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Cinematographic analysis of bovine embryo development in serum-free oviduct-conditioned medium

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Development of bovine embryos produced *in vitro* from the one-cell to the blastocyst stage in serum-free oviduct-conditioned medium was investigated for 8 days consecutively by time-lapse cinematography. Three movies were analysed (130 embryos). The following observations were made. (1) Development under cine-recording conditions was similar to that in a classical incubator. (2) The highest proportion of embryos at the two-cell, three–four-cell, five–eight-cell, 9–16-cell, morula and blastocyst stages were recorded at 34, 46, 61, 115, 149 and 192 h after insemination, respectively. Cleavage asynchrony between blastomeres within individual embryos started at the two-cell stage. (3) The duration of the first three cell cycles was 35 h, 14 h and 11–62 h, respectively. (4) Detailed analysis of 13 embryos revealed that developmental arrest ('Lag-phase') occurred at the four-cell (1 of 13), five-cell (2 of 13), six-cell (3 of 13), seven-cell (3 of 13) or eight-cell stage (4 of 13); this phase lasted about 59 h. Embryos arrested at the eight-cell stage developed into morula–blastocysts (3 of 4) at a higher rate than did those arrested at earlier stages (2 of 9). (5) The faster the embryos cleaved into early stages (two-cell, three–four-cell and five–eight-cell), the higher the probability that they developed into morula–blastocyst: 70% of the embryos reaching the two-cell stage before 30–31 h after insemination developed into morula–blastocyst. We suggest that the lag-phase as well as the link between early cleavage kinetics and further development could be related to the transcriptional activity of the embryo at about the 5–8-cell stage.

Introduction

In vitro methodology to obtain large numbers of bovine embryos is routinely used, and even on an industrial scale to produce beef embryos of desired genetic quality (Gordon, 1991; Lu and Polge, 1992). This method is also an excellent tool for fundamental investigations of early embryo development. In this respect much work remains to be done in livestock species such as cattle. At least three critical stages in embryo development can be influenced by culture conditions: (1) the transition from maternal to zygotic control of development; (2) compaction at the morula stage; and (3) blastocyst formation. Serum-free oviduct-conditioned media support development from the one-cell to the blastocyst stage (Mermillod *et al.*, 1992a, b, 1993).

An accurate study of the timing of embryo development in this kind of media would provide interesting information on preimplantation features, such as cell-cycle duration and the lengthening of the cell cycle at the time when genomic expression resumes.

Few reports have been published on cleavage kinetics of bovine embryos produced *in vitro* (Sirard and Lambert, 1985; First and Barnes, 1989; Pollard *et al.*, 1991; Barnes and First, 1991; Van Soom *et al.*, 1992) because these investigations require frequent observations out of the incubator and could affect normal embryo development. The only way to obtain accurate data is by continuous cinematographic recording of embryos in culture. This method has been used previously to observe cow embryos harvested *in vivo* (Massip and Mulnard, 1980; Massip *et al.*, 1982, 1983a, b).

In the present study time-lapse cinematography was used to analyse the behaviour of embryos cultured in serum-free oviduct-conditioned medium. The aim was to obtain information on the developmental characteristics of individual embryos and thus to establish a correlation between the early behaviour of an embryo and its further development *in vitro*.

Materials and Methods

Source of embryos and culture

Embryos were produced by *in vitro* maturation and *in vitro* fertilization of oocytes from ovaries of slaughtered cows, according to the method described by Mermillod *et al.* (1992b). Intact cumulus–oocyte complexes were matured in tissue culture medium 199 plus 10% heat-treated fetal calf serum supplemented with 0.5 μg pure pig FSH ml^{-1}, 5 μg pure pig LH ml^{-1} (Beckers, 1987) and 1 μg oestradiol ml^{-1} (Sigma Chemical Company, St Louis, MO). One hundred complexes

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were placed in 500 µl of this medium in four-well plates (Nunc, Roskilde). After 24 h at 39°C in a humidified atmosphere containing 5% CO_2, cumulus–oocyte complexes with expanded cumulus masses were transferred to fertilization medium in four-well plates.

This medium consisted of modified Tyrode's medium supplemented with 6 mg fatty-acid-free BSA ml^{-1}, 4 mg sodium lactate ml^{-1}, 0.11 mg sodium pyruvate ml^{-1} (Sigma Chemical Company) (TALP) and 10 µg heparin ml^{-1} (Calbiochem, San Diego, CA). To each 500 µl of fertilization medium 2×10^6 spermatozoa, separated using Percoll (Pharmacia, Uppsala), were added. The same ejaculate from one Belgian Blue bull was used throughout the experiments. Cumulus–oocyte complexes were removed from the fertilization medium after 18 h, and oocytes were denuded from cumulus cells by vortexing at medium speed for 2 min. About 100 ova were handled together in 2 ml PBS containing 0.25% trypsin (Gibco BRL, Paisley). Only zygotes that were completely cumulus-free were placed in culture. This was to avoid possible depletion of the culture medium by granulosa cells.

After washing in medium 199, naked zygotes were cultured at 39°C in droplets (50 embryos per 50 µl) of serum-free oviduct-conditioned medium (Mermillod *et al.*, 1992a, 1993) under mineral oil in a humidified atmosphere containing 5% CO_2. The culture was run for 8 days in the same drop of conditioned medium without replenishing the media.

Time-lapse cinematographic equipment

A Plexiglas thermoregulated box (60 cm × 35 cm × 50 cm) was adapted to fit onto an inverted microscope (Nikon Diaphot, Tokyo). A contact thermometer located close to the cinematographic chamber controlled a domestic hair-dryer and maintained an internal temperature of 39°C. A cinematographic chamber placed on the microscope plate was built by first boring a hole, 6.5 cm in diameter, into a Plexiglas sheet 2 cm thick. A bottom sheet 2 mm thick was glued into place; a sheet 6 mm thick was screwed with thumb screws onto the top; and the entire structure secured by a gasket. Embryos were cultured in a tissue culture dish 3.5 cm in diameter (Falcon 3080, Becton Dickinson, Erembodegem) placed directly inside the closed cinematographic chamber. A 5% CO_2:95% air mixture was humidified and warmed by passing it through sterile water at 39°C in a gas bubbler bottle and flushed into the chamber for 15 min every 30 min. The culture was run for 8–10 days without changing the medium.

A Bolex 16 mm cine-camera controlled by a time-lapse system exposed a single frame every minute. The microscope light was switched on a few seconds before and switched off immediately after. The experiment was carried out in a dark room. Video copies of each movie were prepared and every picture was numbered according to the length of time the embryos had been in culture.

Experimental protocol

After fertilization and removal of the granulosa cells, one group of zygotes was cultured in the cinematographic chamber, and a simultaneous control group in a classical incubator. The day culture began was day 0. On day 2, rates of cleavage and of formation of five–eight-cell embryos were recorded for both groups. The rates of formation of blastocysts on days 6, 7 and 8 were also recorded for both groups.

Three movies were performed, one at 10 × magnification (movie 416) and two at 5 × magnification (movies 477 and 483). The higher magnification was used to obtain more accurate data on each embryo (for example cleavage rate and exact number of cells). The same batch of conditioned medium was used throughout.

Statistical analysis

Chi-square analysis ($P < 0.05$) was used to compare the number of embryos at different stages. ANOVA 1 was used to compare mean cleavage time.

Results

Validity of the time-lapse analysis method

A total of 250 zygotes were cultured, 111 in the incubator and 139 in the cinematographic chamber. However, only 130 of the zygotes in the chamber were analysed because some embryos shifted out of the field of the cine-camera in movie 416. The number of embryos reaching the two–four-cell and five–eight-cell stage after 2 days of culture, and the cumulative number of blastocysts on days 6, 7 and 8 were recorded in both groups (Table 1).

On day 2, 37–74% of embryos were at the five–eight cell stage in the cinematographic chamber, compared with 41–55% in the incubator. The proportion of blastocysts at day 8 varied from 15 to 21% under the camera versus 17 to 24% in the incubator. No significant difference ($P > 0.05$) between control and experimental groups was observed at any stage of the three movies. The rates of formation of five–eight-cell stage embryos on day 2 and of blastocysts on days 6, 7 and 8 did not differ significantly from one movie to another in the control group (I) or in the experimental (C) group.

Kinetics of development

Distribution of cleavage divisions. The distribution of embryos at each developmental stage during the culture period is shown (Fig. 1). The distribution curves overlap and the progressive decrease of the height of the peak for the successive stages indicates the progressive block of embryo development. The curve for the five–eight-cell stage (Fig. 1c) is characterized by a plateau and a slow decline. It is followed at the 9–16-cell stage by an important decrease in the peak distribution in relation to the well-known block after the five–eight-cell stage.

The morphology of the corresponding stages, at the time when they occurred most often, is illustrated in Fig. 2. The time between the first appearance of a given stage and its peak occurrence increases with the successive stages (as do the SDs) (Table 2). This reflects an increasing asynchrony of cleavages from the two-cell stage onwards; intermediate three-cell stages were noted for 4 min to more than 4 h.

Table 1. Development of bovine embryos fertilized *in vitro* cultured either in an incubator (I) or under cine-record (C)

Movie	Group	*n*	Day 2[a] Cleaved *n* (%)	Day 2[a] 5–8-cell *n* (%)	Day 6 Blastocysts[b] *n* (%)	Day 7 Blastocysts[b] *n* (%)	Day 8 Blastocysts[b] *n* (%)
416	I1	29	26 (90)	16 (55)	3 (10)	5 (17)	5 (17)
	C1	27	24 (89)	20 (74)	1 (4)	3 (11)	4 (15)
477	I2	38	27 (71)	16 (42)	4 (11)	8 (21)	9 (24)
	C2	53	38 (72)	31 (58)	2 (4)	8 (15)	11 (21)
483	I3	44	25 (51)	18 (41)	4 (9)	9 (21)	9 (21)
	C3	59	36 (61)	22 (37)	5 (8)	10 (17)	11 (19)

[a]Number and percentage (within parentheses) of cleaved embryos and embryos at the 5–8-cell stage at day 2 under cine-record and in the control group for the three experiments. [b]Cumulative number and percentage (within parentheses) of blastocysts at day 6, 7 and 8 in both groups.

Duration of the cell cycles. The mean duration of the first three cell cycles was calculated in movie 416 by measuring the time taken by each embryo to double its number of cells. The first cell cycle lasted (mean ± SD) about 34 h 23 min ± 6 h 50 min ($n = 13$) and the second cycle 13 h 35 min ± 2 h 3 min ($n = 13$). The duration of the third cycle was 11 h 5 min ± 3 h 17 min or 62 h 37 min ± 19 h 36 min, depending on whether the embryo reached the eight-cell stage before ($n = 4$) or after ($n = 6$) the developmental arrest observed between the five-cell and eight-cell stage (see below).

Developmental arrest at early cleavage stages. It was observed in all three movies that the development of most of the cleaving embryos stopped for some time during the third cell cycle, that is, between the four-cell and eight-cell stage. In movie 416 we were able to determine the exact number of cells for each embryo entering this developmental arrest. This phenomenon, termed 'lag-phase', was taken to occur when the interval between two mitoses within the third cell cycle was twice as long as the duration of the second cell cycle, that is, 28 h (2 × 14 h). This type of behaviour was observed for nearly all of the embryos. In movie 416 we recorded accurately at which stage each embryo enters its lag-phase, duration of the lag-phase and the stage of embryo development at day 8 (Table 3).

In spite of the small number of embryos studied ($n = 13$) some observations are noteworthy. (1) The three blastocysts that appear in movie 416 originated from embryos entering the lag-phase at the six-cell, seven-cell and eight-cell stage. (2) A high proportion (3 of 4 versus 2 of 9) of morulae and blastocysts developed from embryos exhibiting a lag-phase at the eight-cell stage. (3) Embryos arrested at earlier stages (four-cell and five-cell stage) cleaved at a slower rate than did those arrested at more advanced stages (six-cell, seven-cell and eight-cell stages), and did not develop into morulae or blastocysts. This suggests that the slower the embryos cleave, the less chance they have of developing to these late stages.

After their developmental arrest, nearly all the embryos (12 of 13) resumed division. At the end of the culture period (day 8) one embryo (8%) was at the four-cell stage, two (15%) at the five-cell stage, two (15%) at the seven-cell stage, three (23%) at

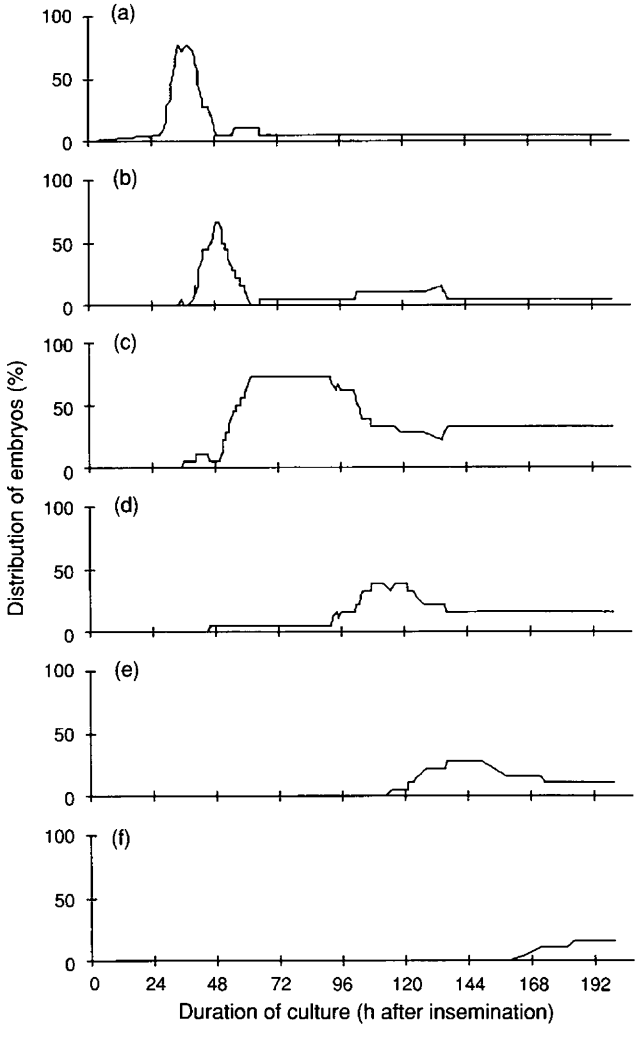

Fig. 1. Distribution (percentage) of cleaved bovine embryos during culture under cine-record (data from movie 416; $n = 18$). Proportion of (a) two-cell embryos, (b) three–four-cell embryos, (c) five–eight-cell embryos, (d) 9–16-cell embryos, (e) morulae and (f) blastocysts during culture are shown.

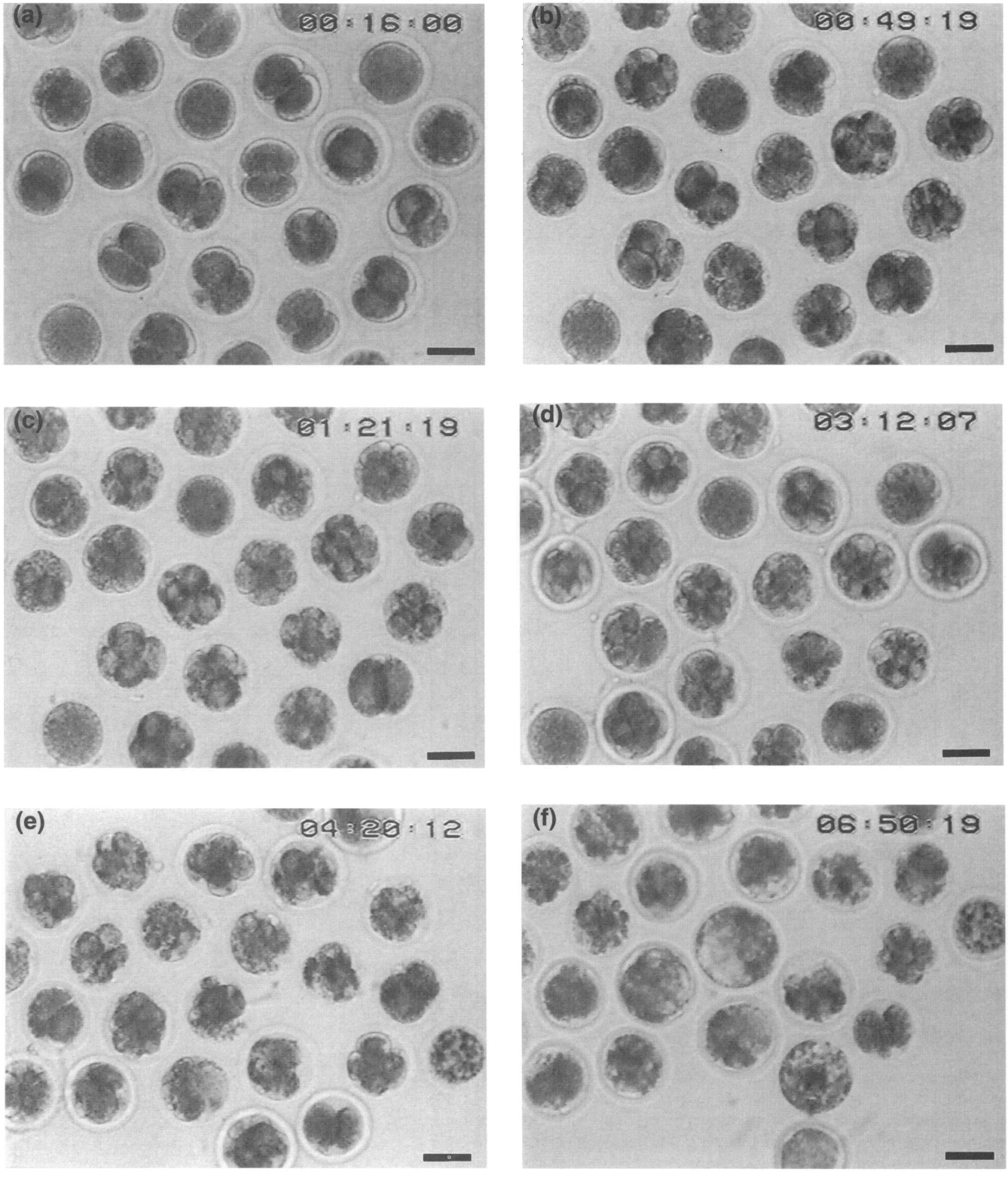

Fig. 2. Pictures from movie 416 taken at the time of peak emergence of (a) two-cell bovine embryos, (b) three–four-cell embryos, (c) five–eight-cell embryos, (d) 9–16-cell embryos, (e) morulae and (f) blastocysts. (Scale bars represent 100 μm.)

Table 2. Time of first appearance and time of peak emergence of early cleavage stages of bovine embryos under cine-recording

Stage	Time of first appearance	Time of peak emergence
Two-cell	26 h 11 min ± 01 h 24 min	33 h 54 min ± 00 h 35 min
Three–four-cell	31 h 17 min ± 03 h 36 min	45 h 28 min ± 03 h 31 min
Five–eight-cell	36 h 44 min ± 00 h 59 min	61 h 03 min ± 05 h 42 min
9–16-cell	59 h 31 min ± 13 h 11 min	115 h 03 min ± 15 h 02 min
Morula	119 h 15 min ± 08 h 02 min	148 h 34 min ± 13 h 48 min
Blastocyst	159 h 13 min ± 06 h 06 min	192 h 07 min ± 12 h 26 min

Values are means ± SD of time after insemination; results are from the three movies representing a total of 130 embryos.

Table 3. Time and duration of the 'lag-phase'[a] and stage reached by day 8, according to the stage at which the phase begins in bovine embryos

Stage at start of phase	*n*	'Lag-phase'		Stage at day 8	
		Start	Duration	Morulae	Blastocysts
Four-cell	1	68 h 48 min	66 h 18 min	0/1	0/1
Five-cell	2	64 h 48 min ± 06 h 29 min	36 h 57 min ± 04 h 57 min	0/2	0/2
Six-cell	3	55 h 48 min ± 02 h 57 min	46 h 06 min ± 03 h 53 min	0/3	1/3
Seven-cell	3	61 h 27 min ± 3 h 47 min	53 h 02 min ± 27 h 53 min	0/3	1/3
Eight-cell	4	59 h 42 min ± 03 h 33 min	42 h 29 min ± 06 h 58 min	2/4	1/4
Total	13			2/13	3/13
Mean		59 h 14 min ± 06 h 16 min	46 h 47 min ± 15 h 06 min		

[a]'Lag-phase': developmental arrest observed within the third cell cycle. Arbitrarily, a lag-phase is present when the time between two mitoses of the third cell cycle exceeds twice the duration of the preceding cell cycle (second cell cycle), that is, 28 h (2 × 14 h).
Values are means ± SD of time after insemination; results are from 13 embryos studied in movie 416.

the 8–16-cell stage, two (15%) at the morula and three (23%) at the blastocyst stage.

The lag-phase was also observed in movies 477 and 483 and its duration was of the same order of duration (55 h). However, at the low magnification used in movies 477 and 483, it was not possible to detect the exact stage of development at which it occurred.

Relationships between early cleavage kinetics and further development of embryos

In movies 477 and 483 the earliest time of appearance of the early cleavage stages (two-cell, three–four-cell, five–eight-cell stages) was noted, as well as the stage reached by each of them at day 8. Only cleaved embryos were taken into account. They were classified into three groups: embryos reaching morula–blastocyst (group 1), embryos reaching the 9–16-cell stage (group 2) and embryos reaching a stage where they had less than nine cells (group 3). In each of these groups, the time at which they cleaved into the two-cell, three–four-cell or five–eight-cell stage was recorded. Embryos that had developed into morula–blastocysts by day 8 cleaved earlier at the two-cell, three–four-cell and five–eight-cell stage than did those that had developed to no more than the nine-cell stage (ANOVA 1) (Table 4). However, no statistical difference could be observed between embryos reaching the 9–16-cell stage (group 2) and those becoming morulae–blastocysts at day 8 (group 1).

In Fig. 3a the time during which two-cell embryos appeared was arbitrarily divided into ten equal intervals. The developmental stage reached by day 8 is related to the kinetics of cleavage at the two-cell stage: the embryos that cleave the fastest are on the left and the slowest are on the right. A similar analysis was carried out using the time at which three–four-cell embryos (Fig. 3b) and five–eight-cell embryos (Fig. 3c) appear.

The first embryos to cleave give a higher rate of morula–blastocyst formation than do the slowest ones (Fig. 3). This is particularly obvious at the two-cell stage and at the three–four-cell stage. More than 70% of the embryos that had reached the two-cell stage before 30–31 h after insemination were morula–blastocysts by day 8. This proportion was significantly higher (χ^2; $P < 0.05$) than that calculated for embryos that reached the two-cell stage later (more than 31 h after insemination).

Discussion

The study reported here shows that bovine embryos matured and fertilized *in vitro* and then cultured in the conditions used

Table 4. Comparison of the time at which embryos cleaved into two cells, three–four cells or five–eight cells, according to the developmental stage they had reached at day 8 (morula–blastocyst, 9–16-cell stage or a stage < nine-cell)

	Stage reached at day 8		
	Morula–blastocyst	9–16-cell stage	< Nine-cell stage
Two-cell stage	30 h 05 min ± 05 h 23 min[a]	30 h 51 min ± 03 h 31 min[a]	36 h 38 min ± 05 h 23 min[b]
Three–four-cell stage	37 h 16 min ± 04 h 16 min[a]	36 h 49 min ± 03 h 52 min[a]	46 h 29 min ± 11 h 05 min[b]
Five–eight-cell stage	48 h 01 min ± 05 h 56 min[a]	47 h 28 min ± 05 h 37 min[a]	54 h 05 min ± 07 h 08 min[b]

Values are means ± SD of time after insemination; results are from two-cell stage ($n = 69$), three–four-cell stage ($n = 69$) and five–eight-cell stage ($n = 58$) embryos from movies 477 and 483.
Values within the same row with different superscripts are significantly different (ANOVA 1).

for cinematography develop at the same rate as do control embryos placed in serum-free oviduct-conditioned medium. The cinematographic method, therefore, is a good tool for analysis of the kinetics of development and behaviour of embryos *in vitro*. This method has already proved useful in investigating the behaviour of mouse embryos (Mulnard, 1967), hamster and rhesus monkey embryos (Bavister, 1988) and cow blastocysts produced *in vivo* (Massip and Mulnard, 1980; Massip *et al.*, 1982, 1983a, b).

The timing of cleavages is in the range reported by First and Barnes (1989); Pollard *et al.* (1991) and Van Soom *et al.* (1992). A comparison with development *in vivo* is difficult because of variations in the time of ovulation and in the subsequent fertilization. It is generally recognized that in bovine embryos produced *in vitro* development is slower than in those that develop *in vivo*, especially after the eight-cell stage has been reached (Barnes and Eyestone, 1990). This is also the case in mice (Bowman and McLaren, 1970). Our results are in agreement with these observations, as the delay in appearance of 9–16-cell embryos, morulae and blastocysts is about 30–40 h when compared with embryos produced *in vivo* (Thibault, 1966; McGaugh *et al.*, 1974; Prather and First, 1988). However, our data correspond to those of Hamilton and Laing (1946) on non-superovulated embryos recovered *in vivo*. Thus, caution is recommended when data obtained from *in vitro* and *in vivo* studies are compared, as the accuracy of cleavage analysis *in vivo* is about ± 24 h. Moreover, such comparisons do not take into account the fact that blastocysts *in vitro* often have significantly fewer cells (Iwasaki *et al.*, 1990), so that the developmental rate is probably slower than it appears by morphological evaluation.

The most unusual finding in our movies was certainly the developmental arrest ('lag-phase') observed at the four-cell stage but mainly at the five-cell, six-cell, seven-cell or eight-cell stages. This phenomenon was suspected in control groups where five–eight-cell embryos present at day 2 were still at the same stage on day 3 but had developed further by day 4. As this lag-phase occurs at about the stage of initiation of zygotic transcription, it is possible that these events are linked. Early transcription can be detected from only the eight-cell stage onwards in embryos produced *in vivo* (Camous *et al.*, 1986; King *et al.*, 1988). However, it appears that transcription could occur earlier in bovine embryos produced *in vitro*: tritiated uridine incorporation into neosynthesized RNA is detected at the two-cell stage but mainly in the five–eight-cell embryos (Plante and King, 1993; B. Grisart, A. Massip and F. Dessy, unpublished).

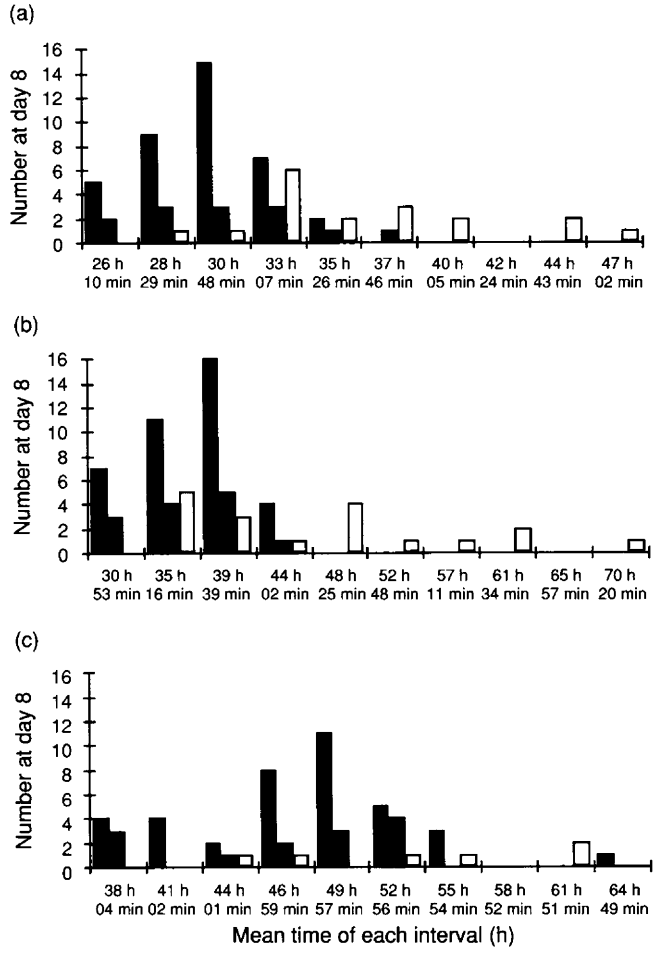

Fig. 3. Proportion of bovine morulae-blastocysts (black), 9–16-cell embryos (grey) and embryos with less than nine cells (white) at day 8 of culture, in relation to the time at which embryos in each category had reached (a) the two-cell stage ($n = 69$), (b) the three–four-cell stage ($n = 69$) and (c) the five–eight-cell stage ($n = 57$). The fastest dividing embryos are on the left while the slowest are on the right. Data are from movies 477 and 483.

The changes in nucleolar morphology associated with activation of rRNA synthesis at the four–eight-cell stage (King *et al.*, 1989) and treatment with α-amanitin to inhibit mRNA synthesis (Barnes and First, 1991) also support the theory that genome expression could start earlier *in vitro*. A link between transcriptional activity of the embryo and cleavage rate seems to be an important feature of early development, particularly between the four-cell and eight-cell stages that are known to be very sensitive to environmental conditions (Eyestone and First, 1991). First and Barnes (1989) put forward an interesting model linking cleavage rate with first genomic expression. Briefly, they state that 'the fixed transcriptional capacity of the early embryo may be the factor that lengthens the cell cycle by slowing the production of specific proteins needed for metaphase . . . thus the lengthening of the cell cycle may allow for zygotic transcription to occur'. This model is supported by some results obtained in the frog (Kimelman *et al.*, 1987), in which artificial lengthening of early cell cycles before the mid-blastula stage by inhibition of DNA replication induces premature expression of the genome.

A possible premature transcription resumption could result in unphysiological conditions that impair further development. Such a model would fit well with our observations: embryos that entered into lag-phase at the eight-cell stage (Table 3) gave more morulae and blastocysts than did those arrested at earlier stages (for example the five-cell and six-cell stages). Furthermore, embryos showing a lag-phase at the four-cell or five-cell stage cleaved more slowly than did those exhibiting a lag phase at the seven-cell or eight-cell stage. The first embryos reached their lag-phase stage after the second embryos had already reached more advanced stages.

The reasons for the lag-phase could be related to the amount or the quality of the RNA or the proteins stored in the oocytes. Some oocytes may have been harvested before the end of their growth phase. These would then be unable to develop to the eight-cell stage, resulting in a lengthening of the cell cycles. The lag-phase may be the manifestation of this deficiency, which could induce premature resumption of transcription. The fact that development *in vitro* of zygotes recovered *in vivo* is better than that of zygotes produced by maturation and fertilization *in vitro* suggests that oocytes ovulated *in vivo* are more competent than are those collected from ovaries from the abattoir (Van Soom and De Kruif, 1992). Suboptimal culture conditions could also have an influence on the lag-phase, but this would not explain why some embryos develop normally to the eight-cell stage and others do not. It is likely that both the status of the oocytes and culture conditions are involved in this phenomenon.

Some previous results (Plante and King, 1992; Van Soom *et al.*, 1992) suggest that the first embryos to reach a given early cleavage stage have a greater chance of developing into morulae–blastocysts. The faster the embryos cleaved, the more chance (up to 70%) they had of becoming morulae–blastocysts by day 8. More slowly dividing embryos develop at a very low rate and reach only the 9–16-cell stage or even earlier stages.

In other experiments embryos were separated into fast, medium and slow cleaving at the two-cell, three–four-cell and five–eight-cell stage (B. Grisart, A. Massip and F. Dessy, unpublished). We obtained similar results: two-cell embryos selected 30 h after insemination, three–four-cell embryos selected 36 h after insemination and five–eight-cell embryos selected 48 h after insemination resulted in 47%, 38% and 43% becoming blastocysts, respectively. As morulae were not taken into account in these experiments, we assume that the percentage of morulae–blastocysts could have been higher. On the basis of these results a future experiment would be to preselect embryos that had reached the two-cell stage before 30–31 h after insemination in order to obtain a more homogeneous sample of embryos with a more predictable development. This may be very useful for the study of the regulation of early development (for example, expression of specific genes involved). It seems that differences in early development (for example, at the level of gene expression) result in variation in later development. Gene expression studies in heterogeneous embryo samples produced by fertilization *in vitro* could lead to some erroneous conclusions. Preselection as suggested above would allow the investigation of this hypothesis. The importance of the developmental competence of early bovine embryos *in vitro* has already been emphasized (Rieger *et al.*, 1992). It would be particularly important to take this into account in such a study.

In conclusion, the cinematographic method is a useful tool for the study of early development of bovine embryos. (1) The kinetics of development was accurately established. (2) A developmental arrest (lag-phase) was observed during the third-cell cycle, which seems to be related to the ability of the embryo to develop up to the morula–blastocyst stage. (3) A clear relationship between the kinetics of early cleavage and further development was observed, allowing the selection of a more homogeneous embryo population in terms of its further developmental capacity.

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