

## FOLLICULAR DYNAMICS ITS REGULATION AND HEAT STRESS EFFECTS ON FOLLICULOGENESIS: A REVIEW

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Follicular dynamics is a continuous process of growth and regression of a group of antral follicles, one of which develops to attain pre-ovulatory stage (Lucy et al 1992). Follicles are formed when primary oocytes are enveloped by a single layer of flattened granulosa cells during pre-natal period. Hence, the primordial follicle is a simple structure consisting of primordial oocytes surrounded by a single layer of flattened granulosa cells demarcated from the stroma by the non-cellular basal lamina. This primordial follicle reserve formed during fetal life or soon after birth remains in the resting stage until they are stimulated (Erickson, 1966). Follicular growth pattern proceeds through integrated stages of follicular recruitment, selection and dominances. A group of primordial follicles are recruited periodically to grow and pass through a phase of growth till either ovulate or degenerate. Follicular growth is believed to commence in a centrifugal pattern within the ovary (Byskov et al., 1977).

Rajakoski (1960) examined cattle ovaries from slaughter house and observed that the number of follicles >5mm in diameter were not evenly distributed throughout the estrous cycle but appropriately exposed to two growth waves. He proposed the term follicular wave for this observed phenomenon. Later on with the advent of ultra-sonography technique to monitor follicle development on daily basis, several workers reported that follicle grow in a wave pattern (Savio et al 1988, Ginther et al 1989). However the follicular wave begins with the recruitment of a cohort of follicles from which a single follicle continues to grow while the other follicles undergo atresia (Wolfenson et al, 1997). Periovarian follicle stimulating hormone (FSH) appears to be critical for the recruitment of follicles (Adams et al, 1994) and the variability in the responsiveness to gonadotropic stimulation among follicles probably determines which follicle continues to grow (selection) from the recruited pool. Selected follicles exerts its dominance through inhibition of recruitment of additional follicles for the next wave (Adams et al 1992, Savio et al, 1993) and the dominant follicle appears to remain active until approximately days 10-11 of estrous cycle (Ginther et al, 1989). Under normal circumstances, the first dominant follicle (DF) regresses and initiation of the second follicular wave results in growth of the second active dominant follicle in the ovary. Maturation of the second dominant follicle of estrous cycle is often associated with regression of corpus luteum and, therefore, this follicle is ovulated after luteolysis.

Variation in number of follicular waves per cycle is detected in cattle 2 or 3 wave pattern (Ginther et al., 1995; Bartlewski et al., 2000), goat 2-5 waves (Ginther and Kot., 1994; Menchaka and Rubians, 2002; Meidan et al., 2003) and buffalo 2 or 3 wave (Baruselli et al., 1997; Lohan et al., 2003). Buffaloes have estrous cycle with one wave (3.3%), two waves (63.3%) or three waves (33.3%) of follicular growth (Baruselli et al., 1997), with the first wave beginning on day 1, the second around day 9-11 while the third wave appeared on day 17 of the estrous cycle. It is unclear whether two or more follicular waves reflect environmental conditions, a casual occurrence or are a specific characteristic of an individual.

### Regulators of follicular dynamics

The ovarian follicle is a balanced physiological unit whose structure and function depends on extra-cellular factors and complex system of intra-follicular relationship (Hafez and Hafez, 2000). However follicular development, differentiation and growth are controlled by intra-ovarian, intra-follicular and extra-ovarian endocrine and growth factors (Gurya,

1997). Follicular growth is regulated under the dynamic interaction between FSH and LH produced by pituitary (Greenwald and Roy, 1994). The pituitary gonadotrophin, ovarian steroids and non-steroidal factors interact to regulate the initiation of meiosis in fetal ovary, recruitment of primordial follicles and selection of ovulatory or atretic follicles (Tsafiriri and Adashi, 1994; Revahand and Butler, 1996; Rhodes et al., 1995). Cyclic growth and atresia of follicles occur during the mid luteal phase (Spicer et al., 1987), which characterized by high levels of progesterone in plasma and the concomitant alteration in gonadotropin production. Studies indicated that alterations in secretion of FSH and LH are related to follicle growth and recruitment of antral follicles in cattle. There is a minimal threshold of FSH concentration below which recruitment cannot proceed. This threshold appears to be variable between animals (Picton et al., 1991). A rise in the levels of both the gonadotropin (FSH & LH) prior to ovulation appears to be related to the formation of a large pool of small antral follicles during the post-ovulatory and early luteal periods (Driancourt et al., 1993). It is well established that gonadotropins are needed for the development of dominant follicles and production of estradiol -17- $\beta$  from these follicles (Driancourt et al., 1991 Driancourt et al., 1993). However it has been reported that Mean LH concentration, its frequency and amplitude were significantly higher during the follicular phase than during the luteal phase of the cycle ( Rao and Pandey, 1983).

All the follicles of a cohort develop in the same endocrine environment, however, the dominant follicle survives and the neighboring sub-dominant follicle die by apoptosis. It seems possible that individual follicles respond to their endocrine environments, thereby, controlling degeneration or continued growth. Prior to selection, there is a surge of circulating FSH that stimulates follicular recruitment (Evans et al., 1997) and LH appears to be minimally involved in the control of recruitment. Within 1 to 2 days the FSH levels drop significantly, which is key mechanism in selection. This decline was coincidental with dominant follicle selection, it was believed that the dominant follicle was selected because it contained more FSH receptors and could compete more effectively for gonadotropin than other subordinate follicles (Mihm et al., 1997). Follicles produce intra-follicular growth factors that contribute to the success of folliculogenesis (Webb et al., 1999) and the later stages of follicle development are reliant upon the gonadotropic hormones LH and FSH (Webb et al, 1992; Campbell et al, 1995). There is general agreement that LH is the key hormone involved in the final growth of the dominant follicle while other follicles in the cohort complete atresia. In contrast, estradiol production does not appear to be pre-requisite for follicular dominance. FSH induces aromatase activity within granulosa cells resulting in follicles to gain the ability to produce estradiol. It also stimulates the production of inhibin and follistatin. Low serum FSH levels maintained by inhibin and estradiol production in dominant follicle, are believed to be the indirect cause of atresia in the sub-ordinate follicles (Fortune, 1994).

Dominant follicles are exquisitely sensitive to LH, thus it is no surprise that change in the pattern of pulsatile LH secretion may alter their fate. Hence, inducing wave turnover (Stock and Fortune, 1993), reduction in dominant follicle could be induced by doubling concentration of circulating progesterone, which results in a 50% reduction in LH frequency. Dominant follicle finally either ovulates (during final wave) or loses its dominance and declines due to the feedback inhibition by elevated oestradiol levels which permits FSH surge and the recruitment of new cohort of follicles (Fortune, 1994). If regression of corpus luteum (CL) occurs coincident with

dominance phase, the dominant follicle ovulates in response to a pre-ovulatory gonadotropin surge. If luteal regression does not coincide with the dominance phase, the dominant follicle undergoes atresia, probably in response to rising titers of intra-ovarian factors involved with atresia.

### Heat Stress and follicular dynamics

Heat stress can be defined as the sum of forces external to a homeothermic animal that acts to displace body temperature from the resting stage (Yousef, 1984). The increase in body temperature caused by heat stress has direct adverse consequences on cellular function. The follicle destined to ovulate emerges as an antral follicle 41.5 days before ovulation (Lussier et al., 1987). Therefore, heat stress during the period of follicular growth has the potential to compromise the oocyte, either because of direct actions of elevated temperature on the oocyte or because of alterations in follicular function that could compromise oocyte quality. Some investigators employed ultrasonography to examine follicular growth. Badinga et al (1993) observed that acute heat stress reduced the size of the first wave dominant follicle by day 8 of the estrous cycle and the follicle contained less follicular fluid than that of non-heat stressed cow. Wilson et al (1998) also observed reduction in follicular size in heat stressed cattle and related it to decreased steroidogenesis within the theca cells, granulosa cells or both. The possible mediators of heat stress response were reduced variability of granulosa cells or more specific changes in steroidogenic enzyme aromatase activity in granulosa cells (Wolfenson et al, 1997).

Badinga et al (1994) observed follicular growth in lactating Holstein cows and reported that first follicular wave was characterized by development of a single layer follicle, which was consistently larger in April as compare to June, August and November. The first wave dominant follicle in April was associated with an earlier regression of the largest follicle and a sharper decrease in medium size follicles by day 9 of the estrous cycle. In contrast, the growth of the first wave dominant follicle was slower and largest sub-dominant follicle was more persistent in August than in April, June and November. This resulted in increase in number of medium size follicles between day 4 to 12 of the estrous cycle in August than in April, June and November. However Wolfenson et al., (1997) observed no difference in follicular diameter and follicular fluid of cows during summer, autumn and winter season, but the winter heat stressed cows had low follicular fluid volume than winter control cows.

Wolfenson et al., (1995) reported decrease number of small follicles to heat stressed cows. Perhaps the population of small follicles is particularly sensitive to physiological changes that occur during heat stress. Approximately 40 days are required for follicles to grow through the antral stage (0.13 to 8.56mm) in bovine, Lusier et al., 1981). A direct effect of heat stress on small follicles may explain why the effects of heat stress on fertility are observed in the late summer and fall (Thatcher and Collier, 1986). Developing small follicles that are damaged by heat stress during summer may ovulate an infertile oocyte or develop sub-functional corpus luteum (Howel et al., 1994).

Wilson et al., (1998) studied follicular growth from day 11 to 21 of the estrous cycle and reported that heifers subjected to heat stress tended to have smaller second wave dominant follicles than heifers in thermo-neutral environments. The second largest follicle increased in diameter till day 15 in both groups. The size of the follicle decreased rapidly between days 15 to 21 in heifers kept under thermo-neutral environment, however, in heat stressed heifers the follicle size decreased between days 15 to day 17 of the estrous cycle and remained unchanged thereafter.

Heat stress compromised ovarian follicular dynamics (Badinga et al., 1993) and the ability of the dominant ovarian follicle to exert dominance (Wilson et al, 1998). Heat stress induced depression of dominance of the largest follicle of first wave found to be associated with 2-3 days earlier emergence of the second wave dominant follicle / pre-ovulatory follicle (Wolfenson et al., 1995) and they suggested that this earlier emergence of pre-ovulatory follicle may result in ovulation of older follicles. The loss of follicular dominance may be related to reduced plasma concentrations of estradiol-17 $\beta$

(Wolfenson et al, 1995) and inhibin (Roth et al, 2000) and increased plasma concentration of FSH (Roth et al, 2000). The duration of dominance of pre-ovulatory follicle was found to be negatively co-related with fertility of cattle (Mihm et al, 1997). Goeloglu et al (2001) reported reduced follicular dominance during a follicular cycle in heat stressed dairy cows. The acute decrease in follicular dominance was reflected by a transit increase in number of class 3 follicles of days 7 and 8 of the follicular cycle that occurred immediately after the period of heat exposure for a 8 day period. The heat stressed induced decrease in follicular dominance as reflected by the acute increase in the number of class 3 follicles, which was not reflected in class 2 follicles on day 4 of the follicular cycle. Accordingly, Wolfenson et al (1995) observed an increase in the number of large follicles in heat stressed cows during days 6-10 of the estrous cycle. Collectively, these results and those of Wolfenson et al (1995) and Badinga et al (1994) indicate less follicular dominance in terms of higher environmental temperature. Perhaps this is due to a heat stress induced decrease in inhibin secretion as suggested by Wolfenson et al, 1995.

Elevated body temperature may also directly affect follicular function. Culture of theca cells at 40.50C reduced androstenedione production from cultured theca cells but generally had no effect on estradiol-17 $\beta$  production from cultured granulosa cells (Wolfenson et al, 1997). It is not clear whether effects of heat stress on follicular development are sufficient to alter subsequent fertility. Al Katnani et al., (1998) reported that indigenous cows experiencing air temperature > 20°C on day 10 before breeding had lower 90-d non-return rate than those cows experiencing air temperature > 20°C. Torres et al., (2008) demonstrated that in Gir cows (*Bos indicus*) heat stress exerted a delayed effect on reproductive functions, manifested by an increased incidence of large follicles, more follicular co-dominance and reduction in estrous cycle length, progesterone concentration and oocyte developmental capacity. Heat stress did not affect follicular recruitment, since the population of > 3 mm follicles and numbers of cumulus oophores complexes (COCs) were not affected. However, heat stress increased the number of large follicles and number of follicles > 9 mm characterized as follicular co-dominance. Heat stressed induced co-dominance which may compromise oocyte quality has been reported in cow (Sartori et al., 2004) and goats (Vikash et al., 2007).

### CONCLUSION:

Heat stress inhibited follicular growth and follicular dominance, decrease proestrus rise in estradiol-17 $\beta$  and induce smaller size of the second wave dominant follicle and longer luteal phase, decrease biosynthetic capacity of theca cells but has less effect on granulosa cells. These changes can account for the well documented low breeding efficiency during warm months in sub-tropical environments.

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