

# SEVERE HYPERTENSION DURING RAT PREGNANCY ALTERS PLACENTAL DEVELOPMENT AND FETAL GROWTH



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## Background and aims

Arterial hypertension (HT) affects 1-5% of pregnant women worldwide, with a rising prevalence due to increasing maternal age, obesity, and type 2 diabetes. Pregnancies complicated by HT increase the risk of superimposed preeclampsia, fetal growth restriction (FGR), preterm delivery, and perinatal death. So far, there is scarce information available on placental development and function in severe HT animal models such as the Stroke-Prone Spontaneously Hypertensive Rat (SHR-SP) strain. Thus, the present study was conducted in order to characterize pregnancy in the SHR-SP focusing on the impact of blood pressure changes on placental development and fetal growth.

# Experimental design and methods

SHR-SP and Wistar Kyoto (WKY) females (10-12 weeks old, N= 5animals/day analysed) were mated to congenic males and checked daily for vaginal plugs, denoted as gestation day (GD) 1. Blood pressure (BP) was determined by the tail-cuff method pre-mating and on the morning of GD1, 7, 9, 13, 17 and 19. On the selected dates, females were housed in metabolic cages for collection of 24 h urine samples. Following collection of blood samples, animals were euthanized and implantation sites isolated for morphological analyses. Fetal and placental weights were recorded on GD18 and GD20. Morphological analyses of placental development were performed in hematoxilin-eosin and Masson's Trichrome stained sections from GD14 and GD20.

# **Results**

# Breeding Efficiency is impaired in SHR-SP matings

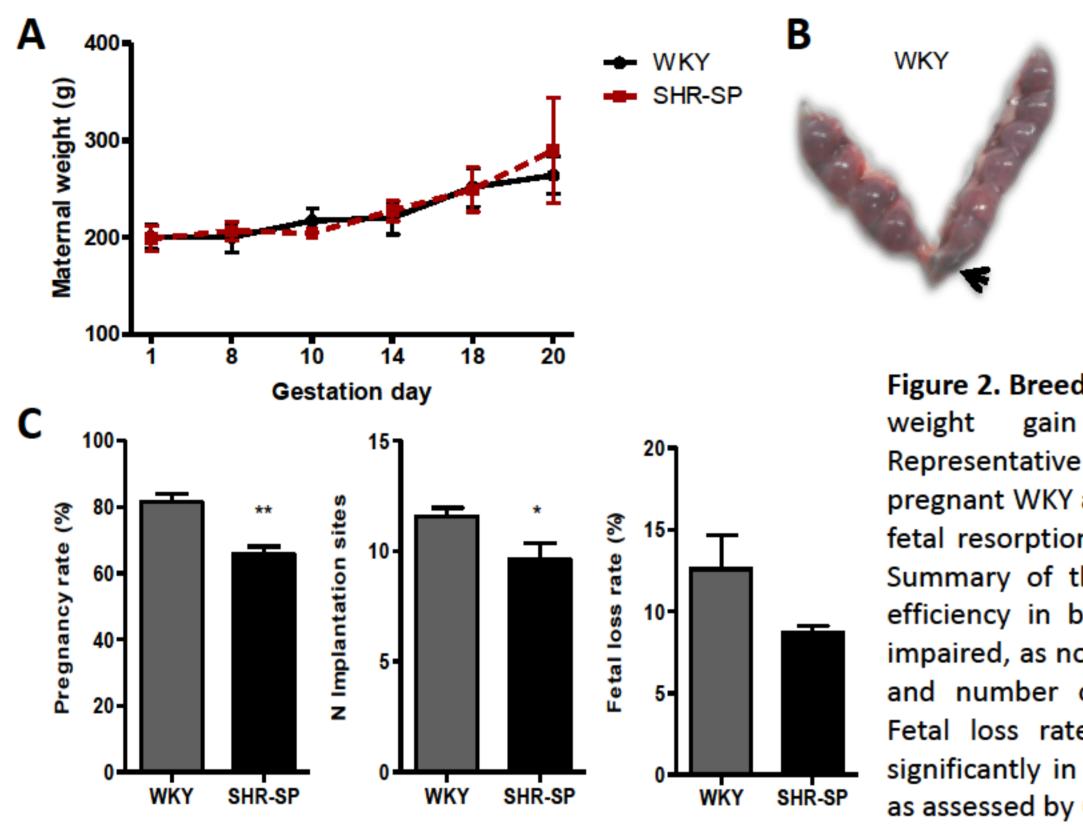
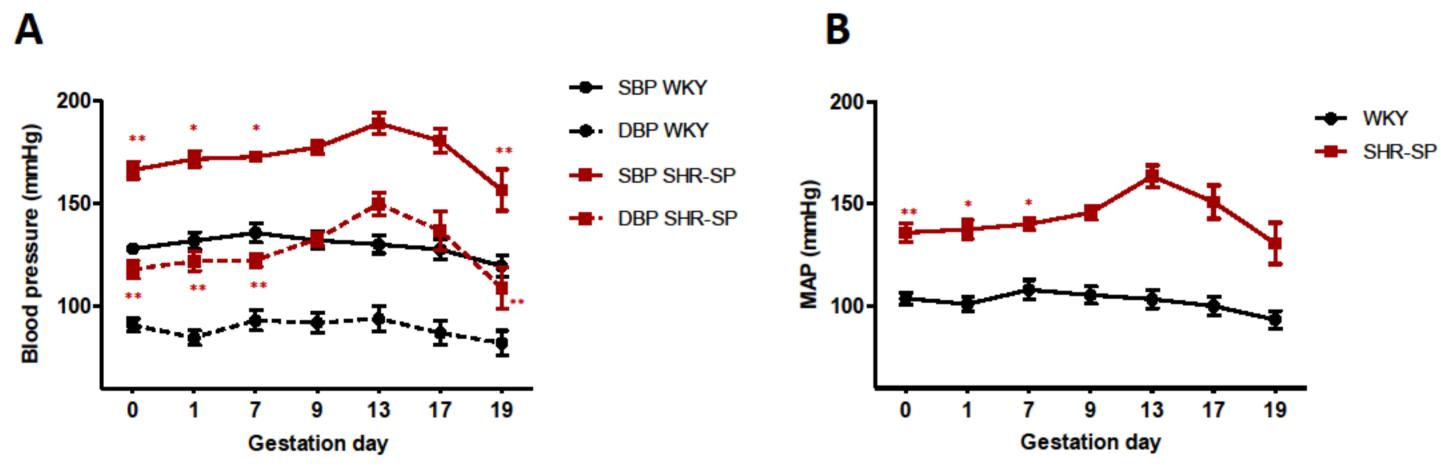


Figure 2. Breeding efficiency analysis (A) Maternal weight gain throughout pregnancy. (B) Representative images of uteri isolated from pregnant WKY and SHR-SP on GD14. Arrows depict fetal resorptions (necrotic implantation sites). (C) Summary of the statisitical analysis of breeding efficiency in both models. Fertility in SHR-SP is impaired, as noted by a decreased Pregnancy Rate and number of implantation sites per female. Fetal loss rates (% resorptions) did not differ significantly in both models. \* p<0.05, \*\* p<0.01 as assessed by unpaired t test



SHR-SP females remain severely hypertensive throughout pregnancy

Figure 3. Blood pressure kinetics throughout pregnancy. (A) Systolic (SBP) and diastolic blood pressure (DBP) records, assessed by the Tail-cuff method. Pregnant SHRSP remained hypertensive throughout the course of the experiment. While BP in WKY females showed slight variations, both SBP and DBP rose in SHRSP, peaking on GD13 and returning to prepregnancy levels at term. (B) Mean arterial pressure (MAP). \* p<0.05, \*\* p<0.01 vs. GD13 as assessed by two-way ANOVA

# MATING MODELS Control (normal pregnancy) Severe Hypertension model WKY X WKY♀ SHR-SP X SHR-SP♀ Individualization of females with vaginal plug (GD1) Read-out parameters (GD1 − GD20) Breeding Efficiency Biochemical data (renal function) Blood Pressure Placental development

Figure 1. Summary of the experimental design to analyse the effect of severe hypertension in pregnant SHRSP rats

WKY

SHR-SP

# Proteinuria is increased at term pregnancy in SHR-SP females

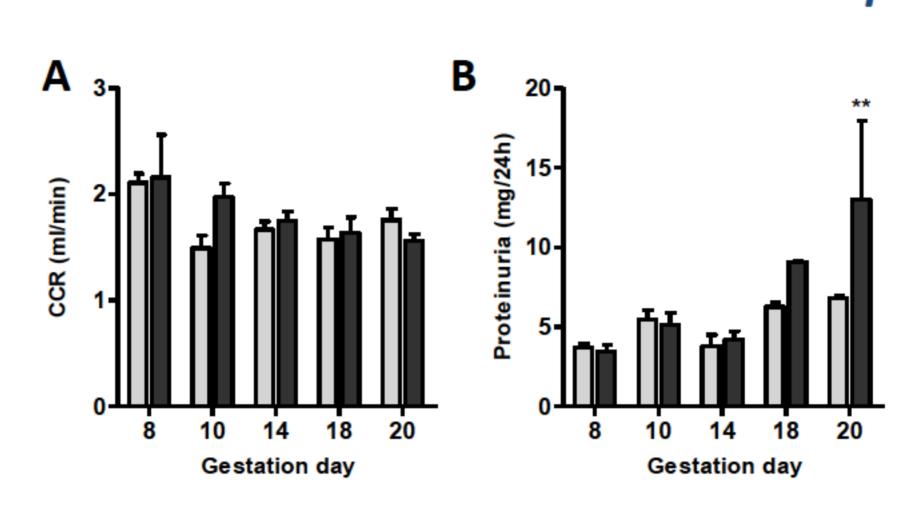


Figure 4. Biochemical analysis of renal function.

(A) Kinetics of the creatinine clearance rate (CCR) during the course of pregnancy. (B) 24h urinary protein content. SHR-SP showed physiological proteinuria throughout pregnancy, which was significantly increased respect to controls at term (GD20). \*\* p<0.01 as assessed by two way ANOVA

# SHR-SP dams show signs of placental insufficiency and FGR

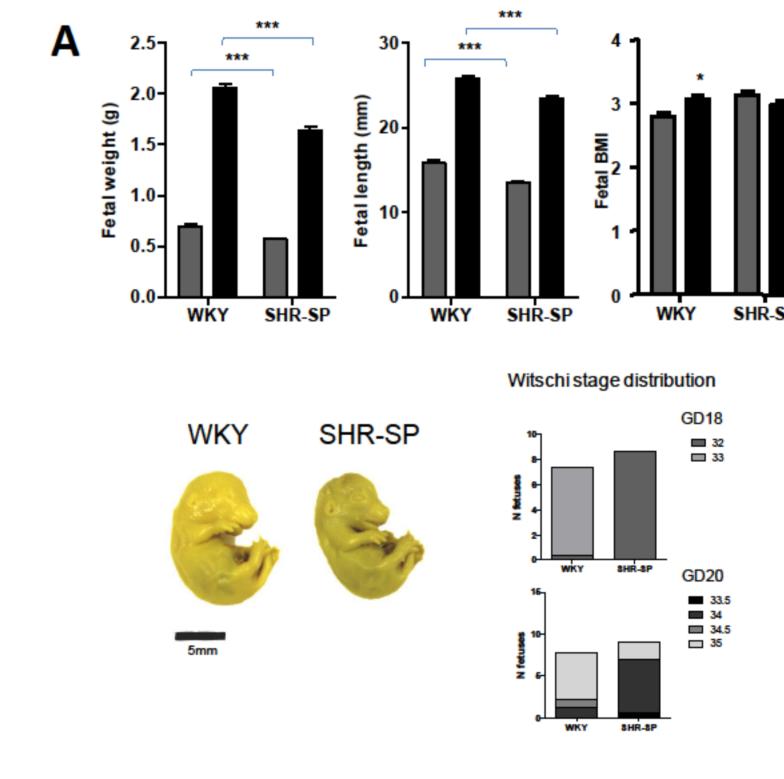
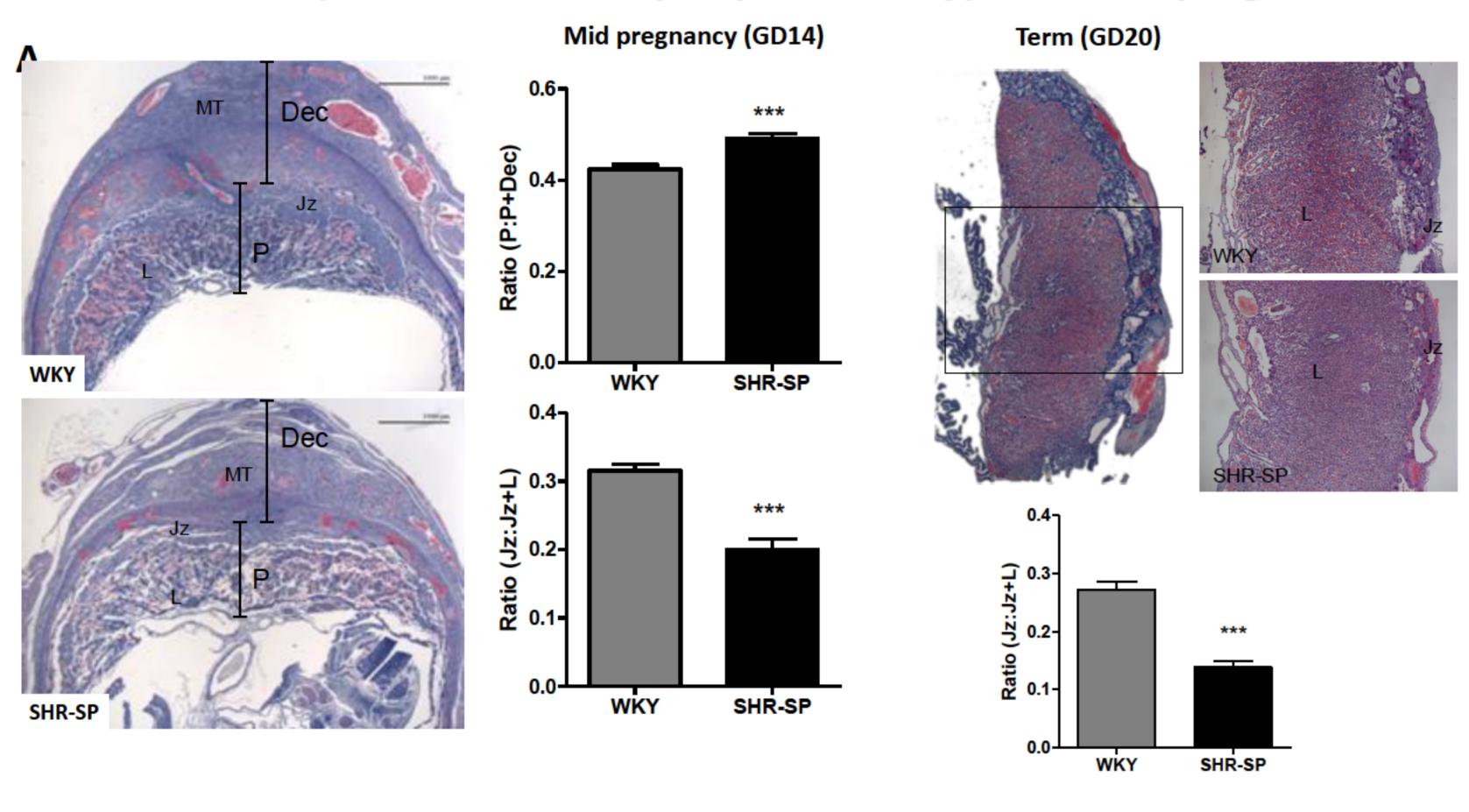


Figure 5. Signs of placental insufficiency and FGR in SHR-SP matings. (A) Fetal growth parameters assessed on GD18 and GD20. SHR-SP fetuses had reduced weights and lengths (left and middle panels) and did not increase their BMI towards GD20 (right panel). FGR was also

SHR-SP matings. (A) Fetal growth parameters assessed on GD18 and GD20. SHR-SP fetuses had reduced weights and lengths (left and middle panels) and did not increase their BMI towards GD20 (right panel). FGR was also evident as a delayed maturation according to the Witschi scale (bottom panels). (B) Placental growth parameters assessed on GD18 and GD20. Placental insufficiency was evident in SHR-SP as a decreased placental weight on GD20 (left) and reduced fetal to placental (F/P) ratio on GD18. \* p<0.05, \*\* p<0.01 and \*\*\*p<0.005 as assessed by two way ANOVA

# Placental development is markedly impaired in hypertensive pregnancies



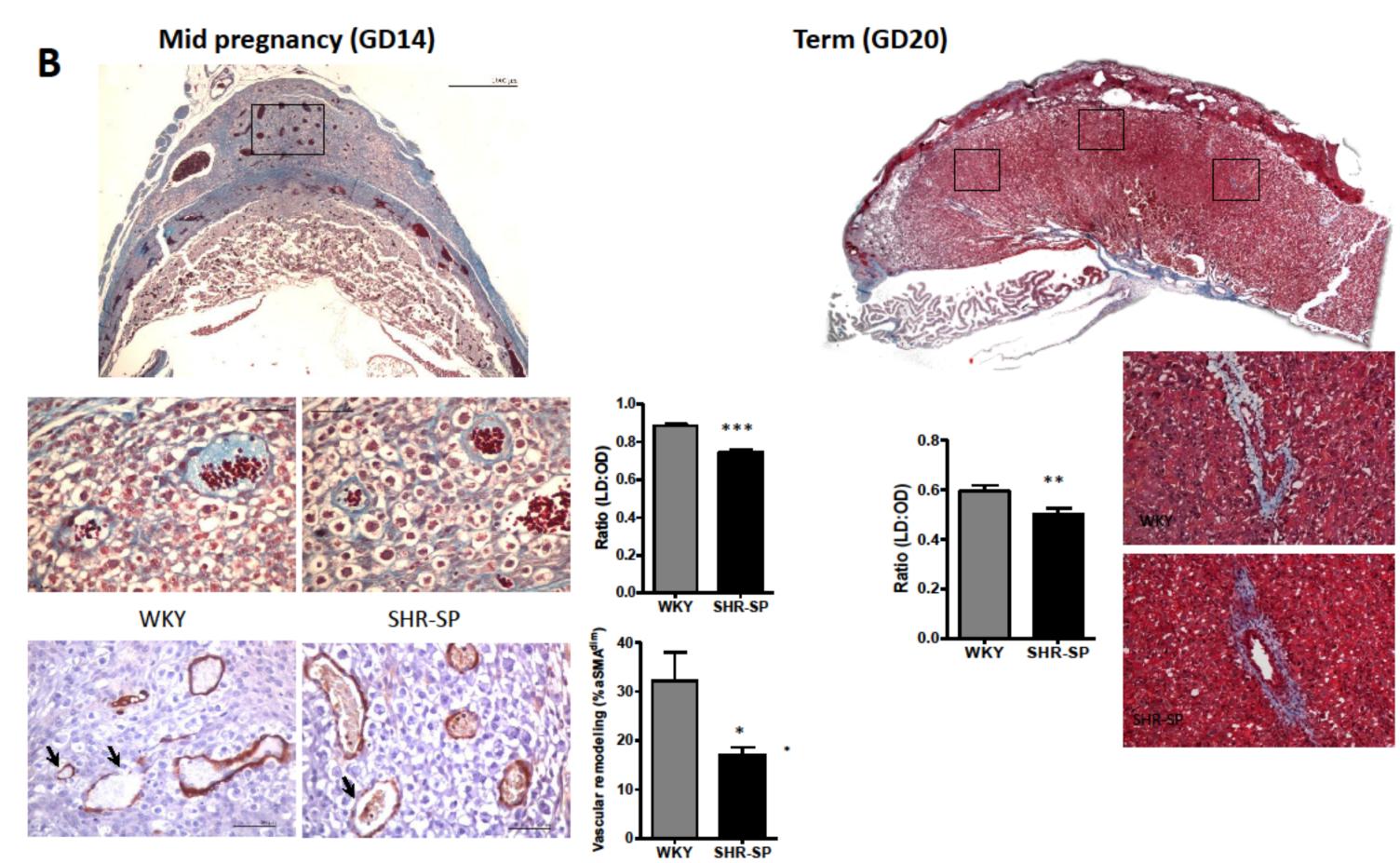


Figure 6. Histological analysis of placental development. (A) Representative images of hematoxylin-eosin stained sections of whole implantation sites isolated on GD14 (left panel) and term placenta (GD20, right panel). Morphometrical alterations were evident in SHR-SP females, incuding an increased placental depth (P:P+Dec ratio) and a decreased fractional area occupied by the junctional zone (Jz:Jz+L ratio). This ratio indicates an hyperplasia of the placental labyrith layer, which persisted towards GD20 (right panel). (B) Masson's trichrome stainings of whole implantations (GD14, left panel) and placentas (GD20, right panel) for the evaluation of vascular remodeling processes. At GD14, SHR-SP showed an impaired remodeling of the maternal spiral arteries irrigating the mesometrial triangle (MT), which showed narrowing of their lumen (decreased LD:OD ratios) and persistence of mural smooth muscle (as evidenced on αSMA immunostainings, lower panels). At term (GD20, right, panel) the maternal vessels irrigating the labyrinth were also thickened in SHR-SP placentas. \* p<0.05, \*\* p<0.01, \*\*\* p<0.005 as assessed by unpaired T test.

# Conclusions

SHR-SP females presented significant alterations in fetal growth probably due to defective placental development and function linked to the hypertensive scenario. Moreover, SHR-SP showed signs of altered renal function near term. These findings together suggest that the SHR-SP strain may be a useful model for further studies on pathogenic mechanisms in hypertensive pregnancies.







