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Preeclampsia-like symptoms induced in mice by feto-placental expression of STOX1 are reversed by aspirin treatment

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1 **Preeclampsia-like symptoms induced in mice by fetoplacental**
 2 **expression of STOX1 are reversed by aspirin treatment.**

3 *STOX1 mice develop preeclampsia symptoms*

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30 **Abstract**

31 Preeclampsia is a common human-specific pregnancy disorder defined by hypertension and
32 proteinuria during gestation and responsible for maternal and foetal morbi-mortality. *STOX1*, encoding
33 a transcription factor, was the first gene identified by positional cloning approaches as associated with
34 preeclampsia. Its overexpression in choriocarcinoma cells mimics the transcriptional consequences of
35 preeclampsia in the human placenta. Here we created transgenic mouse strains overexpressing human
36 *STOX1*. Wild-type female mice crossed with transgenic *STOX1*-males reproduce accurately the
37 symptoms of severe preeclampsia: gestational hypertension, proteinuria, and elevated plasma level of
38 sFlt1 and sEng. Placental and kidney histology were altered. All the symptoms are prevented or
39 alleviated by aspirin treatment. *STOX1*-overexpressing mice constitute a unique model for studying
40 preeclampsia, will allow testing therapeutic approaches, and assessing the long term effects of the
41 preeclamptic syndrome.

42 **Keywords:** hypertension, mouse model, preeclampsia, proteinuria, *STOX1*,

43

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44 Preeclampsia (PE) is a pervasive complication of human pregnancy characterized by a
45 gestational hypertension associated with proteinuria occurring from mid-gestation ¹. Worldwide, this
46 syndrome affects ~5% of pregnant women, and is a leading cause of maternal mortality, especially in
47 low and middle income countries ^{2,3}; it is also a direct cause of iatrogenic prematurity⁴, since the only
48 definitive therapeutic act is the extraction of the foeto-placental unit .

49 *STOX1* is a transcription factor belonging to the enlarged Forkhead Box gene family, that has
50 been associated with preeclampsia ⁵, able to modulate trophoblast proliferation and migration ⁶.
51 *STOX1* is maternally expressed in a specific cell type of the placenta, column extravillous
52 trophoblasts ⁷. Overexpression of *STOX1* in human choriocarcinoma cells induces transcription
53 alterations that mimic those of preeclamptic placentas^{8,9}. Founds and coworkers showed in a
54 transcriptomic analysis that *STOX1* is overexpressed (x 2.1, p = 0.013) during the first trimester in
55 pregnancies that will have a preeclamptic outcome ¹⁰.

56 In the present work, we generated mice overexpressing human *STOX1*. Wild-type female mice
57 crossed with transgenic males overexpressing *STOX1*, have a severe gestational hypertension,
58 proteinuria, an increased plasma level of soluble antiangiogenic factors as well as kidney and placenta
59 histological alterations. We also demonstrate a beneficial effect of low-dose aspirin treatment on the
60 maternal symptoms. Hence, *STOX1* overexpressing mice constitute a unique model for studying
61 severe preeclampsia.

62

63

Methods

64 Some more details of the methods are provided as supplemental data.

65 **Animals and Transgenesis and aspirin treatment**

66 The complete ORF of the human *STOX1* (A isoform) cDNA was used to generate transgenic
67 animals ¹¹. The number of copies of the transgene was assessed by qPCR from the mouse DNA
68 relative to a single copy gene. Transmission analysis through generations showed on the three strains
69 that were analyzed that there was a single locus of insertion.

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70 Animals (FVB/N) were bred in the animal facility of INRA (Jouy en Josas, France) in a
71 controlled environment (light/dark cycle, temperature, free access to food and water). Blood
72 collection, urine collection, blood pressure measures and kidney perfusion were performed according
73 to standardized protocols, as well as supplementation of aspirin in the drinking water (See
74 supplemental methods)

75 **RNA extraction and Quantitative RT-PCR conditions**

76 Total RNA from collected tissues was extracted and quantified by spectrophotometry. Quality
77 of the RNA was systematically verified by Bioanalyzer and RIN consistently > 8 . After reverse
78 transcription, qRT-PCR was performed. The efficiency of the PCR was checked for every primer
79 couple and estimated (Supplemental Methods). The Ct values were normalized by the Ct values obtained for
80 the murine succinate dehydrogenase subunit A (*Sdha*) used as a reporter gene and shown previously to
81 be stable and highly expressed in the placenta¹².

82 All primers are presented as [Supplemental Table 1](#).

83 **Blood pressure measurements**

84 Blood pressure was measured repeatedly in the tail artery in mice before, during and after pregnancy
85 (for a total of around 35 consecutive days). Measurements were performed using a computerized, non-
86 invasive tail-cuff plethysmography method (Letica 5001, Bioseb), using thermostatically warmed
87 restrainers designed for mice and adapted to the size of the animal. This system uses volume-pressure
88 recording technology to detect changes in tail volume that corresponds to systolic and diastolic
89 pressures during each measurement cycle. Unanesthetized mice previously accustomed during one
90 week to the manipulation (systolic blood pressure stabilized), were placed in plastic holders and our
91 protocol consisted of at least 5 acceptable measurements daily, which means without detectable
92 movement of the mice. Thirty-six gestations were followed, which represents more than 5000
93 independent measurements of blood pressure by two independent researchers (systolic, diastolic and
94 median always displayed a highly similar profile). In addition, invasive measures were performed on
95 14 WT mice mated with WT or transgenic males at the end of gestation (14.5 to 17,5 dpc). The

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96 catheter was inserted into the left common carotid artery under xylazine/ketamine anesthesia. Mice
97 were kept on a heating pad during the experimentation. Blood Pressure was recorded and analyzed
98 from continuous measurements using BIOPAC MP36 system. The extraction of the Systolic Blood
99 Pressure (SBP) started when cardiovascular parameters were stable.

100 **Assessment of Albumin/creatinine ratio (ACR)**

101 Urine samples were collected daily during gestation and were pooled at early (1-5 dpc),
102 middle (9-12 dpc) and late (15-18 dpc) gestation times separately for each mice. Albumin-to-
103 creatinine ratio was measured in these urine specimens using the kit ELISA of Exocell (Philadelphia,
104 PA).

105 **sFlt1 and sEng plasmatic concentration assessment**

106 Enzyme-linked immunosorbent assays were used to determine the concentration of sFlt1 and
107 sEng in mouse plasma with commercial kits (R&D Systems).

108 **Histological analysis**

109 Placentas and kidneys were sampled from 10 transgenic and WT mice (after heart perfusion
110 by 20 ml of PBS in the case of the kidneys). Anti-fibrin immunostaining of slides from paraffin-
111 embedded kidneys was performed using the anti-human fibrinogen at 1/400 from Dako (A0080) and
112 the LSAB revelation system (Dako, K0679) according to the manufacturer's instructions (standard
113 conditions). Placental histology was carried out after HES or PAS staining. The number of vacuolated
114 foci present in the labyrinth was counted after HE staining, a 1-5 grade was given according to the
115 level of PAS intensity, and the PAS positive areas were measured in the labyrinth.

116 **Statistical analysis**

117 During this study, parametric approaches were used using ANOVA followed by post-hoc
118 Dunnett T-tests for comparison with the controls. The StatistXL add-in of Excel™ was systematically
119 used. P-values below 0.05 were considered significant.

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121

Results**1. Generation of STOX1 transgenic mice**

122 Three independent transgenic lines (called TgSTOX13, TgSTOX34 and TgSTOX42) were
 123 generated by introducing the human STOX1A (complete isoform) under the control of a ubiquitously
 124 expressed promoter (the cytomegalovirus – CMV- promoter). TgSTOX13 and TgSTOX42 strains
 125 contain a unique copy that was transmitted in a Mendelian fashion. As the expression level of
 126 TgSTOX34 was very similar to that of TgSTOX13, we focused our attention on TgSTOX13 and
 127 TgSTOX42 strains.

129 The expression levels of human and mouse *STOX1* were tested by qRT-PCR in the kidney,
 130 liver, brain and placenta of pregnant TgSTOX13 and TgSTOX42 females, as well as in the testis and
 131 spermatozoa of transgenic males of both strains, using species-specific primers (Table 1); *hSTOX1*
 132 expression was comparatively higher in placental tissue than in any other tissue or in spermatozoa.
 133 qRT-PCR efficiency was maximal for mouse and human STOX, allowing to suggest that the placental
 134 expression level of the human *hSTOX1* was roughly similar to that of *mStox1* in TgSTOX13
 135 transgenic mice (Table 1) and about 13 fold higher in TgSTOX42. In the other tissues tested (kidney,
 136 brain, testis and liver) the transgene expression level was lower than that of the endogenous gene, but
 137 always higher in TgSTOX42 than in TgSTOX13 (Table 1). Since there is a unique transgene insertion
 138 in both strains (as tested by quantitative PCR), we assume that the difference in the expression level
 139 was strictly regulated by the genomic position of this insertion. In transgenic placentas from
 140 WTxTgSTOX42 crosses, where the transgene expression is the highest, the endogenous gene was
 141 overexpressed 4 fold ($p = 2.10^{-11}$ by Student test compared to WTxWT crosses).

142 **2. STOX1 transgenic mice develop a strong gestational hypertension, proteinuria, and**
 143 **present an increased level of circulating sFlt1 and sEng.**

144 Wild type females were crossed with males of three genotypes (WT, TgSTOX13 or
 145 TgSTOX42) to ensure that the transgene expression be restricted to the foeto-placental unit. Blood

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146 pressure was monitored daily in the various crosses (Figure 1A). Increase in blood pressure during
 147 gestation was observed in all the cases of crosses involving transgenic males. The blood pressure
 148 curves reached a plateau in both strains around E10.5, until days 14.5-15.5, when the blood pressure
 149 tends to increase again of an additional 15-20 mm Hg. The maximal increase reached about 80 mm Hg
 150 from a normal pressure of ~116 mm Hg (Figure 1A). Blood pressure returned to normal rapidly (in
 151 about 48 hours) after parturition. In addition, we carried out measurements by catheterization of one
 152 of the carotid at late gestation times, and consistently with human blood pressure data under anesthesia
 153 ¹³, the increase in mice carrying transgenic fetuses was present, albeit reduced; the blood pressure was
 154 12 mmHg more elevated in the ‘transgenic’ crosses (p=0.008, Supplementary Figure 1).

155 Proteinuria was evaluated as a ratio between albumin and creatinin (ACR) (Figure 1B). After
 156 individual normalization, we observed an increase of the ACR comprised between ~1.7 to 1.9 at mid
 157 gestation (p=0.02 and 0.03 for TgSTOX13 and tgSTOX42, respectively) and between ~1.8 to 3.8 fold
 158 at late gestation points (p=0.026 and 0.002, respectively), in females crossed with transgenic males
 159 compared to control crosses.

160 sFlt1, considered as a biomarker for preeclampsia in the human disease ¹⁴⁻¹⁶ was monitored
 161 (Figure 2A). The average concentration in the plasma of pregnant mice was 2075 pg/ml when
 162 TgSTOX13 males have fathered and 2455 pg/ml when crossed with TgSTOX42 males, an increase of
 163 1.9 and 2.3 fold compared to control (1076 pg/ml), respectively (p = 0.02 and 0.03). Soluble endoglin
 164 (sEng) was also measured in the plasma (Figure 2B), and found increased in the crosses involving
 165 TgSTOX13 (x2.0, p=0.04) and TgSTOX42 males (x2.3, p=0.01), compared to crosses with WT males.

166 **3. Wild-type females carrying transgenic foetuses have kidney anomalies that are** 167 **characteristic of preeclampsia**

168 In the kidneys of females crossed with TgSTOX13 mice, three glomerular changes were
 169 observed: first, a glomerular tuft swelling with expansion of the capillary wall, up to the point that the
 170 tuft could occasionally make contact with the Bowman’s capsule; second, we observed, a slight
 171 increase in the glomerular cellularity (42.3 ± 10.1 in TgSTOX13 and 48.1 ± 11.9 in TgSTOX42 versus

172 34.0 ± 11.8 in WT, Figure 3A, $p = 3,4 \cdot 10^{-3}$ and $p = 6.4 \cdot 10^{-7}$ respectively), because of a mild mesangial
173 cell proliferation. Immunohistochemistry revealed subendothelial fibrin deposition, with a
174 predominantly peripheral pattern. These changes are strongly reminiscent of the renal pathology
175 observed in human preeclampsia¹⁷⁻¹⁹ and could not be observed in the kidneys of WT pregnant mice
176 crossed with WT males (Figure 3B upper and middle photographs). No major abnormality was found
177 in the tubulo-interstitial compartment, or in the larger vessels, in either group.

178 **4. Aspirin treatment efficiently improves the maternal phenotype.**

179 WT females crossed with TgSTOX13 or TgSTOX42 males were treated with aspirin at low
180 doses in the drinking water during the whole gestation. As a control, WT pregnant females received
181 aspirin all along their pregnancy after a cross with WT males. Aspirin completely prevented the onset
182 of hypertension and brought the ACR to normal values at mid and late gestation times (Figures 1A and
183 1B). Fibrin immunolabeling of the kidneys showed that the deposit was considerably reduced in
184 aspirin-treated wild type females crossed with TgSTOX13 or TgSTOX42 males, compared with non
185 treated females (Figure 3B).

186 **5. STOX1 transgenic placentas present structural alterations.**

187 The anatomical alterations of hemizygous TgSTOX13 and TgSTOX42 (validated as
188 transgenic by PCR) placentas were studied by histology (Figure 4A). No difference was observed in
189 gross morphology at 16.5 dpc, such as in the ratio between the labyrinth and the junctional zone.
190 Because deregulated imprinted genes expression is known to alter glycogen trophoblasts location, we
191 counted the number of vacuolated foci, graded the PAS staining, specific of glycoproteins, and
192 measured the surface of PAS area in the labyrinth. While in hemizygous TgSTOX13 placentas, no
193 significant difference was detected as compared to measures in wild-type placentas, in hemizygous
194 TgSTOX42 placentas, significant diminution of vacuolated foci in the labyrinth as well as PAS grade,
195 and PAS area (Figures 4B-D) was consistently observed.

196 **6. Litter size is affected by the transgene**

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197 Litter size appeared linked to the genotypes of the males used in the crosses (Supplemental
198 Figure 2, $p=0.0016$ by one-factor ANOVA), with $8.4 (\pm 2.0)$ pups on average in WT crosses. In
199 crosses with TgSTOX13 males, the litter size was reduced to $6.9 (\pm 3.4, p = 0.048)$. Consistent with
200 the higher transgene expression in TgSTOX42, the litter size was reduced to $6.0 (\pm 2.6, p = 0.001)$ in
201 crosses involving a TgSTOX42 male. When aspirin was given to the females during gestation, litter
202 size was $7.8 (\pm 2.4)$ and $9.3 (\pm 3.1)$ in crosses involving TgSTOX13 males TgSTOX42 males,
203 respectively, and in the later case, the difference was significantly different from non treated crosses
204 ($p=0.022$), showing that the drug was able to correct the 'reduced litter size' phenotype.

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208

Discussion

209 Herein, we show that mice overexpressing STOX1 in the embryo-foeto-placental unit
210 reproduce *in extenso* and without further manipulation the major features of preeclampsia: proteinuria
211 and hypertension. A peculiarity of the present mouse model of preeclampsia is the intensity of the
212 gestational hypertension, which reaches values corresponding to severe human preeclampsia at the end
213 of gestation. The lower difference in blood pressure observed under anesthesia compared to vigil
214 animal may presumably be explained by a relaxing effect of the anesthesia, moderating as well the
215 blood pressure level. We also observed an elevation of both sFlt1 and sEng, key anti-angiogenic
216 factors of preeclampsia. The mice also harbored PE-specific kidney alterations.

217 In summary, a unique transcription factor (STOX1) expressed in the foeto-placental unit is
218 sufficient to trigger preeclampsia-like symptoms in WT females, in a dose-dependent manner
219 (illustrated by the more or less severe phenotype observed in crosses involving TgSTOX13 or
220 TgSTOX42 males). While the involvement of STOX1 in human preeclampsia was debated after its
221 discovery²⁰, the present study confirms a growing corpus of evidence indicating an actual contribution
222 of STOX1 to the preeclamptic phenotype.

223 Rodent models of preeclampsia have been considered very important for studying
224 preeclampsia pathophysiological mechanisms. Various models of reduced uterine perfusion were
225 generated by inducing placental ischaemia, administration of angiotensin antagonists, pro-
226 inflammatory drugs or vasoconstrictive agents, or by using the BPH/5 hypertensive mouse strain²¹,
227 and crosses between the CBA X DBA strains²². Gene manipulations have also been used such as gene
228 invalidation experiments for *p57Kip2*, *Comt*, or *Corin*^{23,24}, additive transgenesis of anti-angiogenic
229 factors such as *s-flt1*²⁵, or transgenesis leading to manipulating the renin/angiotensin system²⁶. In the
230 handful of existing mouse models of preeclampsia, gestational hypertension is mild²⁷, probably far
231 below the one of our model and far below what is observed in severe preeclampsia in women.
232 Moreover, we observed that the principal maternal symptom, gestational hypertension, appears very
233 early, from 3.5 days post coïtum. In humans, it has been shown that preeclampsia can exceptionally

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234 occur very early with a complete phenotypic profile before 15 weeks of gestation²⁸ while in general
235 the symptoms are reported to appear from mid gestation (21-22 weeks) at the earliest. The occurrence
236 of the maternal hypertension at the very beginning of gestation in our model suggests that soluble
237 substances produced by the blastocyst, the embryo or even from the ejaculate may initiate the maternal
238 preeclamptic phenotype. It is interesting to compare hypertension curves obtained here with those of
239 most studies (such as^{24, 25}) that show a raise during the last 5 days of gestation or so. This increase is
240 also visible in our model, but is superimposed on a highly elevated blood pressure triggered by
241 gestation. In sum our model cumulates presumably the effects of an early message from the embryo-
242 foeto-placental unit to the mother systems controlling blood pressure (endothelial system and kidney),
243 and the effect of an increased placental/foetal demand occurring at the end of gestation, when the
244 rhythm of weight gain is the highest for the foetuses, this effect being the one reproduced in most
245 mouse models of preeclampsia.

246 Under anesthesia the difference in blood pressure measurement between the control pregnant
247 mice and the one crossed with transgenic males was present, albeit less pronounced than on vigil
248 animals. This is completely consistent with recent human data showing that under anesthesia, there is
249 a steep drop in maternal blood pressure but much more elevated in preeclamptic women than
250 normotensive pregnant women (-10% in normotensive versus -30% in preeclamptic women)¹³. This
251 suggests that blood pressure variations in our model are very close to what is observed in human
252 clinics.

253 To the best of our knowledge, we demonstrate here for the first time in a mouse model, that
254 aspirin treatment at low doses administered in drinking water from the beginning of gestation is
255 efficient for preventing the maternal syndrome. In humans, it appears that a major issue is the timing
256 of aspirin administration: after 16 weeks of gestation it has no effect at all²⁹. A consensus tends
257 nevertheless to emerge, with the idea that aspirin is quite efficient when administered early (before the
258 end of the first trimester of pregnancy)²⁹⁻³³. Aspirin at low doses may change the hemodynamic
259 properties of the blood thus preventing blood clotting, an issue that is sometimes associated with
260 Disseminated Intravascular Coagulation in severe preeclampsia with HELLP syndrome³⁴.

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261 Unexpectedly, mild anomalies of mature placenta (decrease of glycogen trophoblasts surface)
262 was only observed in crosses with TgSTOX42 males. Although we did not perform earlier histological
263 examination of developing placenta, focusing on spiraled arteries invasion for instance, the
264 observation that hypertension occurs before the formation of a placenta indicates that the mechanisms
265 underlying the observed phenotype is not only due to defaults in placentation. This opens the
266 possibility that different causes of preeclampsia may explain the grade of severity of preeclampsia
267 symptoms in Humans as well as the early or late onset of the disease.

268 Imprinted genes and among them the paradigmatic *Igf2* are known to have a crucial role in
269 placental and foetal growth³⁵. In particular, the KO of *Igf2* leads to non-development of glycogen
270 cells³⁶. The observed defaults in glycogen trophoblasts in hemizygous TgSTOX42 placenta may be
271 related to the fact that STOX1 is an imprinted gene in humans and could thus regulate the other
272 members of the imprinted gene networks³⁷.

273

Perspectives

274 The mouse model presented here can help the understanding severe preeclampsia, for which
275 until now, very few experimental alternatives to the direct study of the patients are available. These
276 mice provide the community working on preeclampsia with a tool applicable for cognitive
277 (understanding preeclampsia pathogenesis) as well as applied (drug screening) usage, and even
278 potentially, deciphering how these drugs act.

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290 **Conflict of Interest**

291 None declared

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401 **Novelty and Significance:**

402 **1) What Is New?**

403 The first mouse model of severe preeclampsia

404 Strong clues to prove a role of STOX1 in preeclampsia

405 **2) What Is Relevant?**

406 Aspirin treatment is efficient to revert the syndrome

407 The mice are easy to breed and can be distributed to the researchers interested in

408 preeclampsia or more generally in hypertension

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411 **Figure Legends**412 **Figure 1: Hypertension, elevated Albumin-Creatinin ratio in WT females crossed with STOX1**413 **transgenic males. A)** Systolic arterial pressure is represented for pregnant mice after the indicated

414 crosses, treated or not with aspirin. The values were obtained daily and the curves were smoothed

415 on three consecutive days. Mice overexpressing STOX1 in the foeto-placental units display a strong

416 gestational hypertension, increased by up to 80 mm Hg in the TgSTOX42 strain. Two or three days

417 post-partum the blood pressure goes back to normal. **B)** Albumin/Creatinin ratio (ACR) in the various

418 crosses with (ASP) or without aspirin treatment. The values were normalized for each mouse to the

419 ACR value computed at the beginning of gestation; the value obtained for WT mice was taken as a

420 reference and set to 1. The ACR of females crossed with transgenic males were significantly different

421 from the ACR of females crossed with WT at middle and late gestation times, while aspirin treatment

422 brought back the ACR to values not significantly different from those of controls * refer to significant

423 comparisons with the control after ANOVA and Dunnett test.

424 **Figure 2: Increased plasmatic sFlt1 and sEng concentrations in WT females crossed with**425 **STOX1 transgenic males**

426 sFlt1 (A) and sEng (B) concentrations were elevated in the plasma of pregnant mice (16.5 dpc)

427 carrying transgenic fetuses compared to those carrying WT. Hm refers to homozygous transgenic and

428 ht refers to hemizygous transgenic animals. Statistical analysis was performed by ANOVA followed

429 post-hoc by a Dunnett test (* for $p < 0.05$).430 **Figure 3: Histological kidney alterations in WT females crossed with transgenic males at 16.5**431 **days post coïtum (dpc).** Renal histology of WT females crossed with WT, TgSTOX13 and432 TgSTOX42 males (HES coloration, scale bar = 100 μm); **A)** The glomerular size and the number of

433 cells were significantly augmented in the glomeruli of the mothers (WT) when the foetuses/placentas

434 were transgenic. We also observed a reduction of the endothelial fenestrae (** for $p < 0.01$, see text).435 **B)** Anti-fibrin immunohistochemistry revealed a strong labeling (in brown) of the glomeruli from

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436 females crossed with transgenic animals, while those from females crossed with WT males were
437 completely free of this labeling. TgSTOX42 kidneys were overall more strongly labeled. Under
438 aspirin treatment (+ASP), the intensity of the labeling was dramatically reduced.

439 **Figure 4: Reduced vacuolated foci, PAS staining, and PAS area in hemizygous TgSTOX13**
440 **placentas at 16.5 dpc.** Indexes of glycogen trophoblast mislocation were measured in the labyrinthic
441 zone using vacuolated aspects of the foci under HE coloration and PAS staining (A, see Methods).
442 Several placentas from different crosses were used: 6 placentas from 3 different crosses for ♀WT x
443 ♂WT, ♀ WT x ♂ ht TgSTOX13; and ♀ WT x ♂ ht TgSTOX42. Consistently, numeration of
444 vacuolated foci (B), PAS grade (C) and PAS area (D) were significantly decreased in the labyrinth in
445 hemizygous TgSTOX42 placentas.

446

447 **Table 1**

448

Expression of the endogenous *Stox1* gene and *STOX1* transgene in mice in various tissues

	placenta	kidney	Liver	brain	testis	spermatozoa
WTxWT (endogenous <i>Stox1</i> , arbitrary units)	3000 ± 57	178 ± 4.3	92 ± 2.1	1646 ± 96	882 ± 19	358 ± 14
TgSTOX13 *	2400 ± 90	1.0 ± 0.05	4.2 ± 0.15	1.9 ± 0.04	1646 ± 140	66 ± 4,7
TgSTOX42 *	38088 ± 5540	12 ± 4.6	38.2 ± 11	1384 ± 100	7304 ± 2066	3755 ± 1195

449 *arbitrary units, the kidney expression in TgSTOX13 mice was set to 1 and served as a reference for
 450 the expression of the transgene. The values of the endogenous are corrected by the Ct values of the
 451 reporter gene (SDHA), giving a rough indication of the relative expression of the endogenous
 452 compared to the transgene.

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