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Effect of cigarette smoke condensate on mouse embryo development and expression of pluripotency and apoptotic genes *in vitro*

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Summary

The aim of the present study was to investigate the effect of cigarette smoke condensate (CSC) on *in vitro* development of mouse embryos. In total 3000 NMRI mice 2PN embryos were divided into six groups ($n = 500$). The test group was exposed to 20, 40, 80, 160 or 320 µg/ml of CSC. In the control group, CSC was not added to the culture medium during the development of 2PN embryos. The effects of 20 and 80 µg/ml of CSC on genes involved in pluripotency and apoptosis, and also, the aryl hydrocarbon receptor gene was assessed in the blastocysts. Our results showed that CSC had an adverse effect on the viability of mouse embryos at the concentrations of 80, 160 and 320 µg/ml compared with the control group ($P < 0.05$). In contrast, it had positive effects on the viability of mouse embryos at the concentrations of 20 and 40 µg/ml compared with the control group ($P < 0.05$). The 20 and 80 µg/ml concentrations of CSC increased the expression of pluripotency, apoptotic, and aryl hydrocarbon receptor genes in the blastocyst embryo stage compared with the control group ($P < 0.05$). It can be concluded that concentrations higher than 40 µg/ml of CSC have an adverse effect on mouse embryo development in the preimplantation stages. Also, 20 and 80 µg/ml concentrations of CSC have a significant effect on the expression of pluripotency, apoptotic, and the aryl hydrocarbon receptor genes in the blastocyst embryo stage compared with the control group.

Introduction

Cigarette smoke contains *c.* 7000 chemicals, some of which have toxic effects (Berridge *et al.*, 2010). The detrimental impacts of cigarette smoke lead to a wide range of abnormalities and diseases, including abnormal cell differentiation, cardiovascular diseases, cancers, asthma, degenerative diseases, systemic disorders, inflammation, infertility, and reduced restoration of damaged tissues (Zhang *et al.*, 2005; Zdravkovic *et al.*, 2008; Lin *et al.*, 2009, 2010; Kim *et al.*, 2020). A person who smokes more than one cigarette per day has been reported to have a plasma nicotine concentration greater than 25 ng/dl (Calabrese and Baldwin, 2003; Calabrese, 2013).

The adverse impacts of smoking on females and pregnancy's reproduction system have been reported (Oboni *et al.*, 2016). Smoking increases miscarriage in both natural and laboratory conceptions (Harrison *et al.*, 1990; Hughes and Brennan, 1996; Ness *et al.*, 1999; Zenzes, 2000; Winter *et al.*, 2002). It has been shown that smoking increases the risk of early pregnancy loss in assisted reproductive technology (ART) treatment (Winter *et al.*, 2002). In an *in vivo* study, it has been shown that in mice that were exposed to cigarette smoke condensate (CSC), embryo fragmentation or delayed fertilisation is increased. Also, it has been noted that exposure to cigarette smoke significantly reduces fetal growth (Andreu-Fernández *et al.*, 2017; Kataoka *et al.*, 2018). It has been reported that smoking causes childhood disorders such as low birth weight, increased risk of respiratory disease and pancreatic dysfunction (Kataoka *et al.*, 2018). Different genes are involved in fetal development. *Bcl2* and *Bax* are essential genes involved in mitochondrial apoptosis, in which *Bcl2* is an inhibitor of apoptosis, and *Bax* is the proapoptotic equivalent of *Bcl2* (Andreu-Fernández *et al.*, 2017). Pluripotency is controlled by transcription factors such as *Oct4*, *Sox2* and *Nanog* (Niwa, 2001). Also, aryl hydrocarbon receptor (AhR) is a transcription factor that has a role in the metabolism of toxic compounds and fetal development (Gialitakis *et al.*, 2017).

Table 1. Primer sequences

	Gene	Forward	Reverse	Amplicon (bp)
1	*SOX2*	GGAGAAAGAAGAGGAGAG	GCGATTGTTGTGATTAGT	89
2	*NANOG*	AAGAATAAGTGCTTGAAC	CCACCATATCGTTATACT	141
3	*OCT4*	TAGAGAAGGATGTGGTTC	TGTAGCCTCATACTCTTC	98
4	*BAX*	CGGCGAATTGGAGATGAACTG	GCAAAGTAGAAGAGGGCAACC	162
5	*BCL2*	CCTGTGGATGACTGAGTACC	GAGACAGCCAGGAGAAATCA	140
6	*AhR*	CACTGACGGATGAAGAAGG	CCTTACTTGGGGTTGACTGG	192
7	*β-Actin*	CGTCCCGTAGACAAAATGGT	GAATTTGCCGTGAGTGGAGT	247

In the present research, the impacts of different concentrations of CSC on the *in vitro* development of mouse embryos were assessed. In addition, the expression of pluripotency, apoptotic, and *AhR* genes in the development of mouse embryos in the pre-implantation stages was evaluated.

Materials and methods

Cigarette smoke condensate

CSC was obtained from Murty Pharmaceutical Corporation (Lexington, KY, USA). It was dissolved in dimethyl sulfoxide (DMSO) and prepared at the concentrations of 20, 40, 80, 160 and 320 µg/ml.

Animals

The Pasteur Institute of Iran supplied the outbred NMRI mice that were used in this study. The mice weighed 20–25 g. They had free access to water and food and were housed in cages at the temperature of 22 ± 2°C under a 12-h dark/light cycle.

Experimental groups

In total, 3000 NMRI mice 2PN embryos were divided into six groups ($n = 500$). In the control group, CSC was not added to the culture medium during embryo culture. In test groups, 20, 40, 80, 160 and 320 µg/ml of CSC were added to the culture medium during embryo culture.

Investigating the effect of CSC on in vitro development of mouse embryos

Ovulation was induced in female mice with an intraperitoneal (i.p.) injection of 5 IU pregnant mare serum gonadotropin (PMSG) followed by 5 IU human chorionic gonadotropin (hCG) 2 days later (Sigma, USA). The female mice were housed in cages with the male mice. The next day, the mice with vaginal plugs were selected, and their ampullae were removed. The zygotes were harvested and incubated for 30 min in a human tubal fluid medium (HTF) containing 1% hyaluronidase. The zygotes were washed three times in potassium simplex optimisation medium (KSOM) medium and moved to KSOM medium. The 2PN embryos were exposed to different CSC concentration for 24 h, and the development of the embryo from the 2PN embryo stage to the blastocyst stage was evaluated.

Quantitative real-time polymerase chain reaction

The expression of the genes involved in pluripotency (*Oct4*, *Sox2*, *Nanog*), apoptosis (*Bcl*, *Bax*), and *AhR* processes was evaluated in the *in vitro* development of mouse embryos in three groups: control, 20 µg/ml of CSC, and 80 µg/ml of CSC.

The morulae were exposed to different concentrations of CSC for 24 h. Afterward, the blastocysts were collected. Total RNAs were extracted from all studied groups using an RNA extraction kit (Favorgen Biotech Corp., Taiwan) according to the manufacturer's instructions. A cDNA synthesis kit (SinaClon, Iran) was used to synthesise the complementary DNA (cDNA). *β-Actin* was applied as the reference gene. The $\Delta\Delta C_t$ method was used to calculate the data. Table 1 demonstrates the primers used in this experiment.

Statistical analysis

We used SPSS 16 software for statistical analyses. Results are shown as mean ± standard deviation and a *P*-value lower than 0.05 was considered as statistically significant. Student's *t*-test analysis was applied for comparing the differences between two groups, while the differences among multiple groups were analysed using analysis of variance (ANOVA) and Tukey's test. All the experiments were performed in triplicate.

Results

Effect of CSC on in vitro development of mouse embryos

Compared with the control group (65.5 ± 3.07), the viability increased in 20 µg/ml of CSC (74.3 ± 4.96) and 40 µg/ml of CSC (82.1 ± 2.36) ($P < 0.002$). However, in 80 µg/ml of CSC (47.6 ± 4.64), 160 µg/ml of CSC (11.7 ± 5.69), and 320 µg/ml of CSC (1.8 ± 0.97), viability decreased compared with the control group ($P < 0.002$) (Table 2 and Fig. 1).

Effect of CSC on the expression of pluripotency genes

The results of real-time PCR showed that CSC caused a change in the expression of multiple genes. Compared with the control group, CSC at the concentration of 20 µg/ml did not have any significant effect on *Oct4* gene expression, whereas CSC at the concentration of 80 µg/ml significantly increased *Oct4* expression ($P < 0.05$) (Fig. 2). We found that concentrations of 20 µg/ml and 80 µg/ml significantly upregulated *Sox2* gene expression ($P < 0.05$) (Fig. 2). A similar effect was seen with regards to the *Nanog* gene ($P < 0.05$) (Fig. 2). Also, the expression of *Oct4*, *Sox2* and *Nanog* genes at the concentration of 80 µg/ml CSC were higher than at 20 µg/ml ($P < 0.05$) (Fig. 2).

Effect of CSC on the expression of apoptotic genes

Compared with the control group, CSC at the concentrations of 20 and 80 µg/ml enhanced ($P < 0.05$) the expression of the *Bax/Bcl2*

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Table 2. Effects of CSC on *in vitro* development (IVD)

Groups	IVD (mean ± standard deviation (SD))
Control	65.5 ± 3.07
20 µg/ml	74.3 ± 4.96[a]
40 µg/ml	82.1 ± 2.36
80 µg/ml	47.6 ± 4.64[a]
160 µg/ml	11.7 ± 5.69[a]
320 µg/ml	1.8 ± 0.97[a]
P-value	0.002

[a]Significant differences between treatment groups compared with the control group. ($P < 0.05$) ($n = 5$) (mean ± SD).

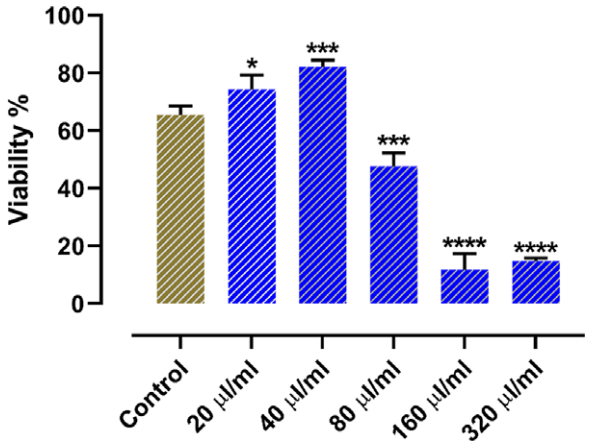

Figure 1. Effects of cigarette smoke condensate (CSC) on *in vitro* development (IVD). The percentage of viability at different concentrations of CSC is shown for IVD of the embryos. *, ***, **** $P < 0.002$.

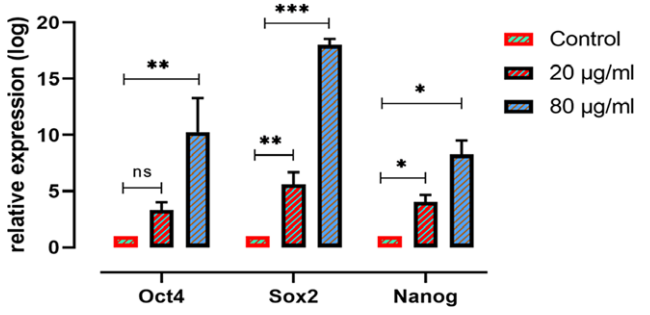

Figure 2. Effects of cigarette smoke condensate (CSC) on the expression of pluripotency genes. The effects of CSC on *Oct4*, *Sox2*, and *Nanog* pluripotency genes are shown in the blastocyst embryos. ($P < 0.05$) ($n = 500$) (mean ± standard deviation (SD)). *, **, *** $P < 0.05$.

ratio in blastocysts (Fig. 3). Also, the expression of *Bax* and *Bcl2* genes at the concentrations of 80 µg/ml CSC were higher than 20 µg/ml ($P < 0.05$) (Fig. 3).

Effect of CSC on the expression of AhR

The results of real-time PCR showed that treatment with both 20 and 80 µg/ml CSC for 24 h significantly upregulated ($P < 0.05$) the expression of the *AhR* gene compared with the control group

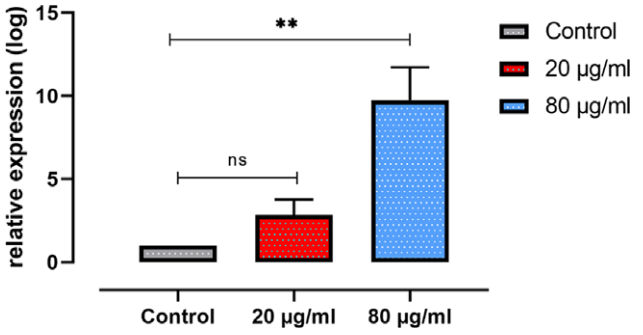

Figure 3. Expression of apoptosis genes. Relative expression of the *Bax/Bcl2* genes in blastocysts is shown. ($P < 0.05$) ($n = 500$) (mean ± standard deviation (SD)). ** $P < 0.05$.

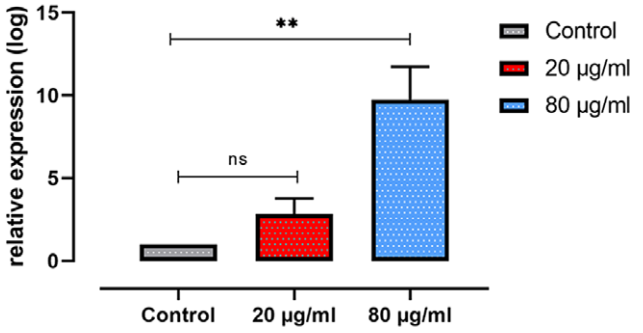

Figure 4. Expression of aryl hydrocarbon receptor (*AhR*). Relative expression of the *AhR* gene is shown. ($P < 0.05$) ($n = 500$) (mean ± standard deviation (SD)). ** $P < 0.05$.

(Fig. 4). We also found that *AhR* expression was higher in the 80 µg/ml treatment group compared with the 20 µg/ml group ($P < 0.05$) (Fig. 4).

Discussion

In this study, it was observed that CSC at concentrations of 80, 160, and 320 µg/ml had an undesirable effect on embryo development. Also, CSC had a significant effect on the expression of pluripotency, apoptotic, and *AhR* genes in the blastocyst embryo stage compared with the control group.

Exposing female mice to cigarette smoke for 3 days had detrimental effects on embryo development in treated mice (Hassa *et al.*, 2007). It has been shown that exposure to cigarette smoke resulted in a higher rate of multinucleated blastomere developed bovine blastocysts (Liu *et al.*, 2008). The effect of cadmium in cigarette smoke on mouse embryos has been investigated, and results have shown that it has no effect on mouse embryo development at low exposure levels. Nevertheless, at high exposure doses, it results in degenerated embryos with a necrotic appearance (Yu *et al.*, 1985). The results of the present research indicated that CSC at the concentrations of 80, 160, and 320 µg/ml reduced embryo development. However, it had beneficial effects at the concentrations of 20 and 40 µg/ml. According to research, a wide variety of drugs and chemicals such as nicotine (Csiszar *et al.*, 2008) and CSC (Assadollahi *et al.*, 2019b) with a low-dose stimulus or beneficial effect and a high-dose inhibitory or harmful effect are called hermetic dose responses (Calabrese, 2013). The results of the current study may be due to the hermetic properties of CSC.

Oct4, *Nanog*, and *Sox2* are transcription factors that regulate self-renewal and pluripotency in normal embryo development

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(Niwa, 2001). The expression of *Oct4* is typically limited to ICM in preimplantation blastocysts which are needed for postimplantation *in vivo* development for embryonic stem cells to be established *in vitro*. Expression of Oct4 is considered as a blastocyst consistency indicator (Liu *et al.*, 2004).

In the current study, CSC at the concentration of 20 µg/ml showed no effect on *Oct4* gene expression, whereas CSC at the concentration of 80 µg/ml increased *Oct4* gene expression. Huang and colleagues showed that CSC could change the expression of *Oct4* in embryos obtained from mice exposed to CSC (Huang *et al.*, 2009). It has been reported that the overexpression of *Oct4* results in mesodermal and endodermal differentiation (Assadollahi *et al.*, 2019a). A study has shown that changes in *Oct4* expression due to CSC reduced the quality of blastocysts (Huang *et al.*, 2009). *Sox2* is involved in deciding the molecular fate of the cell and in controlling the development of the embryo (Kirby *et al.*, 2002), and any alteration of this gene's expression profile will lead to the end of the undifferentiated state and the beginning of differentiation (Kopp *et al.*, 2008). This gene, which is part of the HMG-box family and is also a strong transcription factor due to its DNA-binding properties, is one of the most active genes in the early embryonic stage (Cömertpay *et al.*, 2018). CSC at the concentrations of 20 and 80 µg/ml increased the expression of *Sox2* and *Nanog* genes. In a previous study, we have shown that different doses of cigarette extract could alter the expression of *Sox2* and *Nanog* in the mouse embryonic stem cells (Assadollahi *et al.*, 2019b). Research has shown that nicotine performs its harmful effects by changing *Sox2* master gene expression levels (Cömertpay *et al.*, 2018). *Nanog* is a transcription factor that plays an essential role in regulating pluripotent cells by preserving the pluripotent epiblast and blocking primitive endoderm differentiation. Researchers have demonstrated that tobacco can increase the expression of *Nanog* (Liszewski *et al.*, 2012).

Bcl2 and *Bax* genes have a very significant role in mitochondrial apoptosis. In calculating the cells' sensitivities to apoptosis induction, the *Bax/Bcl2* ratio is considered to be more influential than the individual expression of each gene. The beginning of apoptosis is marked by the increase in the *Bax/Bcl2* ratio (Andreu-Fernández *et al.*, 2017). The current study results demonstrated that CSC at the concentrations of 20 and 80 µg/ml increased the ratio of the *Bax/Bcl2* genes in the blastocysts. As a transcription factor, *AhR* plays a significant role in metabolising toxic compounds (Gialitakis *et al.*, 2017). In the current study, the *AhR* gene had a significantly enhanced expression in the blastocysts treated with 20 and 80 µg/ml of CSC. Exposure to CSC changed the expression of *AhR* in blastocytes developed from fertilised oocytes in the C57BL/6 mice cell line (Assadollahi *et al.*, 2019b). It seems that *AhR* appears gradually during embryonic development. This expression depends on time and explains the gradual effects of cigarette smoke on the development of the embryo (Tscheudschilsuren *et al.*, 1999).

In conclusion, our data revealed that CSC at the concentrations of 80, 160, and 320 µg/ml has an undesirable effect on the development of the embryo. Conversely, CSC at the concentrations of 20 and 40 µg/ml showed some beneficial effects *in vitro*. However, further and more detailed studies in this field are recommended.

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Ethical standards. All animal procedures were conducted according to the Guidelines approved by the Ethics committee of Kurdistan University of Medical Sciences.

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Conflicts of interest. The authors declare they have no competing financial interests.

References

Andreu-Fernández V, Sancho M, Genovés A, Lucendo E, Todt F, Lauterwasser J, Funk K, Jahreis G, Pérez-Payá E, Mingarro I, Edlich F and Orzáez M (2017). Bax transmembrane domain interacts with prosurvival Bcl-2 proteins in biological membranes. *Proc Natl Acad Sci USA* **114**, 310–5.

Assadollahi V, Fathi F, Abdi M, Khadem Erfan MB, Soleimani F and Banafshi O (2019a). Increasing maternal age of blastocyst affects on efficient derivation and behavior of mouse embryonic stem cells. *J Cell Biochem* **120**, 3716–26.

Assadollahi V, Mohammadi E, Fathi F, Hassanzadeh K, Erfan MBK, Soleimani F, Banafshi O, Yosefi F and Allahvaisi O (2019b). Effects of cigarette smoke condensate on proliferation and pluripotency gene expression in mouse embryonic stem cells. *J Cell Biochem* **120**, 4071–80.

Berridge MS, Apana SM, Nagano KK, Berridge CE, Leisure GP and Boswell MV (2010). Smoking produces rapid rise of [^{11}C]nicotine in human brain. *Psychopharmacology* **209**, 383–94.

Calabrese EJ (2013). Hormetic mechanisms. *Crit Rev Toxicol* **43**, 580–606.

Calabrese EJ and Baldwin LA (2003). Hormesis: the dose–response revolution. *Ann Rev Pharmacol Toxicol* **43**, 175–97.

Cömertpay G, Lüleyap Ü, Yılmaz MB, Tufan T and Pazarcı P (2018). Investigation of the effects of nicotine and resveratrol on expression levels of *Sox2* and *Sox4* genes in human amniotic cell culture by real time PCR. *Cukurova Med J* **43**, 7–12.

Csiszar A, Labinskyy N, Podlutsky A, Kaminski PM, Wolin MS, Zhang C, Mukhopadhyay P, Pacher P, Hu F, de Cabo R, Ballabh P and Ungvari Z (2008). Vasoprotective effects of resveratrol and SIRT1: attenuation of cigarette smoke-induced oxidative stress and proinflammatory phenotypic alterations. *Am J Physiol. Heart Circ Physiol* **294**, H2721–35.

Gialitakis M, Tolaini M, Li Y, Pardo M, Yu L, Toribio A, Choudhary JS, Niakan K, Papayannopoulos V and Stockinger B (2017). Activation of the aryl hydrocarbon receptor interferes with early embryonic development. *Stem Cell Rep* **9**, 1377–86.

Harrison KL, Breen TM and Hennessey JF (1990). The effect of patient smoking habit on the outcome of IVF and GIFT treatment. *Aust N Z J Obstet Gynaecol* **30**, 340–2.

Hassa H, Gurer F, Tanir HM, Kaya M, Gunduz NB, Sariboyaci AE and Bal C (2007). Effect of cigarette smoke and alpha-tocopherol (vitamin E) on fertilization, cleavage, and embryo development rates in mice: an experimental *in vitro* fertilization mice model study. *Eur J Obstet Gynecol Reprod Biol* **135**, 177–82.

Huang J, Okuka M, McLean M, Keefe DL and Liu L (2009). Effects of cigarette smoke on fertilization and embryo development *in vivo*. *Fertil Steril* **92**, 1456–65.

Hughes EG and Brennan BG (1996). Does cigarette smoking impair natural or assisted fecundity? *Fertil Steril* **66**, 679–89.

Kataoka MC, Carvalheira APP, Ferrari AP, Malta MB, de Barros Leite Carvalhaes MA and de Lima Parada CMG (2018). Smoking during pregnancy and harm reduction in birth weight: a cross-sectional study. *BMC Pregnancy Childbirth* **18**, 67.

Kim CW, Lee SM, Ko EB, Go RE, Jeung EB, Kim MS and Choi KC (2020). Inhibitory effects of cigarette smoke extracts on neural differentiation of mouse embryonic stem cells. *Reprod Toxicol* **95**, 75–85.

Kirby PJ, Waters PD, Delbridge M, Svartman M, Stewart AN, Nagai K and Graves JA (2002). Cloning and mapping of platypus SOX2 and SOX14: insights into SOX group B evolution. *Cytogenet Genome Res* **98**, 96–100.

Kopp JL, Ormsbee BD, Desler M and Rizzino A (2008). Small increases in the level of Sox2 trigger the differentiation of mouse embryonic stem cells. *Stem Cells* **26**, 903–11.

Lin S, Fonteno S, Weng JH and Talbot P (2010). Comparison of the toxicity of smoke from conventional and harm reduction cigarettes using human embryonic stem cells. *Toxicol Sci* **118**, 202–12.

https://doi.org/10.1017/S0967199421000460 Published online by Cambridge University Press

Lin S, Tran V and Talbot P (2009). Comparison of toxicity of smoke from traditional and harm-reduction cigarettes using mouse embryonic stem cells as a novel model for preimplantation development. *Hum Reprod* **24**, 386–97.

Liszewski W, Ritner C, Aurigui J, Wong SS, Hussain N, Krueger W, Oncken C and Bernstein HS (2012). Developmental effects of tobacco smoke exposure during human embryonic stem cell differentiation are mediated through the transforming growth factor-β superfamily member, Nodal. *Differ Res Biol Divers* **83**, 169–78.

Liu L, Czerwiec E and Keefe DL (2004). Effect of ploidy and parental genome composition on expression of Oct-4 protein in mouse embryos. *Gene Expr Patterns* **4**, 433–41.

Liu Y, Li GP, Sessions BR, Rickords LF, White KL and Bunch TD (2008). Nicotine induces multinuclear formation and causes aberrant embryonic development in bovine. *Mol Reprod Dev* **75**, 801–9.

Ness RB, Grisso JA, Hirschinger N, Markovic N, Shaw LM, Day NL and Kline J (1999). Cocaine and tobacco use and the risk of spontaneous abortion. *New Engl J Med* **340**, 333–9.

Niwa H (2001). Molecular mechanism to maintain stem cell renewal of E.S. cells. *Cell Struct Funct* **26**, 137–48.

Oboni JB, Marques-Vidal P, Bastardot F, Vollenweider P and Waeber G (2016). Impact of smoking on fertility and age of menopause: a population-based assessment. *BMJ Open* **6**, e012015.

Tscheudschilsuren G, Küchenhoff A, Klonisch T, Tetens F and Fischer B (1999). Induction of arylhydrocarbon receptor expression in embryoblast cells of rabbit preimplantation blastocysts upon degeneration of Rauber's polar trophoblast. *Toxicol Appl Pharmacol* **157**, 125–33.

Winter E, Wang J, Davies MJ and Norman R (2002). Early pregnancy loss following assisted reproductive technology treatment. *Hum Reprod* **17**, 3220–3.

Yu HS, Tam PP and Chan ST (1985). Effects of cadmium on preimplantation mouse embryos *in vitro* with special reference to their implantation capacity and subsequent development. *Teratology* **32**, 347–53.

Zdravkovic T, Genbacev O, LaRocque N, McMaster M and Fisher S (2008). Human embryonic stem cells as a model system for studying the effects of smoke exposure on the embryo. *Reprod Toxicol* **26**, 86–93.

Zenzes MT (2000). Smoking and reproduction: gene damage to human gametes and embryos. *Hum Reprod Update* **6**, 122–31.

Zhang H, Guo D, Wang L, Zhao Y, Cheng Y and Qiao Z (2005). Effect of nicotine on Oct-4 and Rex-1 expression of mouse embryonic stem cells. *Reprod Toxicol* **19**, 473–8.

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