High E2 affects blastocysts implantation directly

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Background and Aims: Although the direct effects of high E2 on the endometrium play a major role in the impairment of blastocyst implantation in previous studies, direct effects of high E2 on blastocysts were not well known. Our aim was to clarify the direct impact of high E2 on the blastocyst and its influence in the implantation and post-implantation embryo development.

Methods: The ICR virgin albino mice, male mice and pregnant mice (8 ± 2 weeks old) were used. Using in-vitro models of day-8 blastocyst culture, immune-fluorescent staining for ER receptor, blastocyst outgrowth assays, differential staining and TUNEL assay of blastocysts, and embryo transfer, we have investigated the main outcomes of E2 dose-response *in vitro* experiments and blastocyst transfer *in vivo* experiments.

Results: In our study, we identified the expression of ERα and ERβ at pre-implantation stages during embryo development by immunofluorescent staining. Using an *in vitro* model, we exposed the blastocysts to E2 (10^{-7} to 10^{-4} M) for 24 h followed by culturing for 8 days to find the severe inhibition of implantation and post-implantation development at the concentration of 10^{-4} M (Table 1) and the ability of embryonic outgrowth was suppressed too (Fig. 1). Besides, we observed that the proliferation of blastocyst was reduced and the apoptotic cells were increased following the exposure of high E2 (Fig. 2). Using an *in vivo* embryo transfer model, we also identified more resorption of post-implanted blastocysts and less implantation sites that had been treated with high E2(Fig. 3).

Table 1. 8-day development *in vitro* of mouse blastocysts exposed to estrogen at the blastocyst stage for 24 hours

Developmental stage	Control group	Estrogen group			
		10^{-7}M	10^{-6}M	10^{-5}M	10^{-4}M
Blastocysts	44	26	28	26	28
Hatched/implantated blastocysts (7-8)	93%	100%	100%	100%	57%***
Early egg cylinder stage (9-10)	55%	42%	54%	42%	0***
Late egg cylinder stage (11-13)	48%	27%*	29%	27%*	0***
Early somite stage (14-15)	27%	8%*	0**	4%*	0**

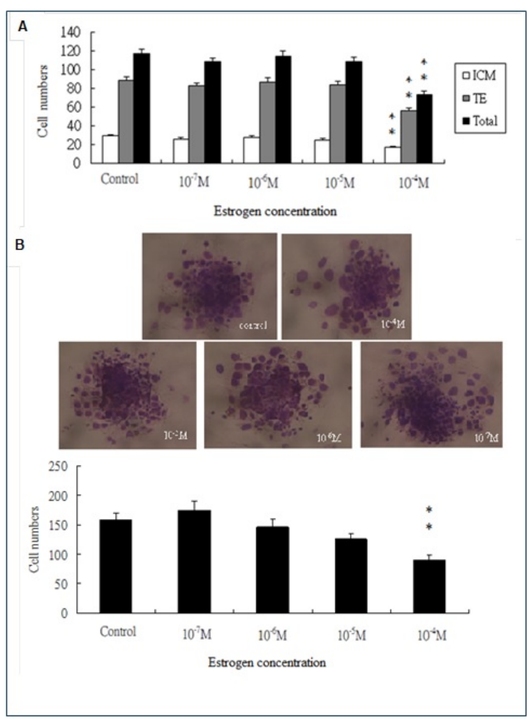

Fig. 1 (A) Cell proliferation of blastocysts by differential Staining; (B) Cell proliferation of outgrowth

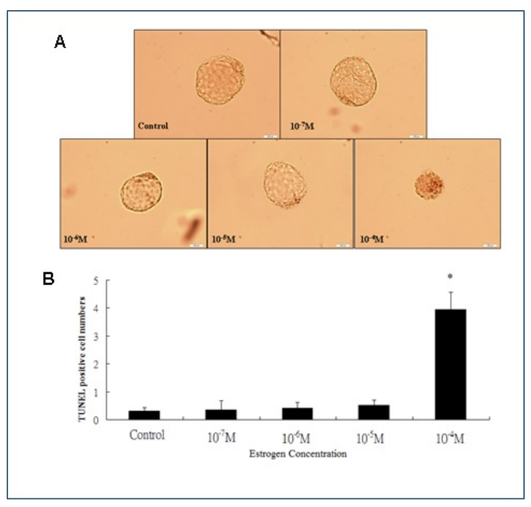

Fig. 2 Apoptotic effects of estrogen on 24-hour exposure at the blastocyst stage.

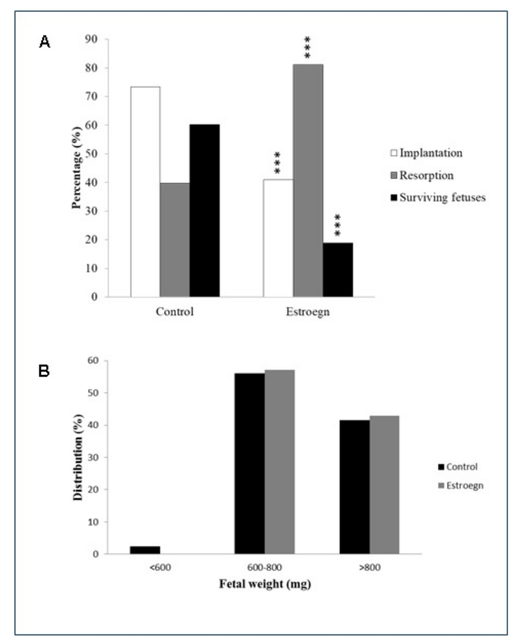

Fig. 2 Effects of estrogen exposure on embryo following transfer model.

CONCLUSION:

High E2 are deleterious to blastocyst implantation as well as early post-implantation development *in vitro*, mainly due to a direct adverse effect of E2 on the implanting blastocysts. It also highlights that high E2 levels in the course of controlled ovarian stimulation of assisted reproductive technologies not only directly impacts on the endometrium but also on the blastocysts clinically.

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