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# Effect of Aesculetin and $O_2$ Concentrations on *In Vitro* Development of Preimplantation Embryo in Hanwoo (Korean Native Cattle)

**Jang, H. Y.[1], K. E. Park[2], C. I. Kim[1], C. K. Park[1], H. T. Cheong[1] and B. K. Yang[1]†**
[1]College of Animal Resource Science, Kangwon National University, Chunchon, 200-701, Korea

# 한우 수정란의 체외발육에 있어서 Aesculetin과 $O_2$농도의 영향

장현용[1] · 박기은[2] · 김정익[1] · 박춘근[1] · 정희태[1] · 양부근[1]†
[1]강원대학교 동물자원과학대학

## ABSTRACT

The present study was examined effects of aesculetin and $O_2$ concentrations on *in vitro* development of Hanwoo (Korean Native Cattle) embryos derived from in-vitro matured and fertilized (IVM-IVF) oocytes. The oocytes were cultured for the first 40~44 h after *in vitro* fertilization, then embryos of 2 to 8 cell stages were cultured under the different culture conditions for another 6 days. In experiment 1, the higher rates of morulae and blastocysts were produced in 5% $O_2$ than in 20% $O_2$ ($P<0.05$). There was significantly ($P<0.05$) higher in embryos cultured with 1 μg/ml than with 0, 5 and 10 μg/ml of aesculetin. In experiment 2, the proportions of embryo developed with blastocysts and morulae plus blastocysts in 5% $O_2$ again was significantly ($P<0.05$) higher in 20% $O_2$ during the culture with aesculetin and/or taurine. In the 5 and 20% $O_2$ atmosphere, the inclusion of 1 μg/ml aesculetin or 2.5 mM taurine increased significantly ($P<0.05$) the percentages of blastocysts and morulae plus blastocysts. In experiment 3, in medium with aesculetin plus PDGF and taurine plus EGF than other treatment groups, significantly ($P<0.05$) higher developmental rates were obtained. Number of blastomeres in balstocyst stage were also higher in medium with that than without aesculetin. However, there were no significant differences in all culture conditions. In experiment 4, the proportions of embryo developed to the morulae and blastocyst stages were significantly ($P<0.05$) higher rates in medium with natural and commercial aesculetin than in control medium. No significant differences, however, were observed in between natural (71%) and commercial (70.0%) aesculetin. Number of blastomere in blastocyst stage were also higher in medium with natural and commercial aesculetin than in control medium. However, there was no effect on the number of blastomeres by these treatment. These data indicate that preimplatation embryos are very sensitive to condition that can cause oxygen concentration and show that efficiency role of aesculetin for improving bovine embryo development *in vitro*.

(Key words : Aesculetin, Bovine, Embryonic Development, $O_2$ concentrations, Taurine)

† Corresponding author : Division of Animal Resource Sciences, College of Animal Resource Science, Kangwon National University, Chunchon, 200-701, Korea, Tel:+82-33-250-8627, Fax: +82-33-244-2532, E-mail: bkyang@kangwon.ac.kr

[2] 일본 오카야마대학교 농학부(Division of Animal Science, Faculty of Agriculture, Okayama University, 700-8530, Japan)

## Ⅰ. INTRODUCTION

*In vitro* culture of mammalian embryos has been widely investigated in efforts to overcome the developmental retardation occurring *in vitro* and to approximate efficiency provided *in vivo*. One of their major differences between the *in vivo* and the *in vitro* environment for the embryo is the oxygen concentration (Mass et al., 1976). The gaseous environment of cultured cells of all types is important in maintaining normal growth characteristics, and commonly, 5% $CO_2$ with 95% humidified air is used (Liu et al., 1995). However, the $O_2$ concentration of the oviduct is about one-third that of the atmosphere (Fischer and Bavister, 1993). Embryos cultured in high oxygen tension may produce more free radicals which are detrimental to embryo development. Reducing oxygen tension to 5 to 8% increases blastocyst development (Li and Foote, 1993).

Toxic metabolities of oxygen, including the superoxide anion radical ($O_2^-$), hydrogen peroxide ($H_2O_2$), and the hydroxyl radical (OH ·), are important mediators of inflammatory tissue injury (Weiss, 1986). Moreover, these highly toxic oxygen metabolites have been found to be the final common mediator of tissue damage in a large number of disparate processes, including inflammation and post-ischaemic re-perfusion injury (Bulkley, 1987). There are therefore striking similarities between many known actions of oxygen-derived free radicals and the events leading to oocyte maturation. Free radicals can be degraded by enzymes such as superoxide dismutase, catalase and taurine. The presence of these anti-oxidant, which serve as radical scavengers in culture medium, has been found to have beneficial effects on embryonic development *in vitro* for bovine (Liu et al., 1995). Although correlations have been reported between the effectiveness of antioxidants and bovine embryos, the importance and action of aesculetin as antioxidants in embryonic development has not been elucidate.

The present study was conducted to determine whether aesculetin might be beneficial when bovine embryos are cultured in the presence of taurine or growth factors under the different $O_2$ concentrations. In this study, we also examined the effects between natural and commercial aesculetin for embryonic development *in vitro*.

## Ⅱ. MATERIALS AND METHODS

### 1. *In Vitro* Maturation and Fertilization

Oocytes aspirated from follicles of 2～6 mm in diameter were cultured in TC-199 medium (Earle's salt) buffered with 25 mM-N-2-hydroxyethylpiperazine N-2-ethane sulphonic acid (Hepes) and supplemented with 10% (v/v) fetal calf serum, 0.5 $\mu$g/ml FSH, 5 $\mu$g/ml LH and 1 $\mu$g/ml Estradiol 17$\beta$ for 20～22 h at 39℃ in 5% $CO_2$ in air. After culture, the oocytes were washed twice and placed into 50 $\mu$l medium with BSA and heparin for insemination. The basic medium, consisting of 112.0 mM-NaCl, 4.02 mM-KCl, 2.25 mM-$CaCl_2$, 0.83 mM-$NaH_2PO_4$, 0.52 mM-$MgCl_2$, 37.0 mM-$NaHCO_3$, 13.9 mM-glucose, 1.25 mM-sodium pyruvate and 31 $\mu$g potassium penicillin G/ml, was used for the treatment of spermatozoa and fertilization of oocytes.

Frozen semen obtained from Korean Native Bulls was thawed by immersion in a 35～37℃ water bath for 30 sec. The spermatozoa were washed twice in a medium containing 1 mM-caffeine-sodium benzoate (Sigma Chemical Co., St Louis, MO USA) by centrifugation at 833 g for a period of 10 min each. The sperm pellet was resuspended in the same medium as used for washing to give a sperm concentration of 5～10×$10^6$/ml. A 50 $\mu$l sample of the sperm suspension was introduced into 50 $\mu$l of the medium that contained bovine serum albumin (BSA; crystallized and lyophilized, essentially glob-

ulin free, No. A-7638; Sigma Chemical Co.), porcine intestinal mucosal heparin(176 USP units/ml; Sigma Chemical Co.) and no caffeine. This medium had been previously covered with warm paraffin oil in a polystyrene culture dish. The mixture gave final concentrations of $2.5\sim5\times10^6$ spermatozoa/ml, 10 mg BSA/ml, 10 $\mu$g/ml heparin and 5 mM-caffeine.

**2. Culture Conditions**

At 40～44 h after *in vitro* fertilization, cumulus cells were removed from the surface of oocytes by repeated pipetting and cumulus-free oocytes were transferred into culture medium. Culture medium was a simple defined medium, $CR_{1aa}$, containing NaCl, KCl, $NaHCO_3$, BSA, L-glutamine, pyruvate, L(+)-lactate, MEM amino acids and BME amino acids. Then, embryos with 2～8 cells were chosen and cultured in each 100-$\mu$l drop, covered with paraffin oil in 5 and 20% $O_2$ at 39℃.

**3. Embryo Evaluation**

The procedure of Papaioannou and Ebert (1988) for the differential staining of inner cell mass and trophectoderm cells was carried out with minor modification. Two chromatin specific fluorochromes with different fluorescent spectra were used with propidium iodide, which is excluded from vital cells, and bisbenzimide, which can enter vital and nonvital cells. Intact embryos with a zona pellucida were treated with 0.5% hyaluronidase to remove the zona pellucida. Zona free embryos were treated in TNBS acid-PBS (1:9) plus 3 mg/ml PVP for 10 min at 4 ℃, and were treated with Anti-DNP-BSA (1:10) for 20 min and Guniea pig complement -PBS(1:3) for 30 min at 37℃. Subsequently, embryos were briefly washed twice in medium with 2.3% citrate-ethanol (1:3) and stained with 10 $\mu$g/ml Hoechst 33342 (Sigma) plus 10 $\mu$g/ml propidium iodide (Sigma) for 4～5 min. Finally, embryos were squashed on a slide and observed under a Ziess fluorescence microscope with an excitation filter of 365 nm and a barrier filter of 410 nm. Inner cell mass cells could be recognized by the blue fluorescence of the Hoechst staining and trophectoderm cells by a red to pink fluorescence due to the accumulated propidium iodide.

**4. Experimental Design**

There were four experiments. In experiment 1, embryos were cultured under an atmosphere of 5 or 20% $O_2$ at 39℃ with different concentrations (0, 1, 5 and 10 $\mu$g/ml) of aesculetin for development. The aesculetin was extracted from ash tree (*Fraxinus rhyncophylla*) in Department of Wood Science and Engineering, Kangwon University. In experiment 2, to evaluate the effects of aesculetin (1 $\mu$g/ml) and taurine (2.5 mM) on *in vitro* development, embryos were cultured under atmosphere of 5 and 20% $O_2$. In experiment 3, the effects of antioxidents plus growth factors on *in vitro* development, embryos were cultured in medium with 1 $\mu$l/ml aesculetin + 1ng/ml platelet-derived growth factor (PDGF), aesculetin + 10 ng/ml epidermal growth factor (EGF), 2.5 mM taurine + PDGF and taurine + EGF. Finally, in experiment 4, to compare the effects of natural and commercial aesculetin on *in vitro* development, embryos were cultured in medium with natural aesculetin (1 $\mu$g/ml) from ash tree and commercial aesculetin (1 $\mu$g/ml) from Sigma Co.

**5. Analysis of Data**

The SAS mixed linear model program was used to analyze the data. Percentage of development was based upon the number of 2- to 8-cell embryos cultured in each treatment. Treatment means were compared for differences through use of Duncan's Modified Multiple Range test. Differences were considered statistically significant at $P<0.05$.

## Ⅲ. RESULTS

### 1. Experiment 1

Table 1 shows the proportions of embryos arrested at the premorulae (2- to 16-cell) and embryos that became either morulae or blastocysts after 6 days of culture. Overall, the percentages of blastocysts and morulae plus blastocysts in 5% $O_2$ were significantly ($P<0.05$) higher than in 20% $O_2$. There was significantly ($P<0.05$) higher in embryos cultured with 1 $\mu$g/ml than with 0, 5 and 10 $\mu$g/ml of aesculetin.

### 2. Experiment 2

The proportions of embryos developed with blastocysts and morulae plus blastocysts stages in 5% $O_2$ again was significantly ($P<0.05$) higher than in 20% $O_2$ in medium with aesculetin and taurine (Table 2). In the 5 and 20% $O_2$ atmosphere, the inclusion of 1 $\mu$g/ml aesculetin or 2.5 mM taurine increased significantly ($P<0.05$) the percentages of blastocysts and morulae plus blastocysts.

### 3. Experiment 3

Embryos were cultured to determine effects of antioxidants and growth factors. When the medium were contained aesculetin plus PDGF and taurine plus EGF than another culture conditions, significantly ($P<0.05$) higher developmental rates were obtained (Table 3). Number of blastomeres were also higher in medium with that than without aesculetin. However, there were no significant differences in all culture conditions.

**Table 1. Effects of aesculetin and $O_2$ concentrations on *in vitro* development of bovine embryos**

| $O_2$ (%) | Aesculetin ($\mu$g/ml) | No. of embryos cultured | No. of embryos developed to (%): Premorulae | Morulae | Blastocysts | Morulae plus blastocysts (%) |
|---|---|---|---|---|---|---|
| 5 | 0 | 40 | $16(40)^a$ | $13(33)^a$ | $11(28)^b$ | $24(60)^b$ |
| | 1 | 43 | $9(21)^b$ | $14(33)^a$ | $20(47)^a$ | $34(79)^a$ |
| | 5 | 40 | $15(38)^a$ | $12(30)^a$ | $13(33)^b$ | $5(63)^b$ |
| | 10 | 40 | $17(43)^a$ | $10(25)^a$ | $13(33)^b$ | $23(58)^b$ |
| 20 | 0 | 41 | $20(48)^a$ | $11(27)^a$ | $10(24)^b$ | $1(51)^b$ |
| | 1 | 41 | $13(32)^b$ | $13(32)^a$ | $15(37)^a$ | $28(68)^a$ |
| | 5 | 41 | $20(49)^a$ | $12(29)^a$ | $9(22)^b$ | $21(51)^b$ |
| | 10 | 40 | $19(48)^a$ | $12(30)^a$ | $9(23)^b$ | $21(53)^b$ |
| Overall means | | | | | | |
| 5 | | 163 | $57(35)^B$ | $49(30)^A$ | $57(35)^A$ | $106(65)^A$ |
| 20 | | 163 | $72(44)^A$ | $48(29)^A$ | $43(26)^B$ | $91(59)^B$ |
| | 0 | 81 | $36(44)^a$ | $24(30)^a$ | $21(26)^b$ | $45(56)^b$ |
| | 1 | 84 | $22(26)^b$ | $27(32)^a$ | $35(42)^a$ | $62(74)^a$ |
| | 5 | 81 | $35(44)^a$ | $24(29)^a$ | $22(27)^b$ | $46(56)^b$ |
| | 10 | 80 | $36(45)^a$ | $22(28)^a$ | $22(28)^b$ | $44(55)^b$ |

[a,b,A,B] Means with different superscripts within treatment groups and embryo stages are significantly different, $P<0.05$.

**Table 2. Effect of $O_2$ concentrations in medium with aesculetin or taurine on *in vitro* development of bovine embryos**

| $O_2$ (%) | Aesculetin (μg/ml) | Taurine (mM) | No. of embryos cultured | No. of embryos to(%) : Premorulae | Morulae | Blastocysts | Morulae plus blastocysts(%) |
|---|---|---|---|---|---|---|---|
| | 0 | 0 | 43 | 23(54)[a] | 11(26)[a] | 9(21)[b] | 20(47)[b] |
| 5 | 1 | 0 | 42 | 15(36)[a] | 13(31)[a] | 14(33)[a] | 27(64)[a] |
| | 0 | 2.5 | 43 | 17(40)[a] | 11(26)[a] | 15(35)[a] | 26(61)[a] |
| | 0 | 0 | 42 | 27(64)[a] | 8(19)[a] | 7(17)[a] | 15(36)[b] |
| 20 | 1 | 0 | 42 | 21(50)[a] | 12(29)[a] | 9(21)[a] | 21(50)[a] |
| | 0 | 2.5 | 43 | 23(54)[a] | 10(23)[a] | 10(23)[a] | 20(47)[ab] |
| Overall means | | | | | | | |
| 5 | | | 128 | 55(43)[A] | 35(27)[A] | 38(30)[A] | 73(57)[A] |
| 20 | | | 127 | 71(56)[B] | 30(24)[A] | 26(21)[B] | 56(44)[B] |
| | 0 | 0 | 85 | 50(59)[a] | 19(22)[a] | 16(19)[b] | 35(41)[b] |
| | 1 | 0 | 84 | 36(43)[a] | 25(30)[a] | 23(27)[a] | 48(57)[a] |
| | 0 | 2.5 | 86 | 40(47)[a] | 21(24)[a] | 25(29)[a] | 46(54)[a] |

[a,b,A,B] Means with different superscripts within treatment groups and embryo stages are significantly different, $P<0.05$.

**Table 3. Effect of antioxidants (aesculetin and taurine) and growth factors(EGF and PDGF) on *in vitro* development of bovine embryos**

| Antioxidants plus growth factors | No. of embryos cultured | No. of embryos developed to(%) : Premorulae | Morulae | Blastocysts | Morulae plus blastocysts(%) | Total cell no. of blastocysts (Mean±S.E) |
|---|---|---|---|---|---|---|
| C[1] | 78 | 40(51)[a] | 2(3)[a] | 36(46)[c] | 38(49)[c] | 117±14.5 |
| AP[2] | 78 | 22(28)[c] | 1(1)[a] | 55(70)[a] | 56(72)[a] | 138±20.0 |
| AE[3] | 78 | 31(40)[b] | 1(1)[a] | 46(58)[b] | 47(60)[b] | 139±30.1 |
| TP[4] | 78 | 33(42)[b] | 1(1)[a] | 44(56)[b] | 45(58)[b] | 121±16.2 |
| TE[5] | 78 | 25(32)[c] | 1(1)[a] | 52(66)[a] | 53(68)[a] | 131±19.3 |

[1] Control, [2] Aesculetin 1 μg/ml + PDGF 1ng/ml, [3] Aesculetin 1 μg/ml + EGF 10ng/ml, [4] Taurine 2.5mM + PDGF 1ng/ml, [5] Taurine 2.5mM + EGF 10ng/ml.

**4. Experiment 4**

The effect of natural and commercial aesculetin was tested for the development of embryos. The embryos cultured in medium with natural and commecial aesculetin showed a significantly ($P<0.05$) higher rates of the development to the morulae and blastocyst stage than control group (Table 4). No significant differences, however, were observed in between natural (71%) and commercial (70.0%) aesculetin. In this experiment, number of blastomere was also higher in medium with natural and commecial aesculetin than control. However, there was no effect on the number of blastomeres by these treatment.

**Table 4. The comparision of natural and commercial aesculetin on *in vitro* development of bovine embryos**

| Treatment | No. of embryos cultured | No. of embryos developed to(%) ; | | | Morulae plus blastocysts(%) | Total cell no. of blastocysts (Mean±S.E) |
|---|---|---|---|---|---|---|
| | | Premorulae | Morulae | Blastocysts | | |
| C[1] | 44 | 21(48)[a] | 6(14)[a] | 17(39)[b] | 23(52)[b] | 138±6.8 |
| NA[2] | 44 | 13(30)[a] | 7(16)[a] | 24(55)[a] | 31(71)[a] | 145±8.3 |
| CA[3] | 43 | 13(30)[a] | 7(16)[a] | 23(54)[a] | 30(70)[a] | 143±4.5 |

[1] Control, [2] natural aesculetin(1 μl/ml), [3] commercial aesculetin(1 μl/ml).

[a,b] Means with different superscripts within treatment groups and embryo stages are significantly different, P<0.05.

## Ⅳ. DISCUSSION

In this study, aesculetin was shown to promote subsequent development of preimplantation bovine embryos with significant effects observed only when exposure closely preceded the expected time of cleavage from the 2-cell to the 8-cell stage. To our knowledge, this is the first report in which an effect of aesculetin exposure has been shown to be dependent on the developmental stage in bovine embryos.

The present study confirmed the report on culturing bovine zygotes that 5% $O_2$ is superior to 20% $O_2$. Short exposure to 20% $O_2$ has been reported to be harmful to bovine embryos (Liu and Foote, 1995). From Table 1 and 2, it is clear that when $O_2$ concentrations were decreased from 20% to 5%, the percentages of blastocysts increased regardless of the aesculetin treatments in various concentrations. The same pattern was observed under the 20% and 5% $O_2$ in experiment 2 with aesculetin, and taurine also increased the proportion of blastocysts when the 5% and 20% $O_2$ was used. There was a greater proportion of blastocysts in medium with 1 μg/ml aesculetin in the presence of 5% $O_2$. These results are consistent with those reported for early embryonic development in previous study of cattle (Tervit et al., 1972; Liu and Foote, 1995). Five percent $O_2$ closely mimics the $O_2$ concentration in the oviducts of rabbits (Fischer and Bavister, 1993) and monkeys (Fischer and Bavister, 1993). However, other studies did not consistently demonstrate beneficial effects of reduced $O_2$ concentration on embryonic development for sheep (Betterbed and Wright, 1985) and cattle (Nakao and Nakatsuji, 1990; Voekel and Hu, 1992). Our interpretation of the beneficial effects of the low $O_2$ concentrations is that they are associated with reduced formation of free radicals in medium with aesculetin or taurine.

The results of the present study suggest that the *in vitro* development of bovine embryos improves during the culture in a culture medium containing aesculetin and 1ng/ml PDGF. Studies by Gandolfi et al. (1991) led to detection of PDGF in bovine oviductal secretions. Larson et al. (1991) found that the addition of this growth factor induced cattle embryos to progress through the 8-16-cell block stage. Keefer et al. (1994) also reported that EGF stimulated hatching in bovine embryos cultured singly from the 8-cell stage. In this study, however, EGF had no synergistic effects on blastocyst development in medium with aesculetin. In the study of Flood et al. (1993), of all the growth factors tested, only EGF had a stimulatory effect in terms of blastocyst rate, although it was not significant. Lee and Fukui (1995) reported that transient expo-

sure of morulae/early blastocysts improved later development, although this effect was not evident for earlier embryonic stages. Here we have demonstrated a stimulatory effect of aesculetin on postfertilization bovine embryonic development in medium with PDGF than that observed when the EGF is added during *in vitro* culture of bovine embryos.

In this study, added natural and commercial aesculetin produced a linear response with increased cell number and developmental rates (Table 4). Culture of bovine embryos in used media with natural and commercial aesculetin resulted in 143～145 cells after a culture periods of 6 days. In contrast, with the control medium alone the average cell count after 6 days in culture was 138. The precise nature of the action of aesculetin on bovine embryonic development is as yet unknown. However, aesculetin has been known as antioxidant to inhibit activity of lipoxygenase and cycloxygenase in mouse and human (Craven et al., 1986; Sekiya et al., 1982). Thus, the effects of aesculetin on preimplantation bovine embryos observed in this study appear to be due to its antioxidant.

In conclusion, our data indicate that reduction of $O_2$ concentration from 20% to 5% results in a major increase in the proportion of the blastocysts formed in a $CR_{1aa}$ medium with aesculetin. This increase, in the presence of aesculetin, was also achieved by the addition of PDGF to the medium. There were not differences on bovine embryo development between natural and commercial aesculetin. It is suggested that aesculetin may serve to inhibit formation of toxic substances through a system independent of any antioxidant activity by taurine.

## Ⅴ. 요 약

본 연구는 aesculetin과 $O_2$농도가 체외에서 성숙 수정된 한우 초기배의 체외발육에 미치는 영향을 검토하였다. 실험을 위하여 체외수정 후 40～44시간에서 2～8세포로 발육한 초기배는 6일간 배양하였다. 그 결과, 실험 1에서 상실배와 배반포기배의 발육은 산소농도 20% 보다는 5%에서 유의적으로 높게 나타났으며 ($P<0.05$), 1 μg/ml의 aesculetin 첨가시 0, 5, 및 10 μg/ml 첨가에 비하여 유의적 ($P<0.05$)으로 높은 발육율을 나타냈다. 실험 2에서, 배반포기 및 상실배 이상 발육한 초기배의 비율은 산소농도 20% 보다는 5%에서 역시 유의적으로 더 높았으며 ($p<0.05$), 1 μg/ml의 aesculetin 또는 2.5 mM taurine 첨가시 배반포기 및 상실배 이상 발육한 초기배의 비율은 다른 실험구에 비하여 유의적으로 높게 나타났다 ($P<0.05$). 실험 3에서, aesculetin과 PDGF 동시 첨가 및 taurine과 EGF를 동시에 첨가한 경우 다른 실험구에 비하여 유의적으로 높은 발육율을 얻었으며 ($P<0.05$), aesculetin 무첨가보다는 첨가시 배반포기배의 세포수가 높게 관찰되었으나 배양조건에 관계없이 유의적인 차이는 인정되지 않았다. 한편, 실험 4에서 자연에서 추출한 것과 상품화된 aesculetin이 초기배의 발육에 미치는 영향을 검토한 결과 무첨가에 비해 유의적으로 높은 발육율을 나타냈으나 ($P<0.05$), 자연에서 추출한 것 (71%)과 상품화 (70%)된 aesculetin 사이에 유의적인 차이는 인정되지 않았다. 또한 자연에서 추출한 것과 상품화된 aesculetin 첨가시 무첨가에 비해 배반포기배의 세포수가 높게 관찰되었으나 이들 사이에 의한 유의적인 차이는 없었다. 본 연구의 결과로부터, 5%의 산소농도하에서 aesculetin은 항산화제와의 공동첨가에 의해 한우 초기배의 체외발육을 증진시키는 것으로 나타났다.

## Ⅵ. REFERENCES

1. Betterbed, B. and Wright, R. W. Jr. 1985. Development of one-cell ovine embryos in two culture media under two gas atmospheres. Theriogenology 23:547-553.
2. Bulkely, G. B. 1987. Free radical-mediated reperfusion injury: a selective review. Br. J. Cancer (Suppl.) 55:66-73.

3. Craven, P. A., Pfanstiel, J. and DeRubertis, F. R. 1986. Role of reactive oxygen in bile salt stimulation of colonic epithelial proliferation. J. Clin. Invest. 77:850-859.
4. Fischer, B. and Bavister, B. D. 1993. Oxygen tension in the oviduct and uterus of the rhesus monkey, hamster and rabbit. J. Reprod. Fertil. 99:673-679.
5. Flood, M. R., Gage, T. L. and Bunch, T. D. 1993. Effect of various growth promoting factors on preimplantation bovine embryo development *in vitro*. Theriogenology 39:823-833.
6. Gandolfi, F., Brevini, T. A. L., Modina, S. and Lauria, A. 1991. Detection and characterization of a growth factor in bovine oviduct secretions. J. Reprod. Fertil. (Abstr.) 7:6 (Abs. 2).
7. Keefer, C. L., Stice, S. L., Paprocki, A. M. and Golueke, P. 1994. *In vitro* culture of bovine IVM-IVF embryos:cooperative interaction among embryos and the role of growth factors. Theriogenology 41:1323-1331.
8. Larson, R. C., Ignotz, G. G. and Currie, W. B. 1991. Platelet derived growth factor (PDGF) initiates completion of the fourth cell cycle of bovine embryo development. J. Reprod. Fertil. (Abstr.) 7:6 (Abs. 1).
9. Lee, E. S. and Fukui, Y. 1995. Effect of various growth factors in a defined culture medium on *in vitro* development of bovine embryos matured and fertilized *in vitro*. Theriogenology 44:71-83.
10. Li, J. and Foote, R. H. 1993. Culture of rabbit zygotes into blastocysts in protein-free medium with one to twenty percent oxygen. J. Reprod. Fertil. 98:163-167.
11. Liu, Z. and Foote, R. H. 1995. Development of bovine embryos in KSOM with added superoxide dismutase and taurine and with five and twenry percent $O_2$. Biol. Reprod. 53:786-790.
12. Liu, Z., Foote, R. H. and Yang, X. 1995. Development of early bovine embryos in co -culture with KOSM and taurine, superoxide dismutase or insulin. Theriogenology 44:741-750.
13. Mass, D. H. A., Storey, B. T. and Mastroianni Jr. L. 1976. Oxygen tension in the oviduct of the rhesus monkey (*Macaca mulatta*). Fertil. Steril. 27:1312-1317.
14. Nakao, H. and Nakatsuji, N. 1990. Effects of co-culture, medium components and gas phase on *in vitro* culture of *in vitro* matured and *in vitro* fertilized bovine embryos. Theriogenology 33:591-600.
15. Papaioannou, V. E. and Ebert, K. M. 1988. The preimplantation pig embryo: cell number and allocation to trophectoderm and inner cell mass of the blastocyst *in vivo* and *in vitro*. Development 102:793-803.
16. Sekiya, K., Okuda, H. and Arichi, S. 1982. Selective inhibition of platelet lipoxygenase by esculetin. Bilchim. Biophys. Acta. 713:68-72.
17. Tervit, H. R., Whittingham, D. G. and Rowson, L. E. A. 1972. Successful culture *in vitro* of ship and cattle ova. J. Reprod. Fertil. 30:493-497.
18. Voelkel, S. A. and Hu, Y. X. 1992. Effect of gas atmosphere on the development of one-cell bovine embryos in two culture systems. Theriogenology 37:1117-1131.
19. Weiss, S. J. 1986. Oxygen, ischemia and inflammation. Acta. Physiol. Scand. (Suppl) 548 :9-37.

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