Cytogenetic analysis of day-7 bovine embryos by cytophotometric DNA measurements

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A Feulgen staining procedure that stains the DNA of individual fixed nuclei stoichiometrically was used to analyse cytophotometrically the incidence of total ploidy and mixoploidy in 28 day-7 bovine embryos that had been fixed after collection ('non-cultured' embryos). The influence of culture on the incidence of chromosome abnormalities was further studied in another group of 24 embryos ('cultured' embryos) by culturing them for 24 h in Whittingham's medium. Of the total 52 embryos studied, two appeared to be entirely abnormal: one embryo was completely haploid, whereas the other embryo was completely triploid. Individual hyperdiploid nuclei and hypodiploid nuclei were frequently observed in the otherwise diploid embryos. As haploid polar bodies can still be present in morulae and blastocysts (to a maximum of three), only embryos with more than three hypodiploid nuclei were considered as abnormal. Of the 'non-cultured' embryos, 33.3% had one or more hyperdiploid nuclei, whereas 51.9% had more than three hypodiploid nuclei. In this latter group, 35.7% of the embryos also had hyperdiploid nuclei. The results also showed that day-7 bovine embryos that are completely haploid, completely triploid or mixoploid cannot be detected only by examining their morphology. It is concluded that the incidence of, especially, mixoploidy in embryos can be better studied by measuring the DNA content of the individual nuclei of an embryo rather than by analysing chromosomes, as in the latter method only dividing cells can be analysed. The presence of hyperdiploid and hypodiploid nuclei may indicate the frequent occurrence of mitotic segregation failures during mitosis in bovine embryos. These segregation failures may cause the death of cells, which in turn may contribute to a reduced embryo viability. This contention is supported by the results of the 'cultured' embryos that show that in this group the mean percentage of hypodiploid nuclei was lower than that in the group of 'non-cultured' embryos, whereas the mean number of nuclei and the mean percentage of degenerated nuclei was approximately the same in both groups of 'non-cultured' and 'cultured' embryos.

Introduction

The usual rate of survival to term after transfer of a single bovine embryo is approximately 60% (Greve and Del Campo, 1986). This is similar to the percentage that is observed after spontaneous ovulation and artificial insemination (Sreenan and Diskin, 1986). Most of the embryos that do not result in a pregnancy are lost during the first 3 weeks of gestation (Sreenan and Diskin, 1986; Kastelic *et al.*, 1991). It is assumed that these losses occur predominantly between days 15 and 18 after oestrus (Sreenan and Diskin, 1986). One of the possible reasons for loss during the preimplantation phase is the presence of chromosome abnormalities in the embryo (reviewed by King, 1990). Of the chromosome abnormalities that have been

found in embryos, mixoploidy (for example diploidy–haploidy, diploidy–triploidy) is the most commonly observed (Kind, 1990). Mixoploidy has been found more often in embryos that were morphologically abnormal than in embryos that were morphologically normal (King *et al.*, 1987, 1988) in cows. Gonadotrophins, which are used for superovulation, may increase (Long and Williams, 1980) or decrease (Murray *et al.*, 1986b) the frequency of these aberrations. In addition, culturing the embryos may increase the number of polyploid cells (Barlow and Sherman, 1972; Johnson and Rossant, 1981).

The cytogenetic evaluation of embryos by chromosome analysis encounters two major problems. First, few cells are in mitosis, even when a metaphase blocker is used (King *et al.*, 1979; Singh and Hare, 1980; Rottman, 1981; King and Linares, 1983; Murray *et al.*, 1985; King *et al.*, 1987). It is therefore not always possible to detect or identify the degree of mixoploidy.

Second, the occurrence of hypodiploid cells has to be interpreted with caution, as chromosomes can be lost during slide preparation (Murray *et al.*, 1985; King *et al.*, 1988; King, 1990).

In the study reported here these two problems have been overcome by measuring quantitatively the DNA content of the individual interphase nuclei. A similar approach has been used for embryos of other species (mouse: Barlow and Sherman, 1972; Barlow *et al.*, 1972; Rossant and Tamura-Lis, 1981; Molls *et al.*, 1982; Bolton *et al.*, 1984; Chrisholm, 1988; human: Angell *et al.*, 1983; O'Rand *et al.*, 1986; rabbit: Fischer and Schumacher, 1991). The various phases of the cell cycle (G1 phase, S phase and G2 phase) are characterized by differences in the DNA content of the cell nucleus. Cells in the G2 phase have the maximum DNA content, which is twice the DNA content of cells in the G1 phase. The DNA content of cells in the S phase is intermediate, as during this phase DNA is being synthesized. Haploid and hypodiploid G1 nuclei will have a DNA content that is less than the DNA content of the diploid G1 nuclei, whereas hyperdiploid and polyploid G2 nuclei will have a DNA content that is more than the DNA content of the diploid G2 nuclei.

The aim of this study was to determine the incidence of aneuploidy in day-7 bovine embryos developed *in vivo*, as these embryos are commonly used for embryo transfer. A Feulgen-Schiff staining procedure was used to stain the DNA in individual nuclei of the embryos. The dye pararosaniline that is used in this procedure binds stoichiometrically to the apurinic acid in hydrolysed DNA to produce a coloured complex (Duijndam and Van Duijn, 1975). The abundance of this coloured complex, which is a measure of the amount of DNA in the nuclei, was measured by using stage scanning cytophotometry. This type of cytometry is highly accurate. This is illustrated in a study by Van der Ploeg *et al.* (1977), who repeatedly measured (four times) Feulgen-stained nuclei of various types of cell. In this study, the nuclei of white blood cells and human fibroblasts had mean integrated absorbance values of the order of 7000 arbitrary units, whereas the coefficient of variance varied only between 0.3 to 2.4. Furthermore, the influence of (residual) cytophotometrical errors is relatively low and methods are available to correct for these errors (Duijndam *et al.*, 1980a, b). Glare is almost negligible (Duijndam *et al.*, 1980a, b; Mares and Van der Ploeg, 1980); the total correction factor for residual distributional and diffraction errors depends upon the size and the local absorbance of the nuclei, but is generally low in cytophotometrical practice. For example, in the study of Mares and Van der Ploeg (1980), this total correction factor was 1.5% and 6.5% for the nuclei of Purkinje cells and granule cells, respectively.

As day-7 bovine embryos are usually stored for several hours in a phosphate-buffered medium prior to transfer, the influence of culture on the incidence of aneuploidy was also investigated. This was studied by comparing freshly collected embryos with embryos that had been cultured for 24 h in a frequently used storage buffer (Whittingham's medium; Whittingham, 1971).

Materials and Methods

Superovulation

Dutch Friesian cows were treated with 3000 i.u. pregnant mares' serum gonadotrophin (PMSG; Folligonan: Intervet B.V.,

Boxmeer) at day 10 of the oestrous cycle (Dieleman and Bevers, 1987). Luteolysis was induced 48 h after PMSG injection with 22.5 mg prostaglandin (PG; Prostaglandin: Intervet B.V.).

A rapid solid-phase radioimmunoassay method (Dieleman and Bevers, 1987) was used to estimate the LH surge in the donor cows. The cows were treated with anti-PMSG (Neutra PMSG: Intervet B.V.) at 6–12 h after the maximum of the LH surge and inseminated 10–12 h after the maximum of the LH surge.

Embryo collection, qualification and culture

Embryos were collected from dissected uteri of slaughtered cows at day 7 after insemination. The uteri were flushed via the oviduct with 50 ml of Dulbecco's phosphate-buffered saline solution (PBS: Difco, Detroit, MI) per uterine horn.

Immediately after collection, embryos were placed in PBS supplemented with 0.33 mmol sodium pyruvate l^{-1} , 5.56 mmol glucose l^{-1} , 3 g BSA l^{-1} (BSA: Fluka Chemie AG, Bucks), 10^5 i.u. penicillin/streptomycin l^{-1} and 100 ml heat-inactivated fetal calf serum l^{-1} (FCS: Gibco, Breda) (Whittingham, 1971) at 37°C. Embryos were classified into four quality groups (excellent, good, fair and poor) according to the criteria of Lindner and Wright (1983), using a stereomicroscope (Wild, $\times 50$, equipped with a 37°C warm plate) and photographed through an inverted phase-contrast microscope (Olympus, CK2, $\times 200$). One group of embryos was fixed immediately after being photographed ('non-cultured' embryos). Another group of embryos was transferred to fresh Whittingham's medium, and cultured for 24 h at 37°C ('cultured' embryos). The latter embryos were photographed for a second time after this culture period.

Preparation of microscope slides

Preparations were made from entire embryos. 'Non-cultured' embryos were cooled to 4°C, and placed in a hypotonic solution (0.075 mol KCl l^{-1}) at 4°C for 1 h. They were further processed as described by Dyban (1983). This procedure includes overnight fixation in a mixture of glacial acetic acid and absolute methanol (1:3, v:v) at -20°C , followed by treatment (at 20°C) with a softening solution (a mixture of 75% acetic acid and absolute methanol, 1:4, v:v) to spread the cells on the slide.

'Cultured' embryos were placed in a hypotonic solution (0.075 mol KCl l^{-1}) at 37°C for 10–15 min. They were further processed by the method described by King *et al.* (1979), which includes fixation and spreading of the embryo on a warm slide (37°C) by adding a 1:1 (v:v) mixture of glacial acetic acid and absolute methanol at 4°C. All preparations were fixed for a second time for 1 h in a freshly prepared mixture of absolute methanol, formaldehyde (35%) and glacial acetic acid (85:10:5, v:v), and were then washed in absolute methanol. After a final rinse in distilled water, the preparations were dried under a stream of N_2 . Both techniques resulted in preparations with mainly the interphase nuclei and metaphase plates left.

In this study, 52 embryos, which had been collected from 20 cows, were analysed. Of these embryos, 28 formed the group of 'non-cultured' embryos and 24 the group of 'cultured' embryos.

Feulgen staining and stage scanning cytophotometry

Preparations were hydrolysed in 5 mol HCl l^{-1} for 1 h at 20°C, and stained in a pararosaniline- SO_2 -Schiff solution,

prepared according to Graumann (1953), for 45 min at 20°C. The preparations were rinsed by subsequent washings in sulfurous acid (3×10 min), 0.1 mol citric acid l^{-1} /0.2 mol Na_2HPO_4 l^{-1} buffer (pH 5.6, 10 min), and distilled water (10 min) (Duijndam and Van Duijn, 1973). Dehydration was obtained with a graded ethanol-xylene series. Preparations were embedded in DePeX (Gurr, BDH, Poole).

The DNA content of the individual interphase nuclei was measured by absorbance scanning at 559 nm with a Cytoscan SMP (Zeiss, Oberkochen) interfaced to a PDP 11/10 computer with a HIDACSYS-ARRAYSCAN program (Van der Ploeg *et al.*, 1977). Measurements were taken at intervals of 0.5 μm . For each nucleus, the integrated absorbance (obtained by summation of the local absorbance values after subtraction of the mean background value) was determined. The integrated absorbance values (DNA values) were calculated in arbitrary units. The correction factor for (residual) cytophotometric errors was determined by measuring 100 nuclei of a representative embryo. The nuclei of this embryo had a mean area of 1489 ± 327 measuring spots of $0.25 \mu m^2$ and a mean local absorbance of 0.07 ± 0.02 . According to Duijndam *et al.* (1980a, b), the correction factor for the combination of diffraction, glare and distributional errors in this case was 1% or less and this was considered as negligible.

Degenerated nuclei were also present in the preparations. These nuclei were either lysed or pycnotic. Only the DNA content of a part of the pycnotic nuclei was measured, as these nuclei still appeared intact.

Actively dividing calf kidney fibroblasts were used to obtain normal diploid DNA values. These cells were cultured in an enriched L-15 medium on cover glasses and were, although not treated with a hypotonic solution, fixed and postfixed with similar solutions to those used to fix the embryos.

Identification of hyperdiploid and hypodiploid nuclei

The minimum and the maximum of the normal G1 and G2 values in a particular embryo could not be calculated, as the number of nuclei per embryo was usually low in each phase of the cell cycle. Instead, for each embryo, these values were estimated from the frequency distribution of the measured DNA values. This estimation was based on the assumption that the G1 values are half of the G2 values and on the assumption that both the G1 and the G2 values are normally distributed, as has been shown for non-dividing G1-phase Purkinje cells (Mares and Van der Ploeg, 1980). The mode of the G1 distribution was determined from the frequency distribution of DNA values. This mode was considered to be the 2C value, since in a normal distribution the median approximates the mode. Although the 4C value was not further used, it was calculated from this 2C value. The 'maximum G2 value' was also determined from the frequency distribution of DNA values. This value was defined by the nucleus with the highest integrated absorbance of less than 5C, since nuclei that have a DNA value of 5C or more can be considered as hyperdiploid or polyploid (Fischer and Schumacher, 1991). This 5C limit was calculated from the observed 2C value. The 'maximum G1 value' was obtained by dividing the 'maximum G2 value' by 2. The 'minimum G1 value' could be estimated from the 'maximum G1 value' by using the 2C value (i.e. the mode) as a mirror. Nuclei with a

Fig. 1. Frequency distribution diagrams of the DNA values of the individual nuclei of: (a) actively dividing calf kidney fibroblasts, (b) an embryo that had been qualified as good, (c) a haploid embryo that had been qualified as good, (d) a triploid embryo that had been qualified as fair and (e) an embryo with hypodiploid nuclei that had been qualified as good. The DNA content is expressed as the integrated absorption (in arbitrary units). ★: hypodiploid nuclei. #: hyperdiploid nuclei.

DNA value that was lower than the 'minimum G1 value' were considered as hypodiploid, whereas nuclei with a DNA content that was higher than 5C were considered as hyperdiploid. Nuclei designated as hypodiploid occasionally included haploid nuclei, and nuclei designated as hyperdiploid occasionally included polyploid nuclei.

The integrated absorbance of a G1 phase nucleus is about 80 arbitrary absorbance units (Fig. 1b). As bovine cells normally contain 60 chromosomes, it can be assumed that the amount of DNA of one chromosome in the G1 phase is represented by 1.3 arbitrary absorbance units. This is only a rough approximation, as bovine chromosomes vary in size (see for an example a bovine karyotype, King and Linares, 1983).

Results

Total haploidy and total triploidy

In general, normal diploid DNA values varied slightly among embryos ($\pm 10\%$ deviation from the DNA values of the

Fig. 2. Photographs of: (a) the haploid embryo (compare Fig. 1c), (b) the triploid embryo (compare Fig. 1d) after collection, (c) the triploid embryo after a culture for 24 h. Bar represents 25 μ m.

fibroblasts (Fig. 1a)). However, completely abnormal DNA values were found in two of the 52 embryos studied (3.8%). One embryo was completely haploid (Fig. 1c), whereas the other embryo was completely triploid (Fig. 1d). No metaphase spreads were present in the preparation of the haploid embryo. The triploidy of the other embryo was confirmed by the number of chromosomes in six metaphase spreads, which was about 84 (the normal chromosome number in cows is $2n = 60$).

The haploid embryo had been graded as a good compact morula (Fig. 2a), and the triploid embryo as a fair compact morula (Fig. 2b). The triploid embryo had been cultured for 24 h, and developed during this period into a morphologically normal blastocyst (Fig. 2c). After fixation, the haploid embryo had 90 nuclei of which 6 (6.7%) were degenerated, while the

triploid embryo had 59 nuclei of which three (5.1%) were degenerated.

Mixoploidy

Hyperdiploid nuclei. Hyperdiploid nuclei, with amounts of DNA in the range of 5C–9C, were regularly observed in the otherwise diploid embryos. They were found in various numbers in nine of the 27 'non-cultured' diploid embryos (33.3%) (Table 1). No hyperdiploid nuclei were found in the group of excellent embryos, whereas such nuclei were observed in seven of the 15 (46.7%) good embryos, in one of the six (16.7%) fair embryos and in one of the three (33.3%) poor embryos. In the group of good embryos, the percentage of hyperdiploid nuclei varied between 1.1 and 7.5. The percentages of hyperdiploid nuclei in the fair and in the poor embryo were 5.0 and 6.3, respectively.

Four of the embryos with hyperdiploid nuclei came from the same donor cow. The mean percentage ($\pm$ SEM) of hyperdiploid nuclei in these embryos was 5.6 ± 1.0 . This percentage is high compared with the mean percentage of hyperdiploid nuclei in the remaining five embryos, which was 2.5 ± 0.8 .

Hypodiploid nuclei. Nearly all (25) of the 27 embryos had hypodiploid nuclei. Because haploid polar bodies can still be present in morulae and blastocysts (to a maximum of three), only embryos with more than three hypodiploid nuclei can be designated as abnormal. Fourteen of the 27 (51.9%) embryos were therefore considered to contain hypodiploid nuclei (Table 1; for a representative example of an embryo with hypodiploid nuclei see Fig. 1e). More than three hypodiploid nuclei were found in two of the three (66.7%) excellent embryos, in seven of the 15 (46.7%) good embryos, in four of the six (66.7%) fair embryos and in one of the three (33.3%) poor embryos. Percentages of these nuclei varied from 3.2 to 4.7 in the group of excellent embryos, from 5.0 to 23.2 in the group of good embryos, and from 7.9 to 48.1 in the group of fair embryos. The poor embryo had 8.0% hypodiploid nuclei. Five (35.7%) of the embryos with more than three hypodiploid nuclei also had hypodiploid nuclei (Table 1).

Culture of embryos

An additional group of embryos was cultured for 24 h. Of the 23 embryos that were analysed, three had been classified as excellent, 14 as good, and six as fair. No DNA measurement could be made of preparations from embryos that had been qualified as poor after collection.

The embryonic stage before culture varied from the morula stage to the early blastocyst stage. Of the group of excellent embryos, one was a compact morula and two were early blastocyst. Of the group of good embryos, two were morulae, four were compact morulae and eight were early blastocysts, and of the group of fair embryos one was a morula, two were compact morulae and three were early blastocysts. Most of the embryos (20) developed at least one stage further during culture. However, one early blastocyst that had been graded as good degenerated, while two early blastocysts that had been graded as fair became arrested during culture.

Table 1. Morphological quality, embryonic stage, total number of nuclei, number of degenerated nuclei, number of normal and pycnotic hyperdiploid nuclei and hypodiploid nuclei of the individual bovine embryos that were fixed immediately after collection ('non-cultured' embryos)

Morphological quality	Embryonic stage	Total number of nuclei	Degenerated nuclei (%)	Hyperdiploid nuclei			Hypodiploid nuclei		
				Normal	Pycnotic	%	Normal	Pycnotic	%
Excellent	CM	127	6 (4.7)	—	—	0	1	—	0.8
	CM	127	14 (11.0)	—	—	0	4	2	4.7
	EB	127	3 (2.4)	—	—	0	4	—	3.2
Good	CM	127	20 (15.8)	—	—	0	—	1	0.8
	CM	141	14 (9.9)	2	—	1.4	—	2	1.4
	CM	86	0 (0)	—	—	0	2	—	2.3
	CM	160	44 (27.5)	—	—	0	17	5	13.8
	CM	99	14 (14.1)	2	—	2.0	18	5	23.2
	CM	118	16 (13.6)	—	—	0	—	3	2.5
	CM	90	2 (2.2)	1	—	1.1	3	—	3.3
	CM	134	13 (9.7)	10	—	7.5	—	3	2.2
	CM	109	6 (5.5)	3	—	2.8	9	—	8.3
	CM	140	14 (10.0)	5	—	3.6	—	7	5.0
	EB	97	6 (6.2)	—	—	0	—	2	2.1
	EB	118	12 (10.2)	—	—	0	10	1	9.3
	EB	153	13 (8.5)	—	—	0	18	2	13.1
	EB	109	3 (2.8)	—	—	0	2	—	1.8
	EB	83	3 (3.6)	4	—	4.8	4	1	6.0
Fair	CM	95	3 (3.2)	—	—	0	—	—	0
	CM	114	5 (4.4)	—	—	0	9	—	7.9
	CM	89	11 (12.4)	—	—	0	8	—	9.0
	CM	106	9 (8.5)	—	—	0	50	1	48.1
	CM	101	16 (15.8)	—	—	0	8	6	13.9
	EB	100	23 (23.0)	4	1	5.0	1	2	3.0
Poor	16–32C	24	2 (8.3)	—	—	0	—	—	0
	M	45	7 (15.6)	—	—	0	2	1	6.7
	M	112	7 (6.3)	7	—	6.3	—	9	8.0

16–32C: 16–32 cell stage, M: morula, CM: compact morula, EB: early blastocyst.

As no DNA measurements could be obtained from the 'cultured' embryos that had been graded as poor after collection, the results of the group of 'cultured' embryos were compared with the results of only those 'non-cultured' embryos that had been graded as excellent, good and fair.

The mean number of nuclei and the percentage of degenerated nuclei in the group of 'cultured embryos' was approximately the same as those in the corresponding group of 'non-cultured' embryos (Table 2). However, the percentage of hypodiploid nuclei was significantly lower in the group of 'cultured' embryos than in the corresponding group of 'non-cultured' embryos. Although the percentage of hyperdiploid nuclei tended to be lower in the group of 'cultured' embryos than in the group of 'non-cultured' embryos, this difference was not significant.

Discussion

In the study reported here the 2C DNA values of the embryos showed a 10% variation compared with those of bovine fibro-

Table 2. Mean number of nuclei and mean percentages of degenerated, hypodiploid and hyperdiploid nuclei in 'non-cultured' and 'cultured' bovine embryos that had been classified as excellent, good and fair

Parameter	Non-cultured embryos	Cultured embryos
Number of embryos	24	23
Mean number of nuclei	114.6 ± 4.4	119.2 ± 7.3
Mean percentage of degenerated nuclei	9.4 ± 1.4	9.7 ± 4.7
Mean percentage of hyperdiploid nuclei	1.2 ± 0.4	0.5 ± 0.2
Mean percentage of hypodiploid nuclei	7.7 ± 2.1	1.9 ± 0.7*

Values are means ± SEM.

*Significantly different ($P < 0.02$) from non-cultured embryos (Student's t test).

blasts. Apart from a normal biological variation, these differences are probably caused by the fact that each embryo was individually treated, as the frequency distribution of the DNA values of the embryos was not affected by higher or lower 2C values. Hence, chromosome abnormalities such as total haploidy and total triploidy could still be detected. Moreover, as a large number of nuclei per embryo could be analysed, these chromosomal abnormalities could be assessed unequivocally. This situation is in contrast to conventional cytogenetic methods, which depend upon the presence of dividing cells. In bovine embryos, there are usually few dividing cells.

Until now, total haploidy has not been reported in day-7 bovine embryos developed *in vivo*. However, in bovine embryos matured, fertilized and developed *in vitro* (Iwasaki *et al.*, 1989) and in porcine (Van der Hoeven *et al.*, 1985) and ovine (Murray *et al.*, 1986b) embryos developed *in vivo*, a low incidence of total haploidy has also been observed. Conversely, in human (Angell *et al.*, 1983) and murine (Whittingham, 1980; Kaufman, 1985; Surani *et al.*, 1987) embryos, complete haploidy, which is thought to be the result of the parthenogenetic activation of the oocyte, occurs more frequently.

The low incidence of triploidy observed here is in agreement with the results of studies in rabbits (Fujimoto *et al.*, 1974), sheep (Murray *et al.*, 1986b), mice (Martin-Deleon and Boice, 1983), pigs (Long and Williams, 1980) and cows (Hare *et al.*, 1980).

Both the haploid and triploid embryos did not differ in morphology from other diploid embryos of the same morphological quality and embryonic stage. This result has also been reported for *in vitro* fertilized and developed human triploid embryos (Dandekar *et al.*, 1990).

The cytophotometric DNA measurements that were used in the study reported here were not sensitive enough to detect abnormalities such as monosomy and trisomy, as the amount of DNA per chromosome was relatively low. Furthermore, as the embryos were treated individually, the measured DNA values varied among embryos. However, this latter variation did not affect the within embryo evaluation, with regard to the ability to detect either individual hypodiploid or hyperdiploid nuclei in the otherwise diploid embryos.

In the present study, relatively high percentages of both hypodiploid and hyperdiploid nuclei were observed. These percentages may even have been underestimated, as the DNA values of part of the hyperdiploid nuclei in the G1 phase and all of the hypodiploid nuclei in the G2 phase were within the range of values of normal diploid G1 and G2 nuclei.

The observed incidence of hyperdiploid nuclei was, in general, higher than reported for mouse (Varmuza *et al.*, 1988), pig (Moon *et al.*, 1975; Van der Hoeven *et al.*, 1985; Long and Williams, 1982), sheep (Murray *et al.*, 1986a) and cow (Hare *et al.*, 1980; King *et al.*, 1987, 1988) embryos after chromosome analysis.

In contrast to the present study, hypodiploidy in embryos developed *in vivo* has been reported only with regard to either monosomy (Long and Williams, 1980; King and Linares, 1983; Murray *et al.*, 1985, 1986a), or the presence of a few haploid cells in otherwise diploid embryos (King *et al.*, 1979; King and Picard, 1985). However, in preparations of bovine embryos developed *in vitro*, hypodiploid metaphase spreads have been found more frequently (King *et al.*, 1988; Iwasaki *et al.*, 1989).

The presence of both hypodiploid and hyperdiploid nuclei in embryos suggests the frequent occurrence of mitotic segregation failures such as non-disjunction and anaphase lagging (King *et al.*, 1988). As a result of these segregation failures, chromosomes are unequally distributed over the two daughter cells. In some cases, chromosomes can be lost during mitosis. These chromosomes can be incorporated into separate micronuclei (Molls *et al.*, 1982; Saito *et al.*, 1984a, b; Pampfer *et al.*, 1989). In the present study, micronuclei were occasionally observed. However, because they were very small, they could not be measured. Alternatively, it is also possible that segregation failures can lead to the formation of multinuclear cells, containing several nucleus-like pseudonuclei as described by Tesarik *et al.* (1987). These pseudonuclei are larger than micronuclei and are probably hypodiploid. Next to segregation failures leading to hyper- and hypodiploid nuclei, polyploid nuclei can be formed as a consequence of the absolute failure of chromosome segregation during mitosis, which is similar to the process of endomitosis (Sarto *et al.*, 1982; King, 1990).

The high incidences of hyper- and hypodiploid nuclei that are observed in the current study may have resulted from the fact that the embryos were collected from excised uteri of slaughtered donor cows (De Leeuw, 1992). However, it is probably the result of the fact that nearly all of the nuclei of an embryo could be analysed, whereas the identification of polyploid and hypodiploid/haploid cells in other studies could only be determined in dividing cells that were in the metaphase of the cell cycle. It is also possible that most of the hypodiploid nuclei that were observed in the present study could not have been detected with the use of conventional cytogenetic methods. Hypodiploid nuclei lack chromosomes, and they may, therefore, lack vital genes. The cells that contain hypodiploid nuclei that lack these vital genes may stop dividing and eventually degenerate. This contention is supported by two observations in the group of 'cultured' embryos. First, the percentage of hypodiploid nuclei, but not that of hyperdiploid nuclei, was lower in the group of 'cultured' embryos than in the group of 'non-cultured' embryos. Second, although most of the 'cultured' embryos had developed during culture, their mean number of nuclei was not significantly greater than the mean number of nuclei in the group of 'non-cultured' embryos.

In general, the presence of hyperdiploid nuclei has been regarded as a normal developmental characteristic (Barlow and Sherman, 1972; Copp, 1978; Hare *et al.*, 1980; Rossant and Tamura-Lis, 1981; Surani and Barton, 1984). In previous studies, it has been suggested that the occurrence of polyploidy may be associated with culture conditions (Barlow and Sherman, 1972; Johnson and Rossant, 1981). This was not observed in the present study, although it is possible that culture conditions may have selected embryos with few abnormal nuclei, as most of the embryos that were analysed had developed during culture. A measurable preparation could be obtained from only one of the embryos that degenerated during culture. The number of nuclei in this embryo was low (45, of which 16 were degenerated; data not shown), and no hypo- or hyperdiploid nuclei were found in this embryo.

In two other studies (King *et al.*, 1987, 1988), it was suggested that the occurrence of polyploid nuclei may be associated with the poor quality of the embryos. The results of the present study, on the contrary, show that neither the

presence of hypodiploid nuclei nor of hyperdiploid nuclei in the embryos was associated with their morphology. However, the results do suggest a possible relationship between the donor cow and the incidence of hyperdiploid nuclei (Hare *et al.*, 1980), as four embryos with a relatively high percentage of hyperdiploid nuclei were all derived from the same donor cow.

It is concluded that the relatively high incidence of hyperdiploid and hypodiploid nuclei may indicate the frequent occurrence of chromosomal segregation failures during mitosis in bovine embryos. These failures may cause the death of cells, and hence may contribute to preimplantation losses.

It is not possible to select for embryos that are mixoploid or even totally haploid or triploid by examining their morphology alone. Furthermore, the presence of hypodiploid nuclei in the blastomeres of the otherwise diploid embryos may have consequences when determining the sex or genetic disorders in biopsies of single blastomeres using DNA amplification techniques (Kirszenbaum *et al.*, 1990).

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