Comparative Aspects of Follicular Development, Follicular and Oocyte Maturation and Ovulation in Cattle and Pigs

Summary
The paper describes comparatively aspects of oogenesis and folliculogenesis, oocyte and follicular growth in cattle and pigs. Moreover, gonadotropin dependent regulatory processes of the wave like pattern of follicular growth are discussed in both species as well as functional and morphological changes during oocyte maturation. Finally, time dependent processes after occurrence of spontaneous or GnRH induced LH surge are discussed.

Key Words: cattle, pigs, oogenesis, folliculogenesis, oocyte growth, follicular growth, gonadotropins, oocyte maturation, ovulation

Oogenesis and Folliculogenesis
The formation of primordial germ cells (PGCs) indicates the commencement of oogenesis. During early fetal life the primordial germ cells which arise in the yolk sac endoderm at the caudal aspect of the embryo (McGEE et al., 1998) migrate via meso- and entoderm to the presumptive gonads. The migration of the PGCs toward the presumptive ovaries based initially on passive transport and later on amoeboid-like movement in response to chemotactic substances like transforming growth factor β (PICTON and GOSDEN, 1998). In cattle the first potential primordial germ cells were identified in an 18-day-old embryo. In 23- to 25-day-old embryos putative primordial germ cells (alkaline phosphatase- and lectin-positive) were situated predominantly in the axial body region at the level of the mesonephros. When the gonadal ridge develops in this region (about day 27) it contains a certain number of primordial germ cells present from the very beginning (WROBEL and SÜSS, 1998). It is well known that PGCs play an indispensable role in the induction of gonadal development (VAN VOORHIS, 1998). In the pig PGCs display pseudopods and
appeared to migrate from the dorsal mesentery of the hindgut (day 18) to the primordium of the gonad (day 23), and enter the genital ridge by day 26. The number of 3-Fucosyl-N-acetyl-lactosamin positive cells associated with the dorsal mesentery and genital ridge markedly increased from the 18-day to the 26-day pig embryo (TAKAGI et al., 1997). During migration of the PGCs to the female gonads cells undergo a species-specific number of mitosis.

PGCs lose their motility after arriving at the developing ovaries. They are called now oogonia and they show a high frequency of mitotic division. In cattle BECKERS et al. (1996) found highest mitotic activity of oogonia from day 45 to 150 of fetal development (approximately 2 million oogonia). Investigation has shown that meiosis starts in oogonia from day 80. The highest number of germ cells (oogonia plus oocytes) was present around day 110 of fetal development. ERICKSON (1966) found that at this time more than 2.7 million germ cells were present in both ovaries. In fetal pigs the highest number of germ cells (approximately 1.1 million) was observed on day fifty (BLACK and ERICKSON, 1968). Similar data (0.9 to 1.4 million germ cells in dependence on breed) were reported by WISE et al. (2001). Until birth the number of germ cells decreased by more than 50 % (BLACK and ERICKSON, 1968). Apoptosis is predominantly involved in this decrease as it was shown in studies on human oogonia and oocytes (De POL et al., 1998; DRIANCOURT et al., 1998).

Oogonial mitotic proliferation is restricted to prenatal development and shortly after birth in cattle and pigs (ERICKSON, 1966; BLACK and ERICKSON, 1968). On day 270 of fetal development in cattle 98 % of all germ cells were oocytes and only 2 % oogonia (ERICKSON, 1966). The process of mitotic division of oogonia leads to the finite number of oocytes for the reproductive life. When oogonia stop mitotic divisions they become arrested in prophase of the first meiotic division. They are called now primary oocytes.

Coincident with the initiation of meiosis oocytes become enclosed in a single layer of pregranulosa cells, surrounded by a thin basement membrane. These very early stages of follicular development are called primordial follicles. Primordial follicles represent the nongrowing pool of the whole follicular population. The size of the store of primordial follicles used for folliculogenesis is the consequence of three processes: (1) oogonia multiplication, (2) time of meiosis initiation and (3) extent of loss of germ cells. Apoptosis is causing this loss, but its mechanisms are poorly documented. Apoptosis also occurs within primordial follicles in adult ovaries and involves oocyte death (DRIANCOURT et al., 1998; MATSUI, 1998). More than 99 % of ovarian follicles endowed at early life are destined to undergo apoptosis and the exhaustion of these follicles serves as a clock for female reproductive senescence. Once a cohort of follicles is recruited to grow, it is destined to undergo apoptosis unless rescued by survival factors (HSUEH et al., 1996). At puberty the pool of primordial follicles will be around 210,000 and 420,000 in cattle and pigs respectively (GOSDEN and TELFER, 1987; TELFER, 1996).

Oocyte and follicular growth

Before oocytes reach their competence to resume nuclear maturation for further fertilization and cleavage they have to grow. During this growth phase several cytological changes occur. Oocytes acquire a complex cytoplasmic organisation dependent on both, new gene products and organelles, and on the modification and
redistribution of existing ones. In growing oocytes from cattle and pigs high levels of RNA synthesis have been observed. The synthesis and uptake of macromolecules into the oocyte serve two purposes. The first is for growth, development and maturation of the oocyte itself. The second one is for the storage of RNA and proteins for the postfertilization development (PICTON and GOSDEN, 1998). During growth phase oocytes increase their diameter from 20 µm to 130 µm in cattle and from approximately 40 µm to 120 µm in pigs. Oocyte and follicular growth are two processes of one functional system. Granulosa cells are coupled by gap junctions with the oocyte and influence actively oocyte growth by providing the oocyte with a variety of biologically active molecules. On the other hand the oocyte actively controls proliferation, morphogenesis and differentiation of the granulosa cells (PICTON and GOSDEN, 1998).

Entry of primordial follicles into the growth phase occurs throughout the reproductive life. The transition of follicles from the non-growing into the growing pool is characterized by conversion of the flattened pregranulosa cells into a single layer of cuboidal granulosa cells. The follicle is called now a primary follicle. Up to now the mechanisms responsible for the initiation of follicular growth are poorly understood although some candidate molecules (gonadotropins, growth factors, c-kit) have been discussed (WEBB et al., 1999). The number of follicles commencing growth in a given time is predictable because it is a function of the size of the primordial store, which declines exponentially with time (WEBB et al., 1999). In the growing follicle the zona pellucida is formed around the oocyte. Whether the zona pellucida is a product of the oocyte, the surrounding granulosa cells or both remains not fully understood (VAN VOORHIS, 1998) although the mRNA for zona antigens were found in oocytes and granulosa cells of porcine follicles (KÖLLE et al., 1996) and bovine follicles (KÖLLE et al., 1998).

Proliferation of granulosa cells accounts for the further development of the primary follicle. Oocytes of these follicles become surrounded by several layers of granulosa cells. The follicles of this developmental stage are called secondary follicles. Secondary follicles are served now by one or two arterioles with capillaries just outside the basement membrane. This blood supply allows the follicle to be exposed to factors circulating in the blood. As secondary follicles enlarge, stromal cells near the basement membrane differentiate and form the theca interna and the theca externa. Laminin, a major component of the basal lamina, is known to be important in the differentiation of epithelial cells. The outer granulosa cell layer of ovarian follicles is attached to a basal lamina surrounding the follicle, and it has been demonstrated that proteins of the basal lamina can alter the steroidogenic capacity and cytoskeletal composition of mature granulosa cells (LEE et al., 1996). In the process of theca cell differentiation cells become epitheloid and acquire organelles characteristic for steroid secreting cells.

In cattle follicles need approximately 180 days for the growth from 0.1 mm to the ovulatory stage (CAMPBELL et al., 1995). Antrum formation was observed at 0.3 mm (PÖHLAND, pers. commun.). In pigs almost all follicles > 0.4 mm had an antrum. Based on estimated growth rates, an activated primordial follicle requires 84 days to reach the antral stage and 14 days in addition to reach a diameter of approximately 3 mm (MORBECK et al., 1992). Table 1 summarizes data about follicular and oocyte populations in cattle and pigs.
Table 1
Follicular and oocyte populations in cattle and pigs (data adapted from LUSSIER et al., 1994 and MORBECK et al., 1992) (Follikel- und Oozytenpopulationen bei Rindern und Schweinen)

<table>
<thead>
<tr>
<th>Stage of follicular development</th>
<th>Parameter</th>
<th>Cow</th>
<th>Pig</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Diameter of follicles (µm)</td>
<td>40 - 100</td>
<td>35 - 100</td>
</tr>
<tr>
<td></td>
<td>Diameter of oocytes (µm)</td>
<td>20 - 45</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>Duration of growth (days)</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Preantral stages</td>
<td>Diameter of follicles (µm)</td>
<td>100 - 150</td>
<td>150 - 300</td>
</tr>
<tr>
<td></td>
<td>Diameter of oocytes (µm)</td>
<td>45 - 60</td>
<td>68 - 90</td>
</tr>
<tr>
<td></td>
<td>Duration of growth (days)</td>
<td>10</td>
<td>26</td>
</tr>
<tr>
<td>Preantral to early antral stages</td>
<td>Diameter of follicles (µm)</td>
<td>150 - 250</td>
<td>300 - 400</td>
</tr>
<tr>
<td></td>
<td>Diameter of oocytes (µm)</td>
<td>60 - 100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Duration of growth (days)</td>
<td>15</td>
<td>10</td>
</tr>
<tr>
<td>Antral stages</td>
<td>Diameter of follicles (mm)</td>
<td>0.2 - 3.7</td>
<td>0.4 - 1.5</td>
</tr>
<tr>
<td></td>
<td>Diameter of oocytes (µm)</td>
<td>110 - 126</td>
<td>105</td>
</tr>
<tr>
<td></td>
<td>Duration of growth (days)</td>
<td>19</td>
<td>11</td>
</tr>
<tr>
<td>Antral preovulatory</td>
<td>Diameter of follicles (mm)</td>
<td>3.7 -&gt; 8.5</td>
<td>1.5 - 3.5</td>
</tr>
<tr>
<td></td>
<td>Diameter of oocytes (µm)</td>
<td>135</td>
<td>115 - 120</td>
</tr>
<tr>
<td></td>
<td>Duration of growth (days)</td>
<td>8</td>
<td>3</td>
</tr>
</tbody>
</table>

In both species the growth of obligatory gonadotropin-dependent follicles occurs in a wave like pattern (PIERSON and GINTHER, 1988; SAVIO et al., 1988; SIROIS and FORTUNE, 1988; DRIANCOURT et al., 1991; FORTUNE, 1994; BURKE et al., 2000). In cattle two (GINHER et al., 1989; KNOFP et al., 1989; RAJAMAHENDRAN and TAYLOR, 1991; AHMAD et al., 1997, BURKE et al., 2000; BELLMANN 2001) three (SAVIO et al., 1988; SIROIS and FORTUNE, 1988; AHMAD et al., 1997, BURKE et al., 2000; BELLMANN 2001) or four waves (RHODES et al., 1995) have been observed during oestrus cycle. Cycles with three waves were on average 1.1 day longer and corpora lutea regressed later than in animals with two waves. Moreover, interval from detection of dominant follicle to ovulation and duration of dominance were shorter in animals with three waves (AHMAD et al., 1997). Normally three to six follicles with a diameter of 4 to 5 mm occur after recruitment of follicles into a follicular wave (SAVIO et al., 1988; SIROIS and FORTUNE, 1988; SUNDERLAND et al., 1994). However, the number of recruited follicles seems to be higher (BELLMANN, 2001).

In contrast pigs express only one wave of follicular activity during oestrus cycle (DRIANCOURT, 1991; RATKY and BRÜSSOW, 1998). Results of LOBCHENKO (1990) and BRÜSSOW et al. (1994) indicate that also in the pig the number of recruited follicles is higher than the number of selected follicles. QUESNEL et al. (1998) report that about 50 follicles are simultaneously recruited. Among the cohort of recruited follicles 15 to 25 follicles are selected to form the ovulatory population.

Gonadotropin dependent regulation of wave like follicular growth

Both in cattle and in pigs, GnRH, FSH and LH are key hormones for the endocrine regulation of follicular wave occurrence. In principal, GnRH is released from the hypothalamus in a pulsatile pattern and binds to the GnRH receptor on the
gonadotroph cell surface of the anterior pituitary gland to stimulate the synthesis and release of FSH and LH. The gonadotropins FSH and LH are heterodimeric molecules comprising a common α-subunit and a hormone-specific β-subunit. Transcriptional regulation of gonadotropin subunits involves basal gene expression and GnRH upregulated gene expression as well. Moreover, synthesis and secretions of FSH and LH are both positively and negatively regulated by steroids and gonadal peptides (BROWN and McNEILLY, 1999). The gonadotropins FSH and LH bind to their receptors on follicular cells and induce processes of proliferation and differentiation.

In cattle waves of follicular activity are characterized by processes of recruitment, selection and dominance (DRIANCOURT, 1991; MIHM et al., 1996). At the beginning of each wave, from a cohort of growing follicles a single follicle continues in growth and becomes dominant. The other follicles of the cohort finish their growth and regress. FSH concentrations increase before follicular wave emergence. During the parallel growth phase of the recruited follicles FSH concentrations decrease and reach a nadir at the end of this phase and the beginning of deviation (GINTEGER et al., 1997, 1999; BERGFELT et al., 2000; GINTHER et al., 1999, 2000). The decline of FSH concentrations to basal levels is attributed to estradiol secretion of the future dominant follicle (GINTEGER, 2000). Results of XU et al. (1995), MIHM et al. (1996, 2000), BAO et al. (1997a,b), SCHAMS et al. (1999), and BEG et al. (2001) indicate that the differentiated expression of the mRNA for steroidogenic enzymes (P450 side chain cleavage enzyme, 3β-hydroxysteroid dehydrogenase, P450 aromatase) in theca and granulosa cells, the expression of the mRNA for the LH receptor in granulosa cells of follicles > 8 mm in diameter and the bioavailability of IGF I in association with IGFBPs play an important role in reaching dominance of one follicle.

As in the rat (RICHARDS et al., 1980), in pigs the increased FSH concentration after ovulation is thought to recruit a cohort of follicles to grow. Additional FSH infusion during the immediate postovulatory period increased the number of recruited follicles (RATKY and BRÜSSOW, 1998). However, data from FOXCROFT and Van de VIEL (1982), SHAW and FOXCROFT (1985) and GUTHRIE and BOLT (1990) demonstrate that an increase in FSH concentration may not be a prerequisite for follicular recruitment. The recruitment occurs between day 14 and 16 of the oestrous cycle, coincident with luteolysis (FOXCROFT and HUNTER, 1985).

Changing the ratio of progesterone and gonadotropin induces further emergence of ovulatory follicles (GUTHRIE and BOLT, 1990). Follicles undergo the process of selection until day 17/19 of the oestrous cycle (CLARK et al., 1982). During this follicular phase FSH concentrations decline to basal levels and remain low until the preovulatory LH surge (GUTHRIE and BOLT, 1990). The growth rate of the selected follicles was approximately 1mm per day (RATKY et al., 1995). FSH application from day 4 to day 11 of oestrous cycle (Tab. 2) can prevent the process of selection and significantly increase the number of follicles on the surface of the ovaries (RATKY and BRÜSSOW, 1998).

Dominance of follicles seems to be less pronounced in pigs as compared to monotocous species. However, studies conducted during the follicular phase of the oestrous cycle by CLARK et al. (1982) and FOXCROFT and HUNTER (1985) suggested that large follicles create a functional block in the development of non-dominant follicles. In addition, GUTHRIE et al. (1993) found abnormal distributions for estradiol-17β and some larger follicles appeared to be less mature. The lack of
atresia in largest follicles coupled with extensive atresia in medium-sized follicles during the follicular phase suggests that the concept of dominance described in monoovular mammals may be also valid in swine (QUESNEL et al., 1998). Data in Table 3 summarize knowledge about regulatory aspects of follicular waves in cattle and pigs.

Table 2
Mean total number of follicles and follicles > 5 mm in diameter in dependence on FSH treatment between day 4 and 11 of oestrous cycle (RATKY and BRÜSSOW, 1998) (Durchschnittliche Gesamtfollikelzahl und Anzahl Follikel > 5 mm Durchmesser in Abhängigkeit von einer FSH-Behandlung zwischen den Zyklustagen 4 und 11)

<table>
<thead>
<tr>
<th>Day of oestrous cycle</th>
<th>Group</th>
<th>Number of follicles (n) mean ± SD</th>
<th>Number of follicles &gt; 5 mm (n) mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>d 4</td>
<td>Control</td>
<td>14.6 ± 5.1</td>
<td>17.2 ± 4.1</td>
</tr>
<tr>
<td></td>
<td>FSH</td>
<td>15.0 ± 7.6</td>
<td></td>
</tr>
<tr>
<td>d 18</td>
<td>Control</td>
<td>23.4 ± 5.0</td>
<td>17.2 ± 4.1</td>
</tr>
<tr>
<td></td>
<td>FSH</td>
<td>42.4 ± 11.8</td>
<td>25.4 ± 16.2</td>
</tr>
</tbody>
</table>

Table 3
Regulatory and numeric aspects of follicular waves in cattle and pigs (Regulatorische und numerische Aspekte von Follikelwellen bei Rindern und Schweinen)

<table>
<thead>
<tr>
<th>Regulated processes</th>
<th>Cattle</th>
<th>Pig</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recruitment</td>
<td>FSH †</td>
<td>FSH †</td>
</tr>
<tr>
<td>Number of follicles (n)</td>
<td>24 to 28 follicles &gt; 1 mm</td>
<td>to 50</td>
</tr>
<tr>
<td>Selection</td>
<td>FSH †/ Estradiol †/ Inhibin †</td>
<td>FSH †/ Progesterone †/ Estradiol †</td>
</tr>
<tr>
<td>Number of follicles (n)</td>
<td>4 to 6 follicles &gt; 4 to 5 mm</td>
<td>20 to 50 follicles 2 to 4 mm</td>
</tr>
<tr>
<td>Dominance</td>
<td>IGF / IGF-BP/FSH-R/LH-R</td>
<td>?</td>
</tr>
<tr>
<td>Number of follicles (n)</td>
<td>1 follicle &gt; 8 to 9 mm</td>
<td>15 follicles &gt; 6 to 9 mm</td>
</tr>
</tbody>
</table>

Our experiments with a long acting GnRH agonist in a depot formulation gave evidence, that LH and its pulsatile secretion is necessary for maturation of follicles in cattle and pigs. In heifers, a GnRH agonist given in depot formulation and acting over 28 day long period caused an immediate LH release (data not shown), which was followed by a significant decrease of LH pulsatility (Fig. 1).

Fig. 1: Characteristics of LH secretion in a control (A) and a GnRH agonist depot treated heifer (B) 15 days after application of solvent or GnRH agonist. Pulses of LH secretion are signed with asterisk (BELLMANN, 2001) (Charakteristika der LH-Sekretion bei einem mit A bezeichnetem Kontrolltier und einem mit B bezeichnetem Versuchstier 15 Tage nach der Applikation eines Lösungsmittels bzw. eines GnRH-Agonisten in Depotformulierung. LH-Pulse sind durch Sternchen gekennzeichnet)
Table 4
Mean number of LH pulses in 6 hours on day 1 to 5, 11 and 15 in control heifers or animals treated with a GnRH agonist in depot formulation (BELLMANN, 2001) (Durchschnittliche LH-Pulszahl in 6 Stunden an den Tagen 1 bis 5, 11 bzw. 15 bei Kontrolltieren bzw. Versuchstieren, die GnRH-Agonist in Depotformulierung erhielten)

<table>
<thead>
<tr>
<th>Day</th>
<th>Control Animals (n)</th>
<th>Pulses (LS-Means ± SEM)</th>
<th>GnRH agonist depot Animals (n)</th>
<th>Pulses</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>13</td>
<td>2.2 ± 0.5</td>
<td>14</td>
<td>0.7 ± 0.4</td>
<td>0.02</td>
</tr>
<tr>
<td>2</td>
<td>11</td>
<td>3.0 ± 0.5</td>
<td>n = 12</td>
<td>0.9 ± 0.5</td>
<td>0.01</td>
</tr>
<tr>
<td>3</td>
<td>9</td>
<td>4.6 ± 0.5</td>
<td>10</td>
<td>1.0 ± 0.5</td>
<td>0.001</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>3.8 ± 0.8</td>
<td>8</td>
<td>1.4 ± 0.7</td>
<td>0.04</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>4.1 ± 0.6</td>
<td>10</td>
<td>0.9 ± 0.5</td>
<td>0.001</td>
</tr>
<tr>
<td>11</td>
<td>7</td>
<td>3.3 ± 0.4</td>
<td>10</td>
<td>1.4 ± 0.4</td>
<td>0.01</td>
</tr>
<tr>
<td>15</td>
<td>7</td>
<td>3.7 ± 0.5</td>
<td>10</td>
<td>0.8 ± 0.4</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Heifers treated with the GnRH agonist had significantly less LH pulses from d 1 to d 5 and on day 11 and 15 after treatment (Tab. 4). As a result of reduced LH pulsatility the diameter of the largest follicle of the second follicular wave was significantly lower in treated (7.2 ± 1.0 mm) than in control animals (14.0 ± 1.6 mm). Similar results were obtained in our experiments with pigs (BRÜSSOW et al. 1994, 1996). After treatment with GnRH in depot formulation follicles reached only a diameter ≤ 4 mm. This is an agreement with results from DRIANCOURT et al. (1995) obtained after application of a GnRH antagonist.

The pulsatile LH secretion with low frequency but high amplitude supports the maintenance of corpora lutea. This is evident in cattle and pigs. However, the luteal phase is shorter in pigs compared to cattle. Progesterone concentrations start to decrease after day 13 in pigs but after day 15 in cattle. LH secretion with high frequency/low amplitude pattern initiates the maturation of follicles and their estradiol synthesis (QUESNEL et al., 1998). This is the prerequisite for the priming of the pituitary and the positive estradiol feedback. In both species, increasing estradiol concentrations present for a longer time induce the preovulatory LH surge.

Oocyte meiotic maturation

The maturation of oocytes depends on communication between follicle cells and oocytes, and processes in the follicle cells are under control of gonadotropins. BALTAR et al. (2000) found an inverse relationship between follicular diameter and binding capacity for LH/hCG. Oocytes are arrested in prophase I at the so-called germinal vesicle stage (GV). They overcome this arrest either after hormonal induction or spontaneously after removal from antral follicles. Both in cattle and pigs cAMP plays an important role in maintaining meiotic arrest in oocytes (RICE and McGAUGHEY, 1981; AKTAS et al., 1995a,b). The control of meiotic maturation involves a complex interplay between somatic and germ cells. In both species meiotic maturation is triggered by the endogenous preovulatory gonadotropin surge. Resumption of meiosis appears to be associated with a decrease in cAMP concentrations within the oocyte caused by elevated LH concentration. However, the reduction in cAMP concentrations is not the only cause for final maturation of the oocyte. There is evidence that the production of active maturation-promoting factor (MPF) in the ooplasm is involved in the processes of nuclear maturation. MPF consist
of two components: a 34 kDa protein and cyclin B. Although oocytes from cattle and pigs do not have the same kinetic of meiotic maturation, MPF activity is low in GV-stage, increases during GVBD, and become high at both metaphase I and metaphase II. The process of meiotic maturation is characterised by nuclear membrane breakdown, which occurs in cattle oocytes 9 to 12 h (HYTTEL et al., 1989) and in pig oocytes 24 h (TORNER et al., 1998) after LH peak. Moreover, during maturation the number of mitochondria decreases and these organelles are located more perinuclear. Cortical granules, originating in the Golgi move to the periphery of the egg complex (STRÖMSTEDT and BYSKOV, 1998).

Together with changes in nuclear configuration (e.g. chromatin condensation, GVBD, spindle formation), meiotic maturation of pig and cattle oocytes is accompanied by extensive phosphorylation/dephosphorylation of cytoplasmic proteins (LEIBFRIED-RUTLEDGE et al., 1989; KASTROP et al., 1990). At least two major kinases or kinase cascades are involved in these processes, namely cdc2 kinase (catalytic part of the maturation promoting factor MPF, with the regulatory subunit cyclin B) and mitogen-activated protein kinase (MAPK). Analysis of bovine and porcine oocytes during IVM show that both kinases become activated during the resumption of meiosis (KUBELKA et al., 1995; TORNER et al., 2001; Fig. 2), however, the physiological targets of these kinases are known only partially. Apart from other substrates, MAP

**Fig. 2: Activity of cdc2- and MAP Kinase during maturation in vitro of bovine oocytes. A: Analysis of the phosphorylation of MAP kinase subclasses ERK1 and ERK2 during IVM by band shift assay. B: Analysis of activities of cdc2 kinase and MAP kinase during IVM by in vitro kinase assay. C: Evaluation of cdc2 kinase (solid line) and MAP kinase (broken line) activities from a gel depicted in Fig 2B (adapted from TORNER et al., 2001) (Aktivität von cdc2- und MAP-Kinase während der meiotischen Reifung boviner Oozyten in vitro. A: Analyse der Phosphorylierung von MAP-Kinasesubklassen ERK1 und ERK2. B: Analyse der Aktivität von cdc2-Kinase und MAP-Kinase. C: Quantifizierung der cdc2- und MAP-Kinaseaktivität)**
kinase can activate transcription factors (JOHNSON and VAILLANCOURT, 1994) and ribosomal subunit S6 kinase (KALAB et al., 1996), and therefore it seems to be involved in the regulation of gene expression.

Cdc2 kinase is believed to be responsible for GVBD and chromosome condensation during the first meiosis, and it was also shown to act as Histone H1 and lamin kinase, at least in vitro (PETER et al., 1990; VERDE et al., 1990). The essential role of protein kinases in resumption of meiosis is also documented by the fact that the inhibition of cdc2 kinase in GV-stage oocytes prevents the onset of meiotic maturation (KRISCHEK and MEINECKE, 2000; KUBELKA et al., 2000).

Meiotic maturation is also characterised by a strong increase in protein synthesis around the time of GVBD. This event is tightly correlated with activation of MAP kinase and with phosphorylation of the translational initiation factor eIF4E (TOMEK et al., 2001). Since no de novo transcription can be observed at this time point, the proteins synthesised must originate from a dormant and subsequently activated mRNA pool. Thus, the regulation of gene expression at this stage of development is regulated at the level of translation. The cytoplasmic polyadenylation of mRNA is one mechanism to activate the transcripts. There is some evidence that this occurs in oocytes and cumulus cells during preovulatory maturation of porcine oocytes (TORNER et al., 1998b) and also during IVM of bovine oocytes (unpublished results), but the mRNAs involved in this activation process remain to be analysed.

Table 5
COC morphology in relation to a hCG application after oestrous synchronisation in pigs (TORNER et al., 1998a)
(COC-Morphologie in Beziehung zu einer hCG-Applikation nach Brunstsynchronisation beim Schwein)

<table>
<thead>
<tr>
<th>Time in relation to hCG (h)</th>
<th>Number of COC (n)</th>
<th>Portion of COC in different classes (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Compact</td>
<td>Slightly expanded</td>
</tr>
<tr>
<td>-2</td>
<td>85</td>
<td>42.2</td>
</tr>
<tr>
<td>10</td>
<td>47</td>
<td>25.5</td>
</tr>
<tr>
<td>22</td>
<td>60</td>
<td>0</td>
</tr>
<tr>
<td>34</td>
<td>59</td>
<td>0</td>
</tr>
</tbody>
</table>

Numbers with different superscripts are significantly different in columns p < 0.05

Table 6
Meiotic configuration in oocytes in relation to hCG application after oestrous synchronisation in pigs (TORNER et al., 1998a) (Meiosestadien in Oozyten von Schweinen in Beziehung zu einer hCG-Applikation nach Brunstsynchronisation)

<table>
<thead>
<tr>
<th>Time in relation to hCG (h)</th>
<th>Number of COC (n)</th>
<th>Portion of oocytes (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>immature (GV)</td>
<td>resumption of meiosis (GVBD-A I)</td>
</tr>
<tr>
<td>-2</td>
<td>81</td>
<td>44.4</td>
</tr>
<tr>
<td>10</td>
<td>42</td>
<td>66.6</td>
</tr>
<tr>
<td>22</td>
<td>58</td>
<td>17.2</td>
</tr>
<tr>
<td>34</td>
<td>57</td>
<td>0</td>
</tr>
</tbody>
</table>

Numbers with different superscripts are significantly different in columns p < 0.01

Although there are some differences between bovine and porcine oocytes in the timing of specific events occurring during meiotic maturation, and also in the sensitivity to some kinase inhibitors, the mechanisms described are generally valid in both species.
In cattle and pigs intrafollicular maturation of cumulus-oocyte-complexes (COCs) are characterized by time dependent changes of COC morphology and oocyte chromatin configuration. (MEINECKE et al., 1984; XIE et al., 1990; TORNER et al., 1998a). TORNER et al. (1998a) observed a close synchronisation between follicular maturation after hCG application and the development of intrafollicular COC morphology and chromatin configuration (Tab. 5 and 6).

Ovulation

In cattle and pigs ovulation is induced by an increase in LH secretion. The LH surge triggers a biochemical cascade that leads to the rupture of the preovulatory follicle(s), the expulsion of the oocyte(s) and the formation of the Corpora lutea. Local acting factors like steroids, prostaglandins and peptides like endothelin 1 are involved in the process of ovulation. An increase of prostaglandins (PGE₂ and PGF₂α) in follicular fluid of preovulatory follicles in the cow has been demonstrated by ALGIRE et al. (1992). More recently ACOSTA et al. (2000) demonstrated an increased local release of PGF₂α and angiotensin II around the time of ovulation.

In cattle PETERS and BENBOULAID (1997) investigated the occurrence of ovulation after PGF₂α/GnRH application in some animals by means of ultrasound. Ovulation occurred between 24 to 48 h after GnRH injection. More recently, we examined the time of ovulation after PGF₂α/GnRH application in heifers. Ultrasonographic examinations of ovaries were done every 6 hours during the periovulatory period. The mean interval from GnRH to ovulation was 25 to 33 hours. Our data and results from PETERS and BENBOULAID (1997) indicate, that ovulatory follicles have a diameter between 15 and 20 mm. The results of KOT and GINTHER (1999) show, that the mean time from beginning to completion of evacuation of ovulatory follicles was 4.3 ± 3.3 min (min. 6 s; max. 14.5 min.). The number of ovulations is constant in cattle.

Duration of ovulation period in pigs assessed with transrectal ultrasound diagnosis varied between 1.8 and 4.6 h. Ovulations took place on average 35 ± 8 h after onset of oestrus (SOEDE et al., 1997). Following GnRH induced stimulation the commencement and completion of ovulation was 35.1 ± 4.1 and 38.3 ± 4.3 h, respectively, and the mean duration of ovulation was 3.3 ± 1.9 h (BRÜSSOW et al., 1993). SOEDE et al. (1997) observed a mean diameter of the ovulatory follicles of 7.1 ± 0.9 mm. This is in agreement with earlier findings of RATKY et al. (1995). They estimated by means of endoscopy a size of preovulatory follicles between 7 to 8 mm. Interestingly, the number of ovulations was significantly increasing in gilts from first to third oestrous cycle (RATKY et al., 1995).

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Intrafollicular health markers during antral follicle wave development in cattle  
(Intrazelluläre Gesundheitsmarker während der Entwicklung von Antralfollikeln beim Rind)

Summary
Ovarian follicle growth in cattle culminating in the ovulation of a single follicle at the end of the oestrous cycle is strictly regulated and has proven to be very difficult to manipulate. This review will describe in detail antral follicle waves and in particular the first follicle wave of the cycle, indicating the specific gonadotrophin dependencies of cohort and dominant follicles, and relating follicle health to steroidogenesis. As intrafollicular growth factors such as proteins belonging to the inhibin family and the insulin-like growth factor (IGF) system have important roles in modifying gonadotrophin response of growing antral follicles, characteristics of healthy or atretic first wave follicles in relation to inhibins, IGFs and IGF-binding proteins will be summarised. Finally, a model for dominant follicle selection will be proposed including very recent data on proteases possibly affecting IGF bioavailability.

Key Words: dominant follicle, FSH, inhibin, insulin-like growth factor binding proteins

Introduction
In cattle, follicles from approximately 300 μm in diameter form an antrum and subsequent growth to a size of 3-5 mm is calculated to take more than 30 days (1). However, growth rates of 0.5 to more than 2 mm per day are seen in larger antral follicles monitored individually with the aid of transrectal ovarian ultrasound scanning during their final stages of development. This review will present recent data which describe the final outcome of antral follicle growth and try to elucidate the regulatory mechanisms involved in limiting the number of the species specific ovulatory quota to one in cattle.

Gonadotrophins and growth of an antral follicle wave in cattle
At the beginning of the oestrous cycle and following ovulation of the last preovulatory follicle, a transient rise in Follicle Stimulating Hormone (FSH) causes emergence of up to 24 small antral follicles 3-5 mm in diameter (the cohort) within 24 hours of the maximum of the transient FSH rise (2-4). During declining FSH and over 3 days more and more follicles from the original cohort become static and regress until only one single follicle is selected to continue to grow and enhance its steroidogenesis, specifically its oestradiol secretion when FSH reaches nadir concentrations (3-5). This is the dominant follicle (DF) and it appears that the DF aids its own selection by suppressing FSH concentrations below the requirement threshold of the other cohort members (6,7). The DF attains a much larger size than all other follicles (up to 15-20 mm), is responsible for ovarian oestradiol secretion during its growth phase and maintains low FSH concentrations to prevent any other cohort growth (6-8). All the other members of its cohort including the second largest follicle undergo rapid atresia via apoptosis during the FSH decline due to their acute FSH-dependency (subordinate...