Nω-Nitro-L-Arginine Methyl Ester Hydrochloride (L-NAME) Induced Hypertension in Pregnant Rat Model: An Analysis on the Effectiveness of Administration Methods to Induce High Blood Pressure in Pregnancy.

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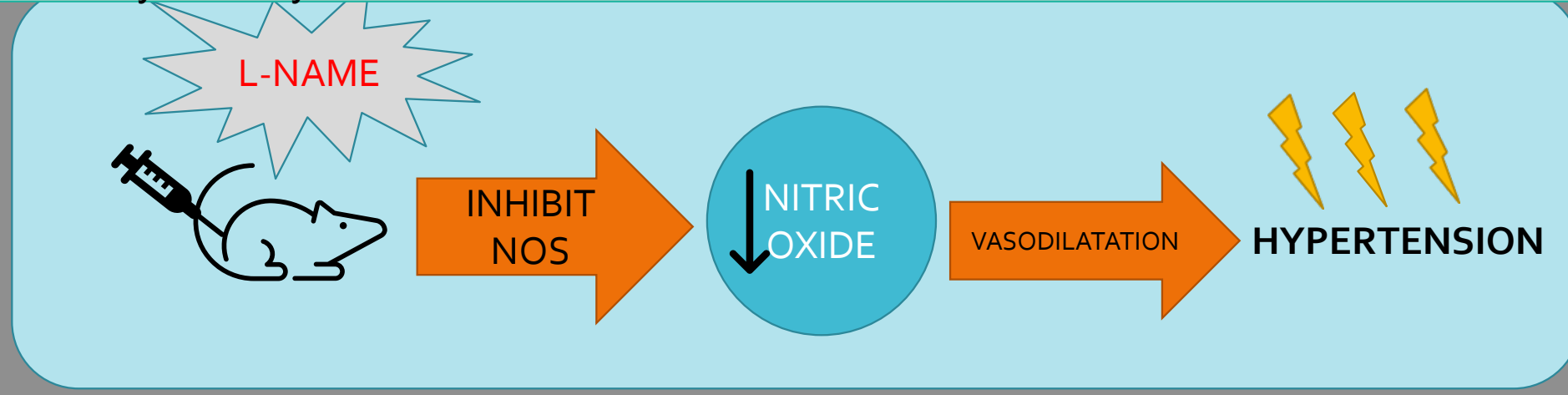International Islamic University Malaysia, Malaysia
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ABSTRACT

Background: Hypertensive disorders of pregnancy (HDP) contribute to high percentage for both maternal and foetal morbidity and mortality and associate with long term complications. A history of having HDP is a known risk factor for women's cardiovascular disease later in their life even though blood pressure normalizes during postpartum period. L-NAME is a drug that has been used to pharmacologically induce hypertension by inhibition of nitric oxide synthase (NOS). In the previous study, there are various administration methods used to induce hypertension in pregnant rat model by using L-NAME, but they produced inconsistent outcomes. **Objective:** This study aims to establish hypertension in the pregnant rat model by using L-NAME. **Materials and Methods:** Twenty-four female Sprague-Dawley (SD) rats were randomly assigned to four groups (n=6 in each): Control- non-pregnant (C), control-pregnant (P), non-pregnant-L-NAME (CL) and pregnant-L-NAME (PL). The pregnant groups were mated with adult male SD overnight and Day 0 of gestation is considered by presence of sperm in the vaginal smear. On Day 10 of gestation, the treated groups (PL and CL) were given 125mg/kg/day of L-NAME via subcutaneous route until the day of delivery and then the animals were sacrificed at Day 30 postpartum. The mean number and weight of the litters were measured via pre-warmed tail-cuff method. The mean number and weight of the litters were recorded. **Results:** Introduction of L-NAME via subcutaneous for 12-13 consecutive days to the treated groups resulted in significant increase of the mean atrial pressure (MAP) on Day 14 and Day 21 of gestation for both treated groups compared to their controls (P and C group respectively). During postpartum period, the MAP of PL and CL decreased but not statistically significant. The mean number and weight of the litters for both P and PL showed no significant differences. **Conclusions:** This method has successfully induced high blood pressure which fits the criteria of HDP definition, and it was relatively safe for the foetus. Based on these findings, we highly recommend the dosing, route, and duration of administration of L-NAME to induce high blood pressure in pregnancy-associated hypertension animal model.
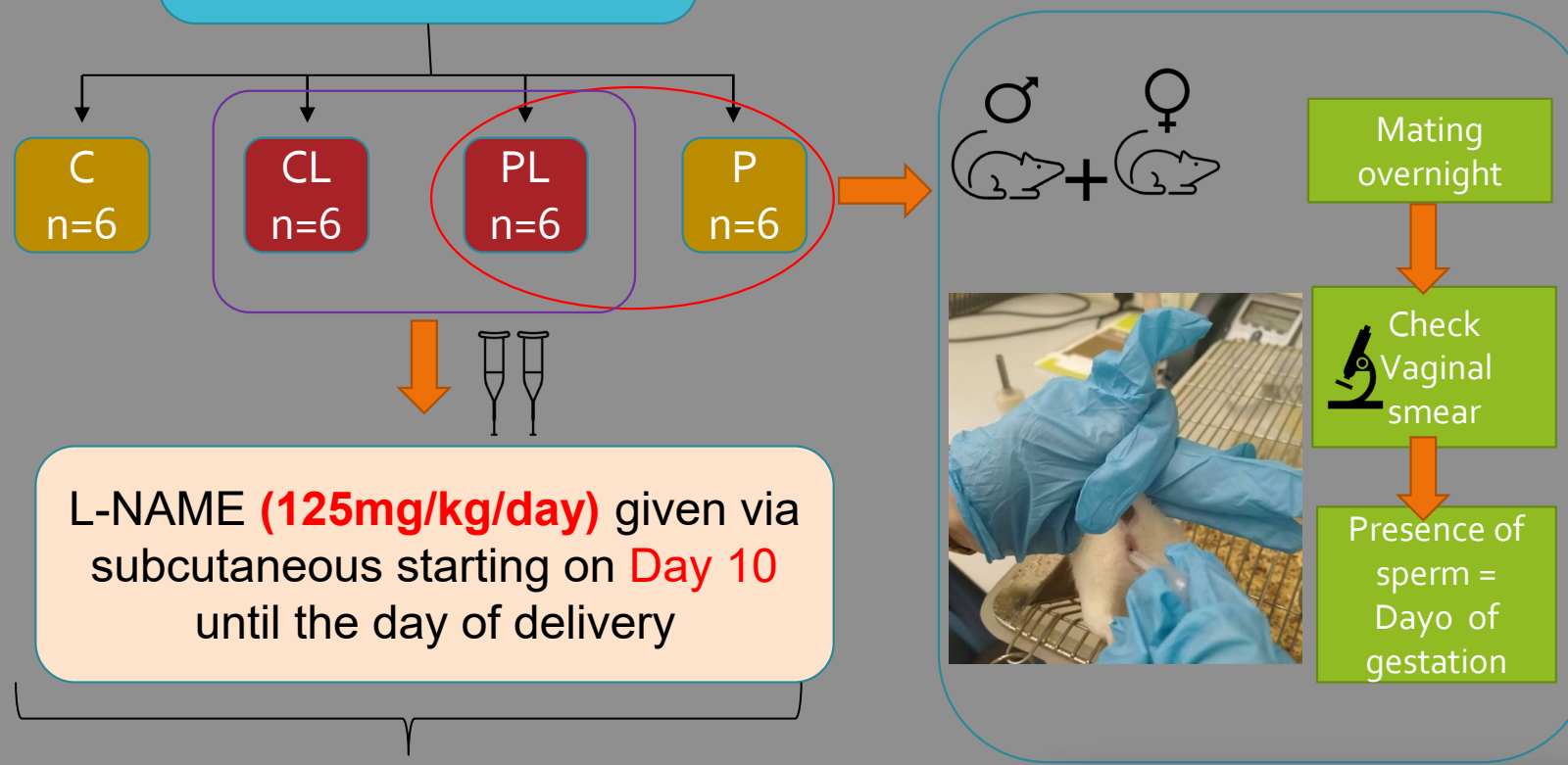
INTRODUCTION

During normal pregnancy in human and animals there are increased production of endothelium-derived nitric oxide[5]. Animal models are reliable tools to study the pathophysiology of hypertensive disorders of pregnancy (HDP)[7]. Nω-Nitro-L-Arginine Methyl Ester Hydrochloride (L-NAME) is a drug that has been used to induce hypertension in pregnant rats model by inhibiting nitric oxide synthase (NOS)[2], resulting in reduction of nitic oxide level which is a potent vasodilator[3]. Even though, L-NAME has been widely used in previous study but they showed inconsistent results[4].

L-NAME → INHIBIT NOS → ↓NITRIC OXIDE → VASODILATATION → HYPERTENSION

METHODS

24 Female Sprague Dawley rats (180-200gram), 5-6 weeks old

C n=6 | CL n=6 | PL n=6 | P n=6

L-NAME **(125mg/kg/day)** given via subcutaneous starting on Day 10 until the day of delivery

Mating overnight → Check Vaginal smear → Presence of sperm = Day 0 of gestation

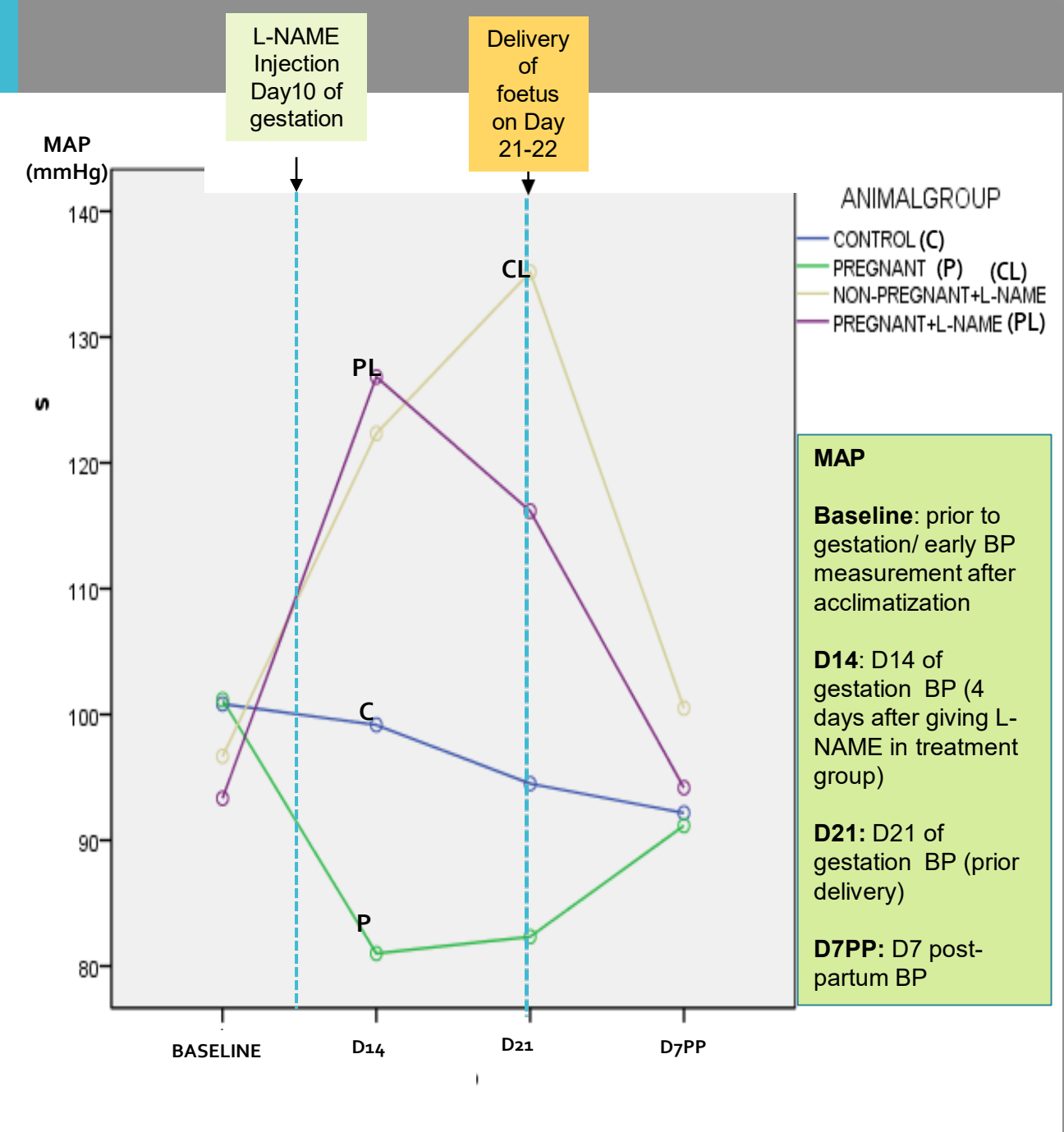**Serial BP Measurement**
- Baseline
- Day 14 of gestation
- Day 21 of gestation

Number of litters

Weight of litters

Vaginal smear

RESULT

L-NAME Injection Day10 of gestation

Delivery of foetus on Day 21-22

ANIMAL GROUP
CONTROL (C)
PREGNANT (P) (CL)
NON-PREGNANT+L-NAME
PREGNANT+L-NAME (PL)

MAP

Baseline: prior to gestation/ early BP measurement after acclimatization

D14: D14 of gestation BP (4 days after giving L-NAME in treatment group)

D21: D21 of gestation BP (prior delivery)

D7PP: D7 post-partum BP

Mean Arterial Pressure (MAP)

Period	Control n=6	Pregnant n=6	Non-pregnant + L-NAME n=6	Pregnant + L-NAME n=6
Baseline	100.83 (7.167)	101.17 (10.998)	96.67 (8.571)	93.33 (7.711)
D14	99.17 (9.683)*	81.00 (10.752)*	122.33 (8.802)*	126.83 (15.892)*
D21	94.50 (8.961)*	82.33 (9.771)*	135.17 (21.498)*	116.17 (23.034)*
D7PP	92.17 (9.968)	91.17 (8.612)	100.50 (4.335)	94.17 (13.075)

*$p < 0.05$ compared with respective controls, standard deviation given in parenthesis.

Foetal Outcome	Pregnant n=6	Pregnant + L-NAME n=6
No. Pups	10.50(2.168)	10.17(2.639)
Pups weight(g)	64.00(9.737)	50.17(14.959)

HDP Definition[1]

BP ≥140/90 mm Hg at least two occasions at least 6 hours apart, but no more than 1 week apart.

- Gestational hypertension: High BP at ≥20weeks of gestation
- Chronic hypertension: Persistent high BP after 6weeks post partum
- Pre-eclampsia: High Proteinuria/ systemic involvement
- Eclampsia

THE IMPORTANT CRITERIA FOR L-NAME INDUCE HYPERTENSION IN PREGNANT ANIMAL MODEL[6]

Routes of administration	Intravenous, **Subcutaneous**, Intraperitoneal, Oral
L-NAME dosage	Low dose, Medium dose, **High dose**
Timing of treatment	Early of gestation, **Middle of gestation**, Late of gestation
Duration of treatment	Depends on the time of starting the treatment

CONCLUSION

- This method has successfully induced high blood pressure which fits the criteria of HDP definition
- It was relatively safe for the foetus.
- We highly recommend the dosing, route, and duration of administration of L-NAME to induce high blood pressure in pregnancy-associated hypertension animal model

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