ABSTRACT  The aim of the present study was to investigate whether protein or macromolecule supplements to in vitro maturation media affect transcript abundance of seven genes (Bax, Bcl2, Hsp70, IGF1, IGF1R, IGF2, and IGF2R) in oocytes and blastocysts. Cumulus–oocyte complexes aspirated from slaughterhouse ovaries were matured in TCM199 medium supplemented either with 10% FBS, 6% fatty acid free BSA (fafBSA) or 4% PVP40, then inseminated and cultured in vitro for 9 days. Transcript abundance analysis was carried out on immature and in vitro matured oocytes, as well as on blastocysts. Total RNA was isolated from pools of oocytes and embryos, reverse transcribed into cDNA and subjected to transcript analysis by real-time PCR. No transcript of IGF1 gene was detected either in oocytes or in blastocysts. Maturation conditions significantly affected transcript levels of investigated loci in blastocysts but not in matured oocytes, with one exception. Only relative abundance (RA) of IGF2 gene was higher in oocytes matured with fafBSA. Moreover, oocyte maturation with fafBSA elevated transcript abundance of IGF1R, IGF2, and IGF2R genes in resulting blastocysts, whereas Hsp70 transcription was stimulated by FBS supplementation. Therefore, developing a completely defined culture medium based on synthetic macromolecules (PVA, PVP) is of big interest. It has to be noticed, however, that different protein or macromolecule supplements affect expression of developmentally important genes in embryos (Wrenzycki et al., 2005).

INTRODUCTION  Despite long-term investigations, the quality of in vitro produced bovine embryos is significantly lower as compared to their in vivo derived counterparts. Many reports demonstrated differences in morphology (Van Soom et al., 2003), metabolism (Khurana and Niemann, 2000), the incidence of chromosomal abnormalities (Viuff et al., 1999), blastocyst yield and quality (Rizos et al., 2002b; Knijn et al., 2003), cryotolerance (Rizos et al., 2003), apoptotic index (Gjorret et al., 2003) and also in the relative abundance (RA) of developmentally important gene transcripts (Wrenzycki et al., 2005). In terms of efficiency, 30–40% of bovine oocytes matured and fertilized in vitro reach the blastocyst stage. Although post-fertilization embryo culture is the most critical period influencing the blastocyst quality (Lonergan et al., 2003a), the conditions of oocyte maturation (IVM) and fertilization should not be disregarded. It is known that oocytes matured in vivo are more competent to develop to the blastocyst stage than those matured in vitro (Lonergan et al., 2003b).

Protein supplementation to culture media was shown to be a crucial factor influencing the efficiency of in vitro culture system. Several studies demonstrated a biphasic effect of serum on embryo development (Langendonck et al., 1997; Thompson et al., 1998), moreover a significant variation among BSA batches were reported (Gray et al., 1992). Nevertheless both, BSA and serum are the most frequently used but still controversial media supplements. Those undefined components contain hormones, growth factors, vitamins and numerous other unknown factors (Gordon, 2003), which make comparisons among experiments difficult. Therefore, developing a completely defined culture medium based on synthetic macromolecules (PVA, PVP) is of big interest.

Gene expression techniques have recently become a powerful tool for analyzing transcripts related to oocyte/
embryo quality. Although monitoring the mRNA level is considered as an indirect way of quality assessment, it generates valuable data on how an embryo survives in a given culture system (Liu et al., 1997; Park et al., 2006). What is interesting, morphology of human preimplantation embryos was attributed to the transcript level of genes coding insulin-like growth factors (IGFs) factors and their receptors (Liu et al., 1997). Moreover, the real-time PCR system provides highly reliable information on mRNA content of even single embryos (Steuerwald et al., 1999). Previously published evidence demonstrated that in vitro culture conditions alter gene expression profile and transcript abundance in bovine embryos (Wrenzycki et al., 2005). However, only a few reports on the effect of oocyte maturation environment on genes expression in developing embryos could be found. It was shown that in vivo or in vitro maturation can alter the RA of gene transcripts in bovine oocytes (Lonergan et al., 2003b; Humblot et al., 2005). Those authors compared TC199 and cSOF maturation media and noticed a significantly higher expression of several genes in oocytes matured in cSOF medium. Watson et al. (2000) demonstrated that IVM media composition influenced the level of certain oocyte transcripts. Similar observations were made by Wrenzycki et al. (1999) for bovine oocytes and embryos.

Apoptosis, a suicidal form of cell death, follows an intrinsic, fully controlled genetic program. It regulates cell number and removes mutated or damaged cells. Presence of apoptotic blastomeres in preimplantation mammalian embryos has been described in a number of species, including mouse, sheep, horse, cattle, pig, and human (Hardy, 1997; Hardy, 1999; Rubio Pomar et al., 2005). Such cells are observed predominantly within the inner cell mass, both in vivo and in vitro produced embryos. However, several studies showed that in vitro culture conditions affect the extent of apoptosis (Byrne et al., 2002; Makarevich and Markkula, 2002; Gjorret et al., 2003; Knijn et al., 2003). Although Watson et al. (2000) concluded that apoptotic index in blastocysts was not related to the oocyte maturation environment, it has been shown recently, that FBS, fatty acid free BSA (fafBSA) or PVP40 supplementation to IVM medium affects the apoptotic index in bovine blastocysts as revealed by TUNEL (Warzych et al., 2006).

In the present study the following loci were analyzed: Hsp70 involved in stress adaptation, two growth factors and their receptors (IGF1, IGF1R, IGF2, IGF2R) as well as pro– (Bax) and antiapoptotic (Bcl2) factors. It has been assumed, that a complex analysis of transcript level of the above gene panel in oocytes and resulting blastocysts should provide the background for the evaluation of applied maturation regimen. This paper for the first time reports the effect of different protein (fafBSA, FBS) and macromolecule (PVP40) supplementation to oocyte maturation medium on the relative mRNA abundance of a set of apoptosis and cell survival related genes in immature and in vitro matured bovine oocytes as well as in hatched blastocysts.

MATERIALS AND METHODS
All reagents are the products of Sigma (Sigma, St. Louis, MO) unless otherwise stated.

Oocyte Collection, In Vitro Maturation and Embryo Production
Bovine slaughterhouse ovaries reached the laboratory within 4–6 hr after animal slaughter. Cumulus–oocyte complexes (COCs) were aspirated with syringe and needle from visible, 2- to 6-mm follicles, washed in Hepes-talp medium and selected with regard to ooplasm and cumulus cells morphology. Only COCs with a homogenous cytoplasm and multilayered, compact cumulus cells were used for maturation and optionally for further fertilization and culture. In each experiment, a sample of immature oocytes suitable for IVM was stripped of cumulus cells by pipetting, washed three times in PBS supplemented with 0.25% PVP and frozen in liquid nitrogen for further gene expression studies. The basic maturation medium was TC199 with glutamax (Gibco, Invitrogen Co., Scotland, UK) supplemented with sodium pyruvate (25 mM), penicillin G (100 IU/ml), streptomycin (100 μg/ml), FSH (2 μg/ml), LH (10 μg/ml) and estradiol (1 μg/ml). The basic IVM medium was further supplemented with either 10% FBS (Gibco, Invitrogen Co., Scotland, UK), 6% fafBSA or 4% PVP40 (PVP—molecular weight 40,000). COCs of good quality were matured in 500 μl of IVM medium in groups of 50–70 oocytes for 24 hr, at 39°C in humidified 5% CO2 atmosphere. IVM droplets were equilibrated for minimum of 2 hr prior to the initiation of oocyte culture. After maturation, a sample of matured oocytes was stripped of cumulus cells, washed three times in PBS supplemented with 0.25% PVP and frozen in liquid nitrogen.

Every IVM experiment was accompanied by a 9-day culture of resulting zygotes according to the protocol described by Makarevich and Markkula (2002). Briefly, after maturation oocytes were inseminated with washed, frozen-thawed semen of a bull of known in vitro fertility (final sperm concentration—1.5 million sperm cells/ml) and co-incubated for 20 hr at 39°C in humidified 5% CO2 atmosphere. After IVF, cumulus cells were removed, presumptive zygotes were transferred to SOF culture medium supplemented with essential and non-essential amino acids as well as with 5% fetal bovine serum (0.7 μl of medium per embryo; Holm et al., 1999) and cultured for 9 days at 39°C in a humidified atmosphere of 5% O2, 5% CO2, and 90% N2. On Day 5 post insemination (p.i.), half of the culture drop volume was replaced with fresh culture medium.

RNA Isolation, Reverse Transcription
Oocytes and Day-9 hatched blastocysts were frozen in groups in liquid nitrogen (approximately 100 oocytes or 10 embryos per group, with variation of maximum 10 oocytes or 2 embryos) according to the maturation groups. RNA isolations were carried out using the GenElute™ Mammalian Total RNA kit according to the manufacturer’s instructions. Afterwards each
sample was treated with DNase (0.5 IU/sample; Promega Biosciences Inc, Madison, WI).

The first step of reverse transcription (RT) was sample incubation at 70°C with a primer mix (random hexamers and oligo-dT, 0.5 µg/µl each) for 10 min followed by 1.5–2 hr incubation in a RT mix (1× reaction buffer, 0.5 mM dNTP, 20 U RNasin, 5 mM MgCl₂, 1 µl Sensiscript, Qiagen, Hilden, Germany). The final volume of each cDNA sample was 20 µl. At the end of the procedure, the enzyme was inactivated (94°C for 5 min) and cDNA was stored at −20°C.

Quantitative RT-PCR

Before the final step of gene expression analysis, each cDNA sample was first amplified with a pair of primers specific for bovine β-actin mRNA (Table 1) in order to screen the samples for contamination with genomic DNA. Due to the small amount of cDNA in analyzed samples, its concentration was not measured and all cDNA was used as a template in gene expression analysis. The quantitation of all gene transcripts was carried out by real-time semiquantitative RT-PCR assay. All genes of interest were analyzed in three replicates using different oocyte/embryo pools (primers—Table 1). To confirm the results obtained from separate pools, each of three replicates was analyzed twice. The duplicates gave one data point for one replicate and three replicates were used to calculate the mean. The gene for bovine histone H2a subunit, which was defined as the most constant transcript across the preimplantation period in cattle (Robert et al., 2002), was used as the internal standard housekeeping gene in RT-PCR. For quantitation, real time PCR was performed using the LightCycler® 2.0 Instrument (Roche Diagnostics GmbH, Germany) and LightCycler FastStart DNA Master SYBR Green I (Roche Diagnostics GmbH, Germany) and LightCycler Gene Sequence (5°C). The PCR reaction mixture (20 µl) contained 1× LightCycler FastStart DNA Master SYBR Green I, 0.3 µM of forward and reverse primers of analyzed gene (Table 1) and 4 µl of cDNA. The cDNA volume used as a template in PCR reaction was an equivalent of a given number of oocytes/embryos. The final volume of each cDNA sample was 20 µl regardless of the oocyte/embryo number. So the equivalent was calculated by dividing the cDNA volume (20 µl) by the actual number of oocytes/embryos (e.g., in 20 µl of cDNA derived from a pool of 10 blastocysts, 4 µl equaled the equivalent of 2 embryos). The PCR protocol included an initial step of cDNA denaturation at 94°C (10 min), followed by 45 cycles of 94°C (10 sec), 55–65°C (10 sec) and 72°C (20 sec). The melting protocol consisted of a hold temperature for 2 min and heating from annealing temperature of analyzed gene to 94°C, holding each temperature for 0.1 sec while monitoring fluorescence.

The method used for gene quantitation was the relative standard curve method. Standard curves were constructed for each individual gene, using tenfold serial dilutions of corresponding PCR products of known concentration. The dilutions were frozen and single one was used in every real time PCR run to be a point of reference to the standard curve. The transcript concentration for each gene calculated by software from the standard curve was normalized according to the relative concentration of the internal H2a standard. The RA of each gene was calculated by dividing the quantity of the target gene by the quantity of the standard gene. The transcript level obtained for each cDNA sample was then related to single oocyte or embryo.

In each experimental group Bax to Bcl2 ratios were calculated by dividing mean RA of Bax mRNA by mean RA of Bcl2 transcript. Amplification efficiency was taken under consideration.

### Table 1. Details of Primers used for Real Time PCR Experiments

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sequence (5′–3′)</th>
<th>Product size (base pairs)</th>
<th>Annealing temperature (°C)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-actin—rev</td>
<td>ACTGGGAGCATGAGGAGAT</td>
<td>441</td>
<td>55</td>
<td>McDougall et al. (1998)</td>
</tr>
<tr>
<td>Bax—rev</td>
<td>TGCTCAGGTCGAAGCGACGT</td>
<td>427</td>
<td>59</td>
<td>Augustin et al. (2003)</td>
</tr>
<tr>
<td>Bcl2—rev</td>
<td>CCAGGCCGCGGTGAAGCTCTTCT</td>
<td>204</td>
<td>65</td>
<td>Pfaffl et al. (2002)</td>
</tr>
<tr>
<td>Hsp70—rev</td>
<td>GCCCAAGCTATGTCGCCTT</td>
<td>76</td>
<td>65</td>
<td>Rief et al. (2002)</td>
</tr>
<tr>
<td>IGF1—rev</td>
<td>TGGCAATCCGCTACTTGTT</td>
<td>240</td>
<td>65</td>
<td>Pfaffl et al. (2002)</td>
</tr>
<tr>
<td>IGF2—rev</td>
<td>GCCGACGGCTGGTTCATTGTT</td>
<td>205</td>
<td>65</td>
<td>Pfaffl et al. (2002)</td>
</tr>
<tr>
<td>H2a—rev</td>
<td>GTCTGTCGCCGCTACTTGTT</td>
<td>201</td>
<td>65</td>
<td>Pfaffl et al. (2002)</td>
</tr>
</tbody>
</table>
Statistical Analysis

Data on mRNA expression was analyzed using the SigmaStat (Jandel Scientific, San Rafael, CA) software package. One-way repeated-measures ANOVA (followed by multiple pair-wise comparisons using Student-Newman-Kleus method) were used for the analysis of differences in mRNA expression assayed by quantitative RT-PCR. Differences of $P < 0.05$ were considered significant. The calculations were made to compare transcript abundance of analyzed genes in: (a) immature oocytes in relation to those matured with tested supplements, (b) all mature oocytes in relation to hatched blastocysts regardless of the maturation regimen, (c) three groups of hatched blastocysts obtained from oocytes matured in tested media.

RESULTS

Altogether, 5,150 oocytes (68 groups) and 460 Day-9 hatched blastocysts (50 groups) were frozen in liquid nitrogen according to the maturation regimen and then subjected to RNA isolation. Each cDNA sample was amplified with $\beta$-actin primers and in case of detection of a DNA-originating product, a given sample was not included into gene expression analysis (4 of 118 analyzed cDNA samples). Transcript level of the control $H2a$ gene was found to be constant in all analyzed stages of preimplantation development (data not shown). Transcript for $IGF1$ gene was not detected in any of the investigated oocyte and blastocyst samples.

With regard to oocytes, transcripts of analyzed genes were detected at constant levels in immature and mature oocytes. Moreover in vitro maturation media applied to this study did not in general affect transcript abundance with one exception. Only RA of $IGF2$ gene was significantly different between immature and in vitro matured oocytes (Fig. 1). The highest transcript level was noticed in oocytes matured in the presence of fafBSA.

Transcript abundance in resulting Day-9 blastocysts were characterized by a high variability. The RA ranged from 10 to 50 for $Bax$ gene to $1.2 \times 10^6$ for $Hsp70$. After considering conditions for oocyte in vitro maturation, significant differences in RA in blastocysts were noticed for $Hsp70$, $IGF1R$, $IGF2$, and $IGF2R$ genes (Fig. 2). The presence of fafBSA significantly elevated the transcript level of $IGF1R$, $IGF2$, and $IGF2R$ genes, whereas FBS increased mRNA level of $Hsp70$. No significant differences in RA of $Bax$ and $Bcl2$ gene transcripts were found. Since the Bax to Bcl2 ratio was previously related to embryo survival (Levy, 2001), appropriate calculations were made for each experimental group of oocytes and blastocysts. A significant elevation has been found in the Bax to Bcl2 ratio only in oocytes matured in fafBSA; however, it was not confirmed at blastocyst stage (Fig. 3).

Interesting observations were made after comparing the two developmental stages: mature oocytes and Day-9 blastocysts without differentiation into treatment groups. The RA of all investigated genes (exclud-
factors, transcript level reflects both, cell response and stress intensity (Neuer et al., 2000). A limited data on Hsp70 gene expression in bovine oocytes is available. Surprisingly, oocytes subjected to heat stress during in vitro maturation did not show elevated expression of Hsp70 (Kawarsky and King, 2001). However, in oocytes matured in serum supplemented medium an increased mRNA level in comparison to PVA supplementation was observed (Wrenzycki et al., 1999). Since elevated temperature is often used as an agent inducing expression of Hsp70 gene, data presented by Kawarsky and King (2001) seems to be controversial. In the present study no variation in RA of Hsp70 gene among mature oocytes was noticed. A tendency for elevation of Hsp70...
transcript was observed in oocytes matured either with serum or albumin in comparison to PVP40.

With regard to bovine embryos produced in vivo and in vitro, some inconsistent results concerning Hsp70 expression have been published. Knijn et al. (2002) did not find any differences, whereas Wrenzycki et al. (2001) detected an increase in transcript level after in vitro culture. In addition, variation in RA of Hsp70 gene in embryos cultured in vitro in different culture media (TCM199, SOF) as well as with different supplements (serum, fafBSA, PVA) was described (Wrenzycki et al., 2001). According to the present results, a significant elevation of Hsp70 transcript level was noticed in blastocysts produced from oocytes matured with FBS. A similar observation was made by Wrenzycki et al. (1999) in blastocysts produced from oocytes matured

![Fig. 2. The effect of maturation medium composition [FBS (black bars), fafBSA (white) or PVP40 (gray)] on RA of pro-proliferative and apoptosis-related genes in bovine blastocysts (values shown as mean ± SEM). Bars with different superscripts within each gene differ significantly (a:b P < 0.05).](image-url)
and subsequently cultured in medium supplemented with FBS or PVA. RA of Hsp70 transcript was also significantly elevated in frozen-thawed bovine embryos (Park et al., 2006). Interestingly, this was accompanied by a rise in apoptotic index. FBS supplementation altered only mRNA content whereas previously described apoptotic index was not affected (Warzych et al., 2006). It may be, therefore, speculated that FBS presence in culture medium acts as a stress factor inducing expression of Hsp70 gene.

Bcl2 and Bax proteins are antagonists and take part in forming ion channels in mitochondria, what results in possible cytochrome c release and cellular death (Betts and King, 2001). In this study, for the first time transcripts of Bax and Bcl2 genes were detected in immature and in vitro matured bovine oocytes. There is a limited published evidence on expression of Bax and Bcl2 genes in bovine embryos. Lonergan et al. (2003b) did not observe mRNA of Bax gene before 8-cell stage in IVP embryos and 16-cell stage in in vivo cultured embryos. IVM media supplementations applied to this work did not affect transcript level of those two loci neither in oocytes nor in blastocysts. Although there is no published data on Bax gene expression in embryos originating from oocytes matured in different media, recently Nemcova et al. (2006) have demonstrated that bovine blastocysts derived from oocytes of high- and low-developmental competence did not differ with regard to Bax gene transcript level. However, an increase in RA of this gene in bovine embryos cultured in serum supplemented medium was described by Rizos et al. (2003). A similar tendency was noticed for SOF medium in comparison to TCM199, ewe oviduct and in vivo conditions (Rizos et al., 2002a). One may suggest that Bax expression, similarly to Hsp70 gene, is a marker of stress. Heat shock protein 70 can inhibit cytochrome c release in heat-stressed cells. Conformational changes induced in Bax protein by heat shock were inhibited in cells with elevated levels of Hsp70, which suppressed Bax activation (Stankiewicz et al., 2005). The interaction observed on the protein level may significantly differ to that for gene transcripts. What may be hypothesized is, that under stress conditions high expression of Hsp70 may be accompanied by a low Bax level.

It has been shown that the Bax/Bcl2 ratio is a marker of oocyte/embryo viability and predictor of the cell fate. In the present study only in oocytes matured with fafBSA a significantly higher ratio was observed what mainly resulted from the reduced Bcl2 transcript abundance. However, when the blastocysts concerned, no significant variation among experimental groups was noticed. In the previous experiment, Warzych et al. (2006) demonstrated, that IVM conditions did not affect the level of apoptosis in mature oocytes. Therefore, a higher apoptotic index previously observed in blastocysts derived from oocytes matured with PVP40 was not confirmed by elevated Bax to Bcl2 ratio. Thus it may be speculated that a Bax-independent type of apoptosis exists, as it was previously suggested (Strasser-Wozak et al., 1998; Yuan et al., 2003).

Transcript level of genes coding various growth factors is considered as an indicator of embryo developmental potential and viability. Insulin-like growth factors have several anabolic effects at the cellular level including stimulation of amino acid and glucose transport, protein and nucleic acid synthesis, and cell multiplication. Moreover, exogenous insulin-like growth factors were found to act as anti-apoptotic agents during in vitro culture (Byrne et al., 2002; Sirisathien and Brackett, 2003; Fabian et al., 2004). The action of IGFs is mediated through cell-surface receptors 1 and 2 (Schultz and Heyner, 1993). Expression of IGF1 and IGF2 genes as well as their receptors in bovine embryos has been well documented (Liu et al., 1997; Lonergan et al., 2000; Yaseen et al., 2001; Makarevich and Markkula, 2002; Pfaffl et al., 2002). In the present study no transcript for IGF1 gene in oocytes and blastocysts was detected. To our knowledge, there is no published evidence on IGF1 expression in bovine oocytes. As far as embryos are concerned, present finding is consistent with the previously published results using endpoint RT-PCR (Yaseen et al., 2001), however, there is also data indicating presence of mRNA for IGF1 in bovine IVP embryos (Watson et al., 1992; Lonergan et al., 2000). The answer to this inconsistency may lay in the detection method applied (primers design, PCR amplification). Lonergan et al. (2000) used RT-nested PCR whereas the other two groups conventional RT-PCR technique. Since
the experiment involved a sensitive real-time PCR system, thus some other still not identified factors may be responsible. Contamination of oocyte mRNA with transcripts derived from follicular cells cannot be ruled out since in this study transcript of IGF1 was observed in those cells (data not shown). Expression of genes coding IGF1R, IGF2, and IGF2R in immature and in vitro matured oocytes as well as in all stages of preimplantation development in cattle was previously reported (e.g., Watson et al., 1992; Yaseen et al., 2001). In this study maturation media supplementation had no significant influence on RA of IGF1R and IGF2R genes in mature oocytes. Only transcript level of IGF2 gene was elevated in oocytes matured with fafBSA and in resulting blastocysts. In vivo produced bovine blastocysts displayed higher expression of IGF1R and IGF2 genes when compared to IVP embryos (Lonergan et al., 2003b). According to Yaseen et al. (2001) serum supplemented TCM199 resulted in higher RA of IGF2R gene in cultured blastocysts in comparison to PVA added to SOF medium. A positive correlation between expression level of genes coding IGFs and their receptors and morphology of embryos was previously described in human (Liu et al., 1997). Thus the transcript abundance of IGF2 gene may be a potential marker of embryonic viability in vitro. Present data suggests that fafBSA may be the optimal supplement to maturation medium under described conditions, due to higher transcript level of GFs coding genes and decreased RA of Hsp70 gene in blastocysts. However, it is rather difficult to unequivocally conclude that PVP40 exerts the most detrimental effect on oocytes/embryos since FBS supplementation was also correlated with decreased RA of IGF1R and IGF2R genes.

Despite a significant elevation in cell number between oocyte and blastocyst stages, the total RNA content increases only 2–3 times (Bilodeau-Goeseels and Schultz, 1997). In this experiment a big variation in transcript level of individual genes noticed between the two analyzed developmental stages may also result from an obvious difference in the cell number (oocyte = 1 cell; blastocyst on average 128 cells). However, it may also exhibit the importance of a given gene during preimplantation development. Thus, basing on the present results, it may be speculated that, for example, IGF1R gene may have a minor significance at blastocysts stage, whereas IGF2R takes over the major obligations as an insulin-like growth factors receptor. This hypothesis seems to be confirmed by other studies, which show that IGF2 receptor binds both IGF1 and IGF2 ligands, however, with different efficiency (Ewton et al., 1987).

In conclusion, considering the previously published data (Warzych et al., 2006), it may be suggested that fafBSA supplementation to IVM medium is the best option in described experimental design, whereas PVP40 supplementation seems to give the lowest quality blastocysts. It is suggested that variation in mRNA level among analyzed blastocysts cannot be attributed to the different blastomere number. With regard to the previously evaluated total blastomere count (Warzych et al., 2006), it varied between 124 and 135 with an average of 128, however, it did not differ among treatments. It must be stressed, however, that in the present work Day 9 hatched blastocysts were analyzed, whereas previously published data considered Day 8 expanded embryos. Although the number of blastomeres within a bovine blastocyst increases as it develops, this phenomenon is not affected by the culture media (Kolle et al., 2002; Neuber et al., 2002). The most valuable contribution of the present work was to demonstrate, that IVM medium composition affects RA of analyzed apoptosis and cell survival related genes in bovine blastocysts but not in mature oocytes (with exception for IGF2 gene). A similar time delay in embryonic response to a suboptimal culture environment was previously reported (Lonergan et al., 2003a; Gardner and Lane, 2005). Gardner and Lane (2005) suggested that oocytes and early embryos are the most sensitive developmental stages for environmental insults but a response to stress factor is often observed later during development, for example, at blastocyst stage. Early embryos with serious defects are not able to develop beyond the MET and transform into blastocyst. The previous results seem to confirm that hypothesis, since in a group of embryos produced from oocytes matured with PVP40 a significant reduction of blastocyst rate was observed (Warzych et al., 2006). Because in the present experiment only hatched blastocysts representing best quality embryos were analyzed, therefore, the observed alterations in transcript level probably did not result from fundamental disturbances in gene expression. This suggestion seems to be supported by Donnison and Pfeffer (2004) who concluded that embryonic developmental competence as a quantitative trait may be regulated by small changes in RNA content of several genes already in oocytes.
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REFERENCES


