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Comparative Study on the Effects of Phenyl Boron and Boric Acid on the Development and Gene Expression (Connexin X43 and E-Cadherin) of Pre-implantationBovine Embryos

*Asmaa M Kandil, **Rings F, **Tesfaye D, **Hölker M, **Schellander K
*Department of pharmacology National Organization for Drug Control and Research.
**Institute of Animal Science, Animal Breeding and Husbandry group Bonn University, Germany.

Abstract

The different actions of Boron (as Phenyl boron (PB) or Boric acid (BA)) on the growth of pre-implantation embryos and their (Connexin X43(CX43) and E-Cadherin) gene expression was investigated. In the first and two experiments, phenyl boron or boric acid was added as 0, 20 and 250 µM/L in embryo culture medium at 18-20 h after in-vitro fertilization (IVF). The percentage of development to blastocyst stage was assessed from day 7 to day 9 post treatment. The experiment was repeated two times using 50 fertilized zygotes /treatment.
The total of two of 10 blastocysts pools from each treatment group was used for mRNA isolation for quantification of Cx43 and E-cadherin gene mRNA, in the embryos of each treatment group was assessed by real time quantitative PCR.
The results indicated that the rate of development to blastocyst is higher in blastocysts group treated with PB than that of BA. The highest development rate was at the 20µM/L PB and the lowest was at the 20µM/L BA treatment. The development of zygotes treated with 250µM/L BA was arressted at 4cell stage then they degenerated.
Relative expression levels of CX 43 were very high at doses of 250µM/L PB and 20 µM/L BA while at the same doses the relative expression levels of E-cadherin were very low.

Introduction

Boron is one of at least 150 different minerals of the earth's crust and is readily Transported into top soil, water and air **(Nemodruk and Karalova 1969 & Basset 1990).** Agricultural soil boron concentrations range from 0.6 to 7.4 mM (**Stevenson 1986**); in rain water it varies from 0.4 µmol/L in rain (**Fogg and Duce 1985, Libes 1992**), to 1.7 µmol/L in fresh water rivers and lakes to 420 µmol/L in the ocean (**Basset 1990)**.
Douglas *et al.,(*1999) reported that exposure of adult Xenopus laevis and embryos to a low boron(B) environment is capable of inducing impaired reproductive performance, abnormal organogenesis and increased embryo lethality and abnormal development of the gut **(Fort *et al.*, 1999).** The early stage of embryo development is especially sensitive to B deficiency as described for mated zebrafish (**Rowe *et al.* 1998 and Rowe and Eckhert, 1999)** and the preimplantation mouse embryo **(Lanoue *et al.* 1999).**

The bovine preimplantation embryogenesis is characterized by various morphological and physiological changes that occur following fertilization. These developmental events include the first cleavage division, the timing of which is known to be an indicator of the activation of embryonic genome **(Memili *et al.*, 1998),**subsequent developmental potential of the embryo **(Lonergan *et al.*, 1999)** as compaction of morula, and formation of blastocyst. These morphological and physiological transitions are known to be accompanied and regulated by differential expression of organogenesis genes (**Zimmermann and Schultz, 1994; Schultz *et al.*, 1999).**
It has been demonstrated that while oocyte quality is the main determinant of the blastocyst development rate in vitro, the post fertilization culture conditions are the main factors affecting the quality of the resulting blastocysts as measured in terms of

cryotolerance and relative transcript abundance (**Rizos *et al.*, 2002a,b, 2003; Lonergan *et al.*, 2003a,b).**

Gap junctions maintain cellular homeostasis by allowing communication between adjacent cells. They span the plasma membrane of two adjacent cells (**Makowski *et al.* 1977**), with each cell contributing half the channel; a hemi channel, or connexon. A connexon from one cell docks in the extra cellular space with a connexon from an opposing cell to form a complete gap-junction channel, allowing adjacent cells to be coupled (reviewed by **Bruzzone *et al.* 1996a**). Each connexon is multimeric assemblies of six proteins, termed connexins .Connexins are integral membrane proteins containing four transmembrane, two extracellular and three intracellular domains. Connexins are classified according to their molecular mass (**Beyer *et al.* 1987**). For example, connexin (Cx43) has a predicted molecular mass of 43 kDa and Cx31 has a predicted molecular mass of 31 kDa. Co expression of connexins is common; hepatocytes express both Cx26 and Cx32 (**Nicholson *et al.* 1987**), as do proximal kidney tubule cells (**Butterweck *et al.* 1994**), whereas vascular endothelium co-expresses Cx37 and Cx40 (**Delorme *et al.* 1997).** Gap junctions may be regulated by hormones and other extracellular signaling molecules such as neurotransmitters, growth factors and cytokines (**Stagg & Fletcher 1990, Bruzzone *et al.* 1996b, Sa´ez *et al*. 1998).** The extent to which cells are coupled depends on several mechanisms: gene transcription, stability of the message, translational and post-translational modifications and assembly of the protein into the membrane (**Sa´ez *et al.* 1998).**

Endothelial cell-cell adhesive junctions are formed by transmembrane adhesive proteins linked to a complex cytoskeletal network. These structures are important not only for maintaining adhesion between endothelial cells and, as a consequence, for the control of vascular permeability, but also for intracellular signaling properties. The establishment of intercellular junctions might affect the endothelial functional phenotype by the down regulation or up regulation of endothelial-specific activities (**Lampugnani and Dejana 1997**).

E-cadherin is a calcium-dependent cell adhesion molecule, which belongs to a family of developmental proteins responsible for maintaining the structural integrity of an epithelial monolayer.

E-cadherin is one of the most important molecules in cell-cell adhesion in epithelial tissues. It is localized on the surfaces of epithelial cells in regions of cell-cell contact known as adherens junctions [**Gumbiner,1996**] .As a member of a large family of genes coding for calcium-dependent cell adhesion molecules (CAMs), the cadherin glycoproteins are expressed by a variety of tissues, mediating adhesion through homotypic binding. Classical cadherins – E- and N-cadherins being the best characterized – play important roles in the formation of tissues during gastrulation, neurulation and organogenesis [**Barth,*et al*.,1997**].

The aim of this study is to investigate the effect of phenyl boron (PB) or boric acid (BA) on development of bovine blastocysts and cell-cell adhesive junctions by studying the Gene Expression (Connexin X43 and E-Cadherin) of Pre-implantation bovine Embryos.

Material and Method

IVM and IVF of Bovine Oocytes

Oocyte collection, In-vitro maturation (IVM) and in-vitro fertilization (IVF) were carried out as described by **Yoshioka and Kamomae, 1996**. Briefly, cumulus-oocyte complexes (COC) were aspirated from small antral follicles (2–5 mm in diameter) on bovine ovaries obtained from a slaughterhouse. After washing of COC with Hepes-buffered Tyrode's medium (TALP-Hepes [**Tesfaye et al., 2007**]), only oocytes with 3–4 layers of intact and unexpanded cumulus cells were selected; these were then cultured for 22 hours (h.) with maturation medium (Hepes-buffered TCM199 [Sigma Chemical Co., St. Louis, MO] + 10% heat-inactivated fetal bovine serum [Gibco Life Technologies, Grand Island, NY] supplemented with 0.2 mM sodium pyruvate, 0.02 U/ml porcine follicular

stimulating hormone (FSH) [Sigma], 1 µg/ml estradiol-17ß [Sigma], and 50 µg/ml gentamicin sulfate [Sigma] at 39°C in a humidified atmosphere of 5% CO_2 in air.

Sperm Capacitation and IVF

Following in vitro maturation (IVM), COCs were transferred into a 4-well dish containing 400 ml fertilization medium (Fert-TALP, **Parrish et al., 1988**), which is supplemented with 6 mg/ ml BSA (Sigma), 2.2 mg/ml pyruvate (Sigma), and 1 mg/ ml Heparin (Sigma). Each milliliter of stock fertilization medium contained 330 mg NaCl, 110 mg CaCl, 1,050 mg NaHCO3, 20 mg NaPO4, 30 mg penicillin G, 50 mg MgCl2, 150 mg CaCl. Ten microliters of PHE (penicilinamin, hypotaurin, epinephrin) was added to each well and covered with mineral oil. A swim-up procedure has been applied to obtain motile sperm cells from frozen-thawed semen. The final sperm concentration in fertilization drop was 2 $x10^6$ sperm cells/ml cells/ ml. Fertilization was initiated during co-incubation of spermatozoa and the matured oocytes for 20 hr in the same incubator under the same temperature and atmospheric CO2 content as used for maturation.

Embryo culture

After 20 h of co incubation with sperm, presumptive zygotes were stripped of cumulus cells by vortex for 4 min in 1 ml of TALP-Hepes, and then washed three times with culture medium. zygotes were cultured in groups of up to 50 zygotes in four-well dishes each containing 400 ml CR1 medium (**Rosenkranz and First, 1994)** until day 7 and 9 after insemination. The CR1 medium is supplemented with 10% OCS, 20 ml/ml BME (amino acids), and 10 ml/ml MEM (nonessential amino acids) (Gibco BRL, Eggenstein, Germany).

Experimental Design:

In the first experiment, groups about 45-50 presumptive zygotes were placed at 18-20 h after insemination in embryo culture medium containing normal medium(CR1), 0(medium free from any traces of boron), 20 and 250 µM/L PBA (phenyl boron). The experiment was replicated two times using 50 presumptive zygotes /treatment.

In the second experiment the presumptive zygotes were placed at 18-20 h after insemination in embryo culture medium containing normal medium (CR1), 0 (medium free from any traces of boron), 20 and250 µM/L BA (Boric acid). The experiment was replicated two times using 50 presumptive zygotes/ treatment. Development proceeded until day 9 post insemination. In vitro culture was also performed in a humidified atmosphere with 5% CO2 at 39 C.

The percentages of cleavage, morulae, blastocysts, and hatched blastocysts were calculated within each group. Differences in the mean percentages of cleavage, morulae, blastocysts, and hatched blastocysts among the experimental groups were analyzed by one-way ANOVA.

Embryo Collection

Morphologically good-quality day 7 , 8 and 9 blastocysts were used for biopsies, as described in **Tesfaye et al. (2004**). All embryos were washed two times with PBS (Sigma) and treated with acidic Tyrode pH 2.5–3 (Sigma) to dissolve the zona pellucida. The zona free embryos were further washed two times in drops of PBS and frozen in cryo tubes containing minimal amounts of lysis buffer [0.8% Igepal (Sigma), 40 U/ll RNasin (Promega, Mannheim, Germany), 5 mM dithiothreitol (DTT) (Promega)]. Until used for RNA isolation all frozen embryos were stored at -80 ºC.

mRNA isolation and reverse transcription

A total of two pools of 10 blastocysts from each treatment group were used for mRNA isolation using oligo (dT) attached magnetic beads (Dynal, Norway, Oslo) following the manufacturer's instructions and described by Tesfaye et al. (2003) and (2004).. Briefly, embryos in lysis buffer were mixed with 40 ml binding buffer [20 mM Tris HCl, pH 7.5, 1 M LiCl, 2 mM ethylene-di-amine-tetra-acetic acid (EDTA), pH 8.0] and incubated at 65ºC for 5 min to obtain complete lysis of the embryo and release of RNA. Ten microlitres of oligo(dT) magnetic bead suspension was added to the samples, and incubated at room temperature for 30 min. The hybridized mRNA and Oligo(dT) magnetic

beads were washed three times with washing buffer (10 mM Tris HCL, pH 7.5, 0.15 mM LiCl, 1 mM EDTA, pH 8.0). Finally, mRNA samples were eluted in 12 µl DEPC treated water and reverse transcribed in a 20 µl reaction volume containing 2.5 µM oligo (dT)12 N (where N=G,A or C) primer, 4 µl of 5x first stand buffer (375 mM KCl,15 mM MgCl2, 250 mM Tris–HCl pH 8.3), 2.5 mM of each dNTP, 10 U RNase inhibitor (Promega) and 100 U of SuperScript II reverse transcriptase (Invitrogen, Karlsruhe, Germany). In terms of the order of adding reaction components, mRNA and oligo (dT) primers were mixed first, heated to 70ºC for 3 min, and placed on ice until the addition of the remaining reaction components. The reaction was incubated at 42ºC for 90 min, and terminated by heat inactivation at 70ºC for 15 min.

Quantitative Real-Time PCR

Quantification of Cx43 and E-cadherin mRNA in the embryos of each treatment group was assessed by real time quantitative PCR. The ABI Prism1 7000 apparatus (Applied Biosystems, Foster City, CA) was used to perform the quantitative analysis using SYBR1 Green JumpStartTM Tag ReadyMixTM (Sigma) incorporation for ds DNA-specific fluorescent detection dye. Quantitative analyses of embryo Cx43 and E-cadherin, cDNA were performed in comparison with heston (H2a) housekeeper gene as an endogenous control (**Robert et al., 2002**) and were run in separate wells. PCR was performed by using 2 µl of each sample cDNA along with specific primers. The primer sequences were designed for PCR amplification according to the bovine cDNA sequence (Table 1) using Primer Express1 Software v2.0 (Applied Biosystems). Standard curves were generated for both target and endogenous control genes using serial dilution of plasmid DNA (101–108 molecules). The PCRs were performed in a 20 ml reaction volume containing 10.2 ml SYBR1 Green universal master mix (Sigma), optimal levels of forward and reverse primers and 2 µl of embryonic cDNA. During each PCR, reaction samples from the same cDNA source were run in duplicate to control the reproducibility of the results. A universal thermal cycle (initial denaturation step at 95ºC for 10 min, 45 cycles of denaturation at 95ºC for 15 sec, and 60ºC for 60 sec) was used to quantify each gene of interest. After the end of the last cycle, a dissociation curve was generated by starting the fluorescence acquisition at 60ºC and taking measurements at 7 sec intervals until the temperature reached 95ºC. Final quantitative analysis was done using the relative standard curve method as used in Tesfaye et al. (2004) and results are reported as the relative expression level compared to the calibrator cDNA after normalization of the transcript amount to the endogenous control.

Table (1) showed the sequences of primers

Genes	primers
Connexin43-623F:	CGTGTGAAGGGAAAGAGCGA
Connexin43-808R:	ATTGCGGCAGGAAGAATTGT
E-cadrhen-776F:	CCTGATGGTCCAAGCAGCA
E-cadrhen -958R:	TCGTCGGCATCAGTCACTGT

Results

Two experiments were conducted in which effects of phenyl (PB) or boric acid (BA) added 18-20 h after IVF were evaluated (Figs 1-3). In the first experiment, the in-vitro development of bovine embryos was monitored at day 2 to assess the cleavage rate and blastocyst rate was monitored until day 9 during culture in the presence or absence of boron at different formula and concentrations in culture medium.

The effects of phenyl boron addition in culture medium.

When the presumptive zygotes were cultured in the absence of boron, the rate of embryo developement to the stage of morula was 22.41 % less than that of the control and all other groups. But the highest rate of embryo development to morula stage at 7th day after IVF was the embryo cultured in medium with 250µM/L PBA (61.54%).

The rate of blastocyst of presumptive zygotes at 7th day after culture in medium with 20µM/LPB were very high (38.89) than the control and the lowest rate was that of zygotes in medium with 250µM/L PB (Fig.1). However the rate of zygotes developed to blastocyst at 8th day was very high in groups cultured in medium with 20µM/LPB and the lowest rate in group without boron.

Effect s of Boric Acid on the development of presumptive zygotes.

All embryos cultured at high dose of BA (250µM/L) arrested at four cell stage (fig. 2). There was no effect on the first cleavage rate of zygotes at control, without boron and with 20µM/L BA groups.

Development to morula and blastocyst stages in control group at 7th and 8th day were high than that of all other groups. In addition the rate of blastocyst in group cultured in mediumwithout boron was more than that in medium with 20µM/L BA (Fig.3).In the present study we have different expression patterns in blastocyst at 8th day after cultured in medium as revealed by real time quantitative PCR.

The relative expression of connexin43mRNA in blastocycts treated with 250uM PBA is very high than blastocyct derived from control medium, without Boron or with 20uM PB (figure 4).

In the second experiment BA was used. The relative expression of connexin43 mRNA in blastocyct cultured in medium containing 20uM BA is very high than the control and than that of medium without BA (figure5). However, the relative expression of connexin43 mRNA in blastocyct cultured in medium without boron was less than that of the control group.

The relative expression of E-cadherin mRNA in blastocycts cultured in medium with 20uM PB is very high than that from medium without PB or with 250uM PB (figure 6).

In the second experiment the relative expression of E-cadherin mRNA in group treated with 20uM BA is higher than that in group without boric acid (figure 7).

The results indicate that PB improved the development of pre-implantation bovine embryo than BA and increased CX43mRNA and E-cadherin mRNA expression of embryo. PB and BA high doses were toxic and the absence of PB or BA decreased blastocyst development of the pre-implantation bovine embryo.

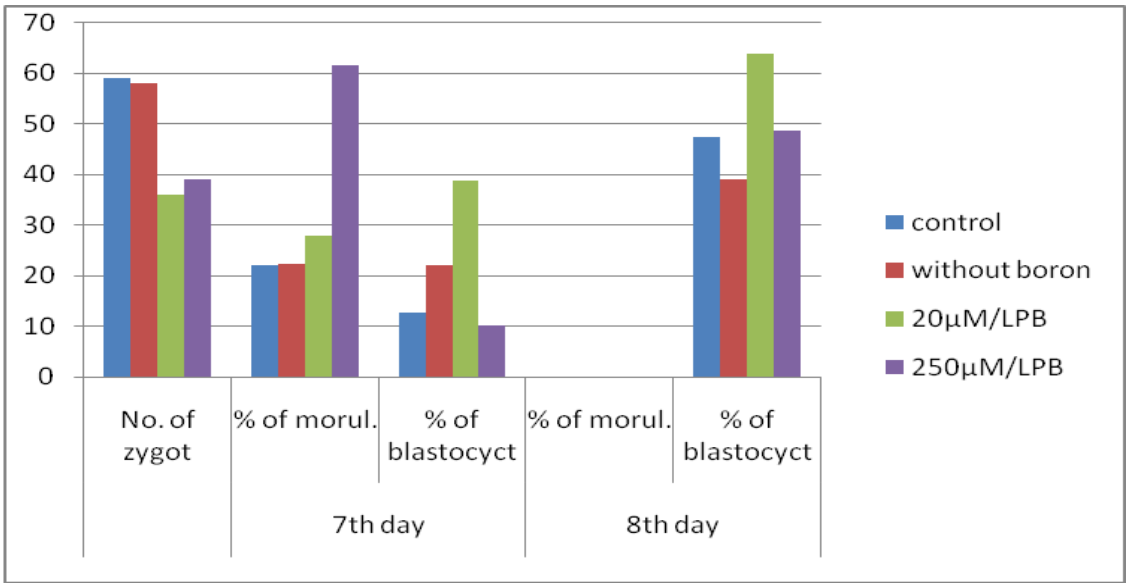

Fig (1) effect of phenyl boron on the rate of the development of pre-implantation bovine embryos

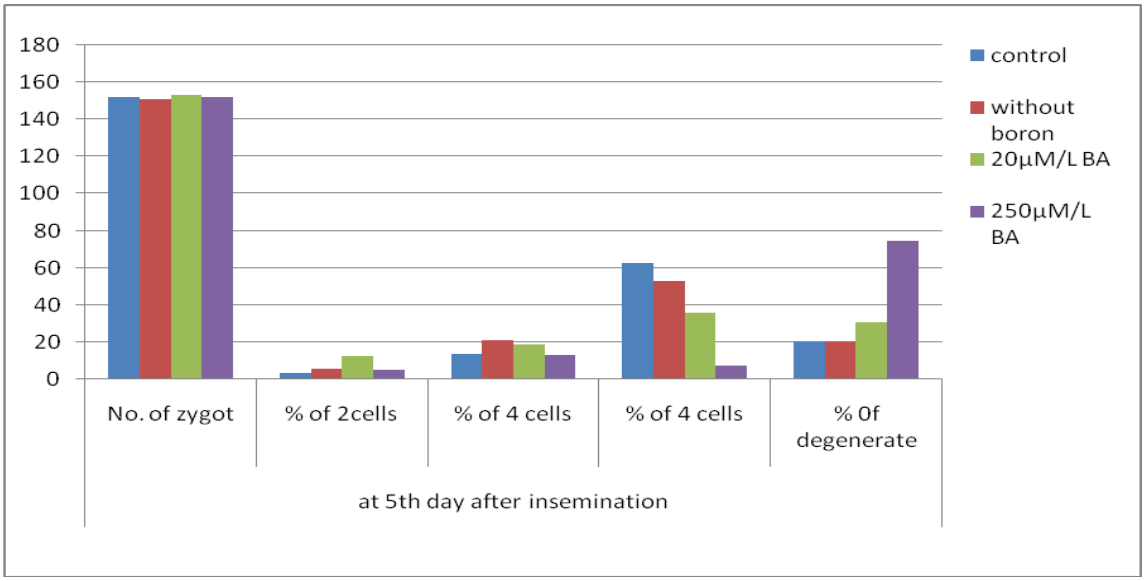

Fig. (2): Effect of Boric acid on the rate of development of pre-implantation bovine embryos at 5th day after In vitro fertilization (IVF)

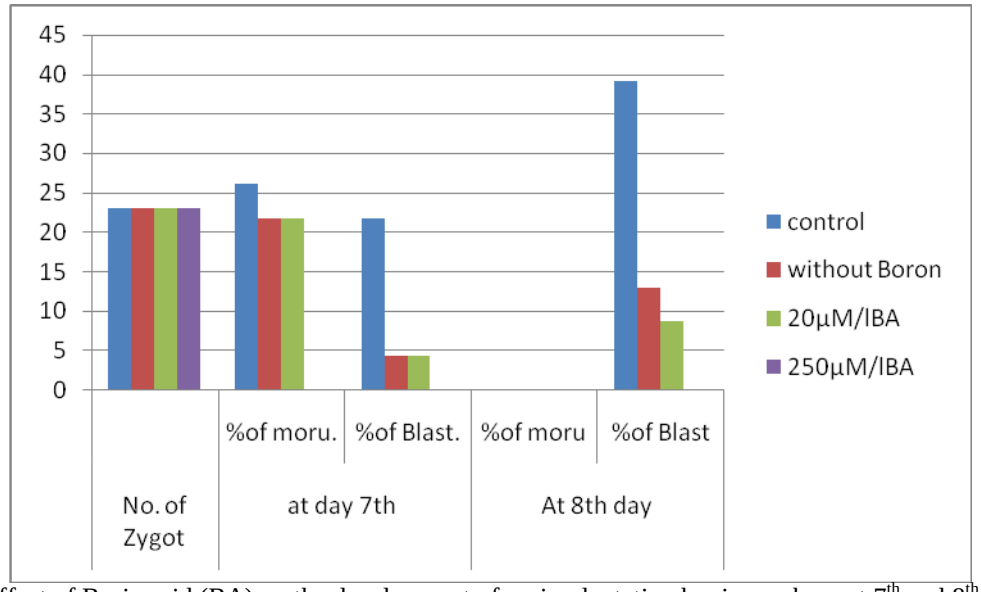

Fig.(3):Effect of Boric acid (BA) on the development of preimplantation bovine embrys at 7th and 8th day after in vitro fertilization (IVF)

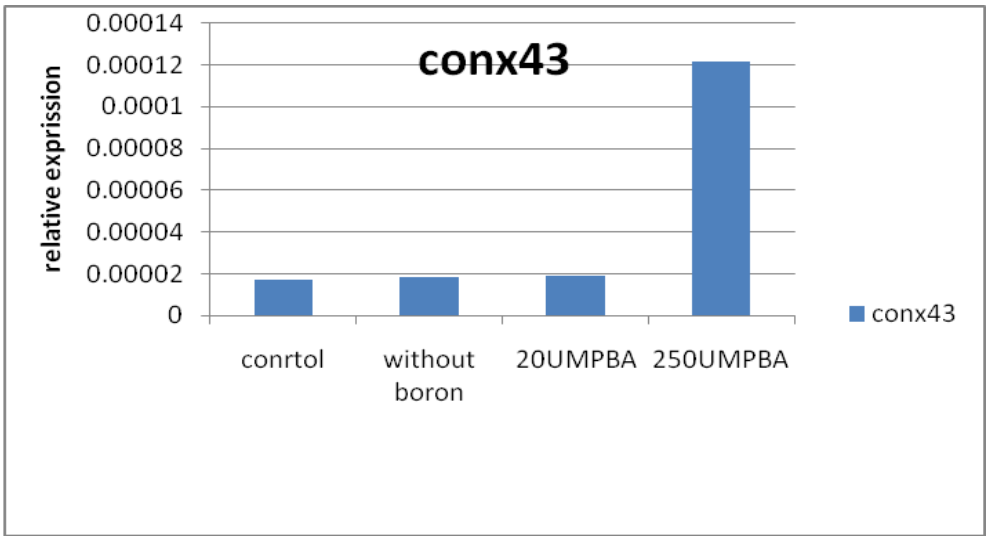

Figure (4): Relative expression level of connexin 43mRNA derived from blastocycts of preimplantation bovine embryo of groups treated with phenyl boron.

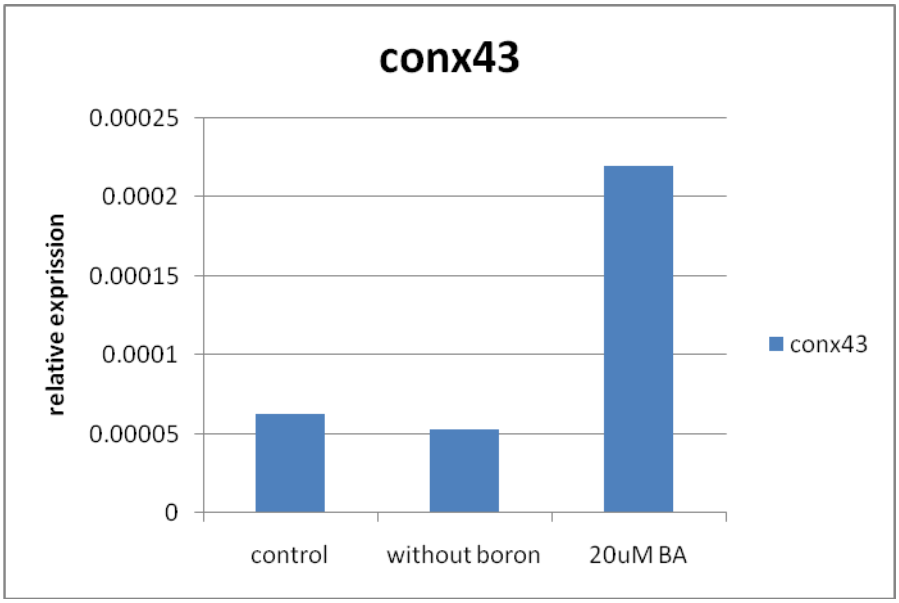

Figure (5): Relative expression level of connexin 43 mRNA derived from blastocycts of preimplantation bovine embryo of groups treated with Boric acid.

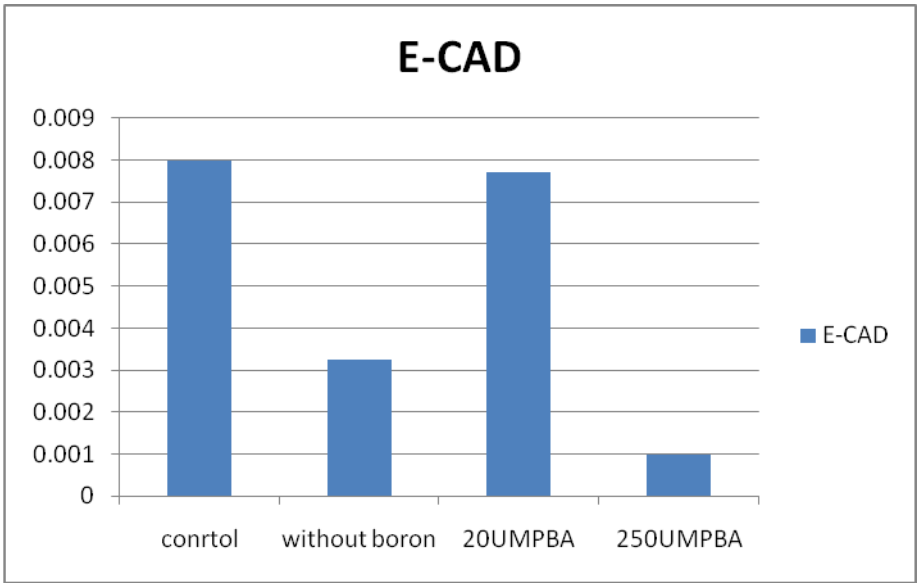

Fig.: (6) Relative expression of E-cadherin mRNA in blastocycts derived from blastocycts of preimplantation bovine embryo of groups treated with phenyl boron.

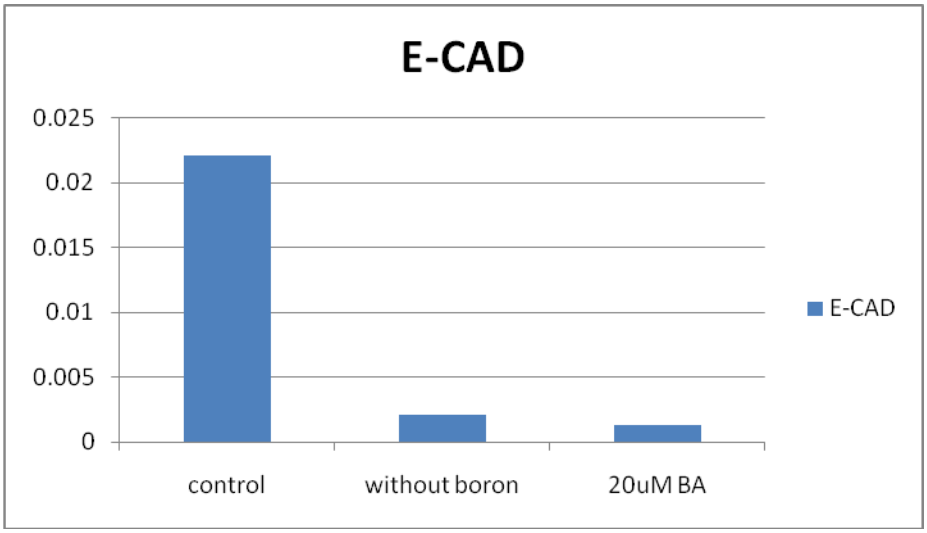

Fig.: (7) Relative expression of E-cadherin mRNA in blastocycts derived of preimplantation bovine embryo of groups treated with boric acid.

Discussion

Earlier reports showed that B was essential for at least for vertebrates embryonic development. When B deprivaion resulted in a high percentage of necrotic eggs and abnormal development of the gut in Xenopus laevis (**Fort *et al.*, 199**9). The early stage of development is especially sensitive to B deficiency as described for mated zebrafish (**Rowe *et al.* 1998 and Rowe and Eckhert, 1999)** and the preimplantation mouse embryo **(Lanoue *et al.* 1999).**

The present results indicate that Phenyl boron improve the development of pre-implantation bovine embryo than the boric acid and increase expression of CX43mRNA and E-cadherin mRNA. In addition the absence of PB or BA decreased the development of the pre-implantation bovine embryo(39.02 and 13.04 respectively compared with 47.37in control group).

According to **Laoue, (1998**) impaired embryonic differentiation and proliferation were observed only when embryos were exposed to high levels of boric acid (>2000microM), reflecting a very low level of toxicity of BA on early mouse embryonic development. Maternal exposure to the low B diet for 10,12, 1nd 16 wk was associated with a reduction in blastocyst formation, a reduction in blastocyst cell number, and an increased number of degenerates. Collectively, these studies support the concept that B deficiency impairs early embryonic development in rodents.

According to **Heindel *et a*l (1994**), boron causes significant increase in both the percentage of embryonic resorption and late fetal death of ratembryo. The percentage of malformed fetuses per litter and the percentage of litters containing at least one malformed fetus were increased at all doses studied. A variety of malformations was noted, including anomalies of the eyes, the central nervous system, the cardiovascular system, and the axial skeleton. They also suggested that boric acid treatment particularly in the high-dose was associated with significant adverse post implantation effects in mice. The most frequently observed malformations in the 1003mg/kg/day boric acid treated litters were skeletal defects, particularly short rib XIII. Malformation were dose dependent as malformations were fewer in low (248mg/kg/day)- and mid dose(452mg/kg/day) group than in the control group. In particular, the incidence of full or

rudimentary lumbar I rib(s) was less frequentl in fetuses of boric acid-treated mice.

The fact that BA react with hydroxyl groups is considered the key to understand boron functions. **Bolanos *et al.*, 2004** stated that "the primary role of boron in biological systems is stabilization of molecules with *cis*-diol groups, independently of their function" and "boron chemistry makes it a perfect candidate for atomic diester bridging". Whether the formation of two-sided complexes is imperative for boron's function(s) is still being debated. On the basis of the stability of known borate monoesters in aqueous systems and the distribution of *cis*-diol-binding sites in cells, it seems less likely that B monoesters play a relevant metabolic function. Chemical conditions in living systems do not favor monoester formation, and only di-esters could achieve stabilities high enough to be of physiological relevance. Using PBA as probes in plant experiments for B-binding ligands forming exclusively one-sided esters makes it possible to search for those functions where B is required for cross-linking ligands with cis-hydroxyl groups as in RGII (**Bassil *et al.*, 2004**).

At physiological pH, B is present as uncharged boric acid which, combined with its relatively high lipid solubility (Raven 1980), allows rapid penetration of biological membranes, as demonstrated by **Stangoulis *et al.* (2001**) and **Hayes and Reid (2004).** Under conditions of adequate B nutrition, active uptake of environmental B would therefore seem unnecessary.

The present results indicated that the high dose of phenyl boron (250µM/L) and absence of PB or BA decreased the development of zygotes and these may be attributed to decrease the expression of CX43and E-cadherin mRNA in blastocyctes of bovine embryo. This is according to Sullivan et al, 1993 whose suggested that Cx43 is a single copy gene that consists of two exons and one intron.

During murine preimplantation development functional gap junctions are first observed at compaction at the eight-cell stage (**Lo and Gilula, 1979; McLachlin *et al,* 1983; Goodall and Johnson, 1984)** and are necessary for the maintenance of compaction, and thus for subsequent blastocyst formation (Lee *et al*, 1987; Bevilacqua *et al*, 1989). Expression of E-cadherin in embryonic development is very early, at the two-cell stage [**Larue *et al.*, 1994 and Rietmacher *et al.*, 1995**]. Epithelial differentiation and polarization (processes fundamental to cell differentiation) occur early in ontogeny in the morula stage, when the embryo compacts and each cell polarizes along its apicobasal axis to generate an epithelial-like phenotype. E-cadherin plays an important role in the adhesion of the blastomeres, and early embryo's ability to compact [**Fleming *et al.*, 1992**]. E-cadherin is expressed in the membrane even before compaction of the morula occurs, is distributed in a non-polar manner, and does not exhibit adhesive function [**Hyafil *et al.*, 1980, and Vestweber&, Kemler,1984**]. The mechanism that renders E-cadherin function is unknown, but it does include phosphorylation of the protein [**Sefton, *et al.*, 1992**]. Controlled epithelial-mesenchymal conversion is the most important exhibit of E-cadherin's function in development [**Thiery, 2002].** Loss of epithelial adhesion and polarity causing mesenchymal cell morphology occurs during mesoderm formation. Rietmacher and co-workers [**Rietmacher *et al.*, 1995**] introduced a targeted mutation in mouse embryonal stem cells and generated a mouse without E-cadherin sequences essential for Ca^{2+} binding and for adhesive function. Heterozygous mutant animals were normal and fertile. In vitro, they were able to form normal blastocysts with normal blastocoels that consequently expanded. On the other hand, the homozygous E-cadherin -/- embryos showed severe abnormalities before implantation which included failure to maintain a polarized and compacted state and also failure to form a trophectoderm epithelium making this mutation lethal. The initial compaction that was observed in -/- embryos is probably due to the presence of E-cadherin proteins from maternal sources [**Sefton, *et al.*, 1992**].

Investigation of zebrafish E-cadherin expression during early embryogenesis confirmed observed expression in

blastomeres, but also led to the detection of a protein expressed in the anterior mesoderm during gastrulation and development epithelial structures [**Babb, *et al.*, 2001**].
Cadherin mediated adhesion is a dynamic process that is regulated by several signal transduction pathways. There is also evidence that cadherins are not only targets for signaling pathways that regulate adhesion, but may themselves send signals that regulate basic cellular processes, such as migration, proliferation, apoptosis and cell differentiation [**Hulsken, *et al.*, 199;Barth, *et al.*,1997and Morin *et al.*, 1997].**

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التأثيرات المختلفة للبورون كفنيل بورون او حمض البوريك على نمو اجنة البقر قبل انغماسها فى جدار الرحم وكذالك على التعبير الجينى للكونكسين 43 والايكادرين يتم دراستها فى هذا البحث.
فى التجربة الاولى والثانية يضاف الفنيل بورون او حمض البوريك 0 – 20 - 250ميكرو مول/لتر فى الوسض الغذائى للجنين بعد 18-20 ساعة من الاخصاب الخارجى . تحسب نسبة تطور الاجنة من اول يوم بعد الاخصاب وحتى اليوم التاسع بعد الاخصاب نسبة الاجنة التى وصلت الى طور البلاستولة فى المجاميع كلها. تكرر التجربة مرتين.
يستخدم خليط من عشرة بلاستوسيست مرتين من كل مجموعة لعزل الحمض النووى الريبوزى لتعين كمية التعبير الجينى لكل من الكزنكسين 43 والايكادرين باستخدام جهاز التعيين الكمى الحقيقى للتعبير الجينى فى الحمض النووى الريبوزى
اوضحت النتائج ان نسبة تطور الاجنة اللى طور البلاستولة فى المجاميع المعالجة بالفنيل بورون اعلى من المعالجة بحمض البوريك. وان اعلى نسبة تطور كانت فى الاجنة المعالجة ب- 20 ميكرومول فنيل بورون واقل نسبة فى الاجنة المعالجة ب- 20ميكرومول حمض البوريك
تطور الاجنة المعالجة ب-250 ميكرومول حمض البوريك توقف عند طور الانقسام اربع خلايا
مستوى التعبير الجينى للكونكسين عالى جدا فى الاجنة المعالجة ب-350 ميكرومول فنيل بوروت و20 ميكرومول حمض البوريك بينما التعبير الجينى للاكادرين لنفس الجرعات منخفض جدا