STUDIES ON ESTROUS BEHAVIOUR AND ESTROUS CYCLE RELATED ENDOCRINE PROFILE OF GADDI GOATS

THESIS

By

SUNIL KUMAR
(V-2013-30-006)

Submitted to

CHAUDHARY SARWAN KUMAR
HIMACHAL PRADESH KRISHI VISHVAVIDYALAYA
PALAMPUR-176062 (H.P.) INDIA

in

Partial fulfillment of the requirements for the degree of

MASTER OF VETERINARY SCIENCE

(VETERINARY GYNAECOLOGY AND OBSTETRICS)

DEPARTMENT OF VETERINARY GYNAECOLOGY AND OBSTETRICS

2015

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2015

Dedicated

to my

beloved Parents

Dr. Pankaj Sood
Professor and Head
(TVCC)

Department of Veterinary Gynaecology and Obstetrics
College of Veterinary and Animal Sciences
CSK Himachal Pradesh Krishi Vishvavidyalaya,
Palampur-176 062 (H.P) INDIA

CERTIFICATE I

This is to certify that the thesis entitled **"Studies on estrous behaviour and estrous cycle related endocrine profile of Gaddi goats"** submitted in partial fulfillment of the requirements for the award of the degree of **Master of Veterinary Science** in the discipline of **Veterinary Gynaecology & Obstetrics** of CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur is a *bonafide* research work carried out by **Dr. Sunil Kumar (Admission No. V-2013-30-006),** son of Smt. Lata Devi and Sh. Manohar Lal, under my supervision and that no part of this thesis has been submitted for any other degree or diploma.

The assistance and help received during the course of this investigation have been fully acknowledged.

Place: Palampur (Pankaj Sood)
Dated: Major Advisor

CERTIFICATE II

This is to certify that the thesis entitled **"Studies on estrous behaviour and estrous cycle related endocrine profile of Gaddi goats"** submitted by **Dr. Sunil Kumar (Admission No. V-2013-30-006),** son of Sh. Manohar Lal to the CSK Himachal Pradesh Krishi Vishvavidyalya, Palampur in partial fulfillment of the requirements for the degree of **Master of Veterinary Science** in the discipline of **Veterinary Gynaecology & Obstetrics** has been approved by the advisory committee after an oral examination of the student in collaboration with an External Examiner.

----------------------- Dr. Pankaj Sood Chair Person	----------------------- External Examiner
------------------------ Dr. Madhumeet Singh Member	----------------------- Dr. P.K Dogra Member
------------------------ Dr. Desh Raj Wadhwa Dean' nominee	

Head of the Department

Dean, Postgraduate studies

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NEEDLESS TO SAY, ALL ERRORS AND OMISSIONS ARE MINE.

DATE:

PLACE: PALAMPUR **SUNIL KUMAR**

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ABBREVIATIONS USED

Abbreviation		**Complete Name**
AUC	:	Area Under Curve
C	:	Confined
G	:	Grazing
I	:	Induced
Ltd	:	Limited
MHz	:	Mega hertz
I_{all}		Overall Induced
S	:	Spontaneous

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ABSTRACT

Ten adult dry and non-pregnant Gaddi does were used in the present study for estrous behaviour and estrous cycle related endocrine profile. The study was conducted during short days under natural photoperiod from October, 2014 to February, 2015. All the does were induced by PG (I_{all} group), four of which were investigated in the subsequent spontaneous estrous cycle (S group). Information on the later set of goats during induced estrus (from the I_{all} group) was also used and categorized in a third group (I) to draw a comparison of estrous behaviour characteristics and endocrinology parameters between S, I and I_{all}. The endocrinology determinants were evaluated in peripheral blood plasma collected from estrus onset and every 3 hours thereafter till termination of estrus; in addition, day 7 and day 14 blood samples were also evaluated. A comparison of estrous behaviour characteristics was also made between confined (C) *versus* grazing (G) conditions. The average time interval from PG administration to estrous onset was 76.1±3.8 hours. Standing to be mounted, biting of estrus female, sniffing of vulva and tail flagging were most frequent signs characterizing onset of estrus, irrespective of PG injection or housing conditions. Unlike G condition, where all estrous signs peaked at 3 to 3.5 hours after estrous onset, all the other estrous behaviour signs attained peak frequency at 6 to 12.5 hours post estrous onset in S, I and I_{all} groups and C condition. Overall, estrous expression was better in S estrus and in C condition compared to their respective corresponding groups. There was no significant difference in estrous duration, estrous onset to ovulation and follicular diameter of S *versus* I *versus* I_{all} groups (28.4±1.4 *versus* 32.0±5.3 *versus* 30.6±4.1 h, 23.9±2.1 *versus* 28.5±2.7 *versus* 24.0±5.6 h and 9.8±0.6 *versus* 9.3±0.5 *versus* 9.5±0.5 mm, respectively). Double ovulation was present in one goat of the I group. There was no significant difference in ultrasonography assisted ovulatory and other associated endocrine determinants between the S, I and I_{all} groups. Estradiol-17β, LH peak concentration, their timings from estrous onset, estradiol- 17β peak to ovulation interval and LH peak to ovulation interval did not differ significantly in all the three groups. The day 7 and day 14 plasma progesterone concentrations did not differ and the average values ranged from 6.5±1.1 to 7.2±1.9 ng/ml at day 7 and 10.9±2.6 to 12.4±2.1 ng/ml at day 14 in S, I and I_{all} groups. Genital discharge fern pattern differed with stage of estrus. Typical fern pattern coincided with peak frequency of estrous behaviour signs except under G condition, where the peak *per se* occurred much early. Induction of estrus did not affect ovulatory and endocrine characteristics.

Student		Major Advisor
(Sunil Kumar)	Head of the department	(Pankaj Sood)

CHAPTER 1

INTRODUCTION

Goats are the earliest domesticated livestock animals compared to cattle and sheep. Goats show a typical grazing and browsing behaviour. They are differ in consumption of vegetation and are advantageous as they often eat many plants that cattle and other livestock do not prefer to ingest. Total population of goats in India is 125.7 million (Prasad 2010), whereas in Himachal Pradesh its count stands to be 12.41 lacks, out of which the number of Gaddi goats is 7.6 lacks (Dogra *et al.* 2015). Gaddi goat is an important breed of Himachal Pradesh. The breed derives its name from nomadic tribe “Gaddi” who originally reared this breed. Due to paucity of fodder, the Gaddi goats are primarily migratory breeds moving to alpine pastures during the summer months. The flock size may vary from 20 to 500. Majority of Gaddi goats have mostly white hair coat, but black, brown or combination of the two are also present. The hair are lustrous and long. Both sexes have large horns, directed upward and backward and occasionally twisted. Ears are medium long and drooping. The nose line is convex. The udder is small and round with small teats placed laterally (German 2013). Gaddi goat is economically important for the local tribes of Kangra, Kullu, Shimla, Sirmour and Chamba districts of Himachal Pradesh. Goats being a multipurpose animal, the latter might be the reason for higher production per unit investment in terms of milk, meat, manure and hide (Prasad 2010). In addition, goats in hilly areas are also used for carrying light loads.

Dairy goats are seasonal breeders. The breeding season is initiated by decreasing day light (Chakraborty *et al. 1993*) beginning from August to last till the end of February in northern hemisphere. The day length affects production, reproduction and behaviour of this breed. (Banerjee 2010).

Reproduction in Gaddi does is characterized by essential single birth and twinning occurs only in 15 to 20 percent goats. Mature body weight of male and female are 30 kg and 20 kg, respectively. Age at first kidding is 19 month (Prasad 2010). Estrous cycle and estrous duration is 21.4 days and 36 hours, respectively (Gupta *et al.* 2005). Estrous behaviour is important to attain knowledge about the breeding season and to provide economic benefit to the farmers (Haenlein *et al.* 1992). Moreover, an idea about time of AI can be drawn.

In general, breeders usually have most trouble in detecting estrus in goats in less number or otherwise in the absence of buck. Does have estrous cycle of 20 days with a range of 16-23 days. Redness of vulva, swelling and clear, thin vaginal discharge is first observed 1-2 days before estrus, becoming maximal on the day of onset of estrus (Llewelyn *et al.* 1993). Does in estrus display tail wagging, bleating and restlessness (Llewelyn *et al.* 1993). In spite of all these manifestations, some does that exhibit estrus in early months of the season show no signs.

As, limited information is available on estrous behaviour and estrous cycle related endocrine profile in Gaddi breed of goat. Hence, the proposed study is designed with following objectives:

1.1 To study estrous behaviour of Gaddi goats.

1.2 To determine endocrine profile during different stages of estrous cycle.

CHAPTER 2

REVIEW OF LITERATURE

Goats have been considered since long as a minor species and were among the earliest domesticated animals (Prasad 2010). However, its minority of existence could not be underestimated as, along with other livestock species, it is a lifeline of 70 and 90 % of the farmers living in rural areas of Himachal Pradesh (Ramachandra *et al.* 2012) and India (Iqubal 2010).

The goat is a multipurpose animal producing milk, hide, fibre and manure. In addition, goats in hilly areas are also used for carrying light loads. They are more tolerant to hot climate and give more production per unit investment. The technical and most important advantage of goat rearing is that consumption of mutton has no restrictions and is therefore relished by all sections of society, which puts goats in never ending demand. The aforementioned benefits render goats to be poor man's cow because of its immense contribution to poor man's economy. Goats show a typical grazing and browsing behaviour. Goats are different in consumption of vegetation, oftentimes eating many plants that cattle and other livestock prefer not to ingest. This preferential eating behaviour of goats favours the management concept of interspecies or rotational grazing (Prasad 2010). The most optimal observed time period for reproductive activity has been reported to be September to November (Noakes *et al.* 2009). Goats are considered to be seasonally polyestrous animals (Hafez 1980). The reproductive activity however varies in different geographical locations. For instance, estrous cycles are manifested several times and continuously during autumn in the goats raised abroad having an extended temperate condition (Chentouf *et al.* 2011), whereas, in some part of India and other nearby countries having tropical or sub-tropical conditions, which are subjected to less change in temperature and photoperiod, the goats show cyclic activity throughout the year (Fatet *et al.* 2011). The later observation is substantiated by recording of longer breeding season in goats raised under tropical and equatorial regions that are subjected to less change in photoperiod and temperature than others exhibiting more seasonal effects under temperate and polar region (Romano and Abella 1997 and Greyling 2000). Another set of authors observed that the seasonality of goats increased gradually from July to December and then declined gradually between January and March under temperate conditions (Chakraborty *et* *al. 1993*); no estrus was observed from April to June (Chentouf *et al.* 2011). In the temperate region of Himachal Pradesh (Sati and Singh 2010), the goat estrous cycles occurs during short days from September (Chemineau 1986) or October (Farshad *et al.* 2008) to March (Fatet *et al.* 2011).The main environmental factors affecting seasonal breeding in small ruminants including goats is the annual change in day length (Fatet *et al.* 2011) although temperature variation has also been reported as the reason *per se* (Chemineau 1986). Breed variation in the goats has been another reason for difference in reproduction activity (Romano and Abella 1997 and Greyling 2000). Irrespective of different geographical conditions, it seems that stressfull seasons of the year (winters or summers) do not favour reproductive activity in goats (Chemineau *et al.* 2004).

2.1 ESTROUS BEHAVIOUR IN GOATS

Reduced homosexual behaviour in goats underscores the use of male to detect estrus (Eiamvitayakorn and Rigor 1988). Different authors have furnished different opinions and conclusions about estrous behaviour in different breeds of goat. The various estrous behaviour traits in present document have been categorised to male specific behaviour and female specific behaviour (Ganesh *et al.* 2014).

2.1.1 MALE SPECIFIC BEHAVIOUR

2.1.1.1 MOUNTING BY BUCK

Mounting of does by bucks is a characteristic feature of estrus presence (Ganesh *et al.* 2014).

Delgadillo *et al.* (2002) in goats reported that mounting instances at the end of breeding period recorded for a 30 min period by long day (18 : 16 h of light : dark) and melatonin treated bucks (75 days followed by insertion of two sub-cutaneous melatonin implants) was 21.3 compared to 4.5 in the bucks kept under natural photoperiod. Higher mounting activity was attributed to a clear stimulation of testosterone secretion. However, exogenous melatonin does not stimulate endocrine activity in bucks as another group given only long day treatment had mounting activity similar to long day plus melatonin group. The average number of mounts by buck during ‘reaction time’ of 44.0±2.1 to 69.6±5.4 seconds was 2.3±0.1 (range of 2.2±0.1 to 2.5±0.1) (Prado *et al.* 2002). Stimulus of estradiol- 17β compared to testosterone treatment in ovariectomized anestrous does have been found to be better in eliciting buck mounting. Flutamide (antiandrogen) injection, followed by estradiol- 17β or testosterone treatment of does yielded 2.4 and 1.2 mounts during 30 min observation. Similarly, administration of flutamide carrier (propylene glycol and ethanol) followed by estradiol- 17β or testosterone treatment of does resulted in 3.2 and 2.8 mounts by buck. More mounting by buck in estradiol- 17β injected does was attributed to activation of androgen receptors by estradiol- 17β (Imwalle and Katz 2004).

Mounting by buck has also been considered to determine the duration (onset to end) of estrus. A doe was considered to be in estrus when it allowed mounting by the buck, till the time when mounting ceased; the time span considered as estrous duration (Widayati *et al*. 2010). In a more recent study, the average mounting activity for a 30 min period was 20 mounts in the bucks exposed to 75 long days compared to 5 mounts in another group given 50 mg of testosterone preparation every three days for 3 weeks before exposure to estrus does. The control group in the evaluation period exhibited 2.5 mounts. Long day exposure resulted in higher mounts probably due to higher testosterone persistence compared to the testosterone supplemented males as the latter caused a negative feedback effect on hypothalamo-pituitary gonadal axis eventually lowering the testosterone production (Luna-Orozco *et al.* 2012). Similar findings of higher mounting activity following long days compared to testosterone treatment has been reported by Perkins and Fitzgerald (1992).

The bucks given an exposure to 75 long days were evaluated for mounting attempts on day 0 (day of estrus), 1, 2 and 8 in fixed (in contact with does throughout the breeding period) or rotated (daily rotation) groups. The mounting frequency in two groups at different days was 12.5 *versus* 2.5, 1 *versus* 2.5, 1.5 *versus* 10 and 2.5 *versus* 35, respectively, during 30 min observation period. As evident, the mounting attempts were greater in the rotated than the fixed group on all days except estrus (day 0). Without assigning a specific reason for reduced mounts by males of rotated group on day 0, the higher mounting activity on the other days by this group was assumed to be a result of a period of separation that improves the sexual activity of male exposed to familiar females (Pepelko and Clegg 1965, Thiery and Signoret 1978, Prado *et al.* 2003, Silvestre *et al.* 2004 and Bailey *et al.* 2005). Furthermore, certain other behaviours such as penile display, flehmen, sniffing and lateral approach were more frequent at different days in rotated than fixed group (Loya-Carrera *et al.* 2014).

2.1.1.2 FLEHMEN

Flehmen reaction, though a sexual dimorphic behaviour (Ladewig *et al.* 1980), is more commonly expressed by males. It is the result of olfaction when male smells the peri-anal area or urine (Fonseca *et al.* 2013) of a doe, especially during estrus (Doty 2012) or after licking the external genitalia of a doe (Obrien 1982). Flehmen response has also been recorded, though with a less intensity, in the bucks in contact with diestrus (Ladewig *et al.* 1980) and anestrous (Delgadillo and Velez 2010) does.

Flehmen reaction was highest in bucks exposed to 75 long days (16: 8 h of light: dark) followed by bucks treated with testosterone injection and then, the untreated one; the respective values for a 30 min period being 20.0, 5.0 and 2.5 (Luna-Orozco *et al.* 2012). The intensity of flehmen reaction is directly related to the concentration of pheromones in the urine of female (Fonseca *et al.* 2013). In a subsequent study, the bucks given an intermittent exposure to different subsets of does (rotated group) exhibited consistent higher flehmen response than the other bucks in regular contact with does (fixed group). The flehmen frequency for a 30 min period on day 0 (day of estrus), 1, 2 and 8 in rotated *versus* fixed group was 10 *versus* 4, 15 *versus* 5, 10 *versus* 6 and 9 *versus* 7.5, respectively (Loya-Carrera *et al.* 2014). In a more recent study, a combined effect of bucks isolated for one year before study than the others housed with does, the progression of breeding season from February to May revealed higher flehmen; 13.91 *versus* 6.75 during 4 h observation period equivalent to 1.8 *versus* 0.8 during 30 min period (Ungerfeld *et al.* 2014).

2.1.1.3 SNIFFING OF VULVA

Incorporation of flutamide in exogenous estradiol- 17β than testosterone protocols given to ovariectomized anestrous does resulted in a higher frequency of sniffing events by male (37.3 *versus* 29.4 per 30 min observation period) during breeding season. However, without flutamide, testosterone protocol was more effective than estradiol- 17β injection in eliciting sniffing events (Imwalle and Katz 2004). Bucks given an artificial exposure of 75 long days (16 h of light: 8 h of darkness) with or without melatonin implant insertion at the end of breeding season were equally effective in increasing the sniffing frequency of buck (23.7 per 30 min observation period) compared to the bucks under natural photoperiod (1.3 per 30 min observation period). The increased sniffing response to the long day exposure in the aforesaid experiment was attributed to the stimulation of testosterone secretion (Delgadillo *et al.* 2002).

The ano-genital sniffing at different days of estrous cycle, except during estrus was higher in rotated than fixed groups. The sniffing frequency at day 0 (day of estrus), 1, 2 and 8 for fixed *versus* rotated groups was 80 *versus* 75, 20 *versus* 125, 37.5 *versus* 120 and 20 *versus* 85, respectively. Sniffing by the buck was in general higher in the rotated than the fixed group on day 2 and 8 (Loya-Carrera *et al.* 2014). Sniffing by male was found to be affected by the stage of the breeding season and continuous presence or isolation of bucks from does. Sniffing frequency during 30 min observation, increased from October to February (0.5 per 30 min) and again during May, the late breeding season (0.7 per 30 min). Introduction of isolated bucks to estrus does revealed a higher sniffing frequency than those kept continuously with does, the corresponding 30 min frequencies being 2.3 and 1.7, respectively (Ungerfeld *et al.* 2014).

In the semi-arid region of Mexico where goat bred throughout the year, a seasonal variation in sniffing frequency had been observed. Sniffing was more during dry period (0.5 per 30 min) starting from January than the rainy period (0.3 per 30 min) beginning from Jun (Dos Santos *et al.* 2015).

2.1.1.4 BITING OF ESTRUS FEMALE

Before the buck mounts, it sniffs the doe's side and genital area. The buck chases her making "gobbling" sounds with his mouth and flicking his tongue, attempts to bite in neck. Following biting, the buck lowers his head and flap his tongue at the side of the doe in estrus (Nolte 2012).

Preceeding biting, the buck runs his tongue in and out of his mouth, appears to be very excited and show intermittently rubbing against the side of doe (Haenlein *et al.* 1992). Biting of estrus female was also seen in the form of exposure of tongue (Fonseca *et al.* 2013 and Dos Santos *et al.* 2015) while monitoring spontaneous cycles in ewes and does, respectively. It was concluded by Dos Santos *et al.* (2015) that exposure of tongue was more frequent in rainy and dry season during the morning as compared to the other times of the day.

2.1.1.5 OTHER BEHAVIOURAL CHARACTERISTICS

Apart from the aforementioned behavioural characteristics, penile exposure and emission of sound by male while in contact with estrus animals were recorded in ewes by Fonseca *et al.* 2013 and Dos Santos *et al.* 2015. Body rubbing and dominance over other bucks have also been reported (Ganesh *et al.* 2014).

2.1.2 FEMALE SPECIFIC BEHAVIOUR

The most common estrus behaviour characteristics reported for estrus presence or detection include tail flagging (Llewelyn *et al.* 1993, Ola and Egbunike 2004 and Widayati *et al.* 2010), bleating (Llewelyn *et al.* 1993, Ola and Egbunike 2004 and Ganesh *et al.* 2014), standing to be mounted (Eiamvitayakorn and Rigor 1988, Ola and Egbunike 2004 and Ganesh *et al.* 2014), interest in male (Eiamvitayakorn and Rigor 1988 and Ola and Egbunike 2004), restlessness (Llewelyn *et al.* 1993 and Ganesh *et al.* 2014) and genital discharge (Eiamvitayakorn and Rigor 1988, Ola and Egbunike 2004, Widayati *et al.* 2010, Bhattacharyya *et al.* 2012 and Ganesh *et al.* 2014).
A variation in the preponderance of difference estrous behaviour characteristics has been recorded by different set of authors. Accordingly, different characteristics have been considered as landmark in estrus detection. However, some of the above authors have reported absence of the aforementioned pertinent signs of estrus in goats. For instance, tail flagging, restlessness, bleating, interest in male and standing to be mounted could not be observed in one of the study (Bhattacharyya *et al.* 2012). Instead, decreased feed intake and urination were the estrous behaviour characteristics. Only tail flagging was present in another study (Widayati *et al.* 2010). Furthermore, tail flagging and interest in male were not recorded (Ganesh *et al.* 2014) and bleating and restlessness were not recorded in estrus does (Eiamvitayakorn and Rigor 1988 and Ola and Egbunike 2004).

No conspicuous difference in estrous behaviour score between spontaneous and induced estrus has been reported; the values being 10.8±0.8 and 10.4±0.8, respectively (Ola and Egbunike 2004).

2.1.2.1 TAIL FLAGGING

Tail flagging occurs in the form of shaking of tail side ward's in does especially when buck is around. It is considered among secondary signs of estrus in does (Nix 2002, Rahman *et al.* 2008 and Ciptadi *et al.* 2014). Tail flagging helps in the spread of pheromones from an estrus female, which probably results in sexual arousal in bucks (Haulenbeek and Katz 2011). The latter is directly related to the frequency of tail flagging (Shank 1972). Compared to bleating and restlessness (Llewelyn *et al.* 1993), tail flagging has been considered as a strong indication of estrus in presence of buck (Ola and Egbunike 2004 and Fakruzzaman *et al.* 2012).

With reference to the stage of estrus, tail flagging in does was recorded from 60 h prior to estrous onset till 36 h after estrous onset. The frequency of tail flagging however, increased 12 h before estrous onset and peaked at estrous onset. The peak frequency at estrous onset was 23.7 during 30 min observation period (Llewelyn *et al.* 1993). Alike sniffing, frequency of tail flagging was higher following exogenous administration of flutamide with estradiol- 17β (80 per 30 min observation period) than with testosterone (39 per 30 min observation period) given to ovarictomized anestrous does. However, without flutamide, testosterone resulted in more frequent tail flagging (76 per 30 minute observation period) than estradiol- 17β (36 per 30 min observation period) (Imwalle and Katz 2004). The average frequency of tail flagging over a 10 min period in 3 ovarictomized does simultaneously in estrus was 61.0±27.0. This frequency was much more higher than 17.0±10.0 recorded in non-estrus hormonally treated females (Haulenbeek and Katz 2011). Tail flagging was more during spontaneous than induced estrus, the values being 2.6±0.1 *versus* 2.4±0.1, respectively (Ola and Egbunike (2004).

However, few non-estrus females also exhibit bouts of tail flagging (Haulenbeek and Katz 2011).

2.1.2.2 RESTLESSNESS

Restlessness, as an estrous indicator, has been observed in several studies (Widayati *et al.* 2010, Fakruzzaman *et al.* 2012, Ciptadi *et al.* 2014 and Ganesh *et al.* 2014). It has been considered among secondary signs of estrus in does (Nix 2002 and Rahman *et al.* 2008). Restlessness in does was recorded from 60 h prior to estrous onset till 36 h after estrous onset. The frequency of restlessness, however, increased 12 h before estrous onset and peaked at estrous onset. The peak frequency at estrous onset was 14 during 30 min observation period (Llewelyn *et al.* 1993).

2.1.2.3 BLEATING

Bleating, as an estrous behaviour sign, has been observed in estrous does (Eiamvitayakorn and Rigor 1988, Rahman *et al.* 2008 and Ganesh *et al.* 2014). Bleating increased 12 h before estrous onset and peaked at estrous onset. Maximum frequency of 13.5 during a 30 min period has been reported at estrous onset (Llewelyn *et al.* 1993). Bleating was more frequent during induced than spontaneous estrus, the average frequencies during day time (three observations) were 0.1±0.1 *versus* 0.1±0.1, respectively (Ola and Egbunike 2004).

2.1.2.4 INTEREST IN MALE

The does in estrus actively seek for the buck in their surroundings and show keen interest in males (Eiamvitayakorn and Rigor 1988, Ola and Egbunike 2004 and Fakruzzaman *et al.* 2012). Ola and Egbunike (2004) also observed that in the absence of response from buck, the estrous does attempt to mount the buck or became aggressive to other does around. Also, the frequency of interest in male was reported to be more during spontaneous than induced estrus (1.7±0.2 *versus* 1.5±0.2).

2.1.2.5 STANDING TO BE MOUNTED

The one true sign of estrus is when doe stands still to be mounted by buck is ‘standing to be mounted’ (Eiamvitayakorn and Rigor 1988, Ola and Egbunike 2004, Farshad *et al.* 2008 and Ganesh *et al.* 2014). Standing to be mounted was more frequent in spontaneous than induced estrus, the frequencies being 2.6±0.1 *versus* 2.5±0.1, respectively (Ola and Egbunike 2004).

2.1.2.6 HOMOSEXUAL BEHAVIOR

Homosexual behaviour was reported as mounting other does (Eiamvitayakorn and Rigor 1988, Ola and Egbunike 2004, Shearer and Katz 2006, Bhattacharyya *et al.* 2012 and Ganesh *et al.* 2014).

Homosexual behaviour, recorded as number of mounts was expressed more during induced than spontaneous estrus (0.46±0.18 *versus* 0.24±0.15). Homosexual behaviour appeared to occur more in maiden (20.5 %) than the primiparous (7.1 %) and multiparous (9.3 %) does (Ola and Egbunike 2004). Mounting behaviour, whether male-female or female-female, increased the total number of sexual behaviour displays, increased ejaculatory frequency and decreased post ejaculatory interval. The male goats are aroused by the visual cues of mounting behaviour and that female-female mounting is proceptive in goats (Shearer and Katz 2006). Homosexual behaviour may not be a consistent feature as it was not recorded in the study of Widayati *et al.* (2010). Bhattacharyya *et al.* (2012) reported 80.95 % homosexual behaviour to be exhibited as mounting or allowing mounting by other nearby goats.

2.1.2.7 GENITAL DISCHARGE AND VULVAR CHANGES

Genital discharge was produced under the influence of estradiol- 17β. Copious genital discharge, congestion and swelling of vulvar region in goats in estrus have been observed (Eiamvitayakorn and Rigor 1988, Ola and Egbunike 2004, Widayati *et al.* 2010, Bhattacharyya *et al.* 2012 and Ganesh *et al.* 2014).

The genital discharge and congestion of vulva were exhibited more frequently in spontaneous than induced estrus, the frequencies being 0.29±0.14 *versus* 0.04±0.04 and 1.19±0.21 *versus* 1.08±0.15, respectively (Ola and Egbunike 2004). The production of genital discharge has been correlated with the concentration of estradiol- 17β. It was maximal at the time of ovulation and was thin, clear and acellular (Widayati *et al.* 2010). Bhattacharyya *et al.* (2012) reported hypermia - swelling of vulva and scanty genital discharge in 71.4 % and 57.14 % local goats of Kashmir in estrus.

Also, at the onset of estrus, 85% of ewes had clear genital discharge and some ewes had incomplete ferning and some had complete ferning. Maximum ferning of genital discharge was observed during mid-estrus. After mid to late estrus, ferning pattern of genital discharge was observed to be degraded. At 24 to 48 hours after estrous onset, 25 % of ewes had a red colour and 75% of ewes had pink vaginal epithelial colour (Leethongdee *et al.* 2007).

2.1.2.8 OTHER BEHAVIOURAL CHARACTERISTICS

Lordosis has been exhibited more frequently in induced than spontaneous estrus, the frequency being 2.3±0.2 *versus* 2.1±0.2 (Ola and Egbunike 2004). Frequent urination (Eiamvitayakorn and Rigor 1988 and Ganesh *et al.* 2014) and low feed intake (Bhattacharyya *et al.* 2012) have also been reported in goats in estrus.

2.3 INTERVAL FROM PG ADMINISTRATION TO ESTROUS ONSET

PG (Greyling and Niekerk 1986, Romano 1998 and Kusina *et al.* 2000), with progestagen (Whitley and Jackson 2004 and Titi *et al.* 2008) or without GnRH (Greyling and Nest 2000 and Alvarez *et al.* 2007) has been used as estrous induction agents in goats. The *ut infra* information restricts to the studies with PG as it was utilized in the present study. Majority of studies reported the time from PG administration to estrous onset (single or double injection 10 to 11 days apart) to be 43.0±5.5 h to 55.7±3.0 h (Bretzlaff *et al.* 1981, Akusu and Egbunike 1984, Mori and Kano 1984, Medan *et al.* 2003, Simoes *et al.* 2008, Vazquez *et al.* 2010 and Esteves *et al.* 2013). Contrarily, in a small sample size of 5 animals, a study observed much earlier estrous onset at 20.4±0.9 (range: 18 to 32 h). The reason *per se* has not been cited (Perera *et al.* 1978). A much longer period of estrous onset of 60.5 to 65 h is also on record (Romano 1998 and Goel and Agrawal 1998).

2.4 ESTROUS DURATION

Majority of studies report estrous duration ranging from 24 to 48 h in goats (Hafez 1980, Rahman *et al.* 2008 and Noakes *et al.* 2009). Some other studies reported average estrous duration of 29.3±14.9 h in Indigenous goats and 28.0±15.7 h in Boer goats, respectively (Greyling and Nest 2000). In another study, it was 38.8±15.1 h (Farshad *et al.* 2008). Contrarily, Angora, the wool breed, had relatively shorter estrous duration of 12 to 24 h (Hafez 1980). Another study also suggested a much shorter estrous duration of 16.3±1.7 h (range: 10 to 22 h) in goats (Llewelyn *et al.* 1993). Short estrous duration in the later study could be attributed to consideration of estrous onset from the trait of peak frequency of tail flagging, restlessness and bleating, which, however begins much earlier around estrus (Rahman *et al.* 2008).

Estrous duration has been longer in induced than spontaneous estrus cycles in goats. Two different studies recorded estrous duration of 30.8±12.1 *versus* 21.4±9.5 h (Akusu and

Egbunike 1984) and 24.4 *versus* 18.8 h (Mori and Kano 1984) in prostaglandin induced versus spontaneous estrus. Difference of season has been another variable affecting estrous duration in goats throughout the year. Estrus lasted relatively longer for 44.0±1.9 h during winters, compared to shorter estrous duration of 36.0±0.7 h and 35.5±0.8 h during summer and rainy season, respectively. Moreover, higher percentage of normal estrous cycles of 17 to 25 days were observed in 79.3 % of goats during rainy season, following by 72.7 % and much less 53.3 % of goats during winters and summers, respectively (Fakruzzaman *et al.* 2012).

2.5 OVULATION

Ovulation is the key process of an estrous cycle, which regulates the time of breeding and conception in goats (Loetz 2013). The onset of breeding season initiates estrous cycle and therefore ovulation. However, during silent estrus, the ovulation occurs in goats not displaying normal estrous behaviour (Etzenhouser *et al.* 1998).

The advent of ultrasonography and its use thorough serial examination has made it possible to closely follow ovulation in goats (Gonzalez-Bulnes *et al.* 2004), although laproscopy (Chakraborty *et al.* 1993) and culdoscopy (Mori and Kano 1984) have also been used. The latter two techniques, however, do not find place in recent studies due to their invasive nature. The ultrasonographic image of pre-ovulatory follicle is characterized by anechoic circumscribed area surrounded by heterogeneous and hyper echoic margins. The ovulation on serial examination is characterized by the disappearance of follicular antrum (Gonzalez-Bulnes *et al.* 2004).

The estrus to ovulation interval in goats ranges from 12 to 36 h (Noakes *et al.* 2009). Slightly longer estrus to ovulation interval of 39.9±12.3 h in different breeds of dairy goats (Esteves *et al.* 2013) and 30 to 48 h in local goats of Assam have been reported (Chakraborty *et al.* 1993).

The PG has been commonly used as a single or double injection 10 to 11 days apart to induce (Mori and Kano 1984) or synchronize (Perera *et al.* 1978, Akusu and Egbunike 1984 and Vazquez *et al.* 2010) estrus in goats. Interestingly, several studies have reported a shorter estrus to ovulation interval in PG induced compared to spontaneous estrus. Administration of two PG injections 10 days apart revealed estrus to ovulation interval to be 28.0±2.5 h in induced estrus compared to 34.7±1.9 h in spontaneous estrous does (Vazquez *et al.* 2010). In another study the interval was found to be affected by type of estrus (induced or spontaneous) and parity of goats. Estrus to ovulation interval in general was relatively shorter in induced compared to spontaneous estrus and in the nulliparous compared to multiparous goats. The estrus to ovulation interval in induced *versus* spontaneous estrus was 30.1±1.1 *versus* 33.4±1.5 h in nulliparous compared to 32.5±1.0 and 36.5±1.1 h in multiparous does (Simoes *et al*. 2008).

In contrast to the findings cited *vide supra,* the ovulations were reported to occur after end of estrus, with a further delay in ovulations in spontaneous cycles (5.6±1.0 h, range of 2 to 8 h in induced cycle; 8.4±0.7 h, range of 6 to 12 h in spontaneous cycle). This variation could be attributed to the culdoscopy, that was preceeded by ketamine and xylazine administration, both of which delay ovulation (Robertson and Rakha 1965, Peet and Lincoln 1977).

Evidence of literature reveals goat to ovulate more than one follicle in an estrous cycle. The earliest study in different Indian breed of goats revealed an ovulation rate of 1.4 for Barbari (Prasad 1971), 1.2 in Kutchi (Shukla *et al.* 1971) and much higher ovulation rate of 4.0 in Black Bengal does (Rao and Bhattacharyya 1980). Studies thereafter also reveal ovulation rate of greater than one in local Assam goats; the ovulation rate was 2.2±0.4 (Rajkhowa *et al.* 1993). Another study using laparotomy on the Assam goats, the ovulation rate recorded at 30, 36 and 40 h from onset of estrus was 0.2, 0.7 and 2.2, respectively. The distribution of ovulation during estrus was speculated to be a result of varying level of LH required for ovulation (Chakraborty *et al.* 1993). Some another studies on goats raised abroad have cited an ovulation rate ranging from 1.3±0.1 to 2.3±0.9 (Eiamvitayakorn and Rigor 1988, Li *et al*. 2001 and De Santiago-Miramontes *et al.* 2008).

Provision of diet with high metabolisable energy to varying extent during the experimental period has indicated an increase in ovulation rate in goats. An ovulation rate of 2.1 *versus* 2.4 (Chemineau 1986) and 1.0±0.2 *versus* 1.6±0.2 (De Santiago-Miramontes *et al.* 2008) have been reported in control *versus* supplemented groups. The latter study confirmed a positive link between nutritional supplementation and ovulation rates in goats; the ovulation rate in the subsequent estrus post dietary supplementation became similar to the control group (1.3±0.1 *versus* 1.3±0.2, respectively).

Another factor influencing the ovulation rate is induced *versus* spontaneous estrus. In two studies, the ovulation rate was higher in induced than spontaneous estrus; the respective values being 2.0±0.2 *versus* 1.5±0.2 (Miller and Martin 1993) and 2.0±0.1 *versus* 1.7±0.2

(Vazquez *et al.* 2010). Primarily, a difference in the FSH concentration might be the reason for variation in the ovulation rate (Miller and Martin 1993). However, another study found that varying FSH concentration is not the absolute governing force for ovulation; instead it was the sensitivity of the hypothalamo-pituitary axis to the steroidal feedback, which, however, varies widely between animals and stage of estrous cycle (Wallace *et al.* 1988).
The size of pre-ovulatory follicle may range from 6 to 7 mm (Noakes *et al.* 2009). However, several other studies have reported larger pre-ovulatory follicles with average size ranging from 6.1±1.1 mm to 8.3±0.4 mm. A variation in progesterone secretion from corpus luteum spurium and therefore the LH secretion from anterior pituitary could influence the development and size of dominant follicle culminating into ovulation (Li *et al.* 2001, Medan *et al.* 2003 and Vazquez *et al.* 2010).

2.6 ENDOCRINOLOGICAL PROFILE

2.6.1 ESTRADIOL- 17B

In spontaneous estrous cycle, the mean basal estradiol- 17β concentration ranged from 10 to 19 pg/ml in the blood samples collected every 8 h during the entire estrous cycle. The estradiol- 17β concentration gradually increased from 60 h prior to LH peak and attained maximum concentration of 26.9±3.18 pg/ml coinciding with the LH peak on the day of estrus in Virgin Alpine goats (Bono *et al.* 1983). Almost similar estradiol- 17β peak concentration of 28.0±6.0 pg/ml was observed on the day of estrus when blood samples were collected once daily in goats having inter-estrus interval of 20 days (Leyva-Ocariz *et al.* 1995). Comparatively, lower estradiol- 17β peak concentration varying from 9 to 16 pg/ml (average of 12 pg/ml) at estrus was observed in another study on dwarf goats (Kausar *et al.* 2005). In contrast, the highest estradiol- 17β concentration of 14.0±1.2 pg/ml was recorded 24 h prior to estrus which decreased to 7.7±1.7 pg/ml at estrus in Dwarf goats with estrous cycle length of 18.2±2.1 days (Khanum *et al.* 2008). In PG induced estrus, peak estradiol- 17β concentration of 30.9±1.0 pg/ml was recorded 2 days before ovulation which decreased to 13.5 pg/ml by day 4 after ovulation in Shiba goats (Medan *et al.* 2003). In spontaneous *versus* induced cycle, the estradiol- 17β concentration were 15 *versus* 11 pg/ml at estrous onset, which increased to 21 and 25 pg/ml after 5 h of spontaneous estrous onset compared to a longer interval of 14.5 h of induced estrous onset, respectively (Mori and Kano 1984). Similarly, in another study, the estradiol- 17β peak in spontaneous *versus* induced estrus was 18 and 24 pg/ml attained at 11.5 and 17.5 h of spontaneous and induced estrus, respectively. A rise in estradiol- 17β coincides with a drop in progesterone confirming the hypothesis of negative feedback (Karsch *et al.* 1979).

2.6.2 PROGESTERONE

Irrespective of spontaneous or induced estrus, majorities of studies have recorded progesterone concentration of less than 1 ng/ml at estrus in different breeds of goat (Bono *et al.* 1983, Kausar *et al.* 2005 and Vazquez *et al*. 2010). However, in one of the study comparing progesterone concentration at spontaneous *versus* induced estrus, the values were 0.2 and 1.8 ng/ml, respectively (Mori and Kano 1984). The progesterone concentration increased gradually and ranged from 3.5 to 10.5 ng/ml on day 7 of the estrous cycle (Vazquez *et al*. 2010 and Leyva-Ocariz *et al.* 1995).

In spontaneous cycles, a peak progesterone concentration of 12 ng/ml was observed at day 15 (Leyva-Ocariz *et al.* 1995), whereas, in another study, it was10.95 ng/ml at day 14 of spontaneous estrous cycle. A comparison between spontaneous and PG induced estrous cycle found a peak progesterone concentration of 17.5 ng/ml at day 12 of spontaneous cycle which was 10 ng/ml at day 14 of induced cycle; the estrus to ovulation interval, respectively, being 34.7±1.9 h *versus* 28.0±2.5 h (Vazquez *et al.* 2010).

2.6.3 LH

Just after rapid progesterone decrease and estradiol- 17β increase, there is a rapid increase in LH. In one of the earlier study, a peak LH concentration of 40.7±10.1 ng/ml was recorded 8 to 24 h after estrus onset. The concentration reached to a basal level of 0.5 to 3.0 ng/ml 16 h later (Bono *et al.* 1983).

In a subsequent study, a breed variation in estradiol- 17β *vis-à-vis* LH concentration was observed. In crossbred goats, LH concentration on day 18 was 10.0±4.9 ng/ml, which decreased to 1.5 ng/ml on day 19 followed by a sharp increase to attain LH peak concentration of 23±6.9 ng/ml a day later (day of estrus). Interestingly, the estradiol- 17β concentration from day 18 to day 20 exhibited a constant increase to a peak of 28.0±6.0 pg/ml. Such a relationship between estradiol- 17β and LH did not exist in Venezuela native goats in which a single LH peak of 30±8.0 and 35±10 ng/ml were observed at first and second spontaneous estrus (Leyva- Ocariz *et al.* 1995).

In contrast, an intimate temporal relationship was observed between LH and estradiol- 17β; with both of these increasing at the same time at estrus, but LH fall occurring earlier than estradiol- 17β fall. After PG induced estrus in Dwarf does, the peak LH concentration ranged from 7.5 to 30 ng/ml (average of 19 ng/ml). The PG to LH peak interval was 70 hours (Kausar *et al.* 2005). A much higher peak LH concentration of 60.1±3.5 ng/ml (range of 18.6 to 151.3 ng/ml) was reported at 13.9±1.0 hours after estrus onset. The LH values 8 h before and after peak were 1.4±0.2 ng / ml and 1.5±0.2 ng/ml, respectively (Simoes *et al.* 2008). Still higher peak LH concentration (102.1±7.8 ng/ml) at 12.0±1.5 h from spontaneous estrus onset (estrous duration of 16.3±1.7 h) is also in record (Llewelyn *et al.* 1993).

A conspicuous difference in LH concentration was recorded in spontaneous *versus* PG induced estrus. LH peak values during spontaneous and induced estrus was 25 ng/ml and 4.5 ng/ml, 6±2.8 hours and 10±1.1 h from estrous onset. The interval between LH peak to end of estrus was 12.8±1.0 and 14.4±1.2 h during spontaneous and induced estrus, respectively. Interval between LH peak to ovulation was 21.2±0.5 and 20±0.9 h for spontaneous and induced estrus, respectively. The interval between LH peak and estradiol-17β peak was 5.5 h and 7.5 h for spontaneous and induced estrus, respectively. Also, the interval between estradiol- 17β peak to ovulation was 15.7 h and 12.5 h during spontaneous and induced estrus, respectively (Mori and Kano 1984).

2.7 INTERESTROUS INTERVAL

Number of studies report interestrus interval in goats to range from 17 to 22 days (Hafez 1980, Rahman *et al.* 2008, Li *et al.* 2001 and Noakes *et al.* 2009). The length of estrous cycle succeeding induced or spontaneous estrus has been slightly different as quoted in different studies. For instance, average estrous cycle length of 21.6±0.4 days following induced estrus (Medan *et al.* 2003) was longer than the range of 18.2±2.1 to 20.9±1.5 days recorded following spontaneous estrus (Farshad *et al.* 2008, Khanum *et al.* 2008 and Li *et al.* 2001). This was reaffirmed, when in the same study, the average estrous cycle length of 19.8±0.5 days after induced estrus was numerically longer than 19.0±0.3 days after spontaneous estrus (Vazquez *et al.* 2010). Season did not have much effect on the length of spontaneous estrous cycle. The average was 23.5±1.5 days in summer followed by

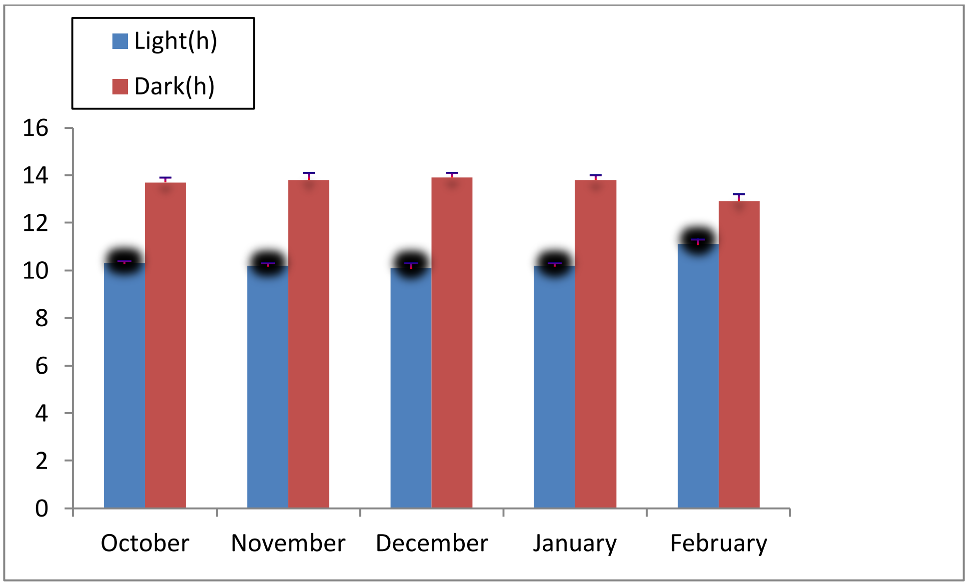
21.3±1.0 days in winters and 20.8±0.6 days in rainy season has been recorded (Fakruzzaman *et al.* 2012).

CHAPTER 3

MATERIALS AND METHODS

The present study was aimed to investigate estrous behaviour, ovulation and endocrine changes during natural photoperiod in Gaddi goats during October, 2014 to February, 2015. The month wise average light: dark hours (h) during the study is presented in Figure 1 (Department of Geo Informatics Research and Training, College of Basic Science, CSKHPKV, Palampur).

Figure 1: Representation of month wise average light: dark h period (October, 2014 to February, 2015) during the study period



The average of light: dark h during the month of October, November- December, 2014 and January and February, 2015 were 10.3±0.1: 13.7±0.2, 10.2±0.1: 13.8±0.3, 10.1±0.2: 13.9±0.2, 10.2±0.1: 13.8±0.2 and 11.1±0.2: 12.9±0.3, respectively.

3.1 ANIMALS AND LOCATION

The adult Gaddi does of 22 to 42 kg body weight and 2 to 3 years age (Prasad 2010) were included in the study. The goats were dry, non-pregnant and belonged to the University dairy farm of Chaudhary Sarwan Kumar Himachal Pradesh Agriculture University, Palampur (latitude30°6 N, longitude 76°3 E).

3.2 HOUSING AND MANAGEMENT

The Gaddi goats were subjected to grazing (G) condition for five hours in a day (9:00 to 12:00 h and from 14:00 to 16:00 h). During the remaining period, the goats were housed under confined (C) conditions in a shed. The shed was half open (9.2×6.4 feet) and half closed (8.5×6.4 feet). The flooring was wooden in the closed area and brick-on-edge in the open area. The shed housed 11 to 13 does. Two bucks were housed in an adjacent shed, except for during the observation periods in C conditions when they were mixed with the does. However, under the G conditions the bucks always accompanied the does. The Gaddi goats were fed as per the standards of National Research Council (NRC, 2001). All goats had round the clock access to the clean drinking water under C condition.

3.3 EXPERIMENTAL DESIGN AND GROUPS

One week prior to the initiation of experiment, all the goats were orally administered a broad spectrum anthelmintic Albendazole (Albomar; 150 mg / tablet; Virbac India Private Limited, Mumbai) at a dose rate of 1 tablet per 25 kg body weight. The investigation began with an intramuscular injection of 187.5 µg of PG (Pragma; Cloprostenol 250mcg/ml; Intas Pharmaceuticals Ltd.) in the selected does. Two does not responding to first PG injection were injected another PG 11 days later. After 36 h of the PG injection, the two aproned bucks were used to tease the does every 3 h for 30 min each under C conditions for detection of estrous onset and recording of different estrous behaviour characteristics. The teasing continued 15 to 18 h after termination of estrous. As mentioned previously, the same bucks were continuously with the does during G conditions.

Induction of estrus was carried out in three different batches. There were three to four does in each batch. The time from PG injection to onset of estrus was recorded. During majority of induced estrus studies two goats were simultaneously in estrus, though at a varying time interval. Four (hereafter designated as I) of the 10 induced (hereafter designated as I_{all}), goats were also investigated during the immediately succeeding spontaneous estrous cycle (hereafter designated as S). There was only one goat in estrus at a time during the S cycle.

3.4 ESTROUS BEHAVIOUR CHARACTERISTICS

Nine different estrous behaviour signs were considered out of which, biting of estrous female, flehmen and sniffing of vulva were categorized under male behavior characteristics, whereas bleating, restlessness, interest in male, tail flagging, standing to be mounted and lordosis were categorized under female behavioral characteristics

The characteristics of different estrous behaviors for consideration in present study were as follows:

Biting of estrous female: The buck chases the doe making “gobbling” sounds with his mouth and flicking his tongue, attempts to bite in neck area (Dalton 2008).

Flehmen: When male smells the peri-anal area or urine (Fonseca *et al.* 2013) or after licking the external genitalia of a doe (Obrien 1982).

Sniffing of vulva: Male sniffs urine and peri-anal area of doe (Dos Santos *et al.* 2015).

Bleating: Increased vocalization (Ola and Egbunike 2004).

Restlessness: Increased movement from one place to another (Fakruzzaman *et al.* 2012).

Interest in male: Does in estrus show great interest and try to come near the buck (Eiamvitayakorn and Rigor 1988).

Tail flagging: Vigorous outward and sideward tail-swishing (Widayati *et al.* 2010).

Standing to be mounted: Immobilization of doe in estrus when mounted by buck (Delgadillo *et al.* 2002).

Lordosis: Arching of back when buck sniffs vulva (Ola and Egbunike 2004).

Each time an estrous behaviour was displayed it was recorded. The frequency of all estrous behaviour characteristics for all the observation periods was recorded to obtain the total and average frequency for each characteristic to be later used in knowing the intensity of estrus. In addition, the area under curve (AUC) was also estimated for each trait using trapezoid rule (Lee and Rosner 2001). The onset of estrus was determined from midway of the observation period immediately preceeding the one in which any estrous sign was detected for the first time. Similarly, the end of estrous was determined from midway of the observation period immediately succeeding the one in which an estrous sign was detected for the last time. The interval between estrous onset and termination was considered as estrous duration. A comparison of estrous behaviour characteristics was made between the S *versus* I groups as well as under C *versus* G conditions.

3.5 GENITAL DISCHARGE

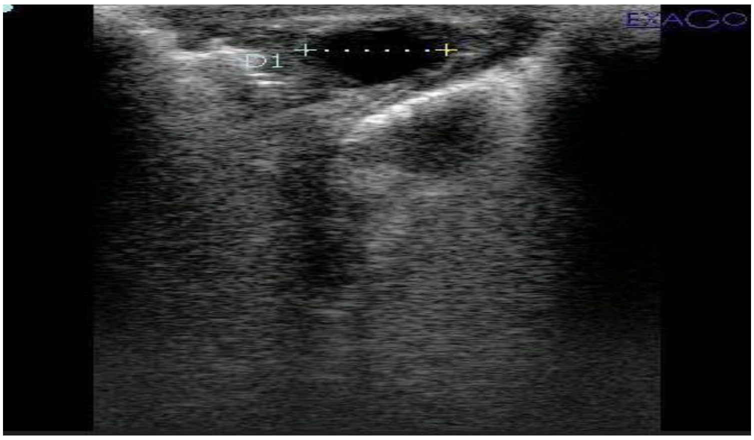
The genital discharge from all the does in estrus was aspirated by a half-cut sterile AI sheath used for cattle AI. A 10 ml syringe was attached to the other end of the sheath for aspiration. Thereafter, the discharge was spread on a clean and grease free glass slide. A smear of the discharge was prepared. After air drying of the slide, it was examined under 40 X magnification of light microscope with photography assembly (BX 40, Olympus; Japan). The fern pattern was observed at the estrus onset as well as 12 h and 24 h later.

3.6 BLEEDING SCHEDULE AND ESTIMATION OF HORMONES

The initiation of blood collection (5 – 7 ml) began from onset of estrus. Thereafter, blood was collected at every 3 hours interval till the end of estrus. In addition, two blood samples at day 7 and day 14 of estrous cycle were also collected in all the does. The blood was collected in a sterile heparinized vacutainer (Peerless Biotech Pvt. Ltd.) and centrifuged at 3000 rpm for 10 minutes. The blood plasma was used to analyze estradiol- 17β (E_2), progesterone (P_4) and leutinizing hormone (LH) in the samples collected during estrus. The P_4 was also estimated in the samples collected at day 7 and day 14. The E_2 and P_4 were estimated using standard ELISA kits (Calbiotech - a life science company, California, US), whereas LH was estimated using EIA (Prakash *et al.* 2002). The analytical sensitivity of E_2 was 8.512 pg/ml and the inter and intra assay coefficient of variation was 6.23 and 3.39 %, respectively. The corresponding values for P_4 were 0.009 ng/ml, 5.63 % and 4.91 %, respectively. The endocrinology determinants were considered as per a previous study (Sood *et al.* 2015).

3.7 OVULATION

Figure 2: Ulrasonographic representation of pre-ovulatory follicle



The ovulation was detected using serial observation at 6 hours interval beginning from 12 h after estrus onset till detection of ovulation. The procedure adopted was similar to De Bulnes *et al.* (1999). Ultrasonography was performed using a Sonosite Vet M Turbo machine having L52×10-5 MHz transducer. The ovulation was said to have occurred after the disappearance of the ovarian follicle which appeared as dark circumscribed anechoic area (Figure 2). All the Gaddi goats were acclimatized to the ultrasonography procedure by subjecting them thrice a day to ultrasonography which began two weeks prior to the induction of estrus. The ovulation was considered to have occurred midway between two consecutive observations, the last being disappearance of follicle from the ovary.

3.8 STATISTICAL ANALYSIS

The average (Mean ± S.E.M) between different values of interest was done using student's t test using SPSS statistical analysis software for Microsoft windows. A statistical significance of $P < 0.05$ (atleast) was considered as significant, whereas $P = 0.10$ was considered as a tendency to differ.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 INDUCTION OF ESTROUS

PG was used to induce estrus in Gaddi does. Out of the 10 animals, 8 responded to first PG injection, whereas the remaining 2 were injected and responded to a similar second dose injected 11 days later. Induction of estrus, coupled with low progesterone at estrus (Section 4.5.2) in present study, confirms 80 % (n= 8) response to first PG followed by remaining 20 % (n= 2) to second PG in the 10 does utilized in the present study. Earlier, the PG induction response has varied from 87.5 % to 100 % (Greyling and Niekerk 1986). The invariable response of PG may be related to the presence of a functional or regressive corpus luteum at the time of PG injection, as the corpus luteum for first 7 days post ovulation does not respond to PG (Esteves *et al.* 2013). The average interval from PG administration to estrous onset was 76.1±3.8 h (range: 57 to 96 h). The available reports recorded the estrous induction interval to range from 43 to 65 h in goats (Bretzlaff *et al.* 1981, Akusu and Egbunike 1984, Mori and Kano 1984, Medan *et al.* 2003, Simoes *et al.* 2008, Vazquez *et al.* 2010, and Esteves *et al.* 2013).

4.2 ESTROUS BEHAVIOUR IN GOATS

Estrous detection is important for successful AI and controlled breeding programs. Even though estrous exhibition is under the direction of estrogen hormone, methods of estrous synchronization (Selvaraju *et al.* 1997) have been reported to influence its exhibition. For instance, induction with PG reduced the time from injection to onset of estrus and resulted in a much better intensity of signs compared to progesterone treatment as also higher fertility with PG (Whitley and Jackson 2004).

4.2.1 ESTROUS BEHAVIOUR UNDER SPONTANEOUS (S) *VERSUS* INDUCED (I) *VERSUS* OVERALL INDUCED (I_{all}) GROUPS

Except for lordosis, all the estrous behaviour signs considered in the present study were displayed at estrous onset in different groups (Table 4.1, Figure 3).

Standing to be mounted followed by biting of estrous female, sniffing of vulva and tail flagging were the most frequent signs characterizing the onset of estrus (Table 4.1).

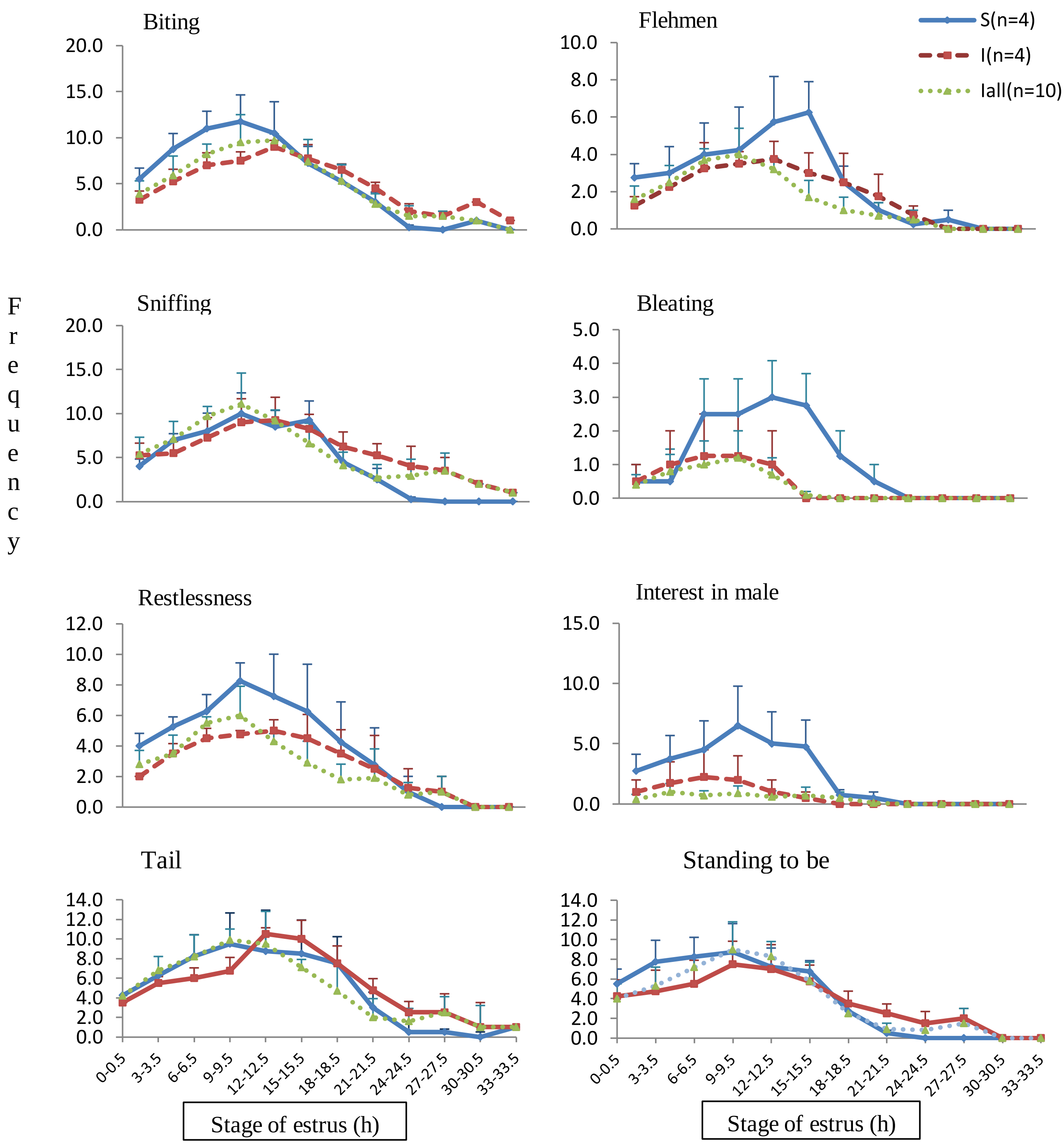
Table 4.1: Average (Mean ± S.E.M) frequency of different estrous behaviour characteristics in Gaddi goats: A comparison between spontaneous (S), induced (I) and overall induced (I_{all}) estrus (combined for confined and grazing conditions)

Time after estrous onset (*)	Male behavioural characteristics									Female behavioural characteristics																	
	Biting of estrous female			Flehmen			Sniffing of vulva			Bleating			Restlessness			Interest in male			Tail flagging			Standing to be mounted			Lordosis		
	(S) n=4	(I) n=4	(I_{all}) n=10	(S) n=4	(I) n=4	(I_{all}) n=10	(S) n=4	(I) n=4	(I_{all}) n=10	(S) n=4	(I) n=4	(I_{all}) n=10	(S) n=4	(I) n=4	(I_{all}) n=10	(S) n=4	(I) n=4	(I_{all}) n=10	(S) n=4	(I) n=4	(I_{all}) n=10	(S) n=4	(I) n=4	(I_{all}) n=10	(S) n=4	(I) n=4	(I_{all}) n=10
0-0.5 (4,4,10)	5.5 ± 1.2	3.3 ± 0.9	3.9 ± 1.4	2.8 ± 0.8	1.3 ± 0.5	1.6 ± 0.7	4.0 ± 0.8	5.3 ± 1.4	5.4 ± 1.4	0.5 ± 0.5	0.5 ± 0.5	0.4 ± 0.3	4.0 ± 0.8^{x}	2.0 ± 0^{y}	2.8 ± 0.9^{zxy}	2.8 ± 1.4	1.0 ± 1.0	0.4 ± 0.4	4.3 ± 0.0	3.5 ± 0.6	4.2 ± 1.4	5.5 ± 1.5	4.3 ± 1.7	4.0 ± 1.5	0	0	0
3-3.5 (4,4,10)	8.8 ± 1.7	5.3 ± 1.3	5.9 ± 2.1	3.0 ± 1.4	2.3 ± 0.8	2.5 ± 0.9	7.0 ± 0.7	5.5 ± 1.3	7.1 ± 2.0	0.5 ± 1.0	1.0 ± 1.0	0.8 ± 0.5	5.3 ± 0.6	3.5 ± 0.6	3.5 ± 1.9	3.8 ± 1.9	1.8 ± 1.8	1.0 ± 0.7	6.3 ± 2.2	5.5 ± 1.0	6.8 ± 2.2	7.8 ± 2.2	4.8 ± 2.1	5.3 ± 1.9	0	0	0
6-6.5 (4,4,10)	11.0 ± 1.9	7.0 ± 1.4	8.2 ± 1.1	4.0 ± 1.7	3.3 ± 1.4	3.7 ± 0.6	8.0 ± 2.0	7.3 ± 2.3	9.7 ± 1.1	2.5 ± 1.0	1.3 ± 1.3	1.0 ± 0.7	6.3 ± 1.1	4.5 ± 0.6	5.5 ± 0.4	4.5 ± 2.4	2.3 ± 2.3	0.7 ± 0.4	8.3 ± 3.1	6.0 ± 1.4	8.2 ± 1.1	8.3 ± 2.0	5.5 ± 2.4	7.2 ± 1.1	0	0	0
9-9.5 (4,4,10)	11.8 ± 2.9	7.5 ± 1.0	9.5 ± 3.0	4.3 ± 2.3	3.5 ± 0.6	4.0 ± 1.4	10.0 ± 2.3	9.0 ± 2.7	11.1 ± 3.5	2.5 ± 1.0	1.3 ± 1.3	1.2 ± 0.8	8.3 ± 1.2^{x}	4.8 ± 0.3^{y}	6.0 ± 1.9^{zxy}	6.5 ± 3.3	2.0 ± 2.0	0.9 ± 0.6	9.5 ± 4.2	6.8 ± 0.6	9.9 ± 3.3	8.8 ± 2.9	7.5 ± 2.3	9.0 ± 2.8	0	0	0
12-12.5 (4,4,10)	10.5 ± 3.4	9.0 ± 0.7	9.7 ± 0.7	5.8 ± 2.4	3.8 ± 0.9	3.2 ± 0.6	8.5 ± 1.8	9.3 ± 2.6	9.2 ± 1.2	3.0 ± 1.1	1.0 ± 1.0	0.7 ± 0.5	7.3 ± 2.8	5.0 ± 0.7	4.3 ± 0.5	5.0 ± 2.6	1.0 ± 1.0	0.6 ± 0.5	8.8 ± 3.4	10.5 ± 1.9	9.5 ± 0.8	7.3 ± 1.9	7.0 ± 2.5	8.3 ± 1.5	0.5 ± 0.5	0	0
15-15.5 (4,4,10)	7.3 ± 1.8	7.8 ± 1.5	7.4 ± 2.4	6.3 ± 1.7	3.0 ± 1.1	1.7 ± 0.9	9.3 ± 2.2	8.3 ± 1.7	6.6 ± 2.3	2.8 ± 0.9^{a}	0^{b}	0.1 ± 0.1^{cb}	6.3 ± 3.1	4.5 ± 1.6	2.9 ± 1.4	4.8 ± 2.2	0.5 ± 0.5	0.7 ± 0.7	8.5 ± 2.7	10.0 ± 1.8	7.1 ± 2.7	6.8 ± 1.1	5.8 ± 1.7	5.8 ± 1.9	1.0 ± 1.0	0	0
18-18.5 (4,4,10)	5.3 ± 1.8	6.5 ± 0.6	5.3 ± 1.8	2.5 ± 0.9	2.5 ± 1.6	1.0 ± 0.7	4.5 ± 1.4	6.3 ± 1.7	4.1 ± 1.5	1.3 ± 0.8	0	0	4.3 ± 2.6	3.5 ± 1.6	1.8 ± 1.0	0.8 ± 0.4^{x}	0^{y}	0.5 ± 0.5^{zxy}	7.5 ± 1.6	7.5 ± 1.2	4.7 ± 1.9	2.8 ± 0.8	3.5 ± 1.3	2.5 ± 1.0	0.3 ± 0.3	0	0
21-21.5 (4,4,10)	3.0 ± 1.1	4.5 ± 0.6	2.8 ± 1.1	1.0 ± 0.4	1.8 ± 1.2	0.7 ± 0.7	2.5 ± 1.3	5.3 ± 1.3	2.7 ± 1.5	0.5 ± 0.5	0	0	2.8 ± 2.4	2.5 ± 2.2	1.9 ± 1.9	0.5 ± 0.5	0	0.1 ± 0.1	3.0 ± 1.1	4.8 ± 1.1	2.0 ± 1.3	0.5 ± 0.3	2.5 ± 1.0	0.9 ± 0.6	0	0	0
24-24.5 (4,4,6)	0.3 ± 0.3	2.0 ± 0.8	1.5 ± 1.1	0.3 ± 0.3	0.8 ± 0.5	0.5 ± 0.5	0.3 ± 0.3	4.0 ± 2.3	2.9 ± 1.9	0	0	0	1.0 ± 1.0	1.3 ± 1.3	0.8 ± 0.8	0	0	0	0.5 ± 0.3	2.5 ± 1.9	1.6 ± 1.6	0	1.5 ± 1.2	0.8 ± 0.8	0	0	0
27-27.5 (2,2,2)	0^{a}	1.5 ± 0.5^{b}	1.5 ± 0.5^{cb}	0.5 ± 0.5	0	0	0^{x}	3.5 ± 1.5^{y}	3.5 ± 2.0^{zy}	0	0	0	0	1.0 ± 1.0	1.0 ± 1.0	0	0	0	0.5 ± 0.5	2.5 ± 2.5	2.5 ± 2.2	0^{x}	2.0 ± 1.0^{y}	1.5 ± 1.5^{zxy}	0	0	0
30-30.5 (1,1,1)	0^{a}	3.0 ± 0.0^{b}	1.0 ± 0.0^{c}	0	0	0	0^{a}	2.0 ± 0.0^{b}	2.0 ± 0.0^{cb}	0	0	0	0	0	0	0	0	0	0^{a}	1.0 ± 0.0^{b}	1.0 ± 0.0^{cb}	0	0	0	0	0	0
33-33.5 (1,1,1)	0^{a}	1.0 ± 0.0^{b}	0^{ca}	0	0	0	0^{a}	1.0 ± 0.0^{b}	1.0 ± 0.0^{cb}	0	0	0	0	0	0	0	0	0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	0	0	0	0	0	0
Total frequency	64.3 ± 5.2	58.3 ± 3.1	56.7 ± 4.0	30.3 ± 2.6	22.0 ± 1.6	18.9 ± 1.7	54.0 ± 4.5	66.5 ± 3.1	65.3 ± 3.9	13.5 ± 1.7	5.0 ± 0.8	4.2 ± 0.5	45.3 ± 3.5	32.5 ± 1.9	30.5 ± 2.5	28.5 ± 2.8	8.5 ± 1.1	4.9 ± 0.5	58.0 ± 4.3	61.5 ± 3.7	58.5 ± 3.9	47.5 ± 4.5	44.3 ± 2.8	45.3 ± 3.5	1.8 ± 0.3	0	0
Average per observation	5.4 ± 1.3	4.9 ± 0.8	4.7 ± 1.0	2.5 ± 0.6	1.8 ± 0.4	1.6 ± 0.4	4.5 ± 1.1	5.5 ± 0.8	5.4 ± 1.0	1.1 ± 0.4	0.4 ± 0.2	0.4 ± 0.1	3.8 ± 0.9	2.7 ± 0.5	2.5 ± 0.6	2.4 ± 0.7	0.7 ± 0.3	0.4 ± 0.1	4.8 ± 1.1	5.1 ± 0.9	4.9 ± 1.0	4.0 ± 1.1	3.7 ± 0.7	3.8 ± 0.9	0.1 ± 0.1	0	0

[a,b,c; x,y,z] adjacent values with different superscripts between S *versus* I *versus* I_{all} group differ at ($p<0.01$) and ($p<0.05$), respectively.

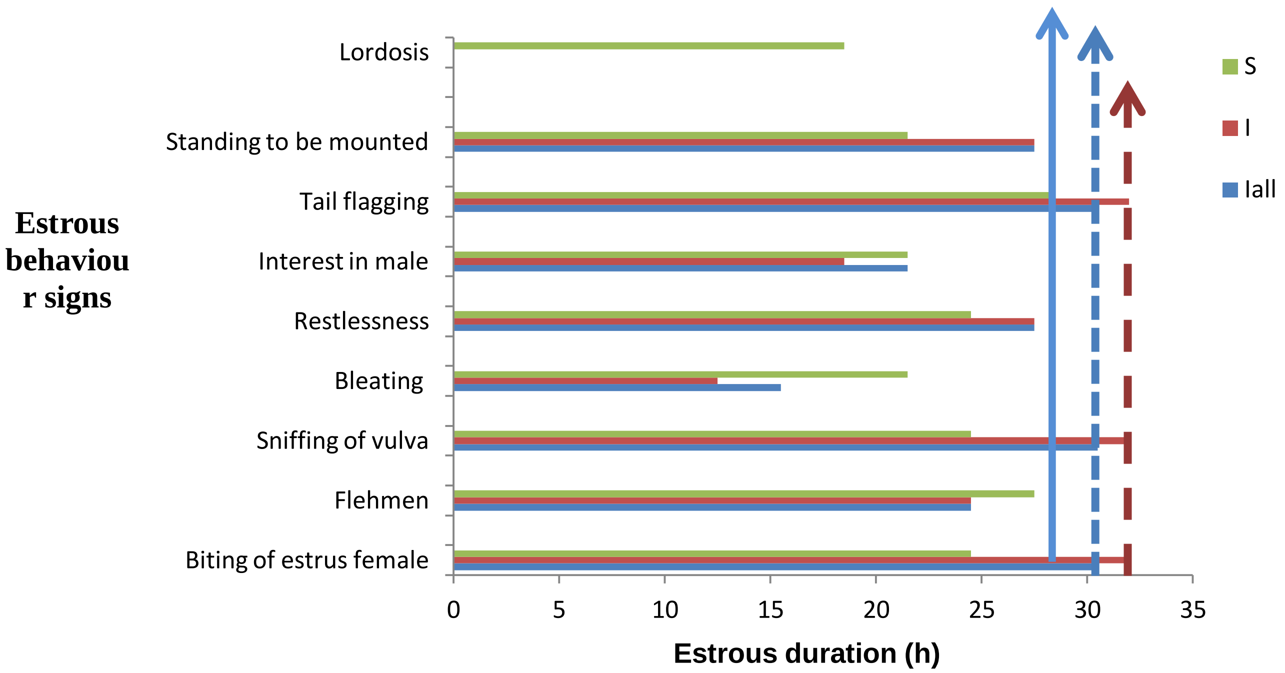
* Number of goats in estrus in S, I and I_{all} groups, respectively.

Figure 3: Estrous behaviour characteristics: A comparison between spontaneous (S), induced (I) and overall induced (I_{all}) estrus (combined for confined (C) and grazing (G) conditions) in Gaddi does



Amongst tail flagging, restlessness and bleating, the formermost has been recorded to be the most useful trait for detecting onset of estrus (Llewelyn *et al.* 1993). In another study that considered bleating, interest in male, tail flagging, standing heat, lordosis and homosexuality, it was either tail flagging or standing to be mounted that were displayed at onset of estrus with tail flagging frequency exceeding the expression of standing events (Ola and Egbunike 2004). It is worth mentioning that the latter and present study observed "standing to be mounted", the golden sign of estrus at its onset unlike the sign present and defining 'mid-estrus' in cattle (Diskin and Sreenan 2000). In an earlier study, much higher average frequency of tail flagging (23.7), restlessness (14.0) and bleating (13.5) were recorded at onset of estrus during a 30 min observation period in British White goats. Hence, a breed variation could be the reason for varying intensity of signs (Llewelyn *et al.* 1993). As evident from Figure 3, bleating, restlessness and interest in male were the prominent estrous behaviour characteristics exhibited at a much higher frequency at different stages of estrus during the S than I cycle (same does). In contrast, sniffing and tail flagging were the two characteristics more frequently expressed in the second half of the estrous period in the I than S group (Figure 4). Further, compared to other signs bleating (24 to 24.5 h in S, 18 to 18.5 h in I and I_{all} groups) and interest in male (after 21.5 h in all groups) terminated earlier. Moreover, bleating was absent in one of the goat during S and I cycle. Interest in male was absent in two of the goats in I group, whereas it was displayed by these goats during S cycles. Accordingly, the total frequency of bleating and interest in male were less than the other signs (Table 4.1). On the basis of total / average frequency during estrus, biting of estrous female, tail flagging and sniffing followed by standing to be mounted and restlessness were the most frequent estrous behaviour signs during S or I estrus periods. During breeding season, much higher average frequency of sniffing (29.4 to 37.3 for a 30 min observation period) has been reported and may be attributed to exogenous treatment with estrogen and testosterone of overictomized anestrous does (Imwalle and Katz 2004). Similarly, other reports after giving different treatments to males (long day exposure, melatonin treatment and fixed *versus* rotated males) during nonbreeding and end of breeding season have also reported much higher average sniffing frequency 23.7 to 80 for a 30 min observation period in different breed of goats (Delgadillo *et al.* 2002 and Loya-Carrera *et al.* 2014).Comparatively lower frequency of sniffing (1.7 in 30 min) compared to present study (4.5±2.0) is also on record (Ungerfeld *et al.* 2014). Tail flagging was the only attribute that was manifested during the entire estrus, both S and I estrous (Table 4.1, Figure 4). This is in consonance to a

Figure 4: Persistence of different estrus signs in spontaneous (S), induced (I) and overall induced (I_{all}) estrous groups of Gaddi does



previous study (Ola and Egbunike 2004). Except for lordosis that was only displayed in S cycles and peaked at 15 to 15.6 h post estrus onset, all the other signs attained peak frequencies within a narrow range of fourth (9 to 9.5 h) to the fifth (12 to 12.5 h) observation after the estrus onset (Table 4.2). It is therefore inferred that 9 to 12.5 h after estrous onset may be considered as mid-estrus. Also, the time of peak frequency of standing to be mounted was similar (9 to 9.5 h) in all the 3 groups, and therefore reaffirms mid-estrus.

Overall, the estrous expression was better during S than I cycle. This was confirmed by significantly higher area under curve (AUC) for all, but sniffing of vulva, which was albeit significantly higher in the I than the S cycles (Table 4.3). A similar observation of a more intense estrus in the S than I cycle is on record (Ola and Egbunike 2004). There is only a single report that compared S *versus* I cycle using bleating, interest in male, tail flagging, standing to be mounted and lordosis as the estrous expression traits in Western African Does (Ola and Egbunike 2004). The latter study, in contrast to the present one, indicated a similar estrous intensity during S and I cycles (estrus behaviour score of 10.8±0.8 and 10.4±0.8, respectively), but with a much lower frequency of estrous expression traits compared to the present study. In S *versus* I cycles, respectively, the average frequency of standing to be mounted (2.6±0.1 *versus* 2.5±0.1) was highest followed by tail flagging (2.6±0.1 *versus* 2.4±0.1), interest in male (1.7±0.2 *versus* 1.5±0.2) and bleating (0.1±0.0 *versus* 0.1±0.0) (Ola and Egbunike 2004). In present study (Table 4.1), the average frequency of tail flagging (4.8±1.1 *versus* 5.1±0.9) was maximum followed by standing to be mounted (4.0±1.1 *versus* 3.7±0.7), interest in male (2.4±0.7 *versus* 0.7±0.3) and bleating (1.1±0.4 *versus* 0.4±0.2). The average frequency of flehmen reaction of 2.5±0.6 and 1.8±0.4 in S and I cycles, respectively, in males in contact with estrous does in present study was less than average frequency of 2.5 in bucks exposed to anestrous does (Luna-Orozco *et al.* 2012). However, another study indicated frequency of flehmen reaction to increase from 4 at estrus (day 0) to 5 (day 1), 6 (day 2) and 7.5 (day 8). Increased flehmen recorded after estrus was attributed to the reason of this being primed by estrus and increasing thereafter. The males were rotated daily between different subgroups of females (Loya-Carrera *et al.* 2014). Intermittent contact with novel or familiar females after a period of separation is known to increase the sexual activity in males (Pepelko and Clegg 1965 and Thiery and Signoret 1978). The peak frequency of bleating, 3.0±1.1 and 1.3±1.3 in S and I groups, respectively, (Table 4.1) in the present study was much lower than 13.5 during a 30 min observation period in mixed breed of goats in U.S. (Llewelyn *et al.* 1993).

Table 4.2: Peak time (from the estrous onset=0h) of different estrous behaviour characteristics under spontaneous (S), induced (I), overall induced (I_{all}), confined (C) and grazing (G) conditions of Gaddi does

Estrous behaviour characteristics	(S)	(I)	(I_{all})	(C)	(G)
Biting of estrous female	9-9.5	12-12.5	12-12.5	12-12.5	3-3.5
Flehmen	15-15.5	12-12.5	9-9.5	12-12.5	3-3.5
Sniffing of vulva	9-9.5	12-12.5	9-9.5	6-6.5	3-3.5
Bleating	12-12.5	6-6.5	9-9.5	9-9.5	3-3.5
Restlessness	9-9.5	12-12.5	9-9.5	6-6.5	3-3.5
Interest in male	9-9.5	6-6.5	3-3.5	9-9.5	3-3.5
Tail flagging	9-9.5	12-12.5	9-9.5	12-12.5	3-3.5
Standing to be mounted	9-9.5	9-9.5	9-9.5	12-12.5	3-3.5
Lordosis	15-15.5	-	-	-	-

Table 4.3: Area under curve (from the estrous onset=0h) of different estrous behaviour characteristics for type of estrus (S *versus* I *versus* I_{all}) and housing (C *versus* G) in Gaddi does

Estrous behaviour characteristics	Type of estrus			Housing	
	(S)	(I)	(I_{all})	(C)	(G)
Biting of estrous female	60.7±1.4^{x}	56.3±0.8^{y}	54.9±0.8^{zy}	42.2±1^{a}	13.7±0.3^{b}
Flehmen	29.1±0.6^{a}	21.7±0.4^{b}	18.1±0.3^{c}	15±0.4^{a}	5.5±0.1^{b}
Sniffing of vulva	48.8±1.2^{a}	63.7±0.7^{b}	62.4±0.7^{cb}	40.5±1^{a}	14.7±0.3^{b}
Bleating	13.4±0.4^{a}	4.9±0.2^{b}	4.8±0.2^{cb}	5.5±0.2^{a}	1.4±0.1^{b}
Restlessness	43.6±0.9^{a}	31.6±0.5^{b}	28.8±0.4^{c}	22.5±0.6^{a}	8.5±0.2^{b}
Interest in male	27.3±0.7^{a}	8.1±0.3^{b}	4.5±0.2^{c}	9.3±0.3^{a}	2.2±0.1^{b}
Tail flagging	55.6±1.1^{a}	42.3±0.7^{b}	56.2±0.8^{ca}	36.5±0.9^{a}	14.2±0.3^{b}
Standing to be mounted	45.1±1.1	42.3±0.7	42.9±0.7	32±0.9^{a}	11.3±0.3^{b}
Lordosis	1.8±0.1^{a}	0^{b}	0^{cb}	0	0

$^{a,b,c:\ x,y,z}$ adjacent values with different superscripts between S *versus* I *versus* I_{all} group and C *versus* G condition differ at ($p<0.01$) and ($p<0.05$) respectively.

S: Spontaneous, I: Induced, I_{all}: Overall induced, C: Confined, G: Grazing

Similarly, the latter investigation has also reported a much higher restlessness frequency of 14 compared to 8.3±1.2 in present study for a 30 min observation period of S estrus. Without quoting frequency in S cycles, biting of estrous female was seen in the form of tongue exposure (Fonesca *et al.* 2013 and Dos Santos *et al.* 2015).

Homosexual behaviour in the present study was unplanned for investigation and its frequency was not recorded. Homosexual behaviour was only observed in the absence of male under C condition during S estrus in all the four does. Tail flagging while mounting and mounting of other non-estrous does were the signs displayed during homosexual behaviour. In other studies, homosexual behaviour was characterised by accepting mounting or mounting other does (Eiamvitayakorn and Rigor 1988, Ola and Egbunike 2004, Shearer and Katz 2006, Bhattacharyya *et al.* 2012 and Ganesh *et al.* 2014). Contrary to our findings, homosexual behaviour was recorded during I cycles and was more prominent than the S cycles; the average frequencies during estrus being 0.5±0.8 and 0.2±0.1, respectively (Ola and Egbunike 2004). Confined condition may be conducive for expression of homosexual behaviour, which was the housing pattern in latter study and was also displayed only in C condition of present study.

4.2.2 ESTROUS BEHAVIOUR UNDER CONFINED (C) *VERSUS* GRAZING (G) CONDITIONS

The onset of estrus occurred either in C (n=10) or G (n=4) conditions. The estrous behaviour was more intense under C than G condition (Figure 5, Table 4.4). Sniffing of vulva, biting of estrous female, tail flagging and standing to be mounted were the most frequently expressed signs, both at estrous onset and overall, under C and G conditions. All these signs were, however, numerically higher in the C than G condition; the corresponding average frequency in the C *versus* G conditions being 3.5±1.0 *versus* 1.3±0.4, 3.5±1.0 *versus* 1.2±0.4, 3.2±0.9 *versus* 1.3±0.4 and 2.8±0.9 *versus* 1.0±0.4, respectively, during a 30 min observation period. Among different traits, bleating and interest in male were the two weakest estrous indicators in G as well as C conditions (Figure 5) as their frequency was not only less, but all these terminated relatively earlier (Table 4.4). Bleating and interest in male can be inter-related as the former activity incited attraction of males, as is also established for cows (Bonner 2015). The most striking feature between the C and G conditions was that the peak frequencies of different estrous behaviour traits under C conditions occurred from 6 - 6.5 to 12 - 12.5 h after estrous onset

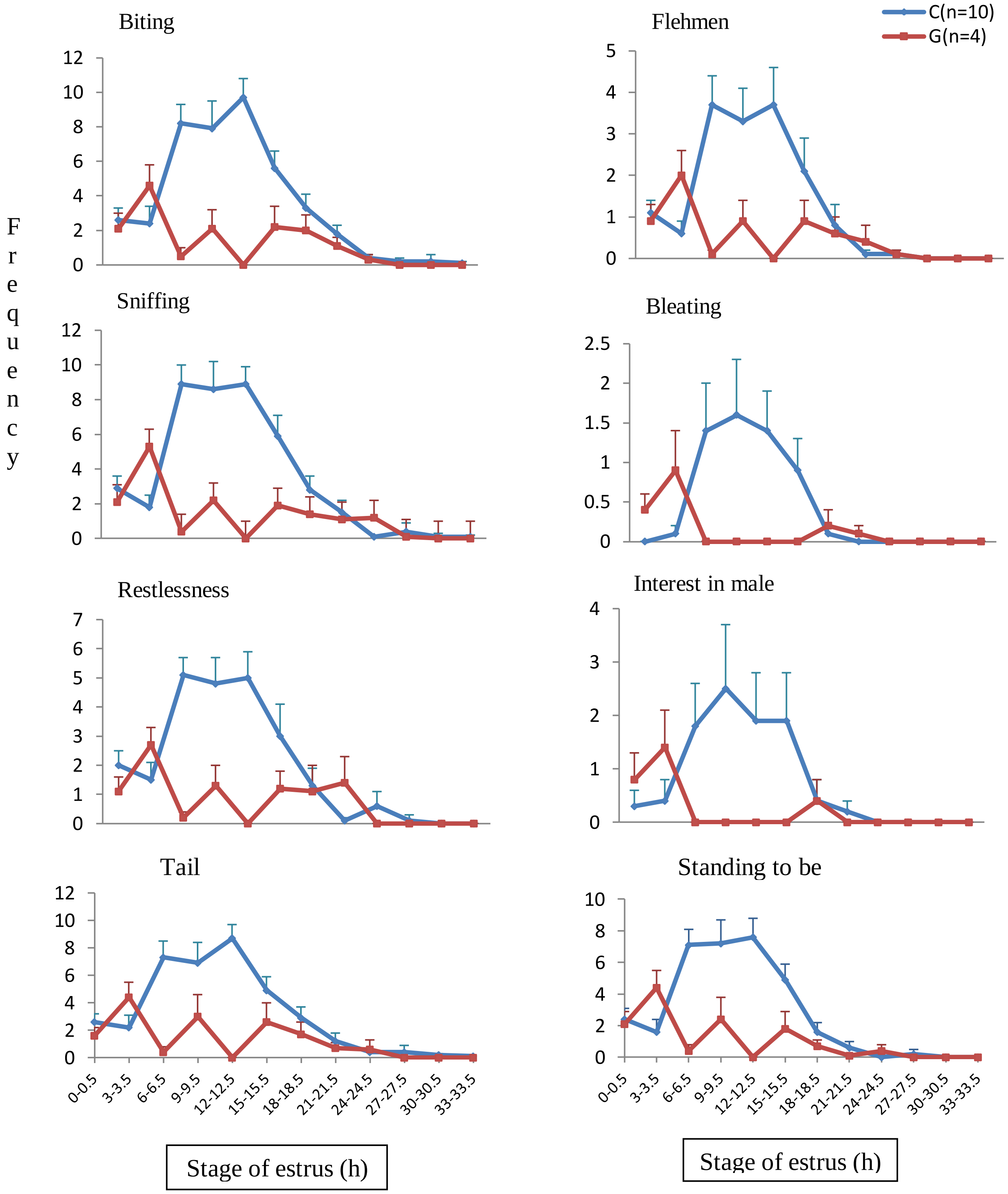
Table 4.4: Average (Mean ± S.E.M) frequency of different estrous behaviour characteristics in Gaddi goats (n=14): A comparison between confined (C) and grazing (G) conditions (combined for spontaneous and induced estrus)

Time after estrous onset (*)	Average																	
	Male behavioural characteristics						Female behavioural characteristics											
	Biting of estrous female		Flehmen		Sniffing of vulva		Bleating		Restlessness		Interest in male		Tail flagging		Standing to be mounted		Lordosis	
	C(n=10)	G(n=4)	C(n=10)	G(n=4)	C(n=10)	G(n=4)	C(n=10)	G(n=4)	C(n=10)	G(n=4)	C(n=10)	G(n=4)	C(n=10)	G(n=4)	C(n=10)	G(n=4)	C(n=10)	G(n=4)
0-0.5 (14)	2.6±0.7	2.1±0.9	1.1±0.3	0.9±0.4	2.9±0.7	2.1±0.8	0	0.4±0.2	2±0.5	1.1±0.5	0.3±0.3	0.8±0.5	2.6±0.6	1.6±0.6	2.4±0.7	2.1±0.8	0	0
3-3.5 (14)	2.4±1	4.6±1.2	0.6±0.3	2±0.6	1.8±0.7	5.3±1.1	0.1±0.1	0.9±0.5	1.5±0.6	2.7±0.6	0.4±0.4	1.4±0.7	2.2±0.9	4.4±1.1	1.6±0.8	4.4±1.1	0	0
6-6.5 (14)	8.2±1.1^{a}	0.5±0.5^{b}	3.7±0.7^{a}	0.1±0.1^{b}	8.9±1.1^{a}	0.4±0.4^{b}	1.4±0.6^{x}	0^{y}	5.1±0.6^{a}	0.2±0.2^{b}	1.8±0.8^{x}	0^{y}	7.3±1.2^{a}	0.4±0.4^{b}	7.1±1^{a}	0.4±0.4^{b}	0	0
9-9.5 (14)	7.9±1.6^{x}	2.1±1.1^{y}	3.3±0.8	0.9±0.5	8.6±1.6^{x}	2.2±1.2^{y}	1.6±0.7^{x}	0^{y}	4.8±0.9^{x}	1.3±0.7^{y}	2.5±1.2^{x}	0^{y}	6.9±1.5	3±1.6	7.2±1.5	2.4±1.4	0	0
12-12.5 (14)	9.7±1.1^{a}	0^{b}	3.7±0.9^{a}	0^{b}	8.9±1^{a}	0^{b}	1.4±0.5^{a}	0^{b}	5±0.9^{a}	0^{b}	1.9±0.9^{x}	0^{y}	8.7±1^{a}	0^{b}	7.6±1.2^{a}	0^{b}	0.5±0.5	0
15-15.5 (14)	5.6±1	2.2±1.2	2.1±0.8	0.9±0.5	5.9±1.2	1.9±1	0.9±0.4^{x}	0^{y}	3±1.1	1.2±0.6	1.9±0.9^{x}	0^{y}	4.9±1	2.6±1.4	4.9±1	1.8±1.1	1.0±1.0	0
18-18.5 (14)	3.3±0.8	2±0.9	0.8±0.5	0.6±0.4	2.8±0.8	1.4±0.7	0.1±0.1	0.2±0.2	1.3±0.6	1.1±0.9	0.4±0.4	0.4±0.4	2.9±0.8	1.7±0.9	1.6±0.6	0.7±0.4	0.3±0.3	0
21-21.5 (14)	1.8±0.5	1.1±0.5	0.1±0.1	0.4±0.4	1.5±0.7	1.1±0.6	0	0.1±0.1	0.1±0.1	1.4±0.9	0.2±0.2	0	1.2±0.6	0.7±0.4	0.6±0.4	0.1±0.1	0	0
24-24.5 (10)	0.4±0.2	0.3±0.3	0.1±0.1	0.1±0.1	0.1±0.1	1.2±0.9	0	0	0.6±0.5	0	0	0	0.4±0.2	0.6±0.7	0	0.4±0.4	0	0
27-27.5 (2)	0.2±0.2	0	0	0	0.4±0.5	0.1±0.2	0	0	0.1±0.2	0	0	0	0.4±0.5	0	0.2±0.3	0	0	0
30-30.5 (1)	0.2±0.4	0	0	0	0.1±0.2	0	0	0	0	0	0	0	0.2±0.1	0	0	0	0	0
33-33.5 (1)	0.1±0.1	0	0	0	0.1±0.1	0	0	0	0	0	0	0	0.1±0.1	0	0	0	0	0
Total frequency	42.4±9.7	14.9±1.7	15.5±4.3	5.9±0.7	42.0±10.4	15.7±1.7	5.5±1.9	1.6±0.3	23.5±5.9	9.0±1.0	9.4±2.7	2.6±0.5	37.8±8.8	15.0±1.7	33.2±8.9	12.3±8.6	1.8±0.9	0
Average frequency per observation	3.5±1.0	1.2±0.4	1.3±0.4	0.5±0.2	3.5±1	1.3±0.4	0.5±0.2	0.1±0.1	2±0.6	0.8±0.2	0.8±0.3	0.2±0.1	3.2±0.9	1.3±0.4	2.8±0.9	1±0.4	0.2±0.2	0

[a,b: x,y] adjacent values with different superscripts between C *versus* G condition differ at (p<0.01) and (p<0.05), respectively.

* Number of goats in estrus under C and G conditions.

Figure 5: Estrous behaviour characteristics: A comparison between confined (C) and grazing (G) condition (combined for spontaneous (S) and induced (I) estrus) in Gaddi does



compared to a peak at 3 - 3.5 h for all the traits under G conditions. Hence, the peak of different signs did not coincide under C and G conditions. Notably, the frequencies of all the traits at 3 to 3.5 h were higher in the G than C condition (Table 4.4). Another distinct feature between the two conditions was that all the estrous behaviour signs were expressed till they vanished completely under C conditions compared to their intermittent expression under G conditions (Table 4.4). Hence, C conditions under the circumstances of the present experiment, appeared to facilitate estrous detection. In contrast, G condition has been strongly relied upon for estrous detection in two previous studies. However, long day treated bucks (Rivas-Munoz *et al.* 2014) or nutritional supplementation of does (Fitz-Rodriguez *et al.* 2009) were the two parameters differing from present study in which the bucks or does were given no specific treatment. The overall reduced appearance of estrus in goats under G conditions may be due to the fact that goats, unlike sheep and cattle, tend to spend more time in eating (as long as 11 hours) and in moving from one plant to another (Haenlein *et al.* 1992). The aforesaid reason may also be an explanation for no exhibition of estrus at 12 - 12.5 h after estrus onset under G conditions, which otherwise was peak estrous period under the C conditions (Table 4.4, Figure 5).

4.3 ESTROUS DURATION

Estrous duration in the S estrus in the does of present study was 28.4±1.4 h, which was numerically less than 32.0±5.3 h in the same group of I does as well as 30.6±4.1 h in the I_{all} group. However, in majority of the estrous periods in all the groups, estrus terminated by 24 to 24.5 h post estrous onset. Most of studies report estrous duration to a range from 24 to 48 h in goats (Hafez 1980, Rahman *et al.* 2008 and Noakes *et al.* 2009). A breed variation in the estrous duration has also been reported. Angora, a wool breed, had relatively shorter estrous duration of 12 to 24 h (Hafez 1980). Relatively shorter estrus duration in the S than I groups in present investigation also corroborates to some previous studies. Two different studies have reported estrous duration of 30.8±12.1 *versus* 21.4±9.5 h (Akusu and Egbunike 1984) and 24.4 *versus* 18.8 h (Mori and Kano 1984) in PG induced *versus* S estrus. An estrous duration of 36.0 h in Gaddi does is also on record (Gupta *et al.* 2005).

4.4 OVULATION

The estrus onset to ovulation interval in the S does was 23.9±2.1 h, which was numerically less than 28.5±2.7 h in the same I goats, but quite similar to the value of 24.0±5.6 h in the I_{all} group. A longer estrus to ovulation interval in the I goats may be attributed to a relatively longer estrous duration in the goats *per se*. The estrus to ovulation interval ranged from 12 to 36 h in goats (Noakes *et al.* 2009). However, breed variation also regulates ovulation. Longer estrus to ovulation interval of 39.9 to 48 h has been reported in Assam goats (Chakraborty *et al.* 1993) and Toggenburg Alpine and Saanen goats (Esteves *et al.* 2013). In contrast to slightly longer estrus to ovulation interval in the I *versus* S group in present study, Vazquez *et al.* 2010 reported the corresponding interval to be 28.0±2.5 *versus* 34.7±1.9 h, respectively. Parity is the other variable regulating the time of ovulation in goats. Nulliparous compared to multiparous ovulate earlier; the difference being attributed to a tendency for a single ovulation than multiple ovulations in multiparous goats (Simoes *et al.* 2008). Irrespective of the S or I estrus, all the ovulations occurred before the termination of estrus in the present study. However, in one of the study ovulation occurred 5.6±1.0 to 8.4±0.7 h after I and S estrus, respectively, which was probably due to use of culdoscopy that was preceeded by ketamine and xylazine administration known to delay ovulation (Peet and Lincoln 1977 and Robertson and Rakha 1965). Only one out of 14 ovulations screened in the present study revealed double ovulation (7.1 % as incidence and 1.1 as the ovulation rate). Goats are known to ovulate more than one follicle in estrous cycle. A much higher ovulation rate ranging from 1.4 (Prasad 1971) to 4 (Rao and Bhattacharyya 1980) has been recorded in different breed of goats. An increase in peripheral FSH, estradiol- 17β and LH have been the associated endocrine milleau with multiple ovulations in goats (Miller and Martin 1993). In present study, the goat exhibiting double ovulation had peak estradiol- 17β and LH concentration of 102.3 pg/ml and 90.3 ng/ml, respectively. Provision of high metabolizable energy (De Santiago-Miramontes *et al.* 2008) and induced estrus (Miller and Martin 1993) favours high ovulation rate. In line with the latter study, double ovulation in the present study was also recorded in one of the I estrus goat.

4.4.1 FOLLICULAR DIAMETER

There was not much difference in the ovulatory follicular diameter among the different groups of does. The average follicular diameter in the S, I and I_{all} groups was 9.8±0.6, 9.3±0.5 and 9.5±0.5 mm, respectively. Previous studies reported the preovulatory follicular diameter in goats to range from 6 to 8.3 mm (Li *et al.* 2001, Medan *et al.* 2003, Noakes *et al.* 2009, and Vazquez *et al.*

2010). A variation in the progesterone secretion regulating LH secretion could influence the development and size of dominant follicle (Noakes *et al.* 2009).

4.5 ENDOCRINOLOGICAL PROFILE

Different endocrinological and estrus/ ovulation related determinants are presented in Table 4.5.

4.5.1 ESTRADIOL- 17B

Estradiol- 17β is involved in estrus expression (Roberts 1971) and in inducing the LH surge by a neural action to evoke a sudden release of GnRH and a pituitary action to maximize response to GnRH (Moenter *et al.* 1990).

The peak estradiol- 17β concentration during S, I and I_{all} estrus were 48.2±6.0, 69.3±19.4 and 63.9±8.8 pg/ml, respectively, which did not differ significantly between groups (Table 4.5). The peak estradiol- 17β concentration in the Gaddi goats was much higher than 18 to 30.9 pg/ml reported earlier (Bono *et al.* 1983, Mori and Kano 1984 and Leyva-Ocariz *et al.* 1995). Breed variation could be a plausible explanation for estradiol- 17β peak concentrations as still lower peak of 14.0±1.2 pg/ml has been cited in Dwarf does (Khanum *et al.* 2008). Another plausible explanation for variation in peak estradiol- 17β concentration could be the difference in intervals of bleeding schedule. In present study, it was every 3 h compared to 8-24 h interval in another studies (Bono *et al.* 1983, Leyva-Ocariz *et al.* 1995 and Kausar *et al.* 2005). A numerically lower estradiol- 17β peak in the S than the I group resembles to earlier findings. Peak estradiol- 17β concentration of 18 *versus* 24 pg/ml (Karsch *et al.* 1979) and 21 *versus* 25 pg/ml (Mori and Kano 1984) have been observed in S *versus* I estrus. The estradiol- 17β surge occurred after estrous onset in all groups; the timing being 6.3±0.9, 6.8±1.3 and 5.1±0.8 h after estrous onset in S, I and I_{all} groups, respectively, without any significant difference (Table 4.5). Several earlier studies have reported estradiol- 17β peak to occur on the day of estrus (Bono *et al.* 1983 and Khanum *et al.* 2008) or 5 to 17.5 h after estrous onset (Karsch *et al.* 1979 and Mori and Kano 1984). However, in one study peak estradiol- 17β concentration of 30.9±1.0 pg/ml has been reported 2 d before ovulation (Medan *et al.* 2003). A precocious estradiol- 17β peak was probably due to high estradiol- 17β production by initial stages of growing follicle rather than the terminal stages of follicular development.

Table 4.5 Endocrine and associated ovulatory determinants in spontaneous (S), induced (I) and overall induced (I_{all}) estrus groups in Gaddi does

Parameter	S	I	I_{all}
Progesterone concentration at estrous onset (ng/ml)	0.2±0.1	0.1±0.1	0.1±0.1
Estrous onset to Estradiol- 17β peak interval (h)	6.3±0.9	6.8±1.3	5.1±0.8
Estrous onset to LH peak interval (h)	$10.1±0.8^{x}$	$9.9±1.1^{yxz}$	$7.1±0.6^{z}$
Estradiol- 17β peak concentration (pg/ml)	48.2±6.0	69.3±19.4	63.9±8.8
LH peak concentration (ng/ml)	66.3±4.1	55.7±8.2	60.1±3.0
Estradiol- 17β peak to LH peak interval (h)	3.7±0.5	3.2±0.1	1.9±0.9
Estradiol- 17β peak to ovulation interval (h)	17.7±1.6	21.8±1.9	18.9±3.3
LH peak to ovulation interval (h)	13.8±1.4	18.5±1.9	16.9±3.1
Progesterone concentration (day 7) (ng/ml)	6.5±1.1	7.2±1.9	6.8±1.4
Progesterone concentration (day 14) (ng/ml)	11.2±1.3	12.4±2.1	10.9±2.6

^{x,y,z} adjacent values with different superscripts between S *versus* I *versus* I_{all} differ at ($p<0.05$).

4.5.2 PROGESTERONE

Following PG administration (Section 3.3), the progesterone concentration at estrus was < 1 ng/ml; average being 0.2±0.1, 0.1±0.1 and 0.1±0.1 ng/ml in S, I and I_{all} estrus groups, respectively (Table 4.5). Several studies, irrespective of S or I estrus, recorded progesterone concentration of < 1 ng/ml in different goat breeds (Bono *et al.* 1993, Kausar *et al.* 2005 and Vazquez *et al*. 2010).

The progesterone concentration at day 7 and day 14 in S, I and I_{all} Gaddi goats was 6.5±1.1, 7.2±1.9 and 6.8±1.4 ng/ml and 11.2±1.3, 12.4±2.1 and 10.9±2.6 ng/ml, respectively (Table 4.5). There was no difference in progesterone concentration among different groups at day 7 or day 14 of estrous cycle. The progesterone concentration of 3.5 ng/ml in Crossbred goats (Leyva-Ocariz *et al.* 1995) and 10.5 ng/ml in Anglo Nubian goats (Vazquez *et al*. 2010) has been reported at day 7. A peak progesterone concentration of 12 ng/ml (simulates to the present study) was, however, was recorded at day 15 while collecting one sample a day (Leyva-Ocariz *et al.* 1995). In contrast to Gaddi goats exhibiting no difference in the progesterone concentration between S and I cycles, one previous study revealed higher peak progesterone concentration of 17.5 ng/ml at day 12 in S cycle compared to 10 ng/ml at day 14 in I cycle in once daily blood samples (Vazquez *et al*. 2010).

4.5.3 LH

LH surge is known to induce ovulation. The time and magnitude of the pre-ovulatory surge of LH are regulated by factors associated with follicular growth. The timing of LH surge also dictates or is strongly related with ovulation timings in cows (Saumande and Humblot 2005) There was no difference in the peak LH concentration of 66.3±4.1, 55.7±8.2 and 60.1±3.0 ng/ml, as recorded in S, I and I_{all} groups, respectively (Table 4.5). There is an evidence of breed variation in the peak LH concentration that ranged from 23.0±6.9 ng/ml in Crossbred goats (Leyva-Ocariz *et al.* 1995) to 40.7±10.1 ng/ml in Alpine goats (Bono *et al.* 1983). However, much lower LH concentration of 7.5 to 30.0 ng/ml (average of 19.0 ng/ml) has been reported in PG induced Dwarf does (Kausar *et al.* 2005). A similar (60.1±3.5 ng/ml) (Simoes *et al.* 2008) or much higher (102.1±7.8 ng/ml) (Llewelyn *et al.* 1993) LH concentration has been reported in PG induced estrous does. In a more recent study, a conspicuous difference in LH concentration, 4.5 *versus* 25.0 ng/ml in I *versus* S estrus has been observed inspite of progesterone concentration being < 1 ng/ml in both groups (Mori and Kano 1984). The LH peak in all the 3 groups of present study occurred after estrous onset, the estrous onset to LH peak interval being 10.1±0.8, 9.0±1.1 and 7.1±0.6 h in S, I and I_{all} groups, respectively (Table 4.5). The findings simulate to a previous study in which LH peak was recorded at 12.0±1.5 h (Llewelyn *et al.* 1993) or 13.9±1.0 h after S estrous onset (Simoes *et al.* 2008). In other studies, LH peak concentration was either attained on the day of estrus (Leyva-Ocariz *et al.* 1995) in crossbred goats or 8 to 24 h after S estrous onset in Alpine goats (Bono *et al.* 1983). Difference between the present investigation and the upper limit of the latter report could be due to a much longer interval of sampling frequency (8 h) and an estrous duration of 32 to 40 h in Gaddi does recorded through survey (Gupta *et al.* 2005). In contrast to the present study, where estrus onset to LH peak interval tended to delay in S than I_{all} group (10.1±0.8 *versus* 7.1±0.6 h) (P= 0.50; Table 4.5), the LH peak was reported to occur much earlier in S (6.3±2.8 h) than I (10.0±1.1h) estrus (Mori and Kano 1984).

No difference was recorded in estrous onset to estradiol- 17β peak / LH peak and LH peak to ovulation in Gaddi goats (Table 4.5). The only available study (Mori and Kano 1984) comparing the said parameters observed a shorter estrus onset to estradiol- 17β peak interval of 5.0 *versus* 14.4 h, with estrous onset to LH peak interval of 6.3±2.8 h *versus* 10.0±1.1 h in S *versus* I estrus, respectively. The latter observation suggests LH peak to precede estradiol- 17β peak in the I goats. An earlier LH peak than estradiol- 17β peak in cattle has also reported by Starbuck *et al.* 2006. In the study by Mori and Kano (1984), the LH peak to ovulation interval was, however, similar in S (21.2±0.5 h) *versus* I (20.0±0.9 h) estrous, with ovulation in both the groups occurring after end of estrus in both the groups. The end of estrus to ovulation interval being 8.4±0.7 h for S and 5.6±1.0 h for I goats. Ovulation occurring after estrus was rare and has also been reported using culdoscopy done under the ketamine and xylazine medication, both of which delay ovulation (Robertson and Rakha 1965, Peet and Lincoln 1977).

4.6 GENITAL DISCHARGE

Genital discharge from all the does was collected at early, mid and late-estrus. The volume of genital discharge aspirated was 0.5 to 1.0 ml and was transparent in all the goats. This is in consonance with the studies of Eiamvitayakorn and Rigor (1988), Ola and Egbunike (2004) and Widayati *et al.* (2010).

Primary branching was seen in the genital discharge collected at early-estrus (Figure 6A), while quaternary branching was seen at mid-estrus (Figure 6B) in all the goats. The fern pattern disintegrates and gave an appearance of primary sort of branching (Figure 6 C). A similar change in fern pattern at different stages of estrous cycle has been observed in Damascus does. Quaternary branching in the genital discharge may be due to high concentration of chloride ion and estradiol- 17β in blood as compared to early and late estrus (Salem *et al.* 2013). Quaternary branching in the genital discharge was recorded when frequency of all the estrus signs was at peak in S, I and I_{all} groups as well as C condition. However, the quaternary branching did not coincide with peak estrus in G condition as all the estrus traits peaked much earlier at 3 to 3.5 h after estrus onset in the latter most group. Hence, under G condition genital discharge evaluation assumes a high relevance from breeding perspective.

Figure 6A: Primary branching at early-estrus in Gaddi does

Figure 6B: Quaternary branching at mid-estrus in Gaddi does

Figure 6C: Degradation of fern pattern at late-estrus in Gaddi does

CHAPTER 5

SUMMARY AND CONCLUSIONS

Estrous behaviour of goats is important to attain knowledge about the breeding season and to provide two fold benefit to the farmers, either economically or providing more efficient management system. Estrous detection is important for successful artificial insemination and controlled breeding programs. Even though estrous exhibition is under the direction of estradiol- 17β hormone, factors such as heat stress and methods of estrous synchronization have been reported to influence its exhibition. Lack of comprehensive details on the subject was lacking in the Gaddi goats. So the present study was undertaken with following objectives:

1.1 To study the estrous behaviour of Gaddi goats.

1.2 To determine endocrine profile during different stages of estrous cycle.

The present study was conducted in 10 adult Gaddi goats during October, 2014 to February, 2015. All the goats were injected an intramuscular injection of 187.5 μg PG. The two non-responsive goats to first PG were repeated with a same dose of PG 11 days later. Eventually all the goats responded to PG (I_{all} group). Teasing of goats with two aproned bucks was initiated from 36 h of the PG injection till 15 to 18 h of termination of estrus and was done for a period of 30 min at every 3 h interval. A group of four does in induced estrus (I group) were also monitored for estrus in the succeeding spontaneous estrus (S group). The visual estrous behavior signs recorded for frequency in the present study were categorized as male (biting of estrous female, flehmen and sniffing of vulva) and female (bleating, restlessness, interest in male, tail flagging, standing to be mounted and lordosis) specific behaviour. The biological samples collected from the Gaddi does comprised of blood (every 3 h interval from estrus onset till termination) and genital discharge (at estrus onset, as well as 12 h and 24 h later). Blood was also collected at day 7 and day 14 to evaluate different hormones (estradiol- 17β, progesterone and luteinizing hormone), whereas fern pattern was evaluated in the genital discharge. Ultrasonography assisted ovulation detection was undertaken at every 6 h interval beginning from 12 h after estrus onset and continued till ovulation was observed. The goats were grazed (G condition) five hours a day, (9:00 to 12:00 h and from 14:00 to 16:00 h), whereas they remained under confined condition in a shed (C condition) during the remaining period of the day. The estrous behaviour was compared separately for S *versus* I *versus* I_{all} groups and C *versus* G conditions.

The average interval from PG administration to estrous onset was 70.2±0.7 h and 76.1±3.8 h in I and I_{all} groups, respectively.

Comparison of estrous behaviour between the S, I and I_{all} groups revealed a much better estrous expression in the S group. The exhibition of different estrous behaviour signs considered in the present study were peculiar at different stages of estrus, including onset, in S, I and I_{all} groups. For instance, relatively higher frequency of standing to be mounted followed by biting of estrous female, sniffing of vulva and tail flagging will prove more reliable indicators of the estrus onset as well as overall estrous expression (restlessness being the other estrous trait) in all the groups. The female behavioural characteristics, namely, bleating, restlessness and interest in male were considerably more frequently expressed than the others in the S compared to I cycles. In contrast, sniffing and tail flagging were more frequently expressed, more so in the second half of estrus, in I and I_{all} compared to S estrus. Tail flagging was the only estrous sign displayed during the entire estrous period in all the groups, whereas bleating terminated relatively earlier, 18 to 24.5 h of estrus onset, in all the three groups. The peak frequency of all the signs, except for lordosis, was attained between 9 to 12.5 h from estrus onset in S, I and I_{all} groups.

Comparison of estrous behaviour between the C and G conditions revealed a better estrous expression under C conditions. Detection of estrous onset under C or G conditions could be relied upon by detecting the presence of sniffing of vulva, biting of estrus female, tail flagging and standing to be mounted as these were the most frequently expressed signs at onset. To recollect, all the four aforementioned signs, though with a reduced frequcncy in C and G conditions, were also the most prominent signs at estrus onset in S, I and I_{all} groups. Bleating terminated earlier in C (18 to 18.5 h) than G (21 to 21.5 h) condition. In contrast, all the other signs terminated earlier in G (18.0 to 27.5) condition unlike some other signs such as, biting of estrus female, sniffing of vulva and tail flagging that persisted for the entire estrus under the C condition. Moreover, all the estrous signs were expressed in an intermittent manner in G condition whereas they were continuously displayed till end of estrus during C condition. All the estrous behaviour signs attained a peak frequency at 12 to 12.5 h from estrous onset under C condition; the frequency for the traits being nil at the corresponding time interval in the G condition. The peak frequency for all traits was attained much earlier, 3 to 3.5 h, under the G condition.

Estrous duration of 28.4±1.4 h, 32.0±5.3 h and 30.6±4.1 h, respectively, in the S, I and I_{all} groups did not vary among different groups, which was also the case with estrous onset to ovulation interval of 23.9±2.1 h, 28.5±2.7 h and 24.0±5.6 h in the respective groups.

The range of average follicular diameter (9.3±0.5 to 9.8±0.6 mm), estradiol- 17β peak concentration (48.2±6.0 to 69.3±19.4 pg/ml), estradiol- 17β peak to LH peak interval (1.9±0.9 to 3.7±0.5 h) and LH peak to ovulation interval (13.8±1.4 to 18.5±1.9 h) did not differ between the S, I and I_{all} groups.

The conclusions drawn from this study are:

1. Irrespective of induced / spontaneous estrus or housing conditions, standing to be mounted, biting of estrous female, sniffing of vulva and tail flagging were the most frequent signs characterizing the estrous onset; the lattermost sign being displayed during the entire estrous length.
2. Majority of the estrous behaviour signs attained peak frequency at 3 to 3.5 h under grazing condition as against 9 to 12.5 h under confined, induced or spontaneous estrus situation.
3. Overall, the estrus expression was better in spontaneous than induced estrus and confined than grazing condition.
4. Induction of estrus did not affect the ovulatory and endocrine characteristics.
5. Genital discharge fern pattern differed with stage of estrus. Typical fern pattern coincided with peak frequency of estrous behaviour signs except for the behaviour recorded under grazing condition.

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