**Follicular dynamic and ovulation in cattle**

Summary

A review is given about follicular populations and aspects of follicular development in cattle. Ovaries of cattle contain two different pools of follicles, the non-growing pool and the growing pool. Entry of primordial follicles into the growth phase occurs throughout the reproductive life. Once follicles are recruited to grow, they are destined to undergo atresia or ovulation. Growth of obligatory gonadotropin-dependent follicles occurs in a wave like pattern. The growth waves are characterized by the processes of recruitment, selection and dominance. The known mechanisms responsible for these three processes are discussed.

 **Follicular populations**

Ovaries of cattle contain two different pools of follicles, the non-growing pool and the growing pool. The non-growing pool contains the primordial follicles, whereas the growing pool contains the primary, secondary and tertiary follicles.

 The primordial follicles continuously leave the arrested pool and undergo the primordial to primary follicle transition. The oocytes increase in size and the surrounding squamous pre-granulosa cells become cuboidal and proliferate to form a layer of cuboidal cells around the growing oocyte.(The follicle is called now a primary follicle).

 The mechanisms responsible for the initiation of follicular growth are poorly understood although some candidate molecules (gonadotropins, growth factors, *c-kit*) have been discussed .

 In vitro studies with rat ovarian tissue showed that leukaemia inhibitory factor (LIF) and basic fibroblast growth factor (bFGF) are able to promote the primordial to primary follicle transition. But the finding that during culture of isolated bovine or primate ovarian cortex almost all primordial follicles activate and develop into primary follicles suggests that non-cortical portions of the ovary may regulate the flow of follicles from the resting reservoir.

During recruitment of follicles into the growing pool theca cells organize into distinct layers around early developing follicles and establish essential cell-cell interactions with granulosa cells. In the process of theca cell differentiation, cells become epitheloid and acquire organelles characteristic for steroid secreting cells.

Once follicles are recruited to grow, they are destined to undergo apoptosis unless rescued by survival factors. The Fas antigen and the Fas ligand system seem to play an important role in mediating apoptosis

**Follicular waves**

In cattle the growth of obligatory gonadotropin-dependent follicles occurs in a wave like pattern. Waves of growth can be observed during the prepubertal period, in pregnant cattle, in postpartum periods and during estrous cycles. Normally three to six follicles with a diameter of 4 to 5 mm occur after recruitment of follicles into a follicular wave**.** From the cohort, one follicle is selected for continued growth and becomes dominant. If luteolysis occurs during the growth phase of dominant follicles, final maturation and ovulation occurs. If luteolysis does not occur during the growing and maintenance phase of follicles, the fate is atresia.

Changes in mRNA expression for the gonadotropin receptors, key steroidogenic enzymes and growth factors (IGF-I and -II) and their binding proteins (IGFBP) have been associated with different stages of follicular growth and atresia.

In general, expression of mRNA for the gonadotropin receptors, steroidogenic enzymes, and steroidogenic acute regulatory protein (StAR) increase with progressive follicular development and is highest when dominant follicles approach maximum size.

**Recruitment**

The concept recruitment is used for the entrance of follicles in the growing pool, but also for the processes associated with the entrance of follicles in a wave like growth pattern. FSH is the key hormone for the endocrine initiation of follicular wave occurrence**.**

two-wave heifers had two FSH surges and three-wave heifers had three apparent FSH surges during the interovulatory interval. Results of the follicular fluid experiments indicated that a surge in FSH necessarily preceded the emergence of a wave. The FSH surges in treated and control heifers began 2 - 4 days before the detectable (ultrasound) emergence of a follicular wave (follicles of 4 and 5 mm), peaked 1 or 2 days before emergence and began to decrease approximately when the follicles of a wave begin to diverge into a dominant follicle and subordinate follicles (follicles 6 - 7 mm).

it is found also that the surge reaches a peak by the time the follicles attain 4 mm in diameter. the emergence of each follicle wave postpartum was preceded by a 2- to 4-day rise in FSH concentrations.

**Selection**

***Selection means that the number of growing follicles is brought into line with the species-specific ovulation number.***

After recruitment fewer and fewer recruited follicles continue in growth until one follicle is selected to become dominant while the remaining members of the recruited follicles become static and undergo atresia via apoptosis.

The processes of selection occur under declining FSH concentrations and take 2 to 3 days. As follicles grew from 3 to 5 mm, they gained the capacity to suppress FSH; however, as follicles grew beyond 5 mm, FSH-suppressing capacity did not increase.

 At the beginning of selection, all of the growing follicles ≥ 5 mm contribute to the decline in FSH.

 It was demonstrated that inhibin neutralization during the early luteal phase produces hypersecretion of FSH with a coincident stimulation of follicular development, indicating that inhibin is an important factor for the negative regulation of FSH secretion during the early luteal phase when secretion of estradiol and progesterone are normally high. It is also stated that inhibin is the primary FSH suppressant at this time.

It isfound that the dominant follicle maintains high amounts of higher molecular weight inhibins while the subordinate follicles have increased amounts of the smallest inhibin. The deviation occurred when the 2 largest follicles were 8.3 ± 0.2 and 7.8 ± 0.2 mm in diameter. This was observed at 61.0 ± 3.7 h after wave emergence.

 It was found that the largest follicles reach means of 8.5 and 7.7 mm in diameter at the end of the selection period. Thereafter they begin to undergo deviation in diameters, which is characterized by continued growth of the largest follicle to become the dominant follicle and reduced or terminated growth of the remaining follicles to become subordinate follicles.

Into the blood, the released estradiol is involved in the continuing depression of FSH concentrations to below the requirement of the smaller follicles but not the largest follicle. In addition aspiration of the dominant follicle at any stage of the cycle affected circulating FSH but did not appear to influence the mean LH concentration. It was reported that apparently both estradiol and inhibin contribute to the continuing FSH decline from this time. Moreover it was found that elevated concentrations of LH and reduced concentrations of FSH were present 32 to 16 h before to at least 24 h after the beginning of follicle deviation.

In heifers, receptors for LH appeared in the granulosa cells of the future dominant follicle about 8 h before the beginning of deviation.

The LH stimulates the production of estradiol and insulin-like growth factor-1. These intrafollicular factors and perhaps others account for the responsiveness of the largest follicle to the low concentrations of FSH. The smaller follicles have not reached a similar developmental stage and because of their continued and close dependency on FSH become susceptible to the low concentrations.

In the past decade, numerous intrafollicular growth factors, such as inhibins, activins, and insulin-like growth factors and their binding proteins, have been identified in follicular fluid of individual bovine follicles. **The IGF stimulate ovarian function by acting synergistically with gonadotropins to promote growth and steroidogenesis of ovarian cells.** Actions of IGF-I and -II are restrained by a series of IGF binding proteins (IGFBP) that either originate from the blood or are synthesized locally within the follicle. Degradation and differential synthesis of IGFBP are important mechanisms regulating IGFBP amounts. The relative amounts of IGFBP may ultimately determine ovarian IGF action. It was found that the future dominant follicle in most cohorts had the highest estradiol and lowest IGFBP-4 concentrations compared with future subordinate follicles. It was concluded that IGFBP-4 and estradiol may have key roles in determining the physiological fate of follicles during selection of the first wave dominant follicle and that both are reliable markers to predict which follicle in a growing cohort of 5 to 8.5 mm follicles becomes dominant. In addition, maintenance of low amounts of intrafollicular IGFBP4 may constitute an important mechanism in the future DF to attain FSH independence. In contrast to that atresia of subordinate follicles appears to be associated with increased expression of the IGFBP2 gene.

invitro studies complete the results obtained in vivo. It was found that granulosa cells from the dominant follicle produce more estradiol than cells from subordinate follicles. Shortly after selection, dominant follicles have higher levels of mRNAs for gonadotropin receptors and steroidogenic enzymes.

In conclusion, the decline in FSH beginning after Day 2 of the heifer oestrous cycle causes differential alterations in FSH dependent growth factors and hormones within the cohort of preselection follicles, simultaneously inducing growth and enhanced estradiol producing capacity of the DF and atresia of subordinate.

**Dominance**

Follicles are functionally dominant (capable of ovulating after luteal regression) while they are still growing and early during their plateau in growth. Follicles acquired ovulatory capacity at about 10 mm, corresponding to about 1 day after the start of follicular deviation, but they required a greater LH dose to induce ovulation compared with larger follicles. It was speculated that acquisition of ovulatory capacity may involve an increased expression of LH receptors on granulosa cells of the dominant follicle and that this change may also be important for further growth of the dominant follicle (SARTORI et al., 2001).

Observations of GINTHER et al. (1997) indicate that the future dominant follicle cannot be identified reliably by either its diameter or estradiol production before the deviation in growth rates between the two largest follicles. Dominance appears to be maintained by negative feedback effects of products of the dominant follicle on circulating FSH. Selection and dominance are accompanied by progressive increases in the ability of theca cells to produce androgen and granulosa cells to aromatise androgen to estradiol. Dominant follicles grow to a much larger size than all the other follicles (from 8.5 mm at the end of selection to 12 – 20 mm). This takes 3 to 4 days.

LH pulses are indispensable for follicle development beyond 9 mm in diameter (GONG et al., 1996; KANITZ et al., 2001). Endocrine pattern of gonadotropins is followed by characteristic changes in follicles. Concentrations of estradiol-17 beta in follicular fluid and aromatase activity of follicular walls were higher in dominant follicles compared to subordinate follicles. Aromatase activity in first-wave dominant follicles was higher at Days 5 and 8 (BADINGA et al., 1992). Follicular fluids were analysed for concentrations of estradiol-17 beta (E2) and progesterone (P4). Dominant follicular growth was associated with increase in the concentration of E2 and P4 in the follicular fluid, which was E2-dominated.

The fate of the dominant follicle depends on function of the Corpus luteum. In the cases of elevated progesterone concentrations the dominant follicle becomes atretic due to the negative influence of the progesterone on pulsatility of LH secretion (IRERLAND et al., 2000).

Under these circumstances functional dominance is lost some time between the early and late plateau phases (between days 7 and 9 of the oestrous cycle), while the follicle is still morphologically dominant (i.e. the largest follicle). Loss of dominance occurs after a decline in estradiol secretion (around day 6) of the dominant follicle of the first follicular wave (SUNDERLAND et al., 1994). A decrease in follicular estradiol and inhibin-A secretion is going in front of the new wave of follicular growth (GINTHER et al., 1996; MIHM et al., 2002).

From the available data following conclusions were drawn (ADAMS, 1999):

(1) follicles grow in a wave-like fashion; (2) periodic surges in circulating FSH are associated with follicular wave emergence; (3) selection of a dominant follicle involves a decline in FSH and acquisition of LH responsiveness; (4) periodic anovulatory follicular waves continue to emerge until occurrence of an LH surge; (5) within species, there is a positive relationship between the duration of the oestrous cycle and the number of follicular waves; (6) progesterone suppresses LH secretion and growth of the dominant follicle; (7) the duration of the interwave interval is a function of follicular dominance, and is negatively correlated with circulating FSH; (8) follicular dominance in all species is more pronounced during the first and last follicular waves of the oestrous cycle and (9) pregnancy, the prepubertal period and seasonal anoestrus are characterized by regular, periodic surges in FSH and emergence of anovulatory follicular waves.