J. Appl. Genet. 39(2), 1998, pp. 193-198

Morphology and developmental potential of bovine parthenotes after spontaneous activation in vitro

Dorota LECHNIAK, Danuta CIEŚLAK, Jarosław SOSNOWSKI*

Department of Genetics and Animal Breeding, Agricultural University of Poznań, Poland

Abstract. Spontaneous parthenogenetic activation of bovine oocytes in an in vitro maturation and fertilization system (IVM/IVF) is described. Altogether, 1403 follicular oocytes, collected by the aspiration method, were matured in vitro and then cultured without insemination in the same conditions as a group of inseminated oocytes. After 48-72 h of additional culture, 141 oocytes (10%) were found to be spontaneously activated. Morphological evaluation revealed that the number of blastomeres within parthenotes ranged from 2 to 16 cells, with a minority (15.7%) comprising of 9-16 blastomeres. According to a cytogenetic analysis, only 1.2% of the analysed parthenotes consisted of more than 9 cells. Parthenotes may not be distinguished from embryos produced in vitro and spontaneous parthenogenetic activation in an IVM/IVF system indicates suboptimal culture conditions. A group of non-inseminated oocytes should be included in each experiment to serve as a control. Spontaneously activated bovine parthenotes only occasionaly developed beyond the 8-blastomere stage in a common IVM/IVF system. The incidence of parthenotes interferes with the efficiency of in vitro embryo production but it is doubtful whether it lowers the pregnancy rate after transfer of IVF embryos.

Key words: bovine, oocyte, parthenogenetic activation.

Introduction

In vitro maturation and fertilization of oocytes as well as subsequent in vitro embryo culture has become of great importance in cattle breeding programmes. Possibilities offered by those technologies include: overcoming infertility of valuable cows, production of transgenic animals and providing

Received: October 1997.

Correspondence: D. LECHNIAK, Department of Genetics and Animal Breeding, Agricultural University of Poznań, ul. Wołyńska 33, 60-637 Poznań, Poland.

*Holder of the Foundation for Polish Science (FNP) 1997 award.

a source of sexed embryos (BRACKETT, ZUELKE 1993). Live-birth of calves derived from embryos produced in vitro from oocytes matured in vitro has been reported already (HENSLEIGH, HUNTER 1985, FUKUDA et al. 1990). However, there are still many problems to overcome in order to achieve a satisfactory birth rate. It has been shown that bovine embryos derived from in vitro matured and fertilized oocytes display a lower quality and developmental potential than embryos produced in vivo so that they may also result in spontaneous parthenogenetic activation and subsequent degeneration (SATO et al. 1987). Various physical and chemical stimuli are known to induce spontaneous activation of oocytes in vitro (WHITTINGHAM 1980, KING et al. 1988, SATO et al. 1988, AYOUB, HUNTER 1993) as well as postmeiotic aging and hence affect the quality of oocytes (WHITTINGHAM 1980, SATO et al. 1987, 1988, CHIAN, SIRARD 1995). Thus, improvement of in vitro culture conditions seems to be necessary. Morphologically, parthenotes are not distinguishable from embryos resulting from fertilization. This phenomenon may also be responsible for the recorded, lower pregnancy rate after transfer of in vitro produced embryos (AYOUB, HUNTER 1993).

The aim of the present study was to assess the level of spontaneous parthenogenetic activation in a common IVM/IVF system.

Material and methods

The IVM/IVF protocol described by LECHNIAK (1996) has been used in the present experiment. Briefly, collection of bovine ovaries at the local slaughterhouse was followed by cumulus-oocyte complex (COC) aspiration. Only oocytes without signs of degeneration and with intact cumulus cell masses were submitted for in vitro maturation. IVM was carried out in 50-μL droplets of maturation medium (TCM-199 Sigma, USA; 20% estrous cow serum; 50 μL mL^{-1} gentamycin; 10 μL mL^{-1} FSH, 1 μL mL^{-1} estradiol 17β) under paraffin oil (10-20 oocytes per droplet). The selected oocytes were incubated for 24 h at 38.5°C in a humid 5% CO_2 atmosphere. Afterwards oocytes were transferred to 50-μL droplets of sperm-Talp medium (PARRISH et al. 1988) containing heparin (3.4 μL mL^{-1}), hypotaurine (4.6 μL mL^{-1}) and epinephrine (7.7 μL mL^{-1}) and cultured for an additional 20 h. The dishes with maturation droplets were put back into the incubator and used later for embryo culture. After 20 h of culture in sperm-Talp medium, oocytes were washed and transferred back into the maturation droplets where the granulosa cell monolayer had already been formed, and cultured for further 48-72 h.

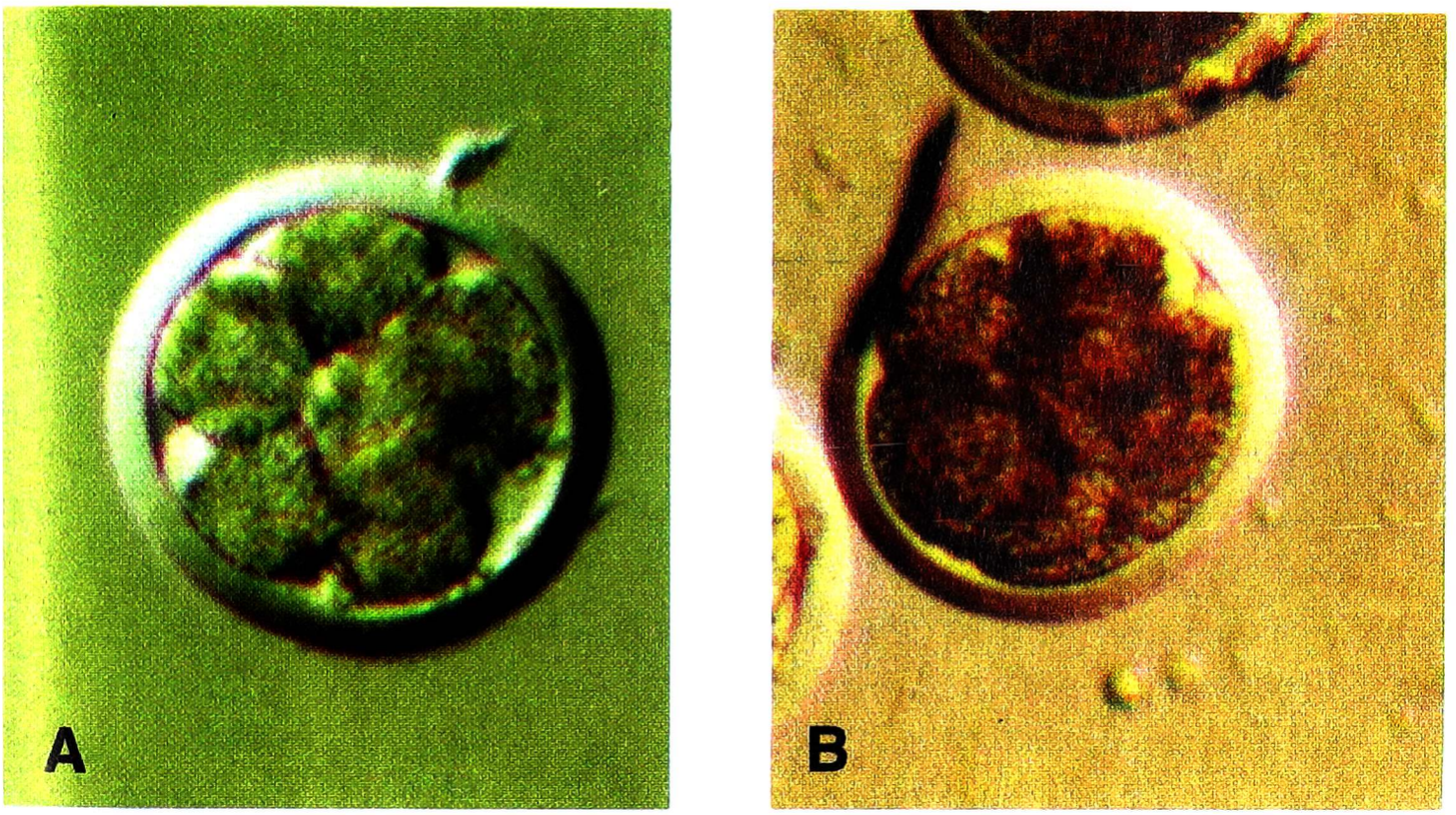

Figure 1. A bovine parthenote (A) and an IVF embryo (B) at 6-8 blastomere stage after 36 h of in vitro culture

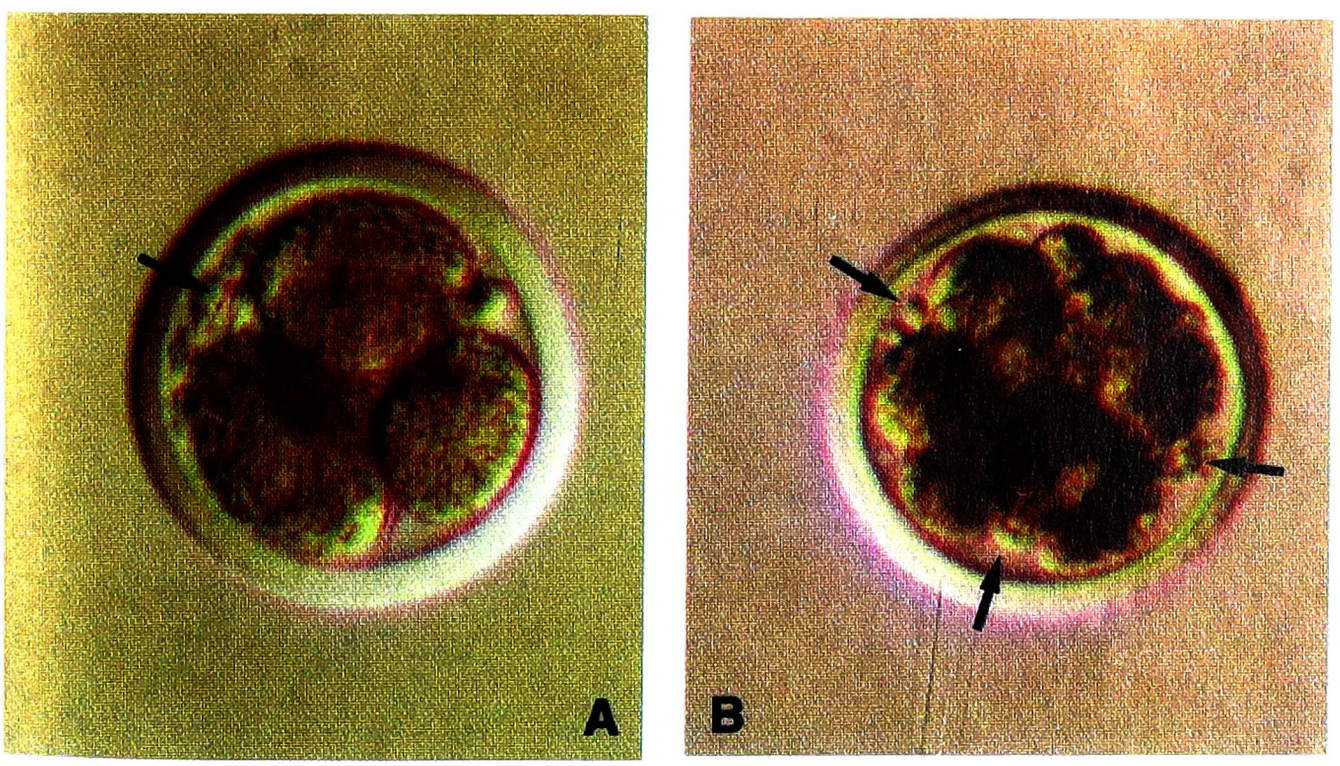

Figure 2. Bovine parthenotes produced in vitro: A – a parthenote at 3-blastomere stage, B – a parthenote comprising of more than 9 blastomere-like structures of unequal size. In both cases the process of vacuolisation is visible (arrows)

Cumulus cells were then removed mechanically by pipetting after a short treatment with trypsin (1 min). Parthenotes were evaluated using a Nikon stereomicroscope (126×). An oocyte was considered as activated when at least two blastomeres were recognized. Only parthenotes with equalsized blastomeres and without signs of degeneration were analysed (Figures 1, 2). To simplify the procedure of blastomere counting, cytogenetic slides were made according to the method descibed by TARKOWSKI (1966) and interphase nuclei or metaphase spreads of blastomeres were counted. The results of the chromosome analysis of the parthenotes were presented elsewhere (LECHNIAK et al. 1998).

Results

Altogether, 1403 oocytes were subjected to in vitro maturation and culture. Among them 141 (10%) were found to be activated according to the morphological evaluation under the stereomicroscope. The number of blastomeres within parthenotes assessed microscopically, ranged from 2 to 16 cells, and 15.7% comprised more than 9 blastomeres (Table 1). Cytogenetic slides were obtained for 84 parthenotes out of 105 submitted for analysis. According to the cytogenetic evaluation, the majority of parthenotes (57.2%) had 2-4 blas-

Table 1. Morphological and cytogenetic evaluation of bovine parthenotes produced in vitro

	Number of parthenotes at various developmental stages (number of blastomeres)			
	total	2-4 (%)	5-8 (%)	9-16 (%)
Morphological evaluation	141	80 (56.7)	39 (27.6)	22 (15.7)
Cytogenetic analysis	84	48 (57.2)	35 (41.6)	1 (1.2)

Table 2. Distribution of metaphase spreads in bovine parthenotes at various developmental stages

	Number of parthenotes with different number of blastomeres		
	2-4	5-8	9-16
With metaphase spreads	18	8	1
With interphase nuclei only	30	27	0
Total	48	35	1

tomeres, 41.6% had 5-8 blastomeres and only one (1.2%) was found to have 9-16 blastomeres (Table 1). Metaphase plates were found in 32.1% of analysed parthenotes (Table 2). The number of metaphase spreads was significantly lower in more advanced parthenotes.

Discussion

The reported spontaneous parthenogenetic activation rate for bovine oocytes in vitro varies markedly from 3.6% to 57% (Table 3). The result of the present study on spontaneous parthenogenetic activation is consistent with previous reports.

Table 3. Incidence of spontaneous parthenogenetic activation in bovine oocytes in vitro

The rate of parthenotes (%)	References
26.0-45.0	KING et al. 1988
3.6-14.1	AYOUB, HUNTER 1993
4.6	PLANTE, KING 1993
5.0	PARK, LIN 1993
6.0-57.0	PRESSICE, YANG 1994
7.5	PLANTE, KING 1996
10.0	present study

Morphological evaluation of blastomere number in parthenotes appeared to be unreliable. The number of interphase nuclei found on cytogenetic slides was not always equal to the number of blastomeres observed under stereomicroscope, especially in the more developed group of parthenotes. In our opinion, this is mainly due to misjudgement caused by cytoplasm fragmentation or vesiculation. Moreover, mitotic activity reflecting embryonic viability drops gradually, reaching the minimum in the most developed group of parthenotes.

The observed differences in the rate of parthenotes are due to various culture factors: media composition (SATO et al. 1988, AYOUB, HUNTER 1993, YAMAUCHI et al. 1996), handling, quality of oocytes and time of culture (KING et al. 1988, PRESSICE, YANG 1994). The spontaneous activation rate of bovine oocytes undergoing postmeiotic aging is higher (6-57%) than that of oocytes

matured for a standard period of time (0-14%) (PRESSICE, YANG 1994). Also the effect of the individual donor may influence the number of matured oocytes (SOSNOWSKI et al. 1996) and this presumably can lead to different activation rates.

Most parthenotes display a low developmental potential and consist of less blastomeres than IVF embryos after the same culture period (DYBAN, BARANOW 1989). In our study we found that the majority of parthenotes were delayed, however at 48-72 hrs of in vitro culture some parthenotes reached the developmental stage of 8-16 blastomeres, equal to embryos produced in vitro at that time (PLANTE, KING 1993). Thus, early cleavage is not a reliable proof of succesful embryonic development. It has been shown previously that parthenogenetic blastocysts can be produced in vitro, however none of them developed to the hatched blastocyst stage and all of them were developmentally delayed (PLANTE, KING 1993, PROCHAZKA et al. 1993). Parthenotes may not be morphologically distinguished from IVF embryos. However, the results of the present study demonstrated that spontaneously activated bovine parthenotes only occasionally developed beyond the 8-blastomere stage in a common IVM/IVF system. Lately, THOMSON (1997) demonstrated a lower quality of in vitro produced bovine embryos when compared with in vivo derived ones, especially with the respect to their survival potential. Spontaneous activation may be one of the factors contributing to this phenomenon. The occurrence of parthenotes interferes with the efficiency of in vitro embryo production but it is doubtful whether it lowers the pregnancy rate after transfer of IVF embryos as suggested by AYOUB and HUNTER (1993).

REFERENCES

AYOUB M.A., HUNTER A.G. (1993). Parthenogenetic activation of in vitro matured bovine oocytes. J. Dairy Sci. 76: 421-429.

BRACKETT B.G., ZUELKE K.A. (1993). Analysis of factors involved in the in vitro production of bovine embryos. Theriogenology 39: 43-64.

CHIAN R.C., SIRARD M.A. (1995). Effect of *cumulus* cells and follicle-stimulating hormone during in vitro maturation on parthenogenetic activation of bovine oocytes. Mol. Reprod. Dev. 42: 425-432.

DYBAN A.P., BARANOW W.S. (1989). Haploidie. In: Die Zytogenetik der Sauger-Embryogenese (Dyban A.P., Baranow W.S. eds). Pareys Studientexte 64, Berlin und Hamburg: Verlag Paul Parey, 1-43.

FUKUDA Y., ICHIKAWA M., NAITO K., TOYODA Y. (1990). Birth of normal calves resulting from bovine oocytes matured, fertilized and cultured with *cumulus* cells in vitro up to the blastocyst stage. Biol. Reprod. 42: 114-119.

HENSLEIGH H.C., HUNTER A.G. (1985). In vitro maturation of bovine *cumulus* enclosed primary oocytes and their subsequent in vitro fertilization and cleavage. J. Dairy Sci. 68: 1456-1462.

KING W.A., XU K.P., SIRARD M.A., GREVE T., LECLERC P., LAMBERT R.D., JAQUES P. (1988). Cytogenetic study of parthenogenetically activated bovine oocytes matured in vitro and in vivo. Gam. Research 20: 265-274.

LECHNIAK D. (1996). The incidence of polyploidy and mixoploidy in early bovine embryos derived from in vitro fertilization. Gen. Sel. Evol. 4: 321-328.

LECHNIAK D., CIEŚLAK D., SOSNOWSKI J. (1998). Cytogenetic analysis of bovine parthenotes after spontaneous activation in vitro. Theriogenology 49: 779-785.

PARRISH J.J., SUSKO-PARRISH J., WINER M.A., FIRST N.L. (1988). Capacitation of bovine sperm by heparin. Biol. Reprod. 38: 1171-1180.

PARK Y.S., LIN Y.C. (1993). Effect of epidermal growth factor (EGF) and defined simple media on in vitro bovine oocyte maturation and early embryonic development. Theriogenology 39: 475-484.

PLANTE L., KING W.A. (1993). Developmental potential of spontaneously activated bovine oocytes. Theriogenology 39: 286.

PLANTE L., KING W.A. (1996). In vitro development of spontaneously activated bovine oocytes. J. Assisted Reprod. Genet. 13: 435-446.

PRESSICE G.A., YANG X.Z. (1994). Nuclear-dynamic of parthenogenesis of bovine oocytes matured in vitro for 20 and 40 hours and activated with combined ethanol and cycloheximide treatment. Mol. Reprod. Dev. 37: 61-68.

PROCHAZKA R., DURNFORD R., FISER P.S., MARCUS G.J. (1993). Parthenogenetic development of activated in vitro matured bovine oocytes. Theriogeneology 39: 1025-1032.

SATO E., ISHIBASHI T., KOIDE S.S. (1987). Preventiom of spontaneous degeneration of mouse oocytes in culture by ovarian glycosaminoglycans. Biol. Reprod. 37: 371-376.

SATO E., KAWAMURA N., ISHIBASHI T. (1988). Chemicals influencing maturation, activation and degeneration of bovine oocytes in culture. J. Dairy Sci. 71: 3482-3488.

SOSNOWSKI J., ŚWITOŃSKI M., LECHNIAK D., MOLIŃSKI K. (1996). Cytogenetic evaluation of in vitro matured bovine oocytes collected from ovaries of individual donors. Theriogenology 45: 865-872.

TARKOWSKI A.K. (1966). An air-drying method for chromosome preparation from moue eggs. Cytogenet. Cell Genet. 5: 394-400.

THOMPSON J.G. (1997). Comparison between in vivo derived and in vitro produced pro-elongation embryos from domestic ruminants. Reprod. Fert. Dev. 9: 341-354.

WHITTINGHAM D.G. (1980). Parthenogenesis in mammals. Oxford Reprod. Biol. 2: 204-231.

YAMAUCHI N., SASADA H., SUGAWARA S., NAGAI T. (1996). Effect of culture conditions on artificial activation of porcine oocytes matured in vitro. Reprod. Fert. Dev. 8: 1153-1156.