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FOLLICULAR DYNAMICS IN GOATS

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ABSTRACT

Goats are among the earliest domesticated animals. They are often described as poor man's cow because of its immense contribution to poor man's economy. Goats are spontaneously ovulating, polyestrous animals, bred in a wide range of production systems. Average duration of the goat estrous cycle is 21 days, its length is highly variable from 18-22 days depending upon the breed differences, stage of breeding season and environmental stress. During the estrous cycle, ovaries undergo a number of morphological, biochemical and physiological changes leading to the ovulation. Application of ultrasonography in veterinary practice and specifically in small ruminants, has developed to become the most efficient diagnostic tool for managing reproduction. With the advent of ultrasonography (USG), our understanding of the dynamics and development of the follicle has increased in recent years. Follicular development occur in wave-like pattern exhibiting between 2 and 6 waves of follicle development during estrous cycles with 3 or 4 waves being the most prevalent. Major point of difference between breeding and non-breeding season is that the last wave being ovulatory in breeding season only. In brief, the ultrasonography has opened up the flood gates of research in the area of follicular dynamics and its further utilization for efficient reproductive management in goats leading to economic upliftment of farming community.

Keywords : Follicular dynamic, Goats, Ultrasonography

Goats are among the earliest domesticated animals. They are often described as poor man's cow because of its immense contribution to poor man's economy. Goats are spontaneously ovulating, polyestrous animals, bred in a wide range of production systems. Average duration of the goat estrous cycle is 21 days, its length is highly variable from 18-22 days depending upon the breed differences, stage of breeding season and environmental stress. During the estrous cycle, ovaries undergo a number of morphological, biochemical and physiological changes leading to the ovulation. Application of ultrasonography in veterinary practice, and specifically in small ruminants, has developed to become the most efficient diagnostic tool for managing reproduction. With the advent of ultrasonography (USG), our understanding of the dynamics and development of the follicle has increased in recent years. Follicular development occur in wave-like pattern exhibiting between 2 and 6 waves of follicle development during estrous cycles with 3 or 4 waves being the most prevalent. Major point of difference between breeding and non-breeding season is that the last wave being ovulatory in breeding season only. In brief, the ultrasonography has opened up the flood gates of research in the area of follicular dynamics and its further utilization for efficient reproductive management in goats leading to economic upliftment of farming community.

INTRODUCTION

Goats have been considered among the earliest domesticated animals. Goats are the lifeline of 70-90 per cent of the farmers living in rural areas of India (Iqbal, 2010; Ramachandra *et al.,* 2012). The goat is a multipurpose animal producing milk, hide, fibre, manure and most importantly is source of chevon relished by all sections of society. They are more tolerant to hot climate and give more production per unit investment. The aforementioned benefits render goats to be the poor man's cow because of its immense contribution to rural economy. Goats are spontaneously ovulating, polyestrous animals, bred in a wide range of production systems. Estrous cycle is a cascade of hormonal events which changes the female reproductive system morphology to prepare animal for pregnancy (Fatet *et al.,* 2011). During the estrous cycle, ovaries undergo a number of morphological, biochemical and physiological changes leading to the ovulation. Ultrasonography is a simple, reliable, non-invasive imaging technique with least side effects. It's applications in veterinary practice has developed, from a limited use for pregnancy diagnosis and detection of some pathological conditions, to the most efficient diagnostic tool for evaluating the reproductive health of both males and females leading to reproductive management of the flock. Limitations associated with the use of ultrasonography include expertise of the operator, and long time to learn the technique (Gonzalez *et al.,* 2010). Transrectal ultrasonic imaging provides a means for repeated, monitoring and measuring of follicles larger than 2 mm, regardless of their depth within the ovary. So the purpose of this review is to utilize the ultrasound based follicular dynamics and its subsequent application to manipulate reproduction in goats.

FOLLICULAR DYNAMICS

A large pool of resting primordial follicles is laid down during fetal development in sheep and goats, with the first follicles being formed about 70 days of gestation (Mariana *et al.,* 1991). It takes about 6 months for a follicle to develop from the resting stage to the pre-ovulatory stage and approximately 34 to 43 days from initial antrum formation to the pre-ovulatory phase (Turnbull *et al.,* 1977; Cahill and Mauleon, 1980). This pool is non-renewable and during the lifetime of the animal, follicles develop to primary, secondary and tertiary stages before ovulating (Armstrong *et al.,* 1998). In

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domestic ruminants, the number of follicles ovulated during a female´s reproductive life is minimal compared to the number of follicles she is born with (Aerts and Bols, 2010) which suggests degeneration or atresia as main fate of the follicle not the ovulation. This seems to follow a selection process to ensure ovulation of only those follicles with oocytes that optimize the fertilization odds (Hsueh *et al.,* 1994).

The dynamics of follicle wave development in goats have received less attention than in cattle or sheep (Rosales-Torres *et al.,* 2012). Follicular development can be divided into two phases-gonadotropin independent (FSH and LH) phase, where the follicle grows from primordial to antral follicle and an another phase, FSH and LH dependent, where the follicle will reach the pre-ovulatory status, which in presence of an adequate frequency and amplitude of LH pulses will ovulate (Webb *et al.,* 2004).

Prior to the use of ultrasonography, monitoring of ovarian follicle development via laparoscopic observations was not able to describe an organised pattern in cyclic goats (Camp *et al.,* 1983). However, ultrasonography has shown that the pattern of follicle development during estrous cycles in goats does follow a wave-like pattern (Rubianes and Menchaca, 2003) and there are between 2 and 6 waves of follicle development during estrous cycles with 3 or 4 waves being the most prevalent (Ginther and Kot, 1994; de Castro *et al.,* 1999; Gonzalez de Bulnes *et al.,* 1999; Schwarz and Wierzchos, 2000; Menchaca and Rubianes, 2001, 2004; Evans, 2003; Berlinguer *et al.,* 2009; Nogueira *et al.,* 2015; Sharma *et al.,* 2018a; Sharma *et al.,* 2018b; Sharma and Sood, 2018).

On any day of the estrous cycle, there are 5 to 10 follicles which are ≥3mm in diameter in the ovaries and follicles ovulate at between 6 and 9mm in diameter (Camp *et al.,* 1983; Ginther and Kot, 1994; de Castro *et al.,* 1999; Gonzalez de Bulnes *et al.,* 1999; Nogueira *et al.,* 2015; Sharma *et al.*, 2018b). Due to the large variation in the number of waves, the onset of each wave and inter wave interval are highly variable (de Castro *et al.,* 1999; Medan *et al.,* 2003, 2005). The number of follicles that start growing in each wave is not well established, however, it is reported that about 4 follicles with a diameter >3mm begin their growth in response to transient peak of FSH (Berlinguer *et al.,* 2009). In Shiba goats, the diameter of the dominant follicle in anovulatory waves ranges from 6 to 6.7mm, while the ovulatory follicle can reach up to 8.2mm in diameter (Medan *et al.,* 2003, 2005). However, Evans (2003) reported that the diameter of the non-ovulatory dominant follicle and ovulatory follicle is similar. Follicular dynamics have been studied by various workers (Cruz *et al.,*2005; Medan *et al.,* 2005; Simões *et al.,* 2006; Delgadillo*,* 2011; Nogueira *et al.,* 2015; Sharma and Sood, 2018; Sharma *et al.,* 2018a; Sharma *et al.,* 2018b) during breeding and non breeding season.

FOLLICULAR DYNAMICS IN BREEDING SEASON

Follicular dynamics during the estrous cycle in cyclic goats suggest that there are between two and six waves of follicular development during an estrous cycle with an average of four waves per cycle (Ginther and Kot, 1994; de Castro *et al.,* 1999; Menchaca and Rubianes, 2002; Medan *et al.,* 2005; Simoes, 2015; Nogueira *et al.,* 2015; Sharma *et al.,* 2018b). Nogueira *et al.* (2015) recorded a mean of 4.5 follicular waves during the estrous cycle. The number of co-dominant follicle, the maximum diameter of the largest follicle within a follicular wave and mean growth rate were greater in the breeding season (Rubianes and Menchaca, 2003; Gonzalez-Bulnes *et al.,* 2005).

During breeding season, the diameter of the largest follicle and duration of a wave differed between follicular waves. In goats, the largest follicle of the first and fourth follicular wave usually attains greater diameter than the largest follicles of second and third follicular wave (Ginther and Kot, 1994). In 71 per cent of goats, the shorter duration of fourth follicular wave was probably due to ovulatory nature as compared to the first, second and third follicular waves (Ginther and Kot, 1994; Nogueira *et al.,* 2015; Sharma *et al.*, 2018b). It could also be explained on basis of coincident occurance of onset of luteolysis and an LH surge with the development of the fourth follicular wave in the majority of goats compared to earlier waves (Ginther and Kot, 1994; de Castro *et al.,* 1999; Simões *et al.,* 2006; Nogueira *et al.,* 2015; Sharma *et al.,* 2018b).

The mean inter-ovulatory interval for Boer goats is 19.7±0.2 days (Nogueira *et al.,* 2015), Najdi goats (20.6±0.89 days; Mohammadi *et al.,* 2010), White Polish goats (20.4±1.1 days), for Saanen goats (20.8±0.89 days, Menchaca and Rubianes, 2002) and Gaddi goats (20.85±0.63 days; Sharma *et al.,* 2018b). Significantly higher inter-ovulatory interval was observed in West African dwarf goats (23.89 days; Akusu *et al.,* 1986).

The lifespan of corpora lutea observed by ultrasound (18 days) was longer than the duration of the luteal phase (16 days; Nogueira *et al.,* 2015) which may be atributed to the visibility of even minute luteal tissues with the ultrasound technique. Similar findings were observed by de Castro *et al.*(1999) who reported the corpora lutea remained detectable by ultrasound after a significant decrease in progesterone concentration.

Ovulatory studies and follicular development has been studied by many authors using serial laparoscopic examinations (Leboeuf *et al.,* 1996; Goel and Aggrawal, 2006; Goel and Kharche, 2012) and this technique is unacceptable due to invasive nature leading to stress affecting the reproductive parameters (Leboeuf *et al.,* 1996; Baril *et al.,* 2000)

FOLLICULAR DYNAMICS IN NON-BREEDING SEASON

Follicular dynamics have also been studied in different goat breeds during non-breeding season by various researchers (Cruz *et al.,* 2005; Nogueira *et al.,* 2015; Sharma *et al.* 2018a). In Anglo-Nubian and Saanen goats, the ovaries remained active and antral follicles continue to grow in a wave-like pattern with the largest follicles within follicular waves reaching the equivalent or larger size of pre-ovulatory follicles during anestrous (Cruz *et al.,* 2005; Sharma *et al.,* 2018a). Nogueira *et al.* (2015) recorded greater number of follicular waves (4.8±0.01) in Boer goats during the non breeding season whereas lower number of waves (3.18±0.12) was observed in Gaddi goats (Sharma *et al.,* 2018a). The mean maximum diameter of the largest follicle during the non-breeding season was 6.5mm in Anglo-Nubian goats, whereas, it was 6.8 mm in Saanen goats (Cruz *et al.,* 2005) and 7.66±0.10 mm in Gaddi goats (Sharma *et al.,* 2018a). Introduction of bucks leads to increase in follicular growth rate from 1.1 mm/day to 1.5 mm/day, in goats raised in subtropical latitude during anestrous (Delgadillo*,* 2011).

COMPARISON OF FOLLICULAR DYNAMICS DURING BREEDING AND NON-BREEDING SEASON

Meager literature (Nogueira *et al.,* 2015; Sharma and Sood, 2018) is available on the comparison of follicular dynamics in same goats during breeding and non-breeding season. Nogueira *et al.* (2015) have compared the follicular dynamics along with progesterone profile in Boer goats during breeding and non-breeding season in Queensland, Australia and likewise in follicular wave characteristics were studied in same set of Gaddi goats the in Himachal Pradesh, India (Sharma and Sood, 2018). Follicular dynamics in the breeding season compared to the non-breeding season was characterized by the development of larger follicles and greater follicular growth rates. The mean duration of follicular waves was similar between the non-breeding and breeding season. The number of follicular waves recorded during a 3-week examination period was greater in the non-breeding season compared to the breeding season. The duration of the estrous cycle observed in Boer goats (20.7±0.2 days) and the number of ovulations (2.1±0.1) after natural estrus were similar to those reported in Boer goats and other breeds (Ginther and Kot, 1994; Greyling, 2000; Medan *et al.,* 2005; Simoes*,* 2015). Whereas in Gaddi goats average number of follicular waves were significantly higher (P<0.01) during breeding season compared to non-breeding season (4.0±0.21 *versus* 3.18±0.12; Sharma and Sood, 2018). During the breeding season, 14.3 per cent of does experienced short cycles and 35.7 per cent of does developed ovarian follicles that resemble follicular cyst (Nogueira *et al.,* 2015). The mean diameters of the largest follicle obtained in the breeding and non-breeding seasons were quite similar (Medan *et al.,* 2005; Nogueira *et al.,* 2015). In Shiba goats, largest follicle of diameter 6.7mm and 8.0mm were found in anovulatory and ovulatory waves, respectively (Medan *et al.,* 2005). However, Nogueira *et al.* (2015) reported large size of anovulatory follicles (11.4±0.3mm) than ovulatory waves (7.4±0.4mm) during breeding season, in Boer goats. Simoes *et al.* (2006) also reported a significant difference (P<0.001) between anovulatory (5.7±0.4mm) and ovulatory (7.1±1.0mm) waves, in Serrana goats. Approximately 1.0mm/day follicular growth rates between the day of emergence and the day of maximum diameter have been reported by Simoes *et al.* (2006), whereas Nogueira *et al.* (2015) reported somewhat lesser (0.8 mm/day) growth rates during breeding season which might be attributed to breed and age variations. Furthermore, in the breeding season, increasing secretion of LH may have maintained faster follicular growth rate compared to the non-breeding season (0.79±0.04 *versus* 0.65±0.03, P<0.05; Gaddi goats, Sharma and Sood, 2018) and other breeds (Ginther and Kot, 1994; Evans, 2003).

Longer inter wave interval (IWI) during non-breeding than breeding season were observed by various workers (de Castro *et al.,* 1999; Menchaca and Rubianes, 2002; Simoes *et al.,* 2006; Sharma and Sood, 2018) which could possibly occur due to endocrine variations during the estrous cycle, especially effect of progesterone concentration on the follicular development. High progesterone concentration terminates a wave earlier causing an emergence of next wave that ultimately reduces the follicular wave emergence interval. Progesterone inhibits follicular development by suppressing the LH pulse frequency. Longer inter wave interval between ovulatory and its immediately preceeding wave (6.16±0.70 days) during breeding season in Gaddi goats (Sharma and Sood, 2018) than any other two successive waves in non-breeding season, corroborates to observations of Simoes *et al.* (2006) supporting the concept of indirect progesterone action on follicular turnover and the fundamental role of natural luteolysis in providing an opportunity for final follicular maturation and ovulation of the dominant follicle of the ovulatory wave.

Significantly greater number of small and total number of follicles were found in the non-breeding season compared to the breeding season (Nogueira *et al.,* 2015), which could be attributed to more follicles being arrested at the gonadotropin independent phase of development and a lower number of follicles are able to progress further in their development because of the lower concentrations of FSH and LH (Driancourt, 2001), which could be one cause of the greater number of smaller follicles being observed during the non-breeding season. Contrarily, Sharma and Sood (2018) observed significantly higher (P<0.05) small and medium follicles in breeding season *versus* non-breeding season (1.12±0.20 *versus* 0.63±0.06, P<0.05; 2.59±0.69 *versus* 1.14±0.07, P<0.01). Accordingly, the average daily number of visible follicles were significantly higher in breeding *versus* non-breeding season (5.50±0.55 *versus* 3.15±0.67, P<0.05) suggesting a pronounced ovarian activity during the breeding season in comparison to non-breeding season (Ginther and Kot, 1994; Medan *et al.,* 2003, 2005; Simoes *et al.,* 2006; Mohammadi *et al.,* 2010).

Twin ovulation could be explained in goats by emergence of ovulatory follicles in goats that ovulated twice, the ovulatory follicles emerged as part of two different follicular waves that emerged at different times (Ginther and Kot, 1994; Gonzalez-Bulnes *et al.,* 2005). Ovulation of dominant follicles from earlier follicular waves can be explained by older dominant follicles losing functional dominance and thus enabling a new ovulatory follicle to emerge, but still appear to retain the ability to ovulate in the presence of a pre-ovulatory LH surge (Gonzalez-Bulnes *et al.,* 2005).

Nogueira *et al.* (2015) observed that the mean maximum concentration of progesterone (13ng/mL) was attained around Day 15 of the estrous cycle in Boer goats, which agrees with de Castro *et al.* (1999) who reported that progesterone profiles in all Saanen goats started to decline on day 15 and attained basal level on day 19 of the inter-ovulatory interval whereas lower progesterone concentration of 8.35±2.60 ng/ml (Jamnunapani goats; Goel and Kharche, 2012) and 9.16-10.95 ng/ml (Markhoz goats; Farshad *et al.,* 2008) and 11.2±1.3 to 12.4±2.1ng/ml (Sharma, 2015) and 11.89±1.55 ng/ml (Sharma *et al.,* 2018b) at day 14 of estrous cycle in Gaddi goats. Mean luteal phase and follicular phase lengths (17.57±0.36 and 3.14±0.26 days; Gaddi goats; Sharma *et al.,* 2018b) and (16.0±0.4 and 5.0±0.3 days; Boer goats; Nogueira *et al.,* 2015) were quite similar in diffferent breeds.

FOLLICULAR DOMINANCE

A higher propensity of goats ovulating >1 follicle raises curiosity on dominance of follicle in a wave. The existence of follicular dominance in small ruminants remained controversial for a long time (Driancourt *et al.,* 1991). However, studies suggests that dominance is also operative in the goat, especially during the wave 1 and the ovulatory wave (Ginther and Kot, 1994; de Castro *et al.,* 1999; Rubianes and Menchaca, 2003). The total number of small follicles decreased in correlation with the development of the largest follicle of wave 1 (Menchaca *et al.,* 2002) and this follicular pattern is one of the characteristics described for the phenomenon of the dominance in cattle (Adams *et al.,* 1993). Furthermore, in goats with mono-ovulatory cycles, the diameter of the two largest follicles differ significantly 3 days before ovulation (de Castro *et al.,* 1999). Nevertheless, follicular dominance in the goats is less absolute than in mono-ovulatory species such as cattle, but the evidence suggests the presence of similar relationships, albeit with two potential ovulatory follicles developing rather than one (Ginther and Kot, 1994). The concept of co-dominance was introduced to explain that two largest follicles can be observed in each wave but specific studies regarding this aspect are still lacking (Ginther and Kot, 1994).

ENDOCRINOLOGICAL PROFILES

Reports on caprine follicular waves and their associations with endocrine profiles in goats during the estrous cycle are scarce. Endocrine events in peripheral blood during the estrous cycle have been studied in detail in ruminants i.e. goats (Chemineau *et al.,* 1982), ewes (Campbell *et al.,* 1990), and cattle (Sirois and Fortune, 1990). However, the temporal relationships between follicular dynamics and hormonal profiles have not yet been clarified throughout the goat estrous cycle. In ewes, a temporal relationship exists between elevations in mean daily serum concentrations of FSH and emergence of successive follicular waves (Ginther *et al.,* 1995; Bartlewski *et al.,* 1999). Surges of FSH were rhythmic and periodic (every 3 or 4 days, Gibbons *et al.,* 1999). The role of inhibin in regulating the production and secretion of FSH has been documented in goats (Medan *et al.,* 2003, 2005).

Follicular recruitment and selection is coordinated by endocrine and paracrine regulation involving changes in the secretion of gonadotropins and numerous growth factors (Hunter *et al.,* 2004). The number of codominant follicles and the number of ovulatory follicles can be increased by decreasing the sensitivity of the hypothalamo–pituitary–ovarian axis to the negative effect of estradiol to maintain the concentration of FSH above the threshold level for longer, thus allowing more time for follicles to pass through the so called, "widened gate" (Baird and Campbell, 1998; Hunter *et al.,* 2004; Scaramuzzi *et al.,* 2011; Nogueira *et al.,* 2015).

Early findings in goats showed that follicle waves developing under continuous and high progesterone concentrations have smaller follicles than those developing when progesterone concentration are low (Ginther and Kot, 1994). de Castro *et al.* (1999) suggests that goats with four follicular waves had higher progesterone levels during the early-mid luteal phase than those with two or three waves. Menchaca and Rubianes (2002) confirmed the relationship between progesterone concentrations and the number of follicular waves and also, documented differences in the estrogen profiles early in the luteal phase between goats with three and four follicular waves. Estradiol concentrations declined earlier in the four-wave goats, probably because progesterone concentrations decreased LH pulsatility and wave 2 emerged earlier. Early decrease of estradiol allowed an early rebound of the FSH concentrations, promoting the emergence of the second follicle wave. In consequence, the day of emergence of wave 3rd was also advanced and a fourth wave occurred in goats having four follicular waves. On the other hand, in the three-wave goats the emergence of wave 2nd was delayed until the decrease of estradiol concentrations, which occured later (Nogueira *et al.,* 2015). The inter-wave intervals between waves 2nd and 3rd did not differ among goats with three or four follicular waves. Serum concentrations of progesterone plays an important role in the control of follicular turnover. The effect is most probably mediated by the control of LH pulsatility as was postulated in cattle (Savio *et al.,* 1993). A shift from FSH dependence to LH dependence occurs in the last stages of follicular development (Baird *et al.,* 1991) and LH support is essential for the survival of the dominant follicle. The strong correlation exists between the CL diameter and the plasma progesterone concentrations (Samartzi *et al.,*1995; Amiridis *et al.,* 2002), which indicates that CL diameter can be used as an index of the peripheral progesterone concentration in goats (Medan *et al.,* 2003). Increasing concentrations of progesterone during the first follicular wave and persistence of elevated concentrations of progesterone during the second and third follicular waves prevents a pre-ovulatory LH surge (de Castro *et al.,* 1999; Menchaca and Rubianes, 2002; Nogueira *et al.,* 2015), resulting in a period of persistence of dominant follicles before atresia ensues. The onset of luteolysis and an LH surge coincident with the development of the fourth follicular wave in the majority of goats would contribute to a shorter duration of the fourth follicular wave compared to earlier waves (Ginther and Kot, 1994; de Castro *et al.,* 1999; Nogueira *et al.,* 2015; Sharma *et al.,* 2018b).

In conclusion, use of ultrasonography has opened up the flood gates of research in the area of follicular dynamics for improving the reproductive efficiency in small ruminants and more and more researchers are now focusing on the use of ultrasonography in different breeds of goats throughout the world on analogy to bovines. Meager literature is available on the correlation of various endocrine events along with ultrasonogphic findings and so the impetus has to be shifted from bovines to the small ruminants and goats in particular for economic development of the farming community as a whole.

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