

Timing of the first zygotic cleavage as a marker of developmental potential of mammalian embryos

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SUMMARY

Embryo quality related to its developmental potential is now one of the most important issues in modern embryology. It has been demonstrated that some *in vitro* produced blastocysts fail to hatch and implant after transfer despite a normal morphology. Although embryos are able to adjust to sub-optimal culture conditions, significant changes in expression profiles of developmentally important genes have been noticed. Timing of the first zygotic cleavage is considered a non-invasive marker of embryo developmental potential and has been successfully used in human IVF programs for identifying embryos of superior quality. Early-cleaving zygotes are more likely to develop to the blastocyst stage than their late-cleaving counterparts. The timing of the first zygotic cleavage has been associated with several parameters that may affect developmental potential of the resulting embryos. The mechanism causing variation in this phenomenon has not been identified. It may be related to culture environment or to some intrinsic factors within the oocyte, the sperm or both. In this paper we discuss some of the important aspects related to the

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INTRODUCTION

A growing body of evidence suggests that whether or not a zygote will transform into a blastocyst is largely established as early as the 2-cell stage. This is rather surprising, since in humans and the majority of domestic animals, the first two or three cell divisions are regulated by factors of maternal origin after which activation of the embryonic genome occurs. Is it possible therefore, that the fate of an embryo may depend only on the maternal factors accumulated in the ooplasm during oogenesis? What can be the role of the spermatozoon? In this paper we discuss some important aspects related to the timing of the first zygotic cleavage and its influence on the developmental competence of resulting embryos.

During the period of preimplantation development, a 1-cell zygote undergoes several mitotic divisions in order to transform into a blastocyst comprised of a minimum of 100 blastomeres (fig. 1). Fertilization is a multistep process involving oocyte penetration by a sperm, oocyte activation, reorganization of the male chromatin, pronuclei formation and establishment of the 1-cell embryo ready to start mitotic divisions. In cattle, the time required for sperm penetration of *in vitro* matured (IVM) oocytes is less than 4 h (time of insemination = 0 h), sperm-head decondensation takes an additional 1-2 h and the male pronucleus appears at 9-11 h post insemination (hpi; [7, 66]). The precise timing of events during the first cell cycle is however hard to establish. The time to the first zygotic cleavage varies by 8 h in humans (between 22 and 30 hpi; [41, 44]) and more than 20 h in cattle (between 22 and 48 hpi; [22, 68]), with a peak of 2-cell stage embryos at 36 hpi [55]. The first human zygotes cleaved 24 h post *in vitro* fertilization (IVF) and 20 h after intra-cytoplasmic sperm injection (ICSI; [41]).

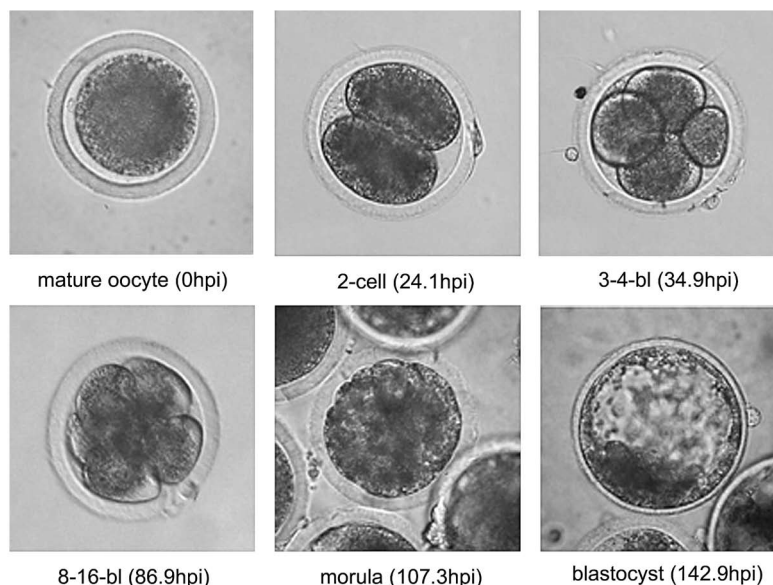


Figure 1. The mean timing of appearance of embryonic subsequent stages [1] during bovine preattachment development *in vitro* (the bar represents 100 µm)

It has been shown that human and bovine zygotes that cleave early after oocyte insemination are more likely to develop to the blastocyst stage than their late-cleaving counterparts [19, 35, 36, 37, 47, 56, 67]. Transfer of fast-cleaving embryos more often resulted in pregnancy than slower embryos [34]. Therefore, early zygotic cleavage may be a useful marker of embryonic developmental potential beyond the blastocyst stage as well as an additional criterion in selection of good quality embryos for transfer or freezing.

EARLY ZYGOTIC CLEAVAGE AS A MARKER OF EMBRYO QUALITY

Humans

In order to improve the success rate in human IVF, the transfer of several embryos is usually carried out which increases the risk of multiple pregnancy. In order to transfer a single embryo with a high success rate,

a reliable selection system is required which would identify embryos of the best viability. Current systems of embryo scoring are based on their morphology (pronuclear morphology, cleavage rate, blastomere number and morphology; [3]) which has been shown to be closely linked to embryo viability [4]. The phenomenon of early cleavage and its impact on pregnancy rate in humans was reported for the first time by Edwards et al. [12]. Several recent studies have confirmed that early zygotic cleavage is a strong indicator of embryonic developmental competence in humans. Salumets et al. [50] showed that the transfer of early-cleaving embryos (EC, 25-27 hpi) yielded higher pregnancy rates (50.0%) than did their late i.e. non early-cleaving (NEC) counterparts (26.4%). In the study by Van Montfoort et al. [54] based on 165 single embryo transfers (SET), transfer of EC embryos resulted in higher pregnancy rates (46%) when compared to the late cleavers (18%). Also the abortion rate was reduced in the early group (20%) when compared to the late group (48%). The timing of the first mitotic cleavage is also variable among zygotes resulting from ICSI [5, 54]. It was shown that such zygotes cleaved 2 h [55] and 4 h [41] earlier compared to IVF counterparts. This was believed to be due to bypassing of the barrier of cumulus cells and zona pellucida.

It should be remembered, however, that EC embryos have to be available in order for selection to occur. The incidence of early cleavers (first mitotic division between 25 and 27 hpi) is rather low and ranges from 15.0% [19, 65] to 38.0% [54]. Usually, EC embryos were observed only in about half of the cycles (37% [37], 46% [19], 57% [5], 61.4% [49]) and the probability of producing EC embryos significantly decreased with the patient's age. Therefore, it was suggested, that the criterion for early cleavage should be extended to 29 hpi [5].

Cattle

It is evident that in cattle as in humans, timing of the first zygotic cleavage is a valuable parameter of intrinsic embryo quality [6, 18, 35, 36, 67]. The experimental design of several studies spans the period of time corresponding to the first division between 24 hpi and 48 hpi with varying

Table 1. Timing of the first zygotic cleavage in selected IVF experiments in cattle

total	Number of zygotes cleaved by 30 hpi early (%)		blast. rate D7/8 (%)	Number of zygotes cleaved by 48 hpi late (%)		blast. rate D7/8 (%)	Reference
716	550	76.8%	53.3%	166	23.2%	21.1%	[35]
1430	1092	76.4%	41.0%	338	23.6%	16.6%	[10]
3164	952	30.1%	45.5%	1354	42.8%	20.0%	[63]
628	278	32.0%	40.3%	222	35.4%	24.8%	[58]
5938	2872	48.4%	45.0%	2080	35.0%	20.6%	

selection time points. Moreover, the rate of bovine zygotes that cleave by 30 hpi also varied significantly among studies (tab. 1). Generally, the best quality embryos arise from zygotes that cleave by 30 hpi and the developmental potential decreases as the time from insemination to the first cleavage increases. Embryos derived from zygotes that cleaved very late (> 42 hpi) did usually not reach the blastocyst stage [35, 56]. Many investigators have shown that the majority of blastocysts develop from EC embryos (99% [35], 96% [58]). Even if a NEC embryo transforms into a blastocyst, its quality is compromised and would be not selected for a routine transfer. According to Holm et al. [25] the optimal interval for selecting viable (i.e. developing beyond the morula stage) 2-3 cell embryos was 32-36 hpi. Interestingly, the timing of the first cleavage significantly influenced an embryo's ability to reach the blastocyst stage, but had no influence either on the timing of blastocyst formation [56] nor its further development in the uterus [35]. In the experiment by Lonergan et al. [35] no difference in the pregnancy rates was noticed after the transfer of Day 7 blastocysts developed from EC and NEC zygotes, respectively. It is therefore evident that in cattle, timing of the first zygotic cleavage affects embryonic competence to transform into a blastocyst, but subsequent development of an embryo may be no longer related to this phenomenon. That hypothesis has to be however treated with caution, since the scope of the bovine experiment is not

comparable with the human data discussed earlier. Altogether, only 107 blastocysts (55 early- and 52 late-cleaving embryos) were transferred and 46 pregnancies resulted [35].

FACTORS AFFECTING TIMING OF THE FIRST ZYGOTIC CLEAVAGE

Although reasons for the observed variation in the timing of the first zygotic cleavage are still not clear, they may be related to culture conditions and some intrinsic factors within the oocyte and/or sperm such as ooplasm maturity, differences in the ability of individual sperm to stimulate calcium transients, paternal effect on the duration of the S-phase or chromosomal abnormalities. It was shown that in humans the timing of the first zygotic cleavage is not influenced either by the timing of fertilization nor by semen parameters [49].

Genetic regulation

In mice, the timing of the first zygotic cleavage as well as the speed of embryonic development is regulated by the *Ped* (*preimplantation embryo development*) gene [64]. Based on the blastomere content, two phenotypes (*fast* and *slow*) were described with the *fast* allele as dominant [21]. The *Ped* gene was localized to the Q region of the murine major histocompatibility complex (MHC). The Q region contains 10 genes (Q1-Q10) of the MHC class Ib antigens. Two genes from the above cluster (Q7, Q9) are responsible for the *fast* phenotype, and encode the Qa-2 antigen. Mice displaying the *Ped slow* phenotype have a deletion in both Q7 and Q9 loci and thus do not express Qa-2 antigen. It has been demonstrated by Jin et al. [27], that transcript for the murine *Ped* gene is present throughout the entire preimplantation period from oocyte to the blastocyst stage. However, the transcription of embryonic genome does not begin until the 2-blastomere stage. Thus, the first zygotic cleavage may be controlled only by transcripts of maternal origin accumulated in the ooplasm. Up to now the search for a bovine homolog of the murine *Ped* gene has not brought satisfactory results [16].

The intrinsic features of the gametes

The oocyte. Several authors note oocyte diameter to be strongly related to its developmental potential. Small bovine oocytes (<100 μm) after fertilization showed lower cleavage and blastocyst rates and slower kinetics of cell divisions (reduced blastomere number) than large oocytes (>120 μm ; [59]). Moreover, the aberrant (diploid) chromosome number was more often observed in smaller (113.16 μm) than larger (116.83 μm) bovine oocytes [33]. The kinetics of oocyte maturation were related to zygotic cleavage [11] and oocyte diameter [2]. Early maturing oocytes (first polar body – Ipb, extruded by 16 h from the onset of culture) were more likely to cleave (75%) and reach the blastocyst stage (47%) in comparison to late maturing oocytes (Ipb extruded by 24 h; 44% and 15% respectively). The speed of sperm chromatin decondensation significantly decreased as the time of the Ipb extrusion increased (oocyte aging). Moreover, oocytes that matured earlier were significantly larger [2]. Thus large oocytes that matured early were able to process the sperm DNA more efficiently and therefore transform into more viable, early-cleaving zygotes. Fast-cleaving embryos derived from large oocytes displayed significantly lower apoptotic cell ratio at all time points. Also it was recently shown by Vandaele et al. [59] that caspase activity reflecting apoptotic processes, was lower in embryos derived from fully grown oocytes.

Early embryonic development is regulated by maternal mRNAs and proteins accumulated during oogenesis. Transcription in bovine oocytes ceases in a follicle of 3 mm in diameter when the oocyte reaches its full size (120 μm ; [15]). As shown by Brevini et al. [6], the polyadenylation status of maternal transcripts differs between early- and late-cleaving bovine zygotes. NEC embryos of reduced quality showed a different pattern of gene-specific polyadenylation than EC embryos. Many recent studies have shown a strong correlation between oocyte competence as related to early zygotic cleavage and abundance of maternal transcripts. Patel et al. [43] observed a significantly higher transcript abundance of follistatin in EC embryos and oocytes derived from adult females (competent) in comparison to late cleavers and oocytes collected from prepubertal females

(non competent). The authors suggested that follistatin of maternal origin may exert a regulatory effect on early embryogenesis in cattle and may serve as a marker of oocyte competence. In addition, adult oocytes and EC embryos were characterized by a similar pattern of follistatin gene expression and therefore were considered as competent. Since adult oocytes are usually larger than their prepubertal counterparts, this finding confirmed the previously described advantages of large over small oocytes in terms of developmental potential.

Another factor of interest that may be related to the developmental competence of mammalian oocytes concerns the mitochondria. A considerable variation in the copy number of oocyte mitochondrial DNA (mtDNA) has been described within a species as well as between species [26, 53]. The important point is that this phenomenon was related to oocyte competence for fertilization [14, 48, 52]. It has been shown [14] that the number of mtDNA copies may vary from 10 000 to 700 000 in porcine oocytes and the probability of an oocyte being successfully fertilized increased with the mtDNA copy number. The rate of cleaving zygotes was over 2-fold higher (46.6% vs. 22.7%) in the group of oocytes with a higher number of mtDNA copies (222 446 vs. 115 352 copies; [14]). In contrast, the number of mtDNA copies in the mammalian sperm cell is very limited and varies from 10 to 1200 [26]. Furthermore, the mtDNA copy number is negatively associated with sperm fertilization ability [9, 39]. May-Panloup [39] showed that the mtDNA content was almost 70 times lower in human sperm with a normal motility than in morphologically abnormal spermatozoa. Thus, since at the time of fertilization the majority of mitochondria is of maternal origin, it may be hypothesized that the timing of the first zygotic cleavage may be related to the number of mitochondria within the oocyte.

The spermatozoa. The paternal effect on the timing of the first cleavage cannot be disregarded. DNA replication has been shown to begin earlier and last longer in zygotes fertilized by sperm produced by bulls with high *in vivo* fertility [8, 13]. The paternal effect can be explained by the size of the sperm aster which is correlated with bull fertility and plays a crucial role in

forming the mitotic spindle [42]. According to Ward et al. [63] the rate of zygotes that cleaved by 33 hpi was the best predictor of bull reproductive potential *in vivo*. The early cleavage rate varied significantly among bulls of the highest (55.3%) and lowest (6.4%) non return rate (NRR represents the rate of inseminated cows which do not return to oestrus before 56 days after the first insemination). Recently, significant differences in the timing of the first zygotic cleavage, speed of embryonic development (blastulation time) and sex ratio were reported [1] for bulls with different fertility under *in vitro* conditions.

In addition, the chromatin status of the spermatozoa may influence early embryonic cleavage. Elevated levels of DNA damage (>27%) detected by flow cytometric chromatin structure assay (SCSA) were related to a significant reduction in fertilization and pregnancy rates in humans [32]. The percentage of sperm cells with damaged DNA was significantly higher (31.1%) in cases of failed pregnancies compared to successful cycles (15.4%). Although sperm cells are transcriptionally silent (transcription ceases when histones are replaced by protamines in elongating spermatides), a fraction of short size RNA was identified in the midpiece, tail and head [20]. After a detailed analysis of the sperm transcriptome the authors suggested, that the contribution of paternal RNA could be associated with epigenetic modifications rather than a special functions in embryogenesis.

***In vitro* culture conditions**

Supplementation of culture media with glucose caused significant changes in cleavage timing of bovine zygotes [45]. In the presence of glucose, male embryos cleaved up to 4-cell stage 125.6 minutes earlier (36.2 hpi) than female embryos (38.2 hpi). On the contrary, in the control culture (no glucose), male embryos cleaved 129.1 minutes later (38.5 hpi) than female ones (37.2 hpi). The observed variation was attributed to the different number of functional X-chromosomes in embryos of both sexes. As suggested by the authors, differences in embryonic growth prior to X-chromosome inactivation which takes place at morula/blastocyst stages,

reflect differential expression of genes linked to this chromosome which controls reactive oxygen species. In the consecutive paper, Peippo et al. [46] demonstrated, that transcripts of two genes (G6PD, HPRT) located on the X-chromosome are present in bovine embryos at 2-cell stage. The authors, however, were not able to quantify the transcripts in 2-blastomere embryos and also to distinguish between maternal and embryo-derived transcripts. Since the bovine fertilized oocyte is transcriptionally silent, the sex-related variation in the timing of the first zygotic division can be attributed rather to the metabolism of the maternal mRNAs than to transcriptional activity of embryonic genome.

THE EXCLUSIVE PROPERTIES OF EMBRYOS DERIVED FROM EARLY-CLEAVING ZYGOTES

Morphology

Assessment of embryo morphology is still the most popular method for embryo selection before transfer or freezing. Several morphological features have been shown to be linked with developmental potential of an embryo [57]. In humans, the incidence of good quality Day-2 embryos was almost 2-times higher among early cleavers (62.5%) when compared to the late cleavers (33.4%; [37]). This phenomenon was also associated with better morphology (degree of fragmentation, uniformity of blastomeres, presence of multinucleated cells) and higher blastomere number [19, 50, 54]. In the study of Salumets et al. [50] who evaluated 178 single embryo transfers, 94.1% of Day-2 EC embryos contained four or more blastomeres and the proportion of embryos with < 20% fragmentation equalled 50.5% in comparison to 44.2% for NEC embryos. Moreover, early-cleaving embryos possessed significantly more evenly sized blastomeres, a parameter strongly related to embryo survival. Interestingly, the presence of irregular blastomeres seemed to be more efficiently tolerated by EC embryos, since transfer of unevenly cleaved EC embryos more often resulted in pregnancy (43.8%) than unevenly cleaved NEC embryos (20.9%; [50]).

In cattle, early-cleaving embryos displayed the following features: a significantly higher rate of embryos developing to the blastocyst stage, less fragmentation at morula stage [56], higher blastomere count [35], lower apoptotic cell ratio (ACR; [58]) and better survival after vitrification [10]. Van Soom et al. [56] has shown, that the extent of compaction and fragmentation at the morula stage has a direct impact on the blastocyst quality in cattle (total cell number, proportion ICM/total cell number). The timing of the first zygotic cleavage significantly affected the incidence of apoptosis in bovine blastocysts [58]. EC embryos cleaved before 30 hpi displayed the lowest apoptotic cell ratio (9.8 ± 7.1) as compared with embryos that cleaved later (by 36 hpi: 11.5 ± 6.9 and by 48 hpi: 14.2 ± 8.4). Embryo survival after vitrification is considered a marker of developmental potential. Day-7 blastocysts derived from EC zygotes survived at a higher rate than did their late-cleaving counterparts [10]. This group of embryos was characterized by better re-expansion, hatching ability and blastocoel maintenance, since 72 h after thawing the blastocoel was observed in 60% of blastocysts (NEC embryos- 40%). Basing on the presented data it may be suggested, that human and bovine embryos which develop from the early-cleaving zygotes are often characterized by a superior morphology and show better cry tolerance than their late-cleaving counterparts.

Chromosomal aberrations

It has been documented that a high proportion of *in vitro*-produced bovine embryos display a chromosomal imbalance, with mixoploidy as the most frequently observed abnormality [60]. Chromosomal aberrations negatively affect embryonic development [29]. The majority of polyploid embryos arrest around the third cell cycle [61], whereas mixoploid ones continue to grow and polyploid cells are located mainly in the trophectoderm of a blastocyst [62]. The published evidence concerning the relationship between early cleavage and chromosomal complement in embryos is rather scarce. Conventional cytogenetic analysis of 205 bovine Day-5 embryos produced *in vitro* revealed that 41.4% of embryos carrying a chromosome abnormality were early cleavers compared to 58.6% of their late-cleaving

counterparts [67]. Also the rate of chromosomally imbalanced embryos was significantly lower in the EC group (17/165, 10.3%) in comparison with NEC embryos (24/100, 24%). In light of the limitations of the conventional cytogenetic analysis (low mitotic index, poor chromosome spreads, chromosome loss) the results presented by Yadav et al. [67] may concern mainly good quality embryos with a majority of live blastomeres. Embryos with reduced quality comprise an elevated number of degenerated or dead cells which are not possible to be analysed by Giemsa staining [30, 51]. Until now no data has been published on embryos analyzed by FISH which would yield a more representative data.

In humans, the timing of the first cleavage was associated with the pronuclear status in the zygote. Only 12% of abnormal, polyspermic zygotes cleaved early which contrasts with 26.9% for normal zygotes [37]. This finding corresponds with an observation published by Hardarson et al. [24] who found that aneuploid embryos significantly often comprised blastomeres of unequal size and showed lower frequency of early cleavage and pregnancy rate. The majority of early cleavers (68.8%) displayed evenly sized blastomeres, whereas only 37.5% of embryos with unevenly sized cells developed from EC zygotes. Moreover, embryos characterized by the presence of uneven blastomeres were significantly more often aneuploid (29.4%, chromosome pairs 13,18,21,X,Y) than embryos with even cells (8.5%). Munne and Cohen [40] demonstrated that significantly more slow-growing embryos were chromosomally abnormal (mainly aneuploid: 57%) when compared to their fast counterparts (29%). It is known, that at least in humans, slow-growing, fragmented embryos are associated with reduced pregnancy rates and increased incidence of chromosomal abnormalities. Recently, Magli et al. [38] described associations between morphology and chromosomal complement in 5 227 human embryos. The authors concluded that embryos developing more slowly or quickly with regard to the expected time frame more often show abnormal chromosome complement and altered morphology. Embryos composed of eight cells at 62 hpi showed significantly less fragmentation and chromosomal abnormalities than their arrested or slow-cleaving counterparts. It may be hypothesized, that the risk of the presence of chromosomally abnormal

blastomeres within an embryo increases with the elevating level of morphological changes. Therefore embryo selection based on the timing of the first zygotic cleavage may increase the chances of selecting embryos free of chromosomal imbalance.

Sex

It is well documented that during *in vitro* culture male embryos grow faster and reach more advanced developmental stages earlier than female embryos [31]. Analysis of association between sex and first zygotic cleavage brought rather contradictory results. Yadav et al. [67] observed significantly more males (3.6:1) among Day 5 morulas derived from EC zygotes, whereas no deviation from the expected male to female ratio (0.93:1) was noticed for NEC embryos. Lonergan et al. [35] observed more males than females at the blastocyst stage. However, the phenomenon was not related to the timing of the first cleavage since no marked deviation in the sex ratio was noticed among 2-blastomere embryos. Holm et al. [25] using time lapse recording did not find any differences in cleavage intervals for the first four divisions between male and female embryos. Furthermore, there was no difference in the sex ratio found after transferring EC and NEC human embryos [28, 37].

More recently, Alomar et al. [1] observed a predominance of males (76%) among blastocysts sired by a bull characterized by the earliest timing of the first cleavage as well as the fastest embryonic development. This phenomenon was observed as early as the 2-cell stage. It is still not evident, if the majority of EC embryos are of male sex since the presented data brought conflicting results. This aspect needs further investigation on a representative pool of embryos cultured in a glucose free medium.

Transcript level of developmentally important genes in embryos derived from EC zygotes

Recent studies brought interesting results on differences in transcript levels (relative transcript abundance - RA) of developmentally important genes in relation to the timing of the first cleavage and the speed of embryonic

development. Early-cleaving, 2-cell bovine embryos were characterized by a significantly higher level of transcript for the histone H3 gene [17]. Since histones are essential for the regulation of gene expression, lower transcript content may indicate some alterations in the process of transcription in the late-cleaving zygotes. Transcript for the *IGFI* gene (growth factor stimulating cell growth and survival) was found in all 2-cell embryos that cleaved by 27-30 hpi, in some embryos cleaved by 33-36 hpi and in none of those cleaved after 36 hpi [36]. Higher RA for the *p66shc* gene associated with apoptosis and cellular senescence was observed in NEC and arrested bovine embryos at 2-4-blastomere stage [18]. Early-cleaving 2-cell bovine embryos contained more transcripts also for the follistatin gene, a marker of oocyte competence [43]. Generally, genes related to growth and metabolism were characterized by higher RA in early- and fast-developing embryos in comparison to late- and slow-cleaving embryos, whereas genes related to cellular stress and apoptosis showed lower RA in EC and fast embryos [17, 18, 23, 36, 43]. Since major genome activation occurs at the 8-16-cell stage in cattle, analysis of transcript abundance in zygotes and early embryos concerns mainly mRNAs of maternal origin and cannot be related to transcriptional activity of the embryonic genome. Thus, alterations in transcript content observed in 2-4-blastomere bovine embryos reflect a variation in synthesis and metabolism (e.g. degradation) of maternal mRNAs.

TIMING OF FIRST ZYGOTIC CLEAVAGE AND SPEED OF EMBRYONIC DEVELOPMENT – WHAT DO THEY HAVE IN COMMON?

Timing of the first zygotic cleavage means the time frame from the oocyte insemination to the first mitotic division, whereas the speed of embryo development is defined as kinetics of subsequent blastomere divisions during the preimplantation development [56]. It is known that early-cleaving zygotes as well as embryos that develop fast in the preattachment period show high developmental competence and significantly more often

transform into viable blastocysts. It is not clear however, if all EC zygotes develop faster in comparison to NEC zygotes and thus create a population of fast-developing embryos. If this hypothesis is true, superior embryos can be selected on the basis of the time of the first zygotic cleavage. In fact, the probability for EC embryo to reach the blastocyst stage is over 2-fold higher (on average 45%) than that of NEC embryos (20.6%, tab. 1). To explain this issue Van Soom et al. [56] selected a pool of embryos that cleaved fast according to the following pattern: 2-cells by 30 hpi, 8-cells by 48 hpi and 16-cells at 72 hpi. Those embryos were classified as “ideal cleavers” and showed a very high potential to reach the morula (88%), blastocyst (57%) and hatched blastocyst (48%) stages. Only 9.5% (131/1376) of embryos exhibited the ideal cleavage pattern, whereas 39.8% (547/1376) of zygotes cleaved by 30 hpi. Thus, only 24% of EC zygotes (131/547) cleaved at high speed. It can be concluded, therefore, that all “ideal cleavers” are in fact EC zygotes, however not all early cleavers may be considered as fast-cleaving embryos. Therefore, “ideal cleavers” form a subpopulation of EC embryos with a superior quality.

Summarizing, it is suggested that early-cleaving, competent embryos arise from large oocytes which extrude the Ipb earlier, are able to process the sperm DNA more efficiently and are equipped with a defined pool of maternal transcripts. Therefore, proper oocyte growth and maturation may have a particular impact on the zygotic competence. In addition, it can be hypothesized that the oocyte may have a more pronounced influence on embryo quality than the spermatozoon since the majority of transcripts and other cytoplasmic compounds in a zygote is of maternal origin. It is possible that early cleavers are derived from oocytes with better synchronized cytoplasmic and nuclear maturation. Paternal factors have been observed to play some role but they are not well documented. There is still a shortage of information concerning the importance and functions of transcripts trapped in spermatozoa. Therefore, a possible involvement of these factors in the regulation of early zygotic cleavage cannot be ruled out. Moreover, it would be important to know if spermatozoa produced by males of high and low *in vivo* fertility show different pools of transcripts that could be associated with male fertility.

Selection based on embryo morphology alone is not sufficient to identify embryos of superior quality and so timing of the first zygotic cleavage is a valuable, non-invasive marker of embryonic competence in cattle. This method of embryo selection may be of special value to IVF procedures which are based on a very limited number of oocytes collected by the ovum pick-up method (OPU) from animals of high breeding value.

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