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Brief Communication

Effect of Prenatal Dexamethasone on Sex-specific Changes in Embryonic and Placental Growth

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To understand the effect of prenatal stress on sex-specific changes in embryonic and placental growth, a synthetic glucocorticoid (dexamethasone) was administered intraperitoneally at a dosage of 1 mg/kg body weight (BW) (Dex1) or 10 mg/kg BW (Dex10) to pregnant ICR mice at the gestational days 7.5, 8.5 and 9.5 post coitum (p.c.). Embryos and placentas were then harvested at days 11.5 and 18.5 p.c., and their body weight and size were measured following the determination of sex through PCR using *Sry* specific primers in tail tissues. As a result, female embryos presented reduced fetal body weight and size in Dex1- and Dex10-treated groups than those of control group at the embryonic day 11.5 p.c. Interestingly, the growth seems to be recovered at day 18.5 as there was no difference in growth between control and dexamethasone treated groups. In the case of males, Dex1 induced a decrease in fetal weight in day 11.5 and this pattern was maintained until day 18.5, whereas their growth was not affected by Dex10 treatment. Placental growth showed similar patterns to fetal growth in both sexes but the extent of reduction was not statistically significant in most cases. Placental weights in Dex1- and Dex10-treated group were decreased significantly in male only. The results imply that the effect of prenatal stress is largely sex dependent due to different strategies for growth and survival in a stressful environment.

Key Words: Dexamethasone, Prenatal stress, Sex bias; Embryonic development, Placental development

Glucocorticoid, which is a steroid hormone, is released by the adrenal gland through the activation of hypothalamic-pituitary-adrenal (HPA) axis in response to stress (Cottrell and Seckl, 2009; Reynolds, 2013). In normal condition, the placenta acts as a barrier between mother and fetus, protecting fetus by the action of enzyme, 11-beta-hydroxysteroid dehydrogenase (11β-HSD). 11β-HSD is secreted from placenta and converts cortisol, an active form of glucocorticoid, into inactive cortisone. However, excessive maternal cortisol can pass through placenta, leading to the activation of fetal HPA axis which is associated with intrauterine growth restriction (IUGR). Maternal exposure to glucocorticoid during pregnancy is also associated with long term adverse programmed consequences including cardiometabolic diseases and neurodevelopmental disorders in adult offspring. The placenta, which is an extra-embryonic tissue in between maternal and fetal compartment, plays a central role in supplying nutrients to fetus, eliminating wastes and preventing fetus from harmful substances (Lee et al., 2012; Rossant and Cross, 2001). Due to the important role of placenta during pregnancy, the normal development of the placenta is essential for fetal development and survival. The adverse effect of prenatal stress on fetal development is possibly mediated by placental responses.

Previous studies with animal models showed that prenatal stress resulted in a reduction of fetal body weight and placenta weight (Ain et al., 2005; Hewitt et al., 2006; Xu et al., 2011). However, sex specific differences in the effect of

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prenatal stress were not known. In many of previous prenatal stress related studies, the results were analyzed regardless of the gender of fetus. Because the sex differences are ignored, there is a possibility that the result from one masks that from the other sex. Thus, we examined the effect of prenatal exposure to glucocorticoids on sex-specific changes in embryonic and placental growth.

In our study, synthetic glucocorticoid dexamethasone was used since it is able to pass through the placenta affecting the fetus directly without being broken down by the enzyme 11β-HSD (Lee et al., 2012). Pregnant ICR mice were purchased from a pathogen-free laboratory

Fig. 1. Gender identification of embryos. Embryos were collected from 1 littermate (Dex1) at day 11.5 p.c. PCR was performed as written in the text. The bands at 451 bp represent the existence of *Sry* gene located on male-specific Y chromosome (M = male and F = female).

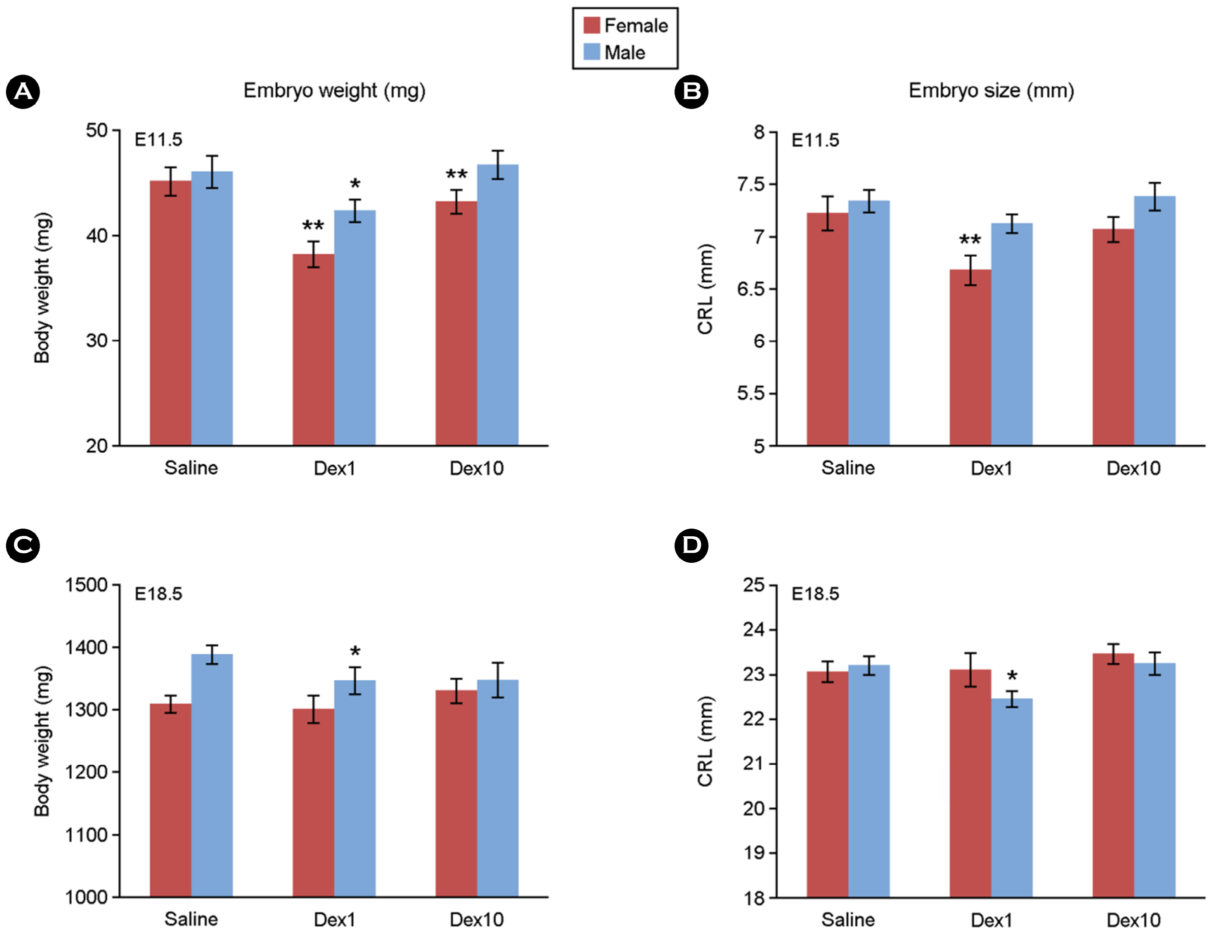

Fig. 2. Effect of prenatal exposure of dexamethasone on sex-specific changes in embryonic growth. Body weight and crown rump length (CRL) of saline, Dex1- and Dex10-treated embryos were measured at gestational days 11.5- (A and B) and 18.5 p.c. (C and D). *$P<0.05$, **$P<0.01$, ***$P<0.001$ unpaired T test comparing Saline and Dex1 or Saline and Dex10. Data are presented as mean ± SEM.

animal company (Orient Bio, Gyeonggi-do, Korea) and then dexamethasone (Sigma-Aldrich, St. Louis, MO) was injected intraperitoneally, with a dosage of 1 mg/kg body weight (BW) (Dex1) or 10 mg/kg (Dex10) for 3 consecutive days (day 7.5, 8.5, and 9.5 p.c.) using 9 mice per group. Equivalent amount of saline was injected to 9 pregnant mice as controls. 5 mice from Dex1 and Dex10 groups and 4 mice from saline treated group were sacrificed by cervical dislocation at day 11.5 p.c. to observe early effect and the remaining mice were sacrificed at day 18.5 p.c. to see later effect. The embryo and placentas were harvested in PBS separately and then their body weights and size, crown rump length (CRL), were measured. For gender identification, a piece of embryos (tail buds) was taken separately in 200 μl of lysis buffer (50 mM KCl, 10 mM Tris-Cl, pH 8.3, 2.5 mM $MgCl_2$, 0.1 mg/ml gelatin, 0.45% Tween-20, and 100 μg/ml Proteinase K) and incubated at 55℃ overnight,+ and boiled for 10 min to inactivate Proteinase K. After centrifugation at 12,000 rpm for 10 min, 1 μl of the lysate was used for PCR to detect Y-chromosome specific gene, using Sry (Sex determining Region Y) specific primers, 5'-CAG CCC TAC AGC CAC ATG AT-3' (sense) and 5'-GAG TAC AGG TGT GCA GCT CTA-3' (antisense) under the following conditions: 95℃ for 2 min, 40 cycles of 94℃ for 40s, 57˚C for 20s and 72℃ for 30s (Fig. 1).

At day 11.5 p.c., Dex1-treated embryos (both male and female) were weighed less compared with the control saline-treated embryos (P = 0.00008 for female and P = 0.0014 for male), whereas Dex10-treated group, the reduction was significant in female embryo only based on the *t*-test

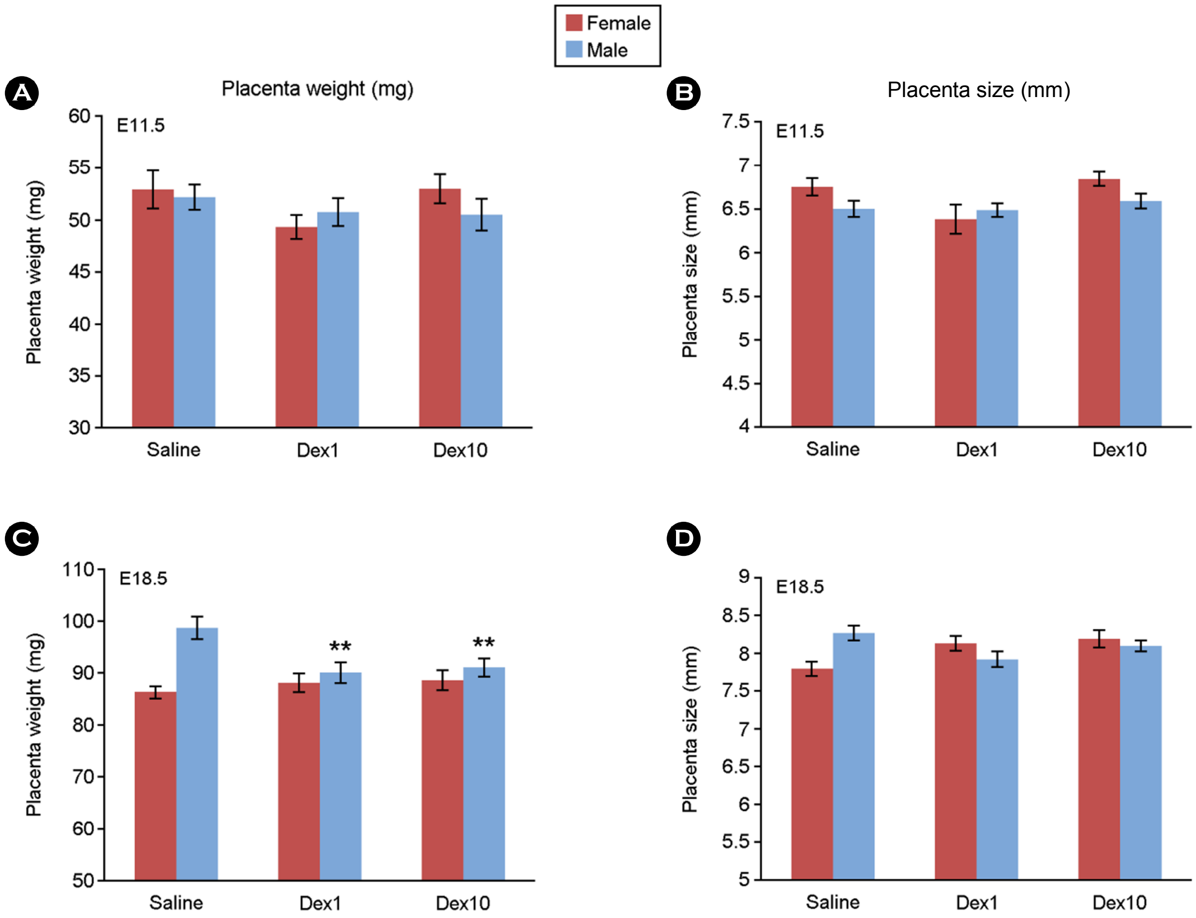

Fig. 3. Effect of prenatal exposure of dexamethasone on sex-specific changes in placental growth. Placental weight and size of saline, Dex1- and Dex10-treated placentas were measured at gestational days 11.5- (A and B) and 18.5 p.c. (C and D). *P<0.05, **P<0.01, ***P<0.001 unpaired T test comparing Saline and Dex1 or Saline and Dex10. Data are presented as mean ± SEM.

analysis ($P = 0.018$ for female; Fig. 2A). Body size was not remarkably affected by dexamethasone exposure except for Dex1-treated female group which was significantly smaller than saline treated controls ($P = 0.0056$; Fig. 2B). At day 18.5 p.c., female embryos treated with dexamethasone appear to recover their growth to normal range. On the other hand, Dex1-treated male embryos remain reduced in both weight and size compared with saline embryos ($P = 0.0381$ for weight and $P = 0.0159$ for size; Fig. 2C and D). In the case of placental growth, the effect of dexamethasone was similar to that in body growth even though the differences were minor (Fig. 3). Placental weights in Dex1 and Dex10 group decreased significantly in male placenta only ($P = 0.004$ for Dex1 and $P = 0.001$ for Dex10; Fig. 3C).

Interestingly, a similar result was reported as well. When dexamethasone 1 μg/kg/h was given for 60 h at day 12.5 p.c., fetal and placental weight decreased temporarily at day 14.5 p.c. and recovered at day 17.5 p.c. in female only (Cuffe et al., 2011). Male fetus and placenta were unaffected by dexamethasone. We could also observe the recovery of fetal and placenta growth at late developmental stage after a temporary restriction at day 11.5 p.c. in female. In contrast, male was less affected than female at day 11.5 p.c. and this pattern was retained until day 18.5 p.c.

Generally, it is widely known that male sex is a risk factor for adverse pregnancy outcome, especially in poor intrauterine environment. A high male-to-female ratio was found in fetal and neonatal morbidity and mortality (Di Renzo et al., 2007). Many of neuropsychiatric diseases associated with prenatal stress also exhibit a sex bias (Bale, 2011). As an example, male offspring from stressed mother during pregnancy have an increased risk of schizophrenia upon adulthood (van Os and Selten, 1998). A recent study reported that postnatal anxiety for offspring can be developed due to maternal depression during pregnancy, particularly in males (Gerardin et al., 2011). In addition, a sex ratio of autism spectrum disease (ASD) is 4.3:1 for male-to-female and a previous study suggested that exposure to maternal stress is a potential contributing factor to ASD (Beversdorf et al., 2005). Furthermore, the expression pattern of placental genes varied depending upon fetal sex (Cuffe et al., 2011). The collection of these studies infer that male and female have a different mechanism under the stress condition, consequently incidence of disorder is different between male and female.

As mentioned above, prenatal exposure to glucocorticoids is known to cause the reduction of fetal body weight and placenta weight (Ain et al., 2005; Hewitt et al., 2006; Xu et al., 2011; Kim et al., 2011; Lee et al., 2012). In this study, we analyzed the result by the gender of fetus to examine sex-specific differences in fetal and placental growth. As most of previous studies ignored gender differences, our results are valuable resources for further investigation to find a mechanism conferring sex difference.

In conclusion, various stress associated disorders are known to be sex bias. Male and female have different strategies of growth and survival in a stressed condition and the difference is visible from the stage of fetal development. Further study to reveal the origin and mechanism that confer sex-specific effect is required.

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REFERENCES

Ain R, Canham LN, Soares MJ. Dexamethasone-induced intrauterine growth restriction impacts the placental prolactin family, insulin-like growth factor-II and the Akt signaling pathway. J Endocrinol. 2005. 185: 253-263.

Bale TL. Sex differences in prenatal epigenetic programming of stress pathways. Stress. 2011. 14: 348-356.

Beversdorf DQ, Manning SE, Hillier A, Anderson SL, Nordgren RE, Walters SE, Nagaraja HN, Cooley WC, Gaelic SE, Bauman ML. Timing of prenatal stressors and autism. J Autism Dev Disord. 2005. 35: 471-478.

Cottrell EC, Seckl JR. Prenatal stress, glucocorticoids and the programming of adult disease. Front Behav Neurosci. 2009. 3: 19.

Cuffe JS, Dickinson H, Simmons DG, Moritz KM. Sex specific changes in placental growth and MAPK following short term maternal dexamethasone exposure in the mouse. Placenta. 2011. 32: 981-989.

Di Renzo GC, Rosati A, Sarti RD, Cruciani L, Cutuli AM. Does fetal sex affect pregnancy outcome? Gend Med. 2007. 4: 19-30.

Gerardin P, Wendland J, Bodeau N, Galin A, Bialobos S, Tordjman S, Mazet P, Darbois Y, Nizard J, Dommergues M, Cohen D. Depression during pregnancy: is the developmental impact earlier in boys? A prospective case-control study. J Clin Psychiatry. 2011. 72: 378-387.

Hewitt DP, Mark PJ, Waddell BJ. Glucocorticoids prevent the normal increase in placental vascular endothelial growth factor expression and placental vascularity during late pregnancy in the rat. Endocrinology. 2006. 147: 5568-5574.

Kim SH, Lee J-Y, Park SJ, Kim MH. Effects of dexamethasone on embryo development and Hox gene expression patterns in mice. J Exp Biomed Sci. 2011. 17: 231-238.

Lee JY, Park SJ, Kim SH, Kim MH. Prenatal administration of dexamethasone during early pregnancy negatively affects placental development and function in mice. J Anim Sci. 2012. 90: 4846-4856.

Reynolds RM. Glucocorticoid excess and the developmental origins of disease: two decades of testing the hypothesis--2012 Curt Richter Award Winner. Psychoneuroendocrinology. 2013. 38: 1-11.

Rossant J, Cross JC. Placental development: lessons from mouse mutants. Nat Rev Genet. 2001. 2: 538-548.

van Os J, Selten JP. Prenatal exposure to maternal stress and subsequent schizophrenia. The May 1940 invasion of The Netherlands. Br J Psychiatry. 1998. 172: 324-326.

Xu D, Chen M, Pan XL, Xia LP, Wang H. Dexamethasone induces fetal developmental toxicity through affecting the placental glucocorticoid barrier and depressing fetal adrenal function. Environ Toxicol Pharmacol. 2011. 32: 356-363.