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Morphokinetics of Porcine and Bovine Embryos and Anomalies in Their Development

Wiesak T[1]*, Goryszewska-Szczurek E[1,2] and Wasielak-Politowska M[1,3]

[1]Department of Gamete and Embryo Biology, Institute of Animal Reproduction and Food Research, Polish Academy of Sciences, Olsztyn, Poland

[2]Department of Hormonal Action Mechanisms, Institute of Animal Reproduction and Food Research, Polish Academy of Sciences, Olsztyn, Poland

[3]Center of Gynecology, Endocrinology and Reproductive Medicine, Artemida, Olsztyn, Poland

***Corresponding author:** Teresa Wiesak, Department of Gamete and Embryo Biology, Institute of Animal Reproduction and Food Research, Polish Academy of Sciences, Tuwima 10, Olsztyn 10-748, Poland

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Abstract

The objective of this study was to evaluate the kinetics of bovine and porcine embryos that failed or developed to the blastocyst stage and anomalies in cleavage using a time-lapse monitoring system.

The timing of early cleavages and their duration were similar for bovine and porcine embryos that developed to the blastocyst stage. There were differences in the time of first and second cell division of the bovine embryos that developed and those that did not develop to blastocyst stage (P=0.004 and P=0.002), respectively. Similarly, in case of porcine embryos such difference was observed only in the time of first cleavage (P=0.0001). Direct cleavage from 1-cell to 3 cells occurred in 13.47% and to more than 3 cells in 3.37% of porcine embryos whereas to 3-cells occurred in 4.23% of bovine embryos. The reverse cleavage was observed in 4.33% of porcine and 8.45% of bovine embryos.

Conclusion: Our study showed: 1) The similarities in timing and duration of early cleavages of bovine and porcine embryos during development to the blastocyst stage, 2) Differences in morphokinetic parameters between bovine and porcine embryos developing or non-developing to the blastocyst, 3) Anomalies in cleaving of *in vitro* developing bovine and porcine embryos.

Keywords: Bovine embryo; Porcine embryo, Blastocyst, Morphokinetics; Direct cleavage; Reverse cleavage

Introduction

The greatest challenge in the management of *In Vitro* Fertilization (IVF) programs (human and animals) is proficiency in selection of the embryo(s) with the highest probability of implantation and normal development in the uterus [1-4]. Periodic observations of embryo development in traditional *in vitro* culture system provide limited information to distinguish differences in the developmental competence of the embryos of the same morphological grade [1]. It is well known, that precise timing of specific events, such as pronuclei formation, pronuclear syngamy, cleavage events, synchrony of cell divisions, cell cycle intervals and initiation of compaction and blastulation are strong indicators of embryo developmental potential [4-8]. Removing embryos from the culture incubator for the "snapshot" inspection in traditional IVF system can perturb their subsequent development due to changes in the temperature and pH of the culture medium [4,8].

Recently, morphological embryo assessment in clinical IVF practice has been boosted by the introduction of noninvasive observation method, Time-Lapse Monitoring System (TLS). Several Time-Lapse Monitoring System are available from the commercial vendors [4]. This system allows without any distractions in culture conditions for continuous observation and recording of the events of embryo development such as:

- Cell-cycle lengths [4,9];
- Differences between embryos that appear morphologically the same by classic evaluation [1];
- Abnormalities in embryo development including abnormal syngamy, cytokinesis and cleavage profiles [5,10,11];
- Abnormal cell divisions such as reverse cleavage (RC, i.e. blastomere fusion causing reduction in the number of blastomeres), direct cleavage (DC, i.e. cleaving directly from 1-cell to 3 or more cells) [11,12];
- Timing of blastocoel cavity appearance [4,8];
- Blastocyst collapse and re-expansion [13].

All of these events are difficult to observe in a conventional culture system and some of them are associated with decreased embryo developmental competence [14], disruption of the genetic and epigenetic constitutions [15] and poor clinical outcomes [16].

Time-lapse technology allows also to collect massive amount of morphokinetic parameters (from thousands of embryos) to create embryo selection algorithms as a possible predictors of embryo competence [4]. Several different algorithms are already in use in human ART that permit reliably and, unbiasedly and, automatically (without human intervention) and, more precisely assess embryo quality [4,17]. In spite of all the benefits listed above, TLS is sluggishly entering into animal IVF programs. The implementation of time-lapse technology and Artificial Intelligence into animal IVF help to predict more consistently embryo quality, improve embryo selection for transfer, and subsequently reduce nonreturn rates. These predictive parameters can also be used as criteria for selection of better culture media for animal IVF systems [18].

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There is abundance of data on embryo developmental kinetics in human [5,6,11,12,19-24], although much less for cattle [10,25-28] and some other species such as hamsters [29,30], mice [31-34] and horses [35]. The precise timing of morphokinetic events of *in vitro* produced porcine embryos has not yet been fully established. On the other hand, there is great interest in producing large quantities of *in vitro* matured oocytes and *in vitro* produced embryos of domestic animals because they could be used in several ways:

- As a research material to further, develop associated reproductive technologies such as Somatic Cell Nuclear Transfer (SCNT) and cryopreservation.
- To advance our understanding of the reproductive processes that will lead to increase the efficiency of embryo production and generate further progress in biotechnology.
- For basic and biomedical research, such as gene targeting tools or to stimulate translational research into stem cell therapies.
- To produce genetically modified animals (such as pigs) to study human diseases or as potential xenograft donors or to produce specific proteins, because of their physiological similarities to humans.

Therefore, it is essential to have a better understanding of all the events occurring during *in vitro* embryo production. The objectives of this study were: 1) to investigate the morphokinetic parameters of bovine and porcine embryos that failed or developed to the blastocyst stage 2) to compare morphokinetic parameters of bovine and porcine embryos, 3) to examine the occurrence of deviations in the cleavage of bovine and porcine embryos during their *in vitro* development.

Materials and Methods

All experiments were performed with the approval from the Local Animal Ethics Committee and Regional Veterinary Control (RF# 151/L014).

Oocyte collection

Porcine and bovine ovaries were collected at a local slaughterhouse and transported to the laboratory in Phosphate Buffered Saline (PBS) within 1h at 38^{0}C. The ovaries were washed several times in PBS supplemented with 50IU/mL of penicillin G (Sigma-Aldrich, St. Louis, USA). Oocytes were aspirated from the follicles (4-6 mm in diameter) using 18 g needle attached to 10ml syringe. Only Cumulus-Oocyte Complexes (COCs) with compact multiple layers of cumulus cells were selected for *In Vitro* Maturation (IVM).

Bovine oocytes maturation, insemination and embryo culture

Bovine COCs were cultured in groups of 20-30 in 4 well culture dishes (Thermo Scientific, Denmark) under mineral oil (Sigma-Aldrich) at 38.5^{0}C in humidified atmosphere of 5% CO_2 in air for 24 hours. Each well contained 500µL of maturation medium (Minitube, Germany) supplemented with 0.02IU/mL FSH and 0.01IU/mL LH (Folligon, Intervet, Poland) and 10% fetal calf serum (FCS, Sigma-Aldrich). *In vitro* matured COCs were washed with a fertilization medium (Minitube, Germany) supplemented with 10% bovine albumin serum (BSA, Sigma-Aldrich), 0.11mg/mL sodium pyruvate (Sigma-Aldrich) and 3IU of heparin (Heparynium, Polfa SA, Poland). Then groups of 20 COCs were placed in 50µL drops of fertilization medium and inseminated with a bull's spermatozoa at a final concentration of 0.5 x 10^6 spermatozoa/mL The straws of frozen semen (from one bull of proven fertility) were used and thawed in a water bath at 35^{0}C for 30 seconds and processed by swim-up as described by Parrish et al. [36]. The COCs and spermatozoa were incubated overnight at 38.5^{0}C in 5% CO_2 in humidified air. After fertilization, the cumulus cells were removed by repetitive pipetting (130µm in diameter pipette), and presumptive zygotes were washed and cultured in a modified synthetic oviduct fluid (SOF, Minitube, Germany) supplemented with the 10µL/mL of MEM essential and 20µL/mL of non-essential amino acids solution (Sigma-Aldrich), 36.3mg/mL sodium pyruvate and 10% fetal calf serum (Sigma-Aldrich) on Primo Vision dishes (Vitrolife, Sweden). The culture media (70µl) were replaced with fresh one every three days.

Porcine oocytes maturation, insemination and embryo culture

The porcine COCs were matured in NCSU-37 medium containing 10% of porcine follicular fluid, 10IU/mL of pregnant mare serum gonadotropin (Folligon, Intervet, Poland), 10IU/mL of hCG (Chorulon, Intervet, Poland), 0.6mM L-cysteine (Sigma-Aldrich) and 1mM dibutyryl cAMP (Sigma-Aldrich). After 20 hours, the COCs were transferred to NCSU-37 medium without hormones and dibutyryl cAMP and cultured for the additional 24 hours. The maturation was performed at 38^{0}C in the atmosphere of 5% CO_2 in air. Then, the COCs were washed and placed in 90µL of fertilization drops (mPig-FM medium [37] covered with mineral oil (approximately 15 COCs per drop), and 10µL of sperm suspension was added to each fertilization drop. The final concentration of sperm was 1x10^7 spermatoza/mL. Fresh porcine semen from Large White boars of known fertility (purchased from the Animal Insemination Center in Bydgoszcz, Poland) was used for insemination matured COCs. Semen was prepared by removal of extender (centrifugation at 800g for 15 minutes), purification on a Percoll gradient (90% and 45%; 1000g for 10 minutes), and washing at 300g for 8 minutes in 6mL of Medium 199 supplemented with 0.68mML-glutamine, 20mM HEPES, 100U/mL penicillin, 0.1mg/mL streptomycin, 2.5mg/mL amphotericin B, 0.91mM sodium pyruvate, 4.12mM calcium lactate, 3.0mM glucose, and 10% FBS (Sigma-Aldrich). The pellet was resuspended in 0.5mL of Medium 199, and the concentration and motility of spermatozoa was determined using a hemocytometer (Thoma chamber). The gametes were co-incubated for 6 hours at 38^{0}C in atmosphere of 5% CO_2 in air. After incubation, the cumulus cells were removed by repetitive pipetting in a hyaluronidase solution (1000IU/mL), and putative zygotes were washed and placed into culture medium, NCSU-37 supplemented with 4mg/mL of BSA (fraction V; Sigma-Aldrich), 0.165mM sodium pyruvate and 2.7mM sodium lactate from Days 0 to 2 or 5.56-mM glucose from Days 2 to 7 on Primo Vision dishes.

Embryo culture

Putative (porcine and bovine) zygotes were washed several times in relevant culture medium and placed into Primo Vision dishes (arrangements of the microwells allowed for easy tracking and identification of each embryo) and cultured for up to 7 days (porcine) or 8 days (bovine) in the atmosphere 5% CO_2, 5% O_2 and 90% N. The images were taken every 5min with seven focal planes by the Primo Vision system (Vitrolife, Sweden) after the monitoring

started. The Primo Vision Analyzer Software v4 was used to analyze the occurrence of important normal and abnormal events (uneven cleavage, direct cleavage, re-absorption of blastomeres or cellular fragmentation). Time zero (t0) was set when spermatozoa were added to the matured oocytes. The exact time-point of fertilization is difficult to determine in traditional IVF compare to Intracytoplasmic Sperm Injection (ICSI) insemination, because spermatozoa penetrate oocytes sometime after the onset of insemination [38,39]. The time of first cleavage duration was determined when the cleavage furrow started to be visible up to the appearance of two or more cells. The duration of the second cleavage was defined when the cleavage furrow at least on one cell was visible to the time of appearance of four or more cells. Time when compaction of embryo cells occurred was assigned to morula stage and time when cavitation started was allocated to blastula stage. In total, 15 (N=215 putative zygotes) bovine and 17 (N=291 putative zygotes) porcine embryo culture experiments using the Primo Vision system were carried out.

Statistical analysis

The results were analyzed using the Statistica 10.0 software (Statsoft, Tulsa, OK, USA. All data were assessed for Gaussian distribution with the Shapiro-Wilk normality test. Because of non-Gaussian distribution of all data, the non-parametric U Mann-Whitney test was applied to determine statistical differences. Statistical differences were considered significant at $P<0.05$.

Results

The time-lapse analysis of embryo in our study showed differences in the timing of the first and second cleavage between bovine embryos that developed and those that did not develop to blastocyst stage ($P=0.004$ and $P=0.002$, respectively). Similarly, timing of the first cleavage of porcine embryos developing to the blastocyst was different from those that not reached the blastocyst stage ($P=0.0001$) (Table 1). The time ranges (min. - max.) of the first and second cleavage and the duration of these cleavages for bovine and porcine embryos developing to the blastocyst were within the corresponding ranges for embryos not developing to blastocyst (Table 1). Additionally, the time and duration of the first and second cleavage for bovine and porcine embryos developing to blastocyst stage were similar (Table 1).

Table 1: The timing of morphokinetic events of the bovine and porcine embryos. The percentages of cleaved embryos are expressed in relation to the total number of presumptive zygotes used for in vitro culture. The blastocysts developmental rate is expressed in relation to the number of cleaved zygotes.

	Cleavage Rate (%)	Bovine Embryos			Porcine Embryos		
		(142/215) 66.05%			(208/291) 71.48%		
		P value for Bovine	Not developed to Blastocyst N=112	Developed to Blastocyst N=30	Developed to Blastocyst N=34	Not developed to Blastocyst N=174	P value for Porcine
First cleavage	Time (hpi)	0.004	29.38 ± 0.46	27.28 ± 0.50	27.10 ± 0.87	41.42 ± 1.67	0.0001
	Median		28,51	26,84	25,33	35,99	
	Interquartile range 25-75%		26,68 – 31,10	25,35 - 28,18	24,26 - 29,25	28,27 - 50,75	
	Time range (hpi)		18.85 - 51.68	23.23 - 36.83	20.37 - 42.23	15.83 - 134.88	
	Duration of cleavage (min)	NS	24.21 ± 1.04	20.83 ± 1.51	19.53 ± 1.21	23.85 ± 1.34	NS
	Range for duration (min)		10.00 - 65.00	10.00 - 45.00	10.00 - 49.00	10.00 - 165.00	
Second cleavage	Time (hpi)	0.002	45.05 ± 2.01	37.91 ± 0.78	45.0 ± 3.19	41.59 ± 1.65	NS
	Median		43,22	37,85	39,35	40,33	
	Interquartile range 25-75%		38,21 - 46,20	34,40 - 40,22	33,27 - 56,16	33,10 - 47,54	
	Time range (hpi)		26.82 - 93.89	37.19 - 47.34	23.16 - 83.55	17.23 - 86.65	
	Duration of cleavage (min)	NS	21.13 ± 1.64	27.69 ± 2.88	28.23 ± 2.05	30.32 ± 1.86	NS
	Range for duration (min)		5.00 - 70.00	10.00 – 80.00	15.00 - 60.00	10.00 – 85.00	
Morula	Time (hpi)		-	137.00 ± 3.85	128.83 ± 2.68	-	
	Median			140,01	126,40		
	Interquartile range 25-75%			132,28 - 147,45	120,46 - 130,25		
Blastocyst	Time (hpi)		-	155.19 ± 3.51	140.38 ± 2.65	-	
	Median			154,61	137,95		
	Interquartile range 25-75%			146,58 - 170,42	132,25 - 142,55		
	Rate of development (%)			21.13%	16.35%		
Expanded Blastocyst	Time (hpi)		-	170.49 ± 3.21	155.64 ± 2.54	-	
	Median			171,73	153,24		
	Interquartile range 25-75%			162,40 - 184,82	146,48 - 163,15		
	Rate of Development (%)			11.27%	14.42%		

Data are expressed as mean ± SEM and medians with interquartile 25-75%. hpi: Hours Post Insemination; NS: Not Statistically Significant.

Table 2: Prevalence of direct and reverse cleavage in bovine and porcine embryos cultured *in vitro*.

Direct Cleavage	Bovine	Porcine
Number of embryos with direct cleavage to 3 c (%)	6 (4.23%)	28 (13.47%)
Time range to start cleavage (hpi)	(27.36 - 31.49)	(23.10 - 75.39)
Time for atretic embryo at hpi, mean ± SEM	128.90 ± 6.53	156.3 ± 28.49
Number of embryos with direct cleavage to >3 c (%)	None	7 (3.37%)
Time range to start cleavage to >3c (hpi)	None	(30.07 - 74.31)
Time for atretic embryo at hpi, mean ± SEM	None	143.13 ± 9.98
Overall number of embryos with direct cleavage (%)	6 (4.23%)	35 (16.82 %)
Overall atretic embryo at hpi, mean ± SEM	128.90 ± 6.53	149.72 ± 19.24
Reverse Cleavage		
Number of embryos with reverse cleavage (%)	12 (8.45%)	9 (4.33%)
Time range for starting the first cleavage (hpi)	(20.28 - 37.54)	(25.35 - 91.31)
Time range for ending the first cleavage (hpi)	(20.48 - 38.18)	(26.05 - 91.51)
Symmetry of the first cleavage	7 symetric	5 symetric
	5 asymetric	4 asymetric

hpi: Hours Post Insemination.

Differences in developmental kinetics between bovine and porcine embryos started to occur at the morula (P=0.001), blastocyst (P=0.0001) and expanded blastocyst stages (P=0.0004) due to already known developmental kinetics: bovine embryos develop to the blastocyst stage in approximately 8 days, whereas porcine embryos do so in about 6 days. The embryos that did not develop to the blastocyst became atretic before reaching morula stage. The data in Table 1 are also presented as mean ± SEM to relate our results to those already published.

Abnormal cleavages

In our study, some embryos showed direct cleavage from 1-cell to 3 cells: 4.23% for bovine and 13.47% for porcine embryos, and from the 1 cell stage to more than 3 cells 3.37% for porcine embryos. None of the bovine embryos directly cleaved to more than 3 cells (Table 2). All of these embryos become arrested and degenerated in culture by approximately 130 hpi (bovine) and 150 hpi (porcine). The reverse cleavage happened in 8.45% of bovine and 4.33% porcine embryos (Table 2). It occurred at any time during bovine and porcine embryo development and these embryos continued to cleave in few cases (Figure 1) but none of these embryos developed to the blastocyst stage in our study.

Discussion

The results of our study present morphokinetic parameters of porcine and bovine embryos development *in vitro* using the time-lapse system. Number of reports on the morphokinetics of porcine embryos are limited. In the study of Mateusen et al. [40] porcine embryos (presumptive zygotes) were recovered 48h post insemination from the reproductive tract and cultured to perform time-lapse monitoring of embryo development (time of *in vivo* insemination was set as t0). The differences between Mateusen et al. [40] and our study can be attributed to the use of different culture media (NCSU-23 *vs.* NCSU-37) and source of zygotes (*in vivo vs. in vitro*). Generally, *in vivo* derived embryos have better developmental potential than those developed from *in vitro* matured oocytes [10,41]. Several studies [4,8,41-45] demonstrated that various culture treatments may alter both the time of first embryo cleavage and its success in reaching the blastocyst stage *in vitro*. Mateusen et al. [40] showed that porcine embryos that failed to reach the blastocyst stage needed on average 6.25h and 5.44h longer to reach the third cell cycle and early morula stage, respectively as compared to the embryos that completed blastocyst development. These authors were not able to establish time of first cleavage due to recovery of fertilized oocytes at 48h post insemination. Dang-Nguyen et al. [46] showed that timing, pattern and evenness of the first cleavage and the timing of the second cleavage affected developmental competence and quality of *in vitro* produced porcine embryos what is in agreement with our study. In our study, the porcine embryos that failed to develop to blastocyst stage exhibited first cleavage later and had broader ranges for the time of first cleavage and its duration compared to the embryos that developed to the blastocyst. Most of these embryos became atretic before reaching the morula stage. Perhaps those embryos underwent anomalous genome activation, therefore failed in the further development [18]. Additionally, Nguyen et al. [47] showed that porcine embryos examined at 48 and 79 hr of culture had high competence for development to the blastocyst despite the moderate or high polyspermy, which is common phenomenon in porcine IVF. The Booth et al. [16] suggested that combining all the morphokinetic parameters with the metabolic criteria (i.e. utilization of amino acids) could provide a better prediction of porcine embryo development to the blastocyst stage.

As for the bovine embryos cultured *in vitro*, there are more data on theirs morphokinetics. The results on the timing of cleavages and attainment of certain stages of embryo development reported in our study are similar to those in previously published reports [10,27,48-52]. We found that, there were differences in the timing of first and second cleavage between bovine embryos that developed to blastocyst stage and non-developing embryos. Based on a retrospective analysis, Holm et al. [27] identified the optimal time frames i.e. 32 to 36 h, 40 to 44 h, 48 to 52 h and 92 to 96 h post insemination for selecting viable 2, 3- to 4, 5- to 8 and 9-to 16 cell bovine embryos, respectively. Park et al. [53] revealed that timing of the first cleavage varied from 7.6 hours

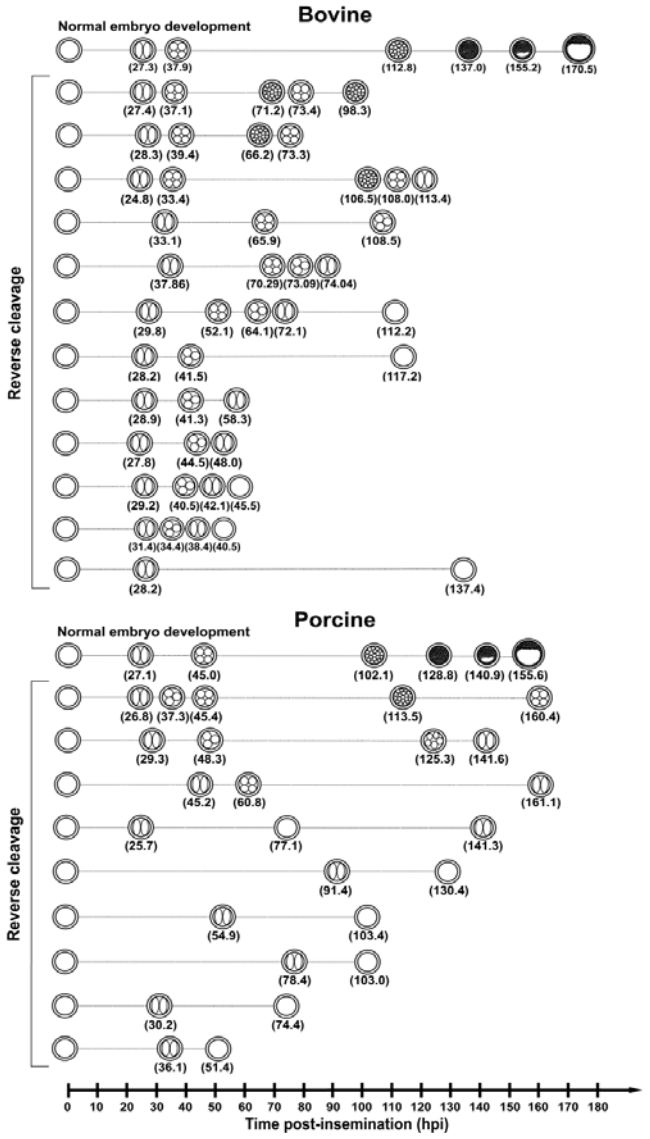

Figure 1: Graphic presentation of reverse cleavage occurrences in bovine and porcine embryos during in vitro development. Numbers in parentheses mean hours post insemination.

in the bovine Parthenogenetic (PA) embryos to 34.5 hours in the two types of somatic cell nuclear transfer embryos (NT-bEC and NT-bTGC) and the timing of expanded/hatching blastocyst appearance varied from 141.6 hours in the PA group to 196.3 hours in the NT-bTGC group. Somfai et al. [10] demonstrated that bovine embryos that developed into blastocyst had significantly shorter first cell cycle time than non-developing embryos, what is consistent with our study results. Similarly, the duration of the first cleavage in case of porcine embryos developing to the blastocyst stage was shorter than for the embryos that did not became blastocyst.

Additionally, our study showed similarities in the time of first cleavage for bovine (27.28 hpi) and porcine (27.10 hpi) embryos that developed to the blastocyst. A similar timing of the first cleavage was also reported by others for bovine embryos (between 25.6 - 32.00 hpi) [43,48,49,52,54], human embryos (24.7 hpi - 27.9 hpi) [19,21,23,55-59], and mouse embryos as 28.2 hpi [32,34] and 32 hpi [34]. Likewise, the semen from different bulls did not affect the time of first cleavage of bovine *in vitro* obtained embryos (25.6, 25.5, 23.1 and 22.7 hpi) or parthenogenetic embryos (25.1 hpi) [49]. The time of first cleavage in human embryos was not affected by the type of gonadotropin [60] and stimulation protocol used during controlled ovarian stimulation in IVF cycles [61], the type of culture medium [24] or abnormal fertilization [62]. The timing of early embryonic developmental events in humans has been successfully linked to euploidy status [1,22], implantation potential [11,19,63] and abnormal cleavage patterns [5,12,20]. Female obesity also did not disturb the developmental dynamics of cultured embryos studied in a time-lapse system [64]. These data suggest that the time of the first cleavage is possibly universal in mammalian embryogenesis.

It has been demonstrated that timing of the first cleavage is highly correlated with embryo potential to develop to the blastocyst stage in cattle [28,65-69], human [70-72], mice [31,33,73] and pigs [16,40,46]. Isom et al. [45] documented this pheonomenon in porcine embryos *in vitro*-fertilized and, Somatic Cell Nuclear Transfer (SCNT), and Parthenogenetic (PA) embryos. Also, blastocyst formation rate was much higher in the bovine early cleaving Parthenogenetic (PA) and two types somatic cell nuclear transfer (NT-bEC and NT-bTGC) embryos (PA, 46%; NT-bEC, 50%; NT-bTGC, 39%) than in the late cleaving groups (PA, 18%; NT-bEC, 23%; NT-bTGC, 28%). However, the percentage of embryos whose development was blocked between the two- and eight-cell stages was increased in the late cleaving groups [53]. Although, embryos that cleave soonest after fertilization are not necessary more developmentally competent than those that cleave later [25,73] what was also confirmed in our study. The ranges for the first and second cleavage timing and their duration of bovine and porcine embryos developing to the blastocyst are within the corresponding ranges for embryos that did not reach the blastocyst stage. A similar observation was reported by Holm [27] for bovine viable *vs.* non-viable embryos. The first cleavage time ranged from 21.4 - 42.50 hpi *vs.* 15.90 - 69.9 hpi, respectively, while the second cleavage time ranged from 2.5 - 18.00 hpi *vs.* 1.5 - 57.00 hpi, respectively. Likewise, Milewski et al. [23] reported the timing range for the first cleavage as 9.7 - 40.10 hpi *vs.* 19.8 - 57.4 hpi for viable and non-viable human embryos, respectively, and the second cleavage timing range as 23.3 - 50.4 hpi *vs.* 23.8 - 84.2 hpi), retrospectively. The mechanisms of relationship between early cleavage and enhanced early development *in vitro* are not currently understood. But the timing of blastocyst formation is also a good marker for embryo quality, and early cavitating embryos are superior in comparison with those cavitating later in regard to the total cell number, allocation of inner cell mass and trophectoderm cells, and cryosurvival [69].

Cleavage anomaly

Little is known about the occurrence and consequences of the cleavage anomalies during *in vitro* development of embryos of farm animals. These phenomena typically go undetected when using traditional assessments (i.e., conventional, static observations) because snap-short observation of embryos once a day does not allow to notice such incidents. In our study, Direct Cleavage (DC) from the 1-cell stage to 3 cells was observed in 4.23% of bovine and 13.47% of porcine embryos and from 1 cell to more than 3 cells in 3.37% of porcine embryos. The prevalence of DC in the literature has been

reported for bovine embryos as 14.1% [10] and for human embryos as 13.7 - 22% [20,74]. These differences probably result from variation in the quality of oocytes used for insemination, the number of embryos studied and perhaps species differences. In our study, none of the embryos showing DC developed to the blastocyst and this is in agreement with a published report that development and implantation were compromised in human embryos exhibiting DC [19]. The implantation frequency of human embryos exhibiting DC was 1.2% to 7.4% [20,75]. The exact mechanisms underlying the phenomenon of DC is still unknown. Therefore, it has been speculated that DC is most likely associated with the presence of surplus centromeres and subsequently a multipolar spindle [76]. All tri-pronucleate oocytes that had undergone DC to three cells were chromosomally abnormal with each containing a varied number of chromosomes [76]. Lagalla et al. [74] reported that when zygotes divided directly into three or more daughter blastomeres, they had a markedly decreased blastocyst formation rate when compared with their normal counterparts. It has also been shown that the ability of embryos exhibiting DC to establish a pregnancy is significantly reduced. Rubio et al. [20] reported that 13.7% of all examined embryos and 6.6% of transferred embryos that underwent DC resulted in 1.2% clinical pregnancies.

Another anomaly in cleavage events is a Reverse Cleavage (RC), the phenomenon of blastomere fusion. Bovine and porcine embryos exhibiting RC in our study did not develop to the blastocyst stage and approximately half of them had asymmetric first cleavage. RC occurred at any time during bovine and porcine embryo development and such embryos only sporadically resumed cleavage. The reported prevalence of reverse cleavage in human embryos was 6.8 - 27.4 % [12,74,75,77,78]. Barrie et al. [75] found that none of nine transferred embryos that exhibited RC implanted after embryo transfer. The embryos that underwent RC had similar fragmentation, cell evenness and morphokinetic profiles as their counterparts that did not show this condition [77]. The etiology of reverse cleavage is unclear but it has been suggested to be related to errors in cell division [79] or to reduced progressive sperm motility [12]. It was also suggested that it could act as an embryo self-correction mechanism. Barbash-Hazan et al. [80] demonstrated that self-correction of aneuploidy, occurred more often during development towards the blastocyst stage. Other studies reported aneuploidy self-correction between cleavage and blastocyst stage of human embryo and some of them could produce healthy babies [80-84]. Further studies on embryos of large animals, could help to elucidate results obtained with human embryos.

Conclusion

In conclusion, the use of time-lapse monitoring system is becoming standard in human IVF but is currently not widely used in animal IVF laboratories, perhaps due to lack of established normalized morphokinetic parameters for animal embryos. Therefore, the results of present study may possibly help to establish guidelines and/or reference standards for developmental kinetics of *in vitro* produced bovine and porcine embryos. They may also provide a basis for the future studies on the etiologies and consequences of anomalies in embryological, cellular and molecular events during embryo development and to understand how such abnormalities impact fetal and postpartum development. Large-scale studies are needed to explore detailed algorithms and equations of morphokinetic variables of bovine and porcine embryos to develop more precise embryo selection criteria. In addition, data are needed to links morphokinetic parameters of animal embryos with their genetic merit. At this moment from a practical point of view, it seems that cinematographic analysis of bovine and porcine embryos development is an excellent method for identifying abnormally developing embryos from cohort of cultured embryos.

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