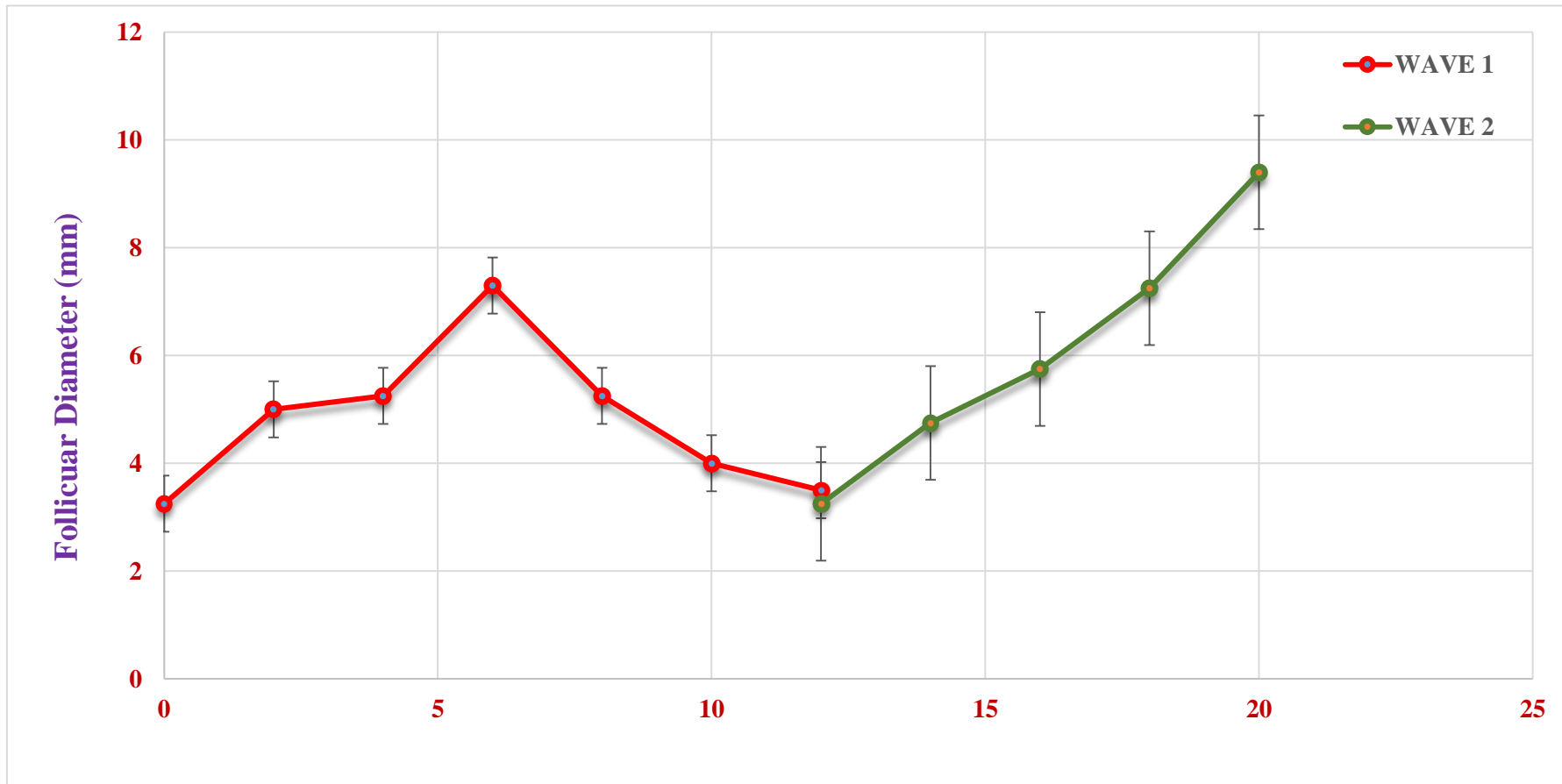
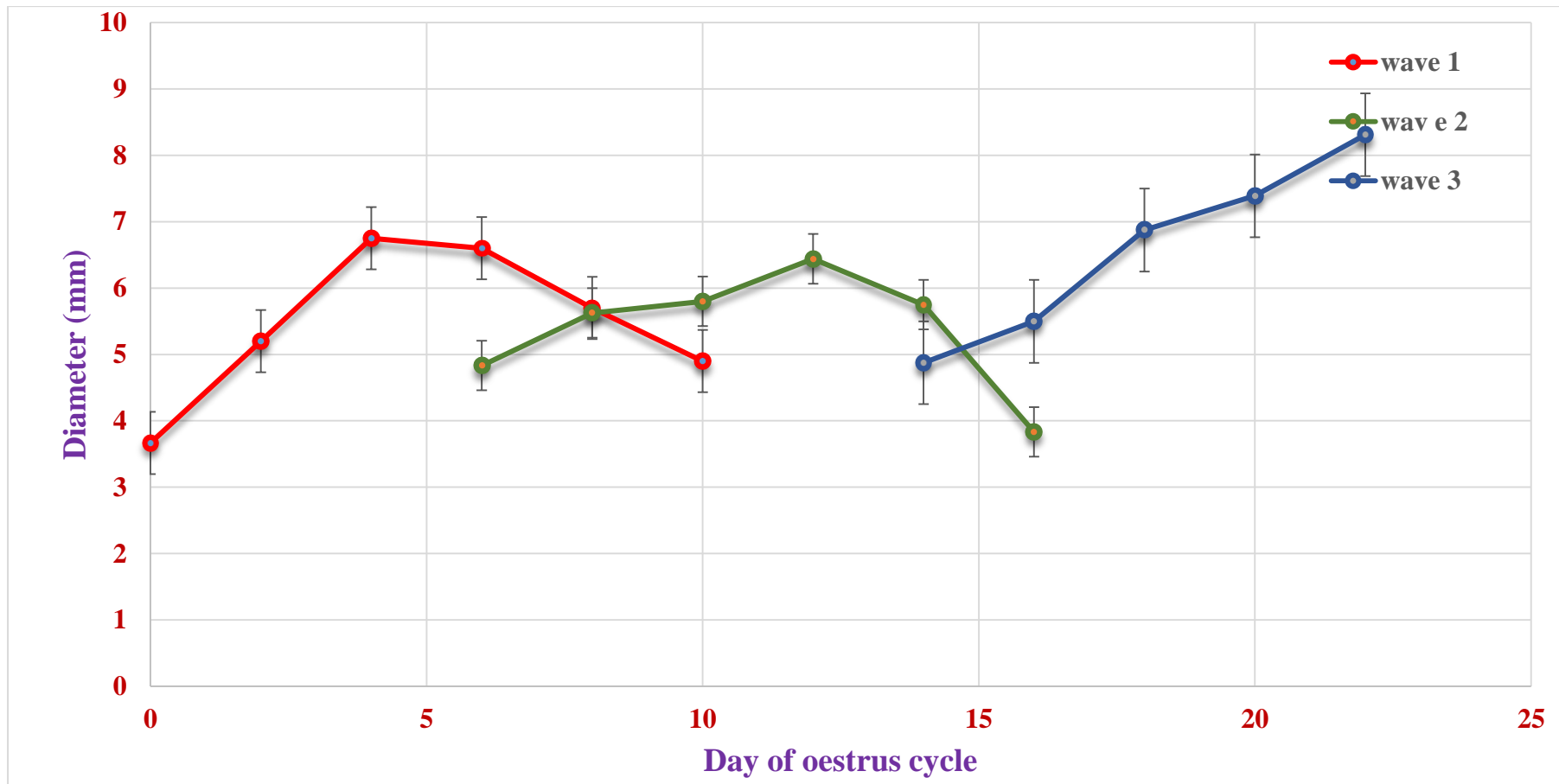


Fig 8: Follicular waves pattern in 3 wave oestrous cycle of Punganur cows.



the same in cows showing 3 follicular wave oestrous cycle was 9.59 ± 1.03 , 15.73 ± 0.76 and 21.70 ± 0.51 during 1st, 2nd and 3rd waves, respectively (Table No. 2 and 3) (Fig.7 and 8).

While, the day of ending of 1st follicular wave in cows showing 3 wave oestrous cycle was insignificantly ($P>0.05$) earlier than 1st wave of 2 wave oestrous cycle but the same was significantly ($P<0.05$) earlier than 2nd wave of 2 wave follicular cycle. The day of ending of 2nd wave of 3 wave cycle was significantly ($P<0.05$) earlier than 2nd wave of 2 wave cycle. The difference between the day of end of 3rd follicular wave and 2nd wave of 2 wave oestrus cycle was insignificant ($P>0.05$) (Table No. 5).

4.2.4 Follicular wave duration

The mean wave duration of follicular wave in cows showing 2 wave oestrous cycle was 11.8 ± 0.49 and 9.25 ± 2.98 days during 1st and 2nd wave, respectively. While, the same in cows showing 3 follicular waves oestrous cycle was 8.16 ± 0.95 , 7.93 ± 0.98 and 7.6 ± 0.25 days during 1st, 2nd and 3rd waves, respectively (Table No. 2 and 3) (Fig.7 and 8). The difference in the period of wave duration within 2 wave and 3 wave oestrous cycle cows was insignificant ($P>0.05$) but the same was significant ($P<0.05$) between 2 wave and 3 wave cycles (Table No. 6).

The duration of 1st and 2nd follicular waves in cows showing 2 wave oestrous cycle was insignificantly ($P>0.05$) longer than in cows showing 3 wave oestrous cycle. While, the duration of 3rd follicular wave (Preovulatory wave) in cows showing 3 wave oestrous cycle was insignificantly ($P>0.05$) shorter than 1st and 2nd wave (Preovulatory wave) of 2 wave oestrous cycle (Table No. 6).

Table No. 5: Day of follicular wave ending analysis during oestrous cycle in Punganur cows

	First wave	Second wave	Third wave
2 wave (day)	12.00 ± 0.68	21.00 ± 0.63 ¹	
3 wave (day)	9.59 ± 1.03	15.73 ± 0.76 ²	21.70 ± 0.51

1, 2 - Values in column with different super scripts differ significantly (P<0.05)

Table No. 6: Follicular wave duration (days) analysis during oestrous cycle in Punganur cows

	First wave	Second wave	Third wave
2 wave (days)	11.80 ± 0.49 ¹	9.25 ± 2.98 ¹	
3 wave (days)	8.16 ± 0.95 ¹	7.93 ± 0.98 ¹	7.60 ± 0.25

1, 2 - Values in column with different super scripts differ significantly (P<0.05)

Table No. 7: Maximum diameter of DF analysis during oestrous cycle in Punganur cows

	First wave	Second wave	Third wave
2 wave (mm)	7.30 ± 0.63 ¹	9.40 ± 0.47 ¹	
3 wave (mm)	6.75 ± 0.45 ^{b1}	6.40 ± 0.29 ^{b,2}	8.33 ± 0.38 ^a

a, b, c - Values in row with different super scripts differ significantly (P<0.05)

1, 2 - Values in column with different super scripts differ significantly (P<0.05)

4.2.5 Maximum diameter of dominant follicle

The mean maximum diameter of DF in cows showing 2 wave oestrous cycle was 7.3 ± 0.63 and 9.4 ± 0.47 mm, respectively in 2 wave cycle. While, the same in cows showing 3 follicular waves oestrous cycle was 6.75 ± 0.45 , 6.4 ± 0.29 and 8.33 ± 0.38 mm, respectively (Table No. 2 and 3) (Fig.7 and 8). The difference in the mean maximum diameter of DF of follicular wave within 2 wave and 3 wave oestrous cycle cows was insignificant ($P>0.05$) except between 1st and 3rd wave and between 2nd and 3rd wave of 3 wave cycle where the difference was significant ($P<0.05$) (Table No. 7).

The maximum diameter of DF of 1st follicular wave in cows showing 2 wave oestrous cycle was insignificantly ($P>0.05$) higher than 1st follicular wave of 3 wave oestrous cycle. While, the same pertaining to 2nd wave was significantly ($P<0.05$) higher in cows having 2 wave oestrous cycle than in cows having 3 wave oestrous cycle. But the maximum diameter of DF of 3rd follicular wave was significantly ($P<0.05$) different with both 1st and 2nd waves of 2 wave oestrous cycle (Table No.7).

4.2.6 Day of maximum diameter of the dominant follicle

The mean day of maximum diameter of DF of follicular wave in cows showing 2 wave oestrous cycle was 6.6 ± 1.21 and 21.6 ± 1.63 during 1st and 2nd wave, respectively. While, the same in cows showing 3 follicular waves oestrous cycle was on day 4.75 ± 0.63 , 11.4 ± 0.49 and 21.4 ± 0.56 during 1st, 2nd and 3rd wave, respectively (Fig.7 and 8).

The day of maximum diameter of DF of 1st follicular wave in cows showing 3 wave oestrous cycle was insignificantly ($P>0.05$) earlier than 1st follicular wave of 2 wave oestrous cycle. But, the same pertaining to 2nd wave was significantly ($P<0.05$) earlier in cows showing 3 wave oestrous cycle than in cows showing 2 wave oestrous

cycle (Table No. 2 and 3). The difference between the day of maximum diameter of 3rd follicular wave and 2nd wave of 2 wave oestrus cycle was insignificant ($P>0.05$) (Table No. 8).

4.2.7 Diameter of preovulatory follicle

The mean preovulatory follicular in Punganur cows showing 2 wave and 3 wave oestrous cycle was 9.4 ± 0.47 and 8.33 ± 0.38 mm, respectively (Table No. 7). The mean diameter of preovulatory follicle was positively and significantly ($P<0.05$) correlated with the corresponding day of oestrous cycle estrogen concentration ($r = 0.94$) but the same was negatively and significantly ($P<0.05$) correlated with the corresponding day of oestrous cycle progesterone concentration ($r = - 0.83$). While, the preovulatory follicle diameter has shown a non-significant positive correlation with maximum diameter of subsequent CL ($r=0.58$) (Table No. 9 and 10).

4.2.8 Day of onset of atresia

The mean day of onset of atresia of follicular wave in cows showing 2 wave oestrous cycle was 8.2 ± 1.24 for 1st wave. While, the same in cows showing 3 follicular wave oestrous cycle was on day 7.16 ± 0.61 and 13.33 ± 0.49 for 1st and 2nd waves, respectively (Table No. 2 and 3).

The day of onset of atresia of 1st follicular wave in cows showing 2 wave oestrous cycle was insignificantly ($P>0.05$) later than in cows showing 3 wave oestrous cycle. While, the same was significantly ($P<0.05$) earlier compared to 2nd wave of 3 wave oestrous cycle (Table No.11).

4.3 Growth rate

The mean growth rate of follicle in each wave in the cows showing 2 wave cycle was 0.64 ± 0.10 and 0.73 ± 0.24 mm per day during 1st and 2nd wave,

Table No. 8: Day of maximum diameter of DF analysis during oestrous cycle in Punganur cows

	First wave	Second wave	Third wave
2 wave (day)	6.60 ± 1.21 ¹	21.60 ± 1.63 ¹	
3 wave (day)	4.75 ± 0.63 ¹	11.40 ± 0.49 ²	21.40 ± 0.56

1, 2 - Values in column with different super scripts differ significantly (P<0.05)

Table No. 9: Correlation co-efficient of diameter of preovulatory follicle with corresponding day steroid hormone concentration in Punganur cow

Correlations	POF	Progesterone	Estrogen
POF	1.00		
Progesterone	-0.83*	1.00	
Estrogen	0.94*	-0.78	1.00

* : Significant (P≤0.05)

Table No. 10: Correlation co-efficient of diameter of POF with subsequent corpus luteum Diameter (Day 14th) in Punganur cows.

Correlation	POF	Corpus luteum
POF	1.00	
Corpus luteum	0.58 ^{NS}	1.00

NS : Non-significant (P≥0.05)

Table No. 11: Day of onset of atresia of follicular wave analysis during oestrous cycle in Punganur cows

	First wave	Second wave
2 wave (day)	8.20 ± 1.24 ¹	
3 wave (day)	7.16 ± 0.61 ¹	13.33 ± 0.49

1, 2 - Values in column with different super scripts differ significantly (P<0.05)

Table No. 12: Growth rate and atresia rate analysis during oestrous cycle in Punganur cows

	First wave		Second wave		Third wave	
	Growth rate (mm/day)	Atresia rate (mm/day)	Growth rate (mm/day)	Atresia rate (mm/day)	Growth rate (mm/day)	Atresia rate (mm/day)
2 wave	0.64 ± 0.10	0.65 ± 0.85	0.73 ± 0.24			
3 wave	0.71 ± 0.10	0.66 ± 0.10	0.68 ± 0.10	0.65 ± 0.10	0.66 ± 0.10	

respectively. While the cows showing 3 waves cycle was 0.71 ± 0.10 , 0.68 ± 0.10 and 0.66 ± 0.10 mm per day during 1st, 2nd and 3rd waves, respectively (Table No. 2 and 3). The difference in the growth rate of follicle of follicular wave within 2 wave and 3 wave oestrous cycle cows was insignificant ($P>0.05$) (Table No. 12).

But the growth rate during 1st follicular wave in cows showing 3 wave oestrous cycle was insignificantly ($P>0.05$) higher than in cows showing 2 wave oestrous cycle. While, the same pertaining to 2nd wave was insignificantly ($P>0.05$) higher in cows showing 2 wave oestrous cycle than in cows showing 3 wave oestrous cycle. The difference between the growth rate of follicle during 3rd follicular wave of 3 wave cycle and 1st and 2nd waves of 2 wave oestrous cycle were insignificant ($P>0.05$) (Table No. 12).

4.4 Atresia rate

The mean atresia rate of follicle of wave in the cows showing 2 wave cycle was 0.65 ± 0.85 per day during 1st wave. While the cows showing 3 wave cycle were 0.66 ± 0.10 and 0.65 ± 0.1 mm per day during 1st and 2nd wave, respectively (Table No. 2 and 3). The difference in the atresia rate of follicle during follicular wave within 2 wave and 3 wave oestrous cycle cows was insignificant ($P>0.05$) (Table No. 12).

The atresia rate of 1st follicular wave in cows showing 3 wave oestrous cycle was insignificantly ($P>0.05$) higher than in cows showing 2 wave oestrous cycle. While, the same pertaining to 2nd wave in cows showing 3 wave oestrous cycle was insignificantly ($P>0.05$) lower than 1st wave of cows showing 2 wave oestrous cycle (Table No. 12).

4.5 Corpus luteum characteristics

The mean CL diameter in Punganur cows having 2 wave oestrous cycle on days 4, 6, 8, 10, 12, 14, 16, 18 and 20 of oestrous cycle was 9.55 ± 1.31 , 11.51 ± 1.07 , 13.81 ± 0.97 , 14.74 ± 0.93 , 15.31 ± 0.31 , 15.52 ± 0.99 , 13.75 ± 0.72 , 12.64 ± 0.81 and 10.93 ± 0.84 , respectively. Same in cow having 3 wave oestrous cycle on days 4, 6, 8, 10, 12, 14, 16, 18 and 20 days of oestrous cycle was 8.14 ± 0.50 , 11.06 ± 0.60 , 12.61 ± 0.51 , 14.37 ± 0.37 , 14.64 ± 0.36 , 15.02 ± 0.51 , 13.2 ± 0.65 , 11.13 ± 0.79 and 10.2 ± 0.52 , respectively. While, the mean overall diameter of CL during 4, 6, 8, 10, 12, 14, 16, 18 and 20 days of oestrous cycle in Punganur cows was 8.49 ± 0.49 , 11.17 ± 0.50 , 12.90 ± 0.45 , 14.46 ± 0.34 , 14.80 ± 0.29 , 15.09 ± 0.46 , 13.34 ± 0.51 , 11.51 ± 0.63 and 10.38 ± 0.43 , respectively (Table No. 13) (Fig.9). The mean maximum diameter of corpus luteum was 15.09 ± 0.46 on 14th day and regression of corpus luteum was initiated on 16th day of oestrous cycle.

The difference in the diameter of corpus luteum between 2 wave and 3 wave oestrous cycle cows and within 2 wave and 3 wave oestrous cycle cows was significant ($P < 0.05$). The increase in the diameter of the CL was significant throughout its growth from the day 4 of oestrous cycle to the day of regression (Table No. 14).

The mean CL diameter was positively and insignificantly ($P < 0.05$) correlated with the progesterone concentration ($r = 0.66$) but the same was negatively and significantly ($P < 0.05$) correlated with the estrogen concentration ($r = - 0.92$) on day 14 of oestrous cycle (Table No. 10 and 15).

Table No 13: Corpus luteum diameter (mm) on various days of oestrous cycle in Punganur cows

Day of oestrous cycle	2 wave oestrous cycle N = 4	3 wave oestrous cycle N = 12	Overall N = 16
	Mean \pm SE		
4 day	9.55 \pm 1.31	8.14 \pm 0.50	8.49 \pm 0.50 ^a
6 day	11.51 \pm 1.07	11.06 \pm 0.60	11.17 \pm 0.51 ^b
8 day	13.81 \pm 0.97	12.61 \pm 0.51	12.91 \pm 0.46 ^{bc}
10 day	14.74 \pm 0.93	14.37 \pm 0.37	14.46 \pm 0.35 ^{bc}
12 day	15.31 \pm 0.31	14.64 \pm 0.36	14.81 \pm 0.29 ^{bc}
14 day	15.52 \pm 0.99	15.02 \pm 0.51	15.09 \pm 0.47 ^{bc}
16 day	13.75 \pm 0.72	13.20 \pm 0.65	13.33 \pm 0.51 ^{bc}
18 day	12.64 \pm 0.81	11.13 \pm 0.79	11.51 \pm 0.63 ^{bcd}
20 day	10.93 \pm 0.84	10.20 \pm 0.52	10.38 \pm 0.44 ^{ab}
Total	13.08 \pm 0.43 ¹	12.23 \pm 0.28 ²	12.44 \pm 0.23

a, b, c : Values in column with different super scripts differ significantly (P<0.05)

1, 2 : Values in row with different super scripts differ significantly (P<0.05)

Table No 14: Analysis of variance of corpus luteum diameter (mm) on various days of oestrous cycle in Punganur cows

Source	Degrees of Freedom	Sum of Squares	Mean Squares	F Value
Between waves	1	18.20	18.20	0.02*
Error	126	448.36		
Between Days	8	457.91	57.24	16.07*
Error	126	448.36		

* : Significant (P \leq 0.05)

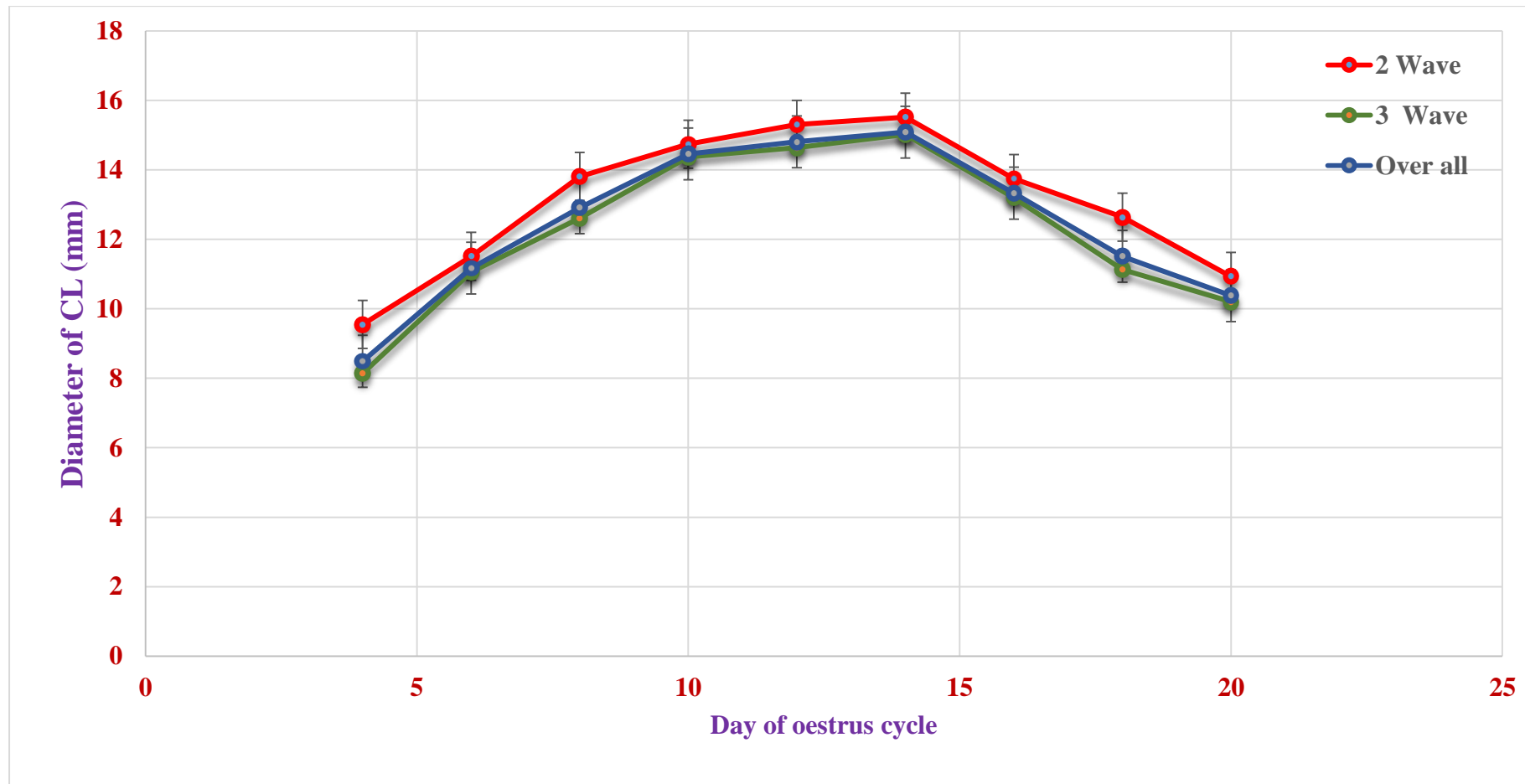
NS : Non-significant (P \geq 0.05)

Table No.15: Correlation co-efficient of diameter of corpus luteum (Day 14th) with corresponding day steroid hormone concentration in Punganur cow

Correlation	Corpus luteum	Progesterone	Estrogen
Corpus luteum	1.00		
Progesterone	0.66	1.00	
Estrogen	-0.92*	-0.49	1.00

* : Significant (P \leq 0.05)

Fig. 9: Corpus luteum diameter (mm) on different days of oestrous cycle in Punganur cows



4.6 Steroids concentration

4.6.1 Progesterone

The mean concentration of serum progesterone in cows having 2 wave cycle during 0, 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20th day of follicular waves oestrous cycle was 0.95 ± 0.83 , 2.07 ± 0.44 , 3.11 ± 0.78 , 4.19 ± 0.38 , 5.21 ± 0.47 , 5.11 ± 0.28 , 5.51 ± 0.52 , 5.74 ± 0.34 , 7.25 ± 1.54 , 3.68 ± 0.38 and 2.04 ± 0.62 , ng/ml respectively. Same in cow having 3 wave oestrous cycle on days 0, 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 days of oestrous cycle was 1.10 ± 0.33 , 3.50 ± 1.19 , 2.14 ± 0.58 , 4.04 ± 0.70 , 4.97 ± 0.83 , 5.72 ± 0.96 , 5.70 ± 0.92 , 7.59 ± 0.80 , 6.97 ± 0.74 , 5.57 ± 1.11 and 2.54 ± 0.84 ng/ml respectively. While, the mean overall concentration of serum progesterone during 0, 2, 4, 6, 8, 10, 14, 16, 18 and 20 days of oestrous cycle in Punganur cows was 1.06 ± 0.29 , 3.14 ± 0.91 , 2.38 ± 0.48 , 4.29 ± 0.50 , 4.49 ± 0.97 , 5.44 ± 0.74 , 5.47 ± 0.74 , 6.30 ± 1.20 , 7.04 ± 0.62 , 5.10 ± 0.87 and 2.47 ± 0.87 ng/ml respectively (Table No. 16) (Fig.10). The mean maximum concentration of serum progesterone was 7.04 ± 0.62 ng/ml on 16th day of oestrous cycle.

The difference in the mean concentration of serum progesterone between 2 wave and 3 wave oestrous cycle cows was not significant ($P > 0.05$) while the same between days of oestrous cycle was significant ($P < 0.05$) and within 2 wave and 3 wave oestrous cycle cows (Table No. 17). The mean progesterone concentration was negatively and significantly ($P < 0.05$) correlated with the corresponding day diameter of preovulatory follicle ($r = - 0.83$) (Table No. 15).

4.6.2 Estrogen

The mean concentration of serum estrogen in cows having 2 wave cycle during 0, 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20th day of oestrous cycle was 43.48 ± 1.04 ,

Fig. 9: Corpus luteum diameter (mm) on different days of oestrous cycle in Punganur cows

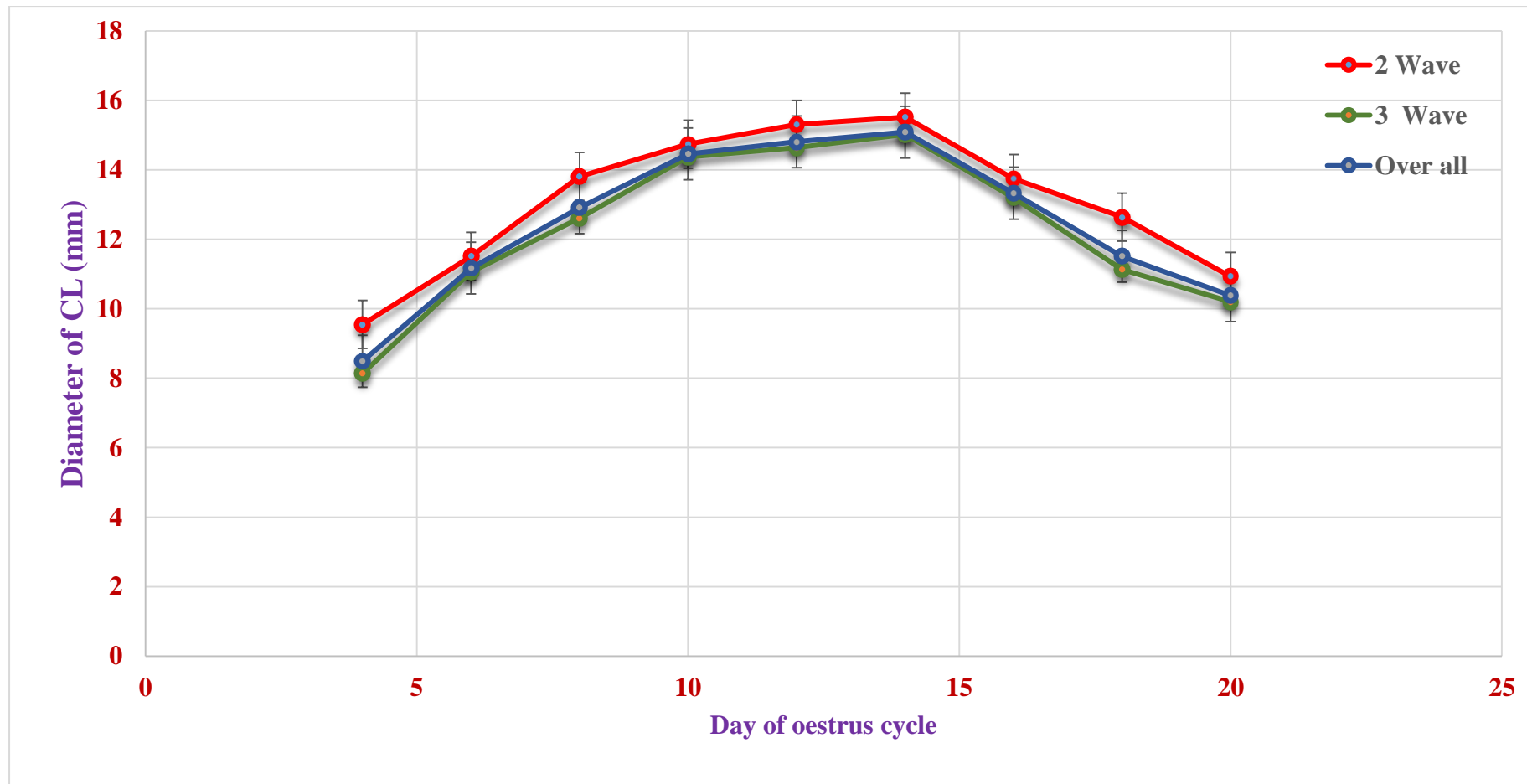


Table No. 16: Mean wave wise and overall progesterone concentration (ng/ml) on different day of oestrous cycle in Punganur cows

Day of oestrous cycle	2 wave oestrous cycle N = 2	3 wave oestrous cycle N = 6	Overall N = 8
	Mean \pm SE		
0 day	0.95 \pm 0.83	1.10 \pm 0.33	1.06 \pm 0.29 ^a
2 day	2.07 \pm 0.44	3.50 \pm 1.19	3.14 \pm 0.91 ^a
4 day	3.11 \pm 0.78	2.14 \pm 0.58	2.38 \pm 0.48 ^a
6 day	4.19 \pm 0.38	4.04 \pm 0.70	4.08 \pm 0.52 ^{ab}
8 day	5.21 \pm 0.47	4.97 \pm 0.83	5.03 \pm 0.62 ^{bc}
10 day	5.11 \pm 0.28	5.72 \pm 0.96	5.56 \pm 0.71 ^{bc}
12 day	5.51 \pm 0.52	5.70 \pm 0.92	5.65 \pm 0.68 ^{bc}
14 day	5.74 \pm 0.34	7.59 \pm 0.80	7.12 \pm 0.66 ^{bcd}
16 day	7.25 \pm 1.54	6.97 \pm 0.74	7.04 \pm 0.62 ^{bcd}
18 day	3.68 \pm 0.38	5.57 \pm 1.11	5.10 \pm 0.87 ^{ab}
20 day	2.04 \pm 0.62	2.54 \pm 0.84	2.42 \pm 0.63 ^a
Total	4.07 \pm 0.41 ¹	4.53 \pm 0.24 ¹	4.41 \pm 0.27

a, b, c : Values in column with different super scripts differ significantly (P<0.05)
1, 2 : Values in row with different super scripts differ significantly (P<0.05)

Table No.17: Analysis of variance of serum progesterone concentrations (ng/ml) on various days of oestrous cycle in Punganur cows

Source	Degrees of Freedom	Sum of Squares	Mean Squares	F Value
Between waves	1	1.33	1.33	0.18 ^{NS}
Error	87	648.06		
Between Days	10	269.58	26.96	5.48*
Error	87	648.06		

* : Significant (P \leq 0.05)

NS : Non-significant (P \geq 0.05)

38.24 \pm 0.94, 40.58 \pm 0.83, 37.93 \pm 3.92, 31.57 \pm 4.19, 38.07 \pm 0.34, 39.46 \pm 0.69, 44.36 \pm 1.45, 42.14 \pm 0.32 39.76 \pm 0.05 and 45.72 \pm 2.98 pg/ml respectively. Same in cow having 3 wave oestrous cycle on days 0, 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 days of oestrous cycle was 45.26 \pm 1.38, 38.93 \pm 2.28, 41.00 \pm 1.95, 41.64 \pm 1.45, 38.50 \pm 6.24, 43.43 \pm 2.31, 44.04 \pm 2.24, 42.26 \pm 1.73, 42.05 \pm 0.88, 43.28 \pm 0.85, 44.51 \pm 0.50 pg/ml respectively. While, the mean overall concentration of serum estrogen during 0, 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 days of oestrous cycle in Punganur cows was 44.82 \pm 1.07, 38.75 \pm 1.68, 41.65 \pm 1.45, 40.72 \pm 1.43, 36.77 \pm 4.77, 42.09 \pm 1.91, 42.89 \pm 1.81, 42.78 \pm 1.34, 42.07 \pm 0.65, 42.40 \pm 0.85 and 44.82 \pm 0.70 pg/ml respectively (Table No. 18) (Fig.11).

The difference in the mean concentration of serum estrogen between 2 wave and 3 wave oestrous cycle cows and days of oestrous cycle was not significant ($P > 0.05$) (Table No. 19). The mean estrogen concentration was positively and significantly ($P < 0.05$) correlated with the corresponding day diameter of preovulatory follicle ($r = 0.94$) (Table No. 15).

Table No. 18: Mean wave wise and overall estrogen concentration (pg/ml) on different day of oestrous cycle in Punganur cows

Day of oestrous cycle	2 wave oestrous cycle N = 2	3 wave oestrous cycle N = 6	Overall N = 8
	Mean \pm SE		
0 day	43.48 \pm 1.04	45.26 \pm 1.38	44.82 \pm 1.07 ^a
2 day	38.24 \pm 0.94	38.93 \pm 2.28	38.75 \pm 1.68 ^b
4 day	40.58 \pm 0.83	41.00 \pm 1.95	41.65 \pm 1.45 ^{ab}
6 day	37.93 \pm 3.92	41.64 \pm 1.45	40.72 \pm 1.43 ^{ab}
8 day	31.57 \pm 4.19	38.50 \pm 6.24	36.77 \pm 4.77 ^{bc}
10 day	38.07 \pm 0.34	43.43 \pm 2.31	42.09 \pm 1.91 ^{abc}
12 day	39.46 \pm 0.69	44.04 \pm 2.24	42.89 \pm 1.81 ^{ab}
14 day	44.36 \pm 1.45	42.26 \pm 1.73	42.78 \pm 1.34 ^{ab}
16 day	42.14 \pm 0.32	42.05 \pm 0.88	42.07 \pm 0.65 ^{ab}
18 day	39.76 \pm 0.05	43.28 \pm 0.85	42.40 \pm 0.85 ^{ab}
20 day	45.72 \pm 2.98	44.51 \pm 0.50	44.82 \pm 0.70 ^{ab}
Total	40.12 \pm 4.35 ¹	42.36 \pm 5.93 ¹	41.80 \pm 0.60

a, b, c : Values in column with different super scripts differ significantly (P<0.05)

1, 2 : Values in row with different super scripts differ significantly (P<0.05)

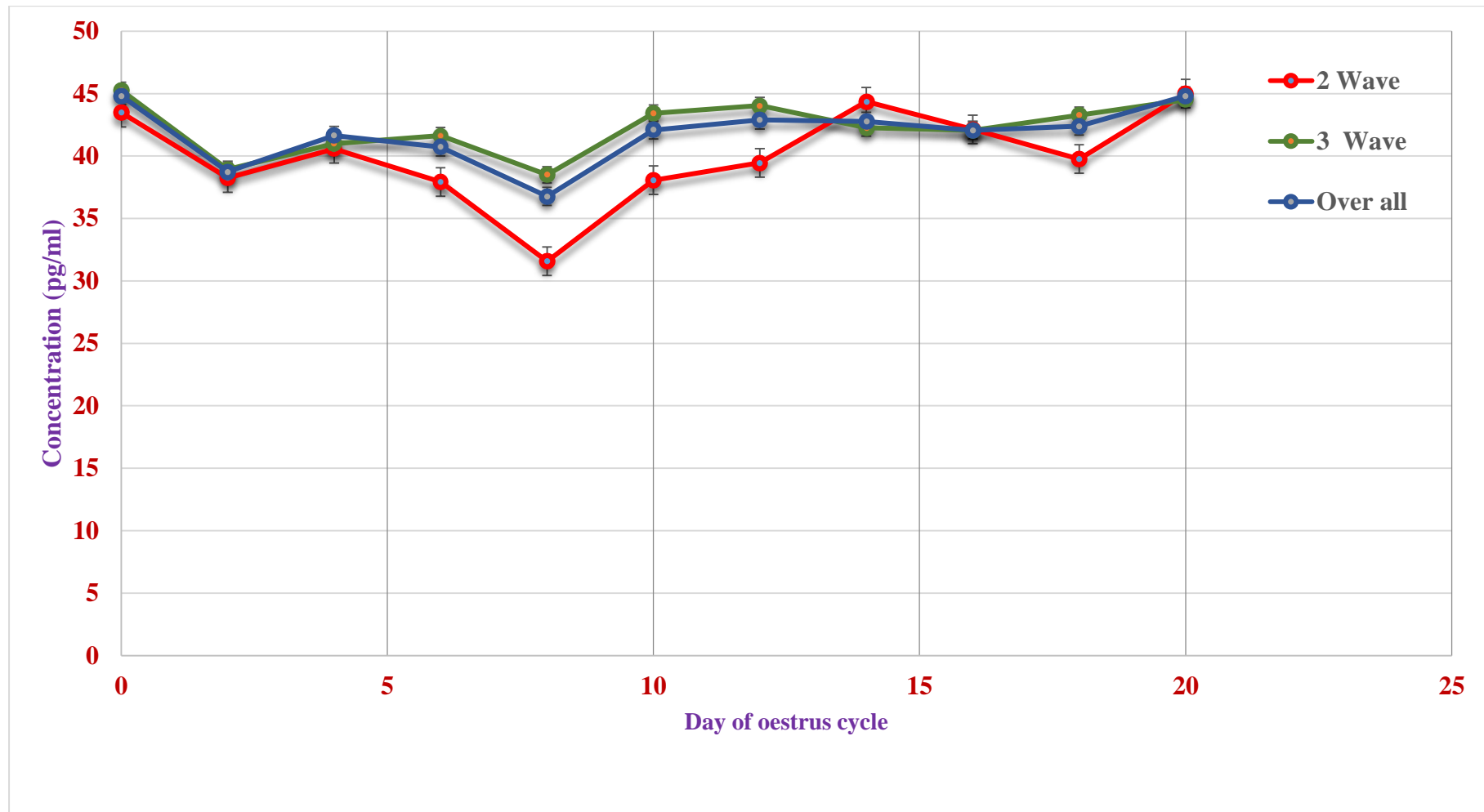
Table No 19: Analysis of variance of serum estrogen concentrations (pg/ml) on various days of oestrous cycle in Punganur cows

Source	Degrees of Freedom	Sum of Squares	Mean Squares	F Value
Between waves	1	82.67	82.67	2.65 ^{NS}
Error	87	2764.799		
Between Days	10	453.56	45.37	1.51 ^{NS}
Error	87	2764.80		

* : Significant (P \leq 0.05)

NS : Non-significant (P \geq 0.05)

Fig 11: Estrogen concentration (pg/ml) on different day of oestrous cycle in Punganur cows



CHAPTER -V

5. DISCUSSION

The present study was undertaken by utilizing eight Punganur cows stationed at Livestock Research Station, Palamaner, Chittoor Dist to study the estrus response and follicular dynamics in Punganur cows during two consecutive oestrous cycles on every alternative day with the help of ultrasonography to study the estrus response by recording the time of onset of estrus, end of estrus signs, duration of estrus and intensity of estrus.

Estrus intensity measured in Punganur cattle was 64.8 ± 0.33 (range from 40 to 85) and 63.7 ± 0.40 (range from 40 to 80) in 2 wave and 3 wave animals, respectively. The estrus intensity was intense in 3 out of 16 estrus period observed (18.75 %); intermediate in 10 out of 16 estrus period (62.50 %) and weak in 3 out of 16 estrus period (18.75 %). The difference in the overall estrus intensity between 2 wave and 3 wave estrus was not significant ($P \geq 0.05$). Similar to present findings of study, Naidu and Rao, (2006) have also noticed the estrus intensity of 17.55 % of intense; 63.30 % of intermediate and 19.80 % of weak intensity in Ongole breed. On contrary to the present findings of study, Layek, (2010) and Gunasekaran *et al.* (2008) have also noticed the high percentage of weak estrus intensity in Sahiwal (52 %) and crossbreed (49.26 %) cattle respectively.

The variations of estrus intensity observed among different studies might be due discrepancy in steroid concentration among individual animals, Genetic predisposition or uncontrolled environmental conditions and also varies with breed, reproductive stage, season, energy balance etc (Roche and Boland, 1991).

The mean duration of estrus in Punganur cattle was 16.02 ± 0.74 h with a range of 11.5 to 34 h. The estrus duration was below 12 h in 14%, between 12-18 h in 42% and 18-24 h in 24%. The estrus duration is shorter to that of the earlier report by Gunasekaran, (1998) in crossbred cattle (34.34 ± 1.99 h) and longer to that recorded by Naidu and Rao (2006) in Ongole cows (15.54 ± 0.28 h) and Layek (2010) in Sahiwal cows (14.53 ± 0.80). This discrepancy among different studies could be attributed to different degrees of responsiveness to different concentrations of the gonadotropins (Ginther *et al.*, 2001), breed (Figueiredo *et al.*, 1997), reproductive stage (Roche and Boland, 1991), season (Badinga *et al.*, 1994), energy balance (Rhodes *et al.*, 1995) and also influenced by sire within genetic group, lactation number, location, time of day of the onset of estrus, and maximum temperature of the day of estrus (Gwazdauskas *et al.*, 1983)

The oestrous cycle length measured in Punganur cattle was 20.25 ± 0.48 (range from 19 to 21) in 2 wave cycle. Similar to the present study, Ginther *et al.* (1989), Figueiredo *et al.* (1997), Taylor *et al.* (1993) and Nosier *et al.* (2003) have also noticed the oestrous cycle length of 20.4 ± 0.3 , 20.6 ± 0.5 , 20.8 ± 1.7 and 19.8 ± 0.6 days, respectively. On contrary to the present findings of study Akter *et al.* (2010) and Imron *et al.* (2016) have recorded higher values pertaining to the Oestrous cycle length i.e., 21.8 ± 0.8 and 22.0 ± 1.6 days, respectively.

The oestrous cycle length measured in Punganur cattle was 21.27 ± 0.50 (range from 19 to 21) in 3 wave cycle. Similar to the present study, Sakhong *et al.* (2011) and Viana *et al.* (2000) have also noticed the oestrous cycle length of 21.63 ± 0.51 and 21.11 ± 1.76 days, respectively. On contrary to the present findings of study, Ginther *et al.* (1989), Figueiredo *et al.* (1997), Taylor *et al.* (1993) and Nosier *et al.*

(2003) have recorded higher values pertaining to the Oestrous cycle length i.e., 22.8 ± 0.6 , 22.0 ± 0.4 , 29.7 ± 4.9 and 22.5 ± 0.8 days, respectively

Ovarian follicular dynamics study during oestrous cycle of Punganur cows has revealed predominance (75.00 %) of 3-wave follicular activity. Similar findings have been also reported in two previous studies (Sirosis *et al.*, 1988, Savio *et al.*, 1988). They demonstrated that only 25% of cows had two follicular waves during each oestrous cycle, and the remainder had three waves. The majority of Nelore heifers (65%) exhibited a three-wave pattern, whereas the majority of cows (83%) exhibited a two-wave pattern (Figueiredo *et al.*, 1997). On the contrary, several studies have shown the prevalence of 2-wave follicular activity during an oestrous cycle in cattle (Knopf *et al.*, 1989, Ginther *et al.*, 1989 and Akter *et al.*, 2010). However, an increase in the proportion of three-wave patterns observed in this study may be attributed to the low plane of nutrition (Murphy *et al.*, 1991 and Rhodes *et al.*, 1995), heat stress (Badinga *et al.*, 1993 and Wolfenson *et al.*, 1995) and no seasonal effect on wave pattern (Zeitoun *et al.*, 1996).

This discrepancy among different studies could be attributed to the high incidence of the 2 or 3 waves of follicular activity might be based on presence of 2 or 3 peaks of gonadotrophic hormones particularly FSH. The peaks of FSH were also related to lower estrogen concentration, which in turn depended on regression in follicular size. Genetic predisposition or uncontrolled environmental conditions may play an important role in regulation of incidence of the 2 or 3 follicular waves within one estrous cycle, through influences on follicular development and the level of their estrogen secretion (Noseir *et al.*, 2003).

The mean onset of follicular wave in cows showing 2 wave oestrous cycle was on 0.8 ± 0.37 and 13.00 ± 1.08 day of 1st and 2nd wave oestrous cycle, respectively.

Similar to the present study, Knopf *et al.* (1989) and Muraya, (2013) have also recorded the onset of follicular wave on day 0.6 ± 0.4 and 0.61 ± 0.06 , respectively during 1st wave in European cows but during 2nd wave Figueiredo *et al.* (1997) and Akter *et al.* (2010) on day 12.0 ± 0.91 and 12.30 ± 1.00 , respectively in zebu cows. Contrary to this study, Savio *et al.* (1988), Figueiredo *et al.* (1997) Guar *et al.* (2007) and Akter *et al.* (2010) have recorded the onset of follicular wave on day 2-4; 1.50 ± 0.15 ; 2.10 ± 0.36 and 3.3 ± 1.0 during 1st wave but the onset is earlier the present study during 2nd wave in the findings of Knopf *et al.* (1989), Savio *et al.* (1988), Muraya, (2013) and Guar *et al.* (2007) on day 9.2 ± 0.7 ; 10-11; 10.40 ± 1.17 and 10.4 ± 0.9 , respectively.

The mean day of onset of follicular wave in cows showing 3 follicular wave oestrous cycle was 1.83 ± 0.27 , 7.6 ± 0.5 and 13.66 ± 0.87 during 1st, 2nd and 3rd waves, respectively. Similar to the present study, Figueiredo *et al.* (1997) and Viana *et al.* (2000) have also noticed the onset of follicular wave on day 1.57 ± 0.20 and 1.25 ± 0.50 , respectively during 1st wave, during 2nd wave Muraya, (2013), Guar *et al.* (2007) and Viana *et al.* (2000) on day 8.15 ± 0.56 ; 7.2 ± 1.0 and 7.25 ± 0.96 , respectively and during 3rd wave Guar *et al.* (2007) on day 13.2 ± 3.4 . On the contrary to the present findings the onset of wave earlier pertaining to 1st wave in the findings of Knopf *et al.* (1989), Ginther *et al.* (1989), Satheshkumar *et al.* (2011), Santos *et al.* (1998), Muraya, (2013) and Guar *et al.* (2007) (on day 0.7 ± 0.4 , 0, 0.89 ± 0.31 , 0, 0.42 ± 0.16 and 0.7 ± 0.5 , respectively). While the 2nd and 3rd follicular waves are noticed later than the onset of present study by Muraya, (2013), Guar *et al.* (2007) and Viana *et al.* (2000) during 2nd wave (on day 8.15 ± 0.56 ; 7.2 ± 1.0 and 7.25 ± 0.96 , respectively) and by Knopf *et al.* (1989), Ginther *et al.* (1989), Satheshkumar *et al.* (2011), Figueiredo *et al.* (1997), Santos *et al.* (1998) and Muraya, (2013) during

3rd wave (on day 16.1 ± 0.1 ; 16; 15.67 ± 0.29 ; 15.14 ± 0.48 ; 15 and 15.00 ± 0.98). But the onset of 3rd follicular wave is earlier compared to the findings of Viana *et al.* (2000) (on day 11.25 ± 0.96) in the zebu cows

The similarities in the onset of wave among different studies might be due to proper responsiveness of follicle to FSH during recruitment phase which is one of the best timing for superovulatory activities as this responsiveness is lost as the follicles get to the next phase of their development (Muraya, 2013). Fluctuations in circulating concentrations of FSH and estradiol are appeared to be in association with initiation and progression of follicular waves (Gordon, 2003). The second wave onset was earlier in three wave oestrous cycle than two wave cycles as reported earlier (Baruselli *et al.*, 1997). The second wave dominant follicle of a three wave cycle becomes incapable of suppressing follicle stimulating hormone (FSH) and thereby allowing FSH to surge for early emergence of third wave (Adams *et al.*, 1992). The dominant follicle directly inhibits growth of small follicles through the secretion of a factor(s) that acts directly on other follicles in the ovary and regardless of the mechanism, destruction or ovulation of a dominant follicle results in a transient rise in circulating concentrations of FSH and subsequent initiation of a new follicular wave (Adams *et al.*, 1992).

Emergence of the second wave occurs on day 13.00 ± 1.08 for two-wave cycles, and on day 13.66 ± 0.87 for three-wave cycles. Under the influence of progesterone, dominant follicles of successive waves are anovulatory and undergo atresia and one that is present at the onset of luteolysis becomes the preovulatory follicle and emergence of the next wave is delayed until the day of the ensuing ovulation (Ginther, 1989).

The mean day of 1st follicular wave ending and preovulatory follicular wave ending in cows showing 2 wave oestrous cycle is later and earlier, respectively than the follicular wave ending in cows having 3 wave oestrous cycle. This in turn has led to the prolongation of oestrous cycle duration in cows having 3 waves (Gordon, 2003) as observed in this study. Relative changes in circulating concentrations of estradiol and progesterone during the estrous cycle will influence whether the follicle go for atresia or ovulation (Gordon, 2003).

While, the day of ending of 1st follicular wave in cows showing 3 wave oestrous cycle was insignificantly ($P>0.05$) earlier than 1st wave of 2 wave oestrous cycle but the same was significantly ($P<0.05$) earlier than 2nd wave of 2 wave follicular cycle. The day of ending of 2nd wave of 3 waves cycle was significantly ($P<0.05$) earlier than 2nd wave of 2 wave cycle. The difference between the day of end of 3rd follicular wave and 1st wave of 2 wave oestrous cycle was significant ($P<0.05$) and insignificant ($P>0.05$) with 2nd wave of 2 wave oestrus cycle.

The mean maximum diameter of DF in cows showing 2 wave oestrous cycle was 7.30 ± 0.63 and 9.40 ± 0.47 mm, respectively in 2 wave cycle. Similar to the present study, Nosier *et al.* (2003) and Guar *et al.* (2007) have also noticed the maximum diameter of DF of follicular wave of 6.80 ± 0.30 and 10.90 ± 0.30 and 11.75 ± 1.59 and 14.65 ± 1.24 mm during 1st and 2nd wave, respectively. On contrary to the present findings of study, Filho *et al.* (2001), Akter *et al.* (2010) and Muraya, (2013) have recorded higher maximum diameter of DF of follicular wave (13.80 ± 1.40 and 13.80 ± 0.70 ; 13.60 ± 0.92 and 14.00 ± 0.85 and 12.10 ± 1.10 and 18.10 ± 0.6 mm during 1st and 2nd wave, respectively) than maximum diameter of Punganur cows follicle in the present study.

The mean maximum diameter of DF in cows showing 3 follicular waves oestrous cycle was 6.75 ± 0.45 , 6.40 ± 0.29 and 8.33 ± 0.38 mm, respectively in 1st, 2nd and 3rd wave of oestrous cycle. Similar to the present study, Nosier *et al.* (2003) have also noticed the maximum diameter of DF of follicular wave of 6.80 ± 0.30 , 7.30 ± 0.70 and 11 ± 0.90 mm during 1st, 2nd and 3rd wave, respectively. On contrary to the present findings of the study, Filho *et al.* (2001), Muraya, (2013) and Siregar *et al.* (2016) have observed higher maximum diameter of DF respective follicular wave as 11.80 ± 0.80 , 12.00 ± 0.60 and 12.40 ± 0.90 ; 10.95 ± 0.34 , 10.63 ± 0.58 and 13.52 ± 0.50 ; 10.00 ± 1.83 , 8.75 ± 0.50 and 13.75 ± 1.71 mm during 1st, 2nd and 3rd wave, respectively.

The variation in the mean maximum diameter of DF of follicular wave observed among different studies might be due to variations in parity, season, variations in the reproductive physiology of zebu cows with European breeds of cow (Purohit *et al.*, 2000) with special reference to changes in LH pulses for follicle development beyond 9 mm in diameter (Kanitz *et al.*, 2001).

The mean day of maximum diameter of DF of follicular wave in cows showing 2 wave oestrous cycle was 6.6 ± 1.21 and 21.6 ± 1.63 during 1st and 2nd wave, respectively. Similar to the present study, Filho *et al.* (2001), Muraya, (2013) and Guar *et al.* (2007) have also noticed maximum diameter of DF of follicular wave on day 6.2 ± 0.9 and 20.2 ± 0.6 ; 5.0 ± 0.63 and 17.5 ± 0.5 and 7.20 ± 0.37 and 19.40 ± 2.01 during 1st and 2nd wave, respectively.

The mean day of maximum diameter of DF of follicular wave in cows showing 3 follicular waves oestrous cycle was 4.75 ± 0.63 , 11.4 ± 0.49 and 21.4 ± 0.56 during 1st, 2nd and 3rd wave, respectively. Viana *et al.* (2000), Filho *et al.* (2001), Muraya, (2013) and Siregar *et al.* (2016) have also recorded similar maximum

diameter of DF of follicular wave on day 6.45 ± 0.88 , 12.89 ± 3.06 and 20.44 ± 1.42 ; 6.7 ± 0.7 , 13.7 ± 0.3 and 20.0 ± 2.60 ; 6.31 ± 0.44 , 14.54 ± 1.22 and 22.31 ± 1.04 during 1st, 2nd and 3rd wave, respectively.

The similarities in the appearance of maximum diameter at specified period observed in Punganur cows with other breeds may indicate that the FSH stimulus provided by the DF is uniform among all the animals (Perez *et al.*, 2003). Slight variations observed in the appearance of maximum diameter during one of the waves among various studies might be due to variations in the parity (Gordon, 2003), season, reproductive physiology of zebu cows with European breeds of cow etc (Purohit *et al.*, 2000).

The mean preovulatory follicular in Punganur cows showing 2 wave and 3 wave oestrous cycle was 9.4 ± 0.47 and 8.33 ± 0.38 mm, respectively. The mean diameter of preovulatory follicle was positively and significantly ($P < 0.05$) correlated with the corresponding day of oestrous cycle estrogen concentration ($r = 0.94$) but the same was negatively and significantly ($P < 0.05$) correlated with the corresponding day of oestrous cycle progesterone concentration ($r = - 0.83$)

The preovulatory follicle diameter has shown non-significant positive correlation with maximum diameter of subsequent CL ($r=0.58$). In consonance with the present study, Rahman *et al.* (2012); George *et al.* (2005) and Echternkamp *et al.* (1973) reported positive correlation between POF and CL diameter wherein follicles diameter has influenced the size of CL. On the contrary, Barile *et al.* (2007) did not find a steady correlation between the size of a POF and that of the subsequent CL. It is to note that size of the preovulatory follicle could influence the estrogen concentration and in turn the estrous behavior and after ovulation same prepares the follicular cells for luteal formation and function (Busch *et al.*, 2008, Wiltbank *et al.*,

2011 and Vasconcelos *et al.*, 2013). The variations in size of CL might be due to alterations in number of granulosa cells, luteinizing hormone receptors on granulosa and thecal cells and capability of granulosa cells to synthesize the amounts of progesterone after luteinisation (McNatty *et al.*, 1979). Preovulatory follicle in turn reduced the duration of follicular wave compared to the previous unovulatory follicular waves of respective cycle because of higher progesterone concentrations and the time lapse for the atresia of dominant follicle.

The mean day of onset of atresia of 1st follicular wave in cows having 2 wave oestrous cycle was 8.20 ± 1.24 . Similar to the present study, Guar *et al.* (2007) have also noticed the onset of atresia of follicular wave on day 10.5 ± 1.11 during 1st wave of 2 wave cycle. On contrary to the present findings of study, Ginther *et al.* (1989), Knopf *et al.* (1989), Muraya, (2013) and Hadiya *et al.* (2016) have recorded higher values pertaining to the onset of atresia of follicular wave on day 11 or 13; 11 or 13; 12.20 ± 1.11 and 14.00 ± 0.41 during 1st wave of 2 wave cycle.

The mean day of onset of atresia of follicle during 1st and 2nd follicular wave of 3 wave oestrous cycle was on day 7.16 ± 0.61 and 13.33 ± 0.49 , respectively. Similar to the present study, Viana *et al.* (2000), Guar *et al.* (2007) and Siregar *et al.* (2016) have also noticed the onset of atresia of follicular wave on day 7.89 ± 0.78 and 15.55 ± 3.36 ; 7.89 ± 0.78 and 15.55 ± 3.36 and 6 and 13 during 1st and 2nd waves, respectively. On contrary, Hadiya *et al.* (2016) and Muraya, (2013) have recorded higher values pertaining to the onset of atresia of follicular wave on day 13.00 ± 1.00 and 17.00 ± 0.00 and 10.15 ± 0.34 and 16.23 ± 1.18 , respectively.

The variation in the day of onset of atresia of DF of follicular wave observed among different studies might be due to the variations in number of waves, progesterone which may cause dominant follicles anovulatory and undergo atresia and

one that is present at the onset of luteolysis becomes the ovulatory follicle (Ginther, 1989).

A follicle wave is characterized by the synchronous growth of a cohort of follicles, one (or a species-specific number) which continues growing while the others regress at variable intervals. The mean duration of follicular wave in cows showing 2 wave oestrous cycle was 11.8 ± 0.49 and 9.25 ± 2.98 days during 1st and 2nd wave, respectively. Similar to the present study, Guar *et al.* (2007) and Figueiredo *et al.* (1997) have also noticed the duration of follicular wave as 13.35 ± 1.72 and 10.45 ± 0.98 and 14.75 ± 0.70 and 9.05 ± 0.69 days during 1st and 2nd waves, respectively. While, Hadiya *et al.* (2016) have recorded lower values than the present study (7.75 ± 0.48 and 12.00 ± 0.70 days during 1st and 2nd waves of 2 wave cycle, respectively).

The mean duration of follicular wave in cows showing 3 wave oestrous cycle was 8.16 ± 0.95 , 7.93 ± 0.98 and 7.6 ± 0.25 days during 1st, 2nd and 3rd waves, respectively. Similar findings were also recorded during 1st, 2nd and 3rd wave by Hadiya *et al.* (2016), Siregar *et al.* (2016) and Guar *et al.* (2007) (8.50 ± 0.50 , 7.00 ± 0.00 and 7.50 ± 0.50 days; 8, 6 and 4 days and 13.00 ± 1.58 , 11.44 ± 2.19 and 7.67 ± 1.80 days, respectively).

Emergence of a follicular wave and selection of the dominant follicle are temporally and causally associated with a rise and fall in circulating concentrations of follicle-stimulating hormone (Adams *et al.*, 1992). Emergence of a follicular wave is preceded by a surge in plasma FSH concentrations in both spontaneous waves and induced waves (Adams *et al.*, 1992 and Adams, 1999). Surges in FSH preceding each wave are of similar magnitude: mid-cycle surges are similar in amplitude and breadth as those of the preovulatory gonadotrophin surge (Adams *et al.*, 1992 and Adams, 1998). Follicular products, especially those from the dominant follicle, are responsible

for suppressing FSH release and therefore the emergence of the next follicular wave (Singh *et al.*, 1998). A direct relationship between the number of follicles emerging in response to the FSH surge and the subsequent degree of FSH suppression documents that all follicles of the new wave contribute to FSH suppression (Gibbons *et al.*, 1997; Burns *et al.*, 2005 and Ginther *et al.*, 2005).

The mean DF growth rate of follicle in the cows showing 2 wave cycle was 0.64 ± 0.10 and 0.73 ± 0.24 mm per day during 1st and 2nd wave, respectively. Similar to the present study, Muraya, (2013) have also noticed DF growth rate of 1.21 ± 0.12 and 0.91 ± 0.07 mm per day, respectively during 1st and 2nd wave, respectively. On contrary to the present findings, Guar *et al.* (2007) have recorded higher values pertaining to DF growth rate of follicular wave i.e., 1.81 ± 0.22 and 1.16 ± 0.22 mm per day during 1st and 2nd wave, respectively.

The mean DF growth rate of follicle of wave in the cows showing 3 wave cycle were 0.71 ± 0.10 , 0.68 ± 0.10 and 0.66 ± 0.10 mm per day during 1st, 2nd and 3rd waves respectively. Similarly, Viana *et al.* (2000) and Guar *et al.* (2007) have also noticed DF growth rate of 1.00 ± 0.50 , 1.00 ± 0.16 and 1.01 ± 0.33 and 1.03 ± 0.24 , 1.07 ± 0.23 and 1.08 ± 0.26 mm per day during 1st, 2nd and 3rd waves, respectively. While, 1st, 2nd and 3rd Satheshkumar *et al.* (2011) have recorded higher values in the DF growth rate follicular wave i.e., 1.58 ± 0.09 , 1.42 ± 0.10 and 1.95 ± 0.30 mm per day during 1st, 2nd and 3rd waves respectively.

The preovulatory follicle is the functional dominant follicle present at luteolysis (Quirk *et al.*, 1986), indicating that luteolysis occurred between selection and the day in which the third DF reached its maximum diameter. The absence of the atresia period of the DF determined the shorter length of this wave ($P < 0.01$).

The mean DF atresia rate of follicle in the cows showing 2 wave cycle was 0.65 ± 0.45 mm per day during 1st wave. Similar to the present study Guar *et al.* (2007) and Muraya, (2013) have also noticed the DF atresia rate of 0.55 ± 0.22 ; 1.01 ± 0.29 mm per day during 1st wave. On contrary to the present findings of study, Hadiya *et al.* (2016) have recorded higher values pertaining to the DF atresia rate of follicular wave i.e., 1.80 ± 0.29 mm per day during 1st wave.

The mean DF atresia rate of wave in the cows showing 3 wave cycle were 0.66 ± 0.10 and 0.65 ± 0.1 mm per day during 1st and 2nd wave, respectively. Similar to the present study, Viana *et al.* (2000), Guar *et al.* (2007) and Muraya, (2013) have also noticed the DF atresia rate of 0.97 ± 0.26 , 0.96 ± 0.33 ; 0.97 ± 0.26 , 0.96 ± 0.33 ; 0.91 ± 0.10 , 1 ± 0.10 mm per day during 1st and 2nd wave, respectively. On contrary to the present findings of study, Hadiya *et al.* (2016) and Satheshkumar *et al.* (2011) have recorded higher values pertaining to the DF atresia rate of follicular wave i.e., 1.39 ± 0.09 , 1.33 ± 0.14 ; 1.5 ± 0.23 , 1.96 ± 0.10 mm per day during 1st and 2nd wave, respectively.

The variation in the growth rate and atresia rate of DF of follicular waves observed among different studies might be due to follicles of different diameters may have different degrees of responsiveness to different concentrations of the gonadotropins (Ginther *et al.*, 2001), breed (Figueiredo *et al.*, 1997), reproductive stage (Roche and Boland, 1991), season (Badinga *et al.*, 1994), energy balance (Rhodes *et al.*, 1995). It may be assumed that lesser maximum diameter of DF present in Punganur cows than other breeds might also be one of the reasons for the lower growth rate and atresia rate of DF observed in this study.

The mean overall maximum diameter of subordinate follicles (n=10) observed throughout the oestrous cycle was 6.00 ± 0.35 mm. Among these subordinate follicles

about 20 % (N=2) subordinate follicles were observed during 1st follicular wave; 50% (N=5) during 2nd follicular wave and 30 % (N=3) during 3rd follicular wave of oestrous cycle. These follicles have initiated the regression on day 7.50 ± 0.5 , 12.75 ± 1.43 and 20.25 ± 0.47 during 1st, 2nd and 3rd waves, respectively. Similar to present finding, Figueiredo *et al.* (1997) and Muraya, (2013) have also noticed the mean overall maximum diameter of subordinate follicle measuring 6.05 ± 0.36 and 6.97 ± 0.84 mm, respectively. On the contrary, Viana *et al.* (2000) and Guar *et al.* (2007) have recorded higher (7.15 ± 0.34 and 7.15 ± 0.16 mm, respectively) mean overall maximum diameter of subordinate follicle than the present study.

Follicular dominance requires the production of inhibin and estradiol by the DF with a consequent reduction in FSH limiting the maximum diameter of subordinated follicles and inducing their atresia. The dominant follicle reached its maximum diameter on day 6.45 ± 0.88 and entered atresia on day 7.89 ± 0.78 , showing a short period of stabilization or plateau. This growth pattern differs from that reported for two wave cows, in which the first DF regression occurs only after days 11 or 13 (Ginther *et al.*, 1989; Knopf *et al.*, 1989; Taylor and Rajamahendran, 1991), but was similar to that observed in cycles with three follicular waves (Savio *et al.*, 1988; Sirois & Fortune, 1988 and Gambini *et al.*, 1998). The second wave emerged on day 7.11 ± 1.05 , with the respective dominant follicle being selected on day 11.33 ± 2.06 , reaching its maximum diameter on day 12.89 ± 3.06 and beginning atresia on day 15.55 ± 3.36 , with intervals among events similar to those observed on the first wave. The third wave (ovulatory) emerged on day 13.22 ± 2.44 and reached its maximum diameter on day 20.44 ± 1.42 . Other studies which evaluated cows with three follicular waves (Sirois and Fortune, 1988 and Ginther *et al.*, 1989) agree with the appearance of waves at intervals of approximately seven days, which occur due to

transient increases on serum FSH concentration (Ginther *et al.*, 1996). There was no difference ($P>0.05$) on dominant or subordinated follicle growth or atresia rates among follicular waves, but the DF maximum diameter of the second wave was smaller ($P<0.05$) than the diameter of the other DFs.

This difference may be related to the fact that the second wave emerges during the period of higher progesterone production by the corpus luteum, whereas the first and third waves emerge, respectively, during the luteogenic and luteolytic periods.

The mean diameter of CL in Punganur cows on day 4, 6, 8, 10, 12, 14, 16, 18 and 20 of oestrous cycle was 8.49 ± 0.49 , 11.17 ± 0.50 , 12.90 ± 0.45 , 14.46 ± 0.34 , 14.80 ± 0.29 , 15.09 ± 0.46 , 13.34 ± 0.51 , 11.51 ± 0.63 and 10.38 ± 0.43 mm, respectively. The mean maximum diameter of corpus luteum was 15.09 ± 0.46 mm on 14th day and regression of corpus luteum was initiated on 16th day of oestrous cycle. Similar to present study findings, Muraya, (2013) have also noticed maximum diameter of CL measuring 15.9 ± 0.64 mm on day 12.7 ± 0.61 of oestrus cycle. On the contrary, Satheskumar *et al.* (2011) and Taponen *et al.* (2000) have recorded higher maximum diameter of CL (21.58 ± 0.36 and 22.7 ± 1.10 mm on day 9th and day 11 or 12 of oestrus cycle, respectively).

The mean CL diameter was positively and insignificantly ($P<0.05$) correlated with the progesterone concentration ($r = 0.66$) but the same was negatively and significantly ($P<0.05$) correlated with the estrogen concentration ($r = - 0.92$) on day 14 of oestrous cycle. Maximum diameter of subsequent CL has shown non-significant positive correlation with preovulatory follicle diameter ($r=0.58$). Similar correlation is observed by Perry *et al.* (1991), Savio *et al.* (1988) and Wolfenson *et al.* (2004) who have explained the role of enhanced progesterone for regular and series of events

or mechanisms that eventually lead to the rise of $\text{PGF2}\alpha$, followed by onset of CL regression (Mann and Lamming *et al.*, 1995 and Goff, 2004).

The variation in the diameter of CL observed among different studies might be due to a positive correlation between size of the preovulatory follicle and size of the CL (Sartori *et al.*, 2002). It seems likely that increased ovulatory follicular size would lead to increased numbers of granulosa cells which differentiate into large luteal cells following the LH surge and subsequent increased size of the CL (Smith *et al.*, 1994). So the variation in size of the CL between the different breed could be at least partially the result of the variation in the size of preovulatory follicles of previous cycles (Satheshkumar *et al.*, 2011).

The mean concentration of serum progesterone in cows during 0, 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20th day of oestrous cycle was 1.06 ± 0.29 , 3.14 ± 0.91 , 2.38 ± 0.48 , 4.29 ± 0.50 , 4.49 ± 0.97 , 5.44 ± 0.74 , 5.47 ± 0.74 , 6.30 ± 1.20 , 7.04 ± 0.62 , 5.10 ± 0.87 and 2.47 ± 0.87 ng/ml. The mean maximum concentration of serum progesterone was 7.04 ± 0.62 ng/ml on 16th day of oestrous cycle. The concentration of progesterone in the present study gained support from the findings of Mutharao *et al.* (2005), Naik *et al.* (2013), Harpreet Singh *et al.* (2006) and Echternkamp and Hansel, (1973) who have also recorded similar concentrations of progesterone at different days of oestrous cycle. But the concentrations of progesterone reported by Rabiee *et al.* (2002) at day '0' and Hamit *et al.* (2005) at different days of oestrous cycle were higher than the present findings.

While, progesterone concentrations in the present study were higher than the reports of Shukla *et al.* (2000), Mondal and Prakash (2003), Mehrotra *et al.* (2005), Venkatesan *et al.* (2005), Christensen *et al.* (1974), Dutta *et al.* (1989) and Selvaraju

et al. (2002) who recorded lower concentrations of progesterone on different days of oestrous cycle as compared to the concentration of progesterone in Punganur cattle.

The progesterone concentration showed significant increase from day '0' to 16. Later, from day 18 onwards, the progesterone concentration showed a significant decreasing trend and finally came down to the lowest concentration. This constant rise in the concentration up to day 16 might be due to the functional activity of corpus luteum and the decreasing trend from day 18 might be due to the initiation of luteolysis (Hafez, 2008).

Further, the lowest progesterone concentrations on the day of estrus and this gained support from the findings of all the authors who have also recorded either similar or higher or lower concentrations on the other days of oestrous cycle than the present study. These inconsistencies in the concentration of progesterone hormone during different days of oestrous cycle might be due to the differences in the size of preovulatory follicle of previous cycle, sampling frequency, steroid concentrations (GnRH), season and climate at the time of sampling, age and physiological stage (lactating or non lactating) of the animals (Alvarez *et al.*, 2000).

The mean concentration of serum estrogen in cows showing during 0, 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20th day of oestrous cycle was 44.82 ± 1.07 , 38.75 ± 1.68 , 41.65 ± 1.45 , 40.72 ± 1.43 , 36.77 ± 4.77 , 42.09 ± 1.91 , 42.89 ± 1.81 , 42.78 ± 1.34 , 42.07 ± 0.65 , 42.40 ± 0.85 and 44.82 ± 0.70 pg/ml oestrous cycle. The mean maximum concentration of serum estrogen was 44.82 ± 1.07 pg/ml on 0 day of oestrous cycle. Similar to the concentration of estrogen in the present study, Mehrotra *et al.* (2005) in Holstein Friesian x Harijana cattle, Mutharao *et al.* (2005) in Ongole cows, Harpreet Singh *et al.* (2006) in Sahiwal crossbred heifers also recorded same concentrations at different days of oestrous cycle.

While, the estrogen concentrations in the present study were higher than the reports of Agarwal *et al.* (1978) in cows, Selvaraju *et al.* (2002), Gupta *et al.* (1998), Purohit *et al.* (2000), Naik *et al.* (2013) and Venkatesan *et al.* (2005) in indigenous cows, Coe and Allrich (1985), Jimenez (1988), Ecthernkamp and Hansel (1973), Christensen *et al.* (1974), Corah *et al.* (1974) and Glencross and Pope (1981) in exotic cows who also recorded lower concentrations of estrogen on different days of oestrous cycle compared to the concentration of estrogen in Punganur cattle. These inconsistencies in the concentration of estrogen hormone on different day of oestrous cycle might be due to the differences in the sampling frequency, seasons and climate at the time of sampling and age and physiological stage (lactating or not) of the animals (Alvarez *et al.*, 2000).

The estrogen concentration showed a significant ($P < 0.05$) decrease from day '0' to day 2 of oestrous cycle which might be due to the ovulation and subsequent development of luteal tissue under the influence of luteinizing hormone (Hafez, 2008) and Noakes *et al.* (2001). Later, the concentration of estrogen from day 3 to day 8 showed a non significant ($P > 0.05$) increase but the concentration of estrogen did not reach the peak levels which indicated the development of first dominant follicle (Alvarez *et al.*, 2000). Immediately after day '8' the concentration of estrogen decreased significantly indicating atresia of dominant follicle developed during first follicular wave (Hafez, 2008 and Noakes *et al.*, 2001). Later, the concentration from day 10 onwards estrogen showed an increasing trend up to subsequent estrus day '0' at which time the concentration of estrogen was at peak level which might be due to the development of preovulatory follicle (Alveraz *et al.*, 2000 and Hafez, 2008).

The mean estrogen concentration was positively and significantly ($P < 0.05$) correlated with preovulatory follicle diameter ($r = 0.94$). Similarly, Nishimoto *et al.*

(2009) also have observed a positive correlation between estrogen concentration and preovulatory follicle since bovine granulosa cells are capable of producing estradiol; thecal cells are the source of androgen in the follicles and androgen secretion is increased by LH, but not by FSH (Gordon, 2003). Androgen is produced by thecal cells under the influence of LH and aromatized to estradiol by P450 arom and 17- β HSD in smooth endo-plasmic reticulum of granulosa cells, under the control of FSH (Gordon, 2003).

The study concluded that follicular dynamics in Punganur cattle is characterized by a higher incidence of 3 wave oestrous cycles with slightly insignificantly longer oestrous cycles and the trend of estrogen and progesterone hormones during oestrous cycle indicates the normal cyclicity like any other Indian cattle breed.

SUMMARY

Follicular dynamics is one of the most important subjects on ovarian physiology, and was largely studied in European breeds. However, the number of reports on Zebu cattle (*Bos indicus*) and its crossbreeds is limited, especially on Indian breeds. Study on follicular dynamics of any breed establishes reference for estrus, follicular wave, follicle and luteal characteristics and steroid profiles during entire oestrous cycle. Punganur breed is one such breed that is poorly characterized in terms of follicular pattern. Hence, the present investigation was carried out at Livestock Research Station, Palamaner, Chittoor district to investigate the follicular dynamics during two consecutive estrous cycles in eight Punganur breed of cows (*Bos indicus*) on every alternative day by using an ultrasound device.

Estrus intensity in Punganur cattle was 64.8 ± 0.33 and 63.7 ± 0.40 in 2 wave and 3 wave cows, respectively without any significant difference between these waves. The estrus intensity was intense in 3 out of 16 estrus period observed (18.75 %); intermediate in 10 out of 16 estrus period (62.50 %) and weak in 3 out of 16 estrus period (18.75 %). The duration of estrus in Punganur cattle was 16.02 ± 0.74 h with an oestrous cycle length 20.25 ± 0.48 days for 2 wave cycle and 21.27 ± 0.50 days for 3 wave cycle.

In the present study, among the eight Punganur cows during 2 consecutive oestrous cycles (n=16) 4 cycles (25.00%) have exhibited two follicular waves and 12 cycles (75.00 %) exhibited three follicular waves.

The mean day of onset of follicular wave in cows showing 2 and 3 waves oestrous cycle was 0.8 ± 0.37 and 13.00 ± 1.08 and 1.83 ± 0.27 , 7.6 ± 0.5 and 13.66 ± 0.87 during 1st and 2nd wave and 1st, 2nd and 3rd waves, respectively..

The mean day of follicular wave ending in cows showing 2 and 3 waves oestrous cycle was 12.00 ± 0.68 and 21.00 ± 0.63 and 9.59 ± 1.03 , 15.73 ± 0.76 and 21.7 ± 0.51 day during 1st and 2nd wave and 1st, 2nd and 3rd waves, respectively.

The mean wave duration of follicular wave in cows showing 2 and 3 waves oestrous cycle was 11.8 ± 0.49 and 9.25 ± 2.98 and 8.16 ± 0.95 , 7.93 ± 0.98 and 7.6 ± 0.25 days during 1st and 2nd wave and 1st, 2nd and 3rd waves, respectively. The difference in the period of wave duration within 2 waves and 3 waves oestrous cycle cows was insignificant ($P > 0.05$) but the same was significant ($P < 0.05$) between 2 waves and 3 waves cycles.

The mean maximum diameter of DF in cows showing 2 and 3 waves oestrous cycle was 7.3 ± 0.63 and 9.4 ± 0.47 mm and 6.75 ± 0.45 , 6.4 ± 0.29 and 8.33 ± 0.38 mm, respectively during 1st and 2nd wave and 1st, 2nd and 3rd waves, respectively. The difference in the mean maximum diameter of DF of follicular wave within 2 waves and 3 waves oestrous cycle cows was insignificant ($P > 0.05$) except between 1st and 3rd wave and between 2nd and 3rd wave of 3 wave cycle where the difference was significant ($P < 0.05$).

The mean day of maximum diameter of DF of follicular wave in cows showing 2 and 3 waves oestrous cycle was 6.6 ± 1.21 and 21.6 ± 1.63 4.75 ± 0.63 , 11.4 ± 0.49 and 21.4 ± 0.56 day, respectively during 1st and 2nd wave and 1st, 2nd and 3rd waves, respectively.

The mean diameter of preovulatory follicle in cows showing 2 wave and 3 wave oestrous cycle was 9.4 ± 0.47 and 8.33 ± 0.38 mm, respectively and was correlated positively and significantly ($P < 0.05$) with the corresponding day of oestrous cycle estrogen concentration ($r = 0.94$).

The mean day of onset of atresia of follicular wave in cows showing 2 and 3 wave oestrous cycle was 8.2 ± 1.24 and 7.16 ± 0.61 and 13.33 ± 0.49 day respectively during 1st wave and 1st and 2nd wave respectively.

The mean growth rate of follicle in each wave in the cows showing 2 and 3 wave cycle was 0.64 ± 0.10 and 0.73 ± 0.24 mm and 0.71 ± 0.10 , 0.68 ± 0.10 and 0.66 ± 0.10 mm per day respectively during 1st and 2nd wave and 1st, 2nd and 3rd waves, respectively. The difference in the growth rate of follicle of follicular wave within 2 waves and 3 waves oestrous cycle cows was insignificant ($P > 0.05$).

The difference in the atresia rate of follicle during follicular wave within 2 waves and 3 waves oestrous cycle cows was insignificant ($P > 0.05$) while atresia rate of 1st follicular wave in cows showing 3 waves oestrous cycle was insignificantly ($P > 0.05$) higher than in cows showing 2 waves oestrous cycle.

The difference in the diameter of corpus luteum between 2 wave and 3 wave oestrous cycle cows and within 2 wave and 3 wave oestrous cycle cows was significant ($P < 0.05$). The increase in the diameter of the CL was significant throughout its growth from the day 4 of oestrous cycle to the day of regression while the mean CL diameter was positively and insignificantly ($P < 0.05$) correlated with the progesterone concentration ($r = 0.66$)

The mean overall concentration of serum progesterone during 0, 2, 4, 6, 8, 10, 12, 14, 18 and 20 days of oestrous cycle in Punganur cows was 1.06 ± 0.29 , 3.14 ± 0.91 , 2.38 ± 0.48 , 4.29 ± 0.50 , 4.49 ± 0.97 , 5.44 ± 0.74 , 5.47 ± 0.74 , 6.30 ± 1.20 , 7.04 ± 0.62 , 5.10 ± 0.87 and 2.47 ± 0.87 ng/ml respectively. The mean maximum concentration of serum progesterone was 7.04 ± 0.62 ng/ml on 16th day of oestrous cycle. The difference in the mean concentration of serum progesterone between 2

wave and 3 wave oestrous cycle cows was not significant ($P>0.05$) while the same between days of oestrous cycle was significant ($P<0.05$) and within 2 wave and 3 wave oestrous cycle cows.

The mean overall concentration of serum estrogen during 0, 2, 4, 6, 8, 10, 12, 14, 18 and 20 days of oestrous cycle in Punganur cows was 44.82 ± 1.07 , 38.75 ± 1.68 , 41.65 ± 1.45 , 40.72 ± 1.43 , 36.77 ± 4.77 , 42.09 ± 1.91 , 42.89 ± 1.81 , 42.78 ± 1.34 , 42.07 ± 0.65 , 42.40 ± 0.85 and 44.82 ± 0.70 pg/ml respectively. The difference in the mean concentration of serum progesterone between 2 wave and 3 wave oestrous cycle cows and days of oestrous cycle was not significant ($P>0.05$). The trend of estrogen and progesterone hormones showed during oestrous cycle of Punganur cow indicates the normal cyclicity of these breed like any other Indian cattle breed.

The study concluded that follicular dynamics in Punganur cattle is characterized by a higher incidence of 3 wave oestrous cycles with slightly insignificantly longer estrous cycles and the trend of estrogen and progesterone hormones during oestrous cycle indicates the normal cyclicity like any other Indian cattle breed.

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