

## Hypobaric Hypoxia as a Tool to Study Pregnancy-Dependent Responses at the Maternal–Fetal Interface

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### Summary

Establishment of proper oxygen and nutrient supply to the fetus is essential for a successful pregnancy. The maternal–fetal interface is the site of vascular modifications, providing a conduit for the delivery of essential nutrients to the developing fetus. Pregnancy-dependent adaptive vascular responses within the uteroplacental compartment can be exaggerated by exposure to a physiological stressor such as hypoxia. A simple procedure for exposing pregnant rats and mice to hypobaric hypoxia is presented.

**Key Words:** Hypoxia; pregnancy; rat; metrial gland; vascular remodeling; trophoblast.

### 1. Introduction

Oxygen is an essential component of life, and is especially important in pregnancy because oxygen tension has a regulatory role in placental development (1). Vascular remodeling at the maternal–fetal interface is necessary in order to deliver an adequate oxygen and nutrient supply to the fetus (2–4). Hypoxia has been implicated in pregnancy-associated diseases such as intrauterine growth restriction (IUGR) and preeclampsia, which elucidates the importance of oxygen regulation in pregnancy.

The rodent has been used as a model to study the role of oxygen in pregnancy. When rats and mice are exposed to hypoxia, gene expression is altered, impacting placental and fetal development (5–7). The effect of low oxygen can vary—it can lead to IUGR and termination of pregnancy at one extreme or, alternatively, it can elicit maternal adaptations that allow a successful pregnancy to be achieved. The outcome of maternal hypoxia is dependent on the level of oxygen restriction, the duration of exposure, and the gestational period in which the insult is made.

During normal human and rat pregnancy, erythrocyte, hematocrit, and hemoglobin concentrations decrease as pregnancy progresses as a result of hemodilution (8,9). There are also many reports of complications in pregnancies at high-altitudes in which chronic hypoxia has a role in the etiology of pregnancy-associated disease (1). High altitude, defined as >2500 meters, has been shown to reduce uterine blood flow and increase the incidence of IUGR and preeclampsia (1,10,11). Blood vessel development is stimulated by low oxygen tension from high altitude, which results in increased vascularity (12,13).

In this chapter, we describe a simple *in vivo* model that allows induction of maternal adaptive responses to hypoxia, sparing the fetus from IUGR. In this model, pregnant rats or mice are exposed to hypobaric hypoxia, which activates maternal uterine vascular remodeling and hematological adaptations (14, Ho-Chen, J. K. and Soares, M. J., unpublished results). This profound maternal response largely protects the fetus from IUGR.

## 2. Materials

1. Animals: Holtzman rats are obtained from Harlan-Sprague Dawley (Indianapolis, IN) and CD-1 mice are obtained from Charles River Laboratories (Wilmington, MA).
2. The hypobaric chamber was designed and constructed by Alt Manufacturing (Kansas City, KS). A hypobaric chamber has four main components: a vacuum pump, a vacuum breaker valve, a differential vacuum gauge, and a chamber (*see Fig. 1*). The first three components are readily available and come from a manufacturer ready to be used. A one-fourth to one-half horsepower continuous duty, oil-less rotary vane vacuum pump that is capable of pumping down to at least 26 inches of Hg and moving at least 3 cfm of air, is required (McMaster-Carr, Elmhurst, IL, cat. no. 9901K64). The vacuum breaker valve is used to maintain a preset vacuum in the chamber. A heavy-duty bronze diaphragm valve with a breaking range of 2-30 in. of Hg is recommended (McMaster-Carr, cat. no. 4614K11). This type of valve ensures a highly controlled and reproducible vacuum. A digital differential vacuum gauge can be purchased from Cecomp Electronics, Inc. (Cecomp Electronics, Inc., Libertyville, IL, cat. no. DPG1000AD). The vacuum gauge can be programmed at the factory to display one of several different units. Construction of the chamber is considerably more involved because it usually requires custom fabrication and has several unique requirements. The chamber can be made to accommodate a wide range of space and weight requirements, as long the chamber can withstand a differential pressure equivalent to 10 psi. It is useful for the chamber to have at least one transparent side, so that animal light-dark cycles are not disrupted. The pump is connected to the chamber using one-half-inch (inner diameter) braid reinforced tubing (McMaster-Carr, cat. no. 55425K33) and generic brass barbed hose fittings. The vacuum breaker valve and vacuum gauge are connected to the chamber using standard copper pipe and various fittings (*see Notes 1 and 2*).

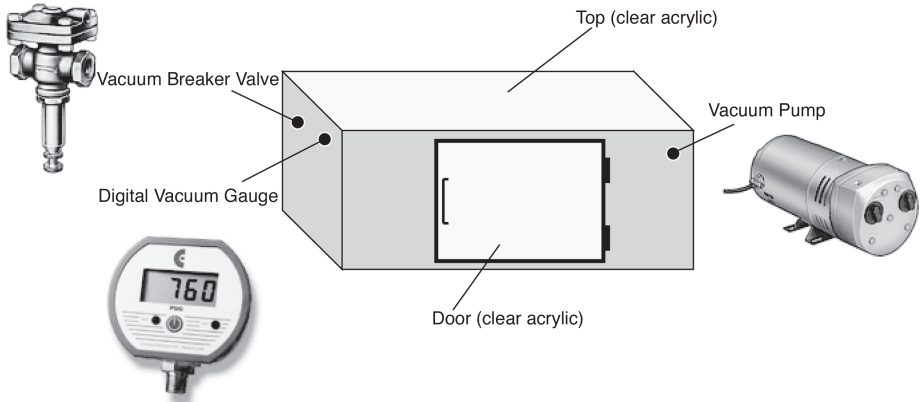


Fig. 1. Diagram of the hypobaric chamber, illustrating the essential components of the chamber.

3. Pediatric BD Vacutainer(tm) Tubes containing spray-dried  $K_2$ -ethylenediamine tetraacetic acid (EDTA) (Becton Dickinson, Franklin Lakes, NJ).
4. Act10 Hematological Analysis System (Beckman Coulter, Miami, FL).

### 3. Methods

#### 3.1. Animal Preparations (see Note 3)

1. Male and female Holtzman rats are kept under controlled conditions of 14 h light and 10 h dark (14 h-L:10 h-D) or, alternatively, 12 h-L:12 h-D photoperiods with access to food and water *ad libitum*. To obtain timed pregnancies, female rats are caged overnight with fertile males. The presence of sperm in vaginal smear is designated as d 0 of pregnancy.
2. Male and female CD-1 mice are housed in 14 h-L:10 h-D photoperiod with access to food and water *ad libitum*. Timed mouse pregnancies are obtained by co-housing females with fertile males. The presence of a seminal plug in the vagina of females is designated as d 1 of pregnancy.

#### 3.2. Chamber Calibration (see Table 1)

The barometric pressure adjustments are presented in **Table 1** and are based on the following equations:

$$P_{I_{O_2}} = (P_C - P_{H_2O}) \times \%O_2 \text{ in air} = (P_B - P_{H_2O}) \times \%O_2 \text{ inspired}$$

where:

$P_{I_{O_2}}$  = Partial pressure of inspired oxygen

$P_C$  = Pressure inside hypobaric chamber

$P_B$  = Barometric pressure at experiment site

$P_{H_2O}$  = Partial pressure of water vapor = 47 Torr

**Table 1**  
**Calibration of the Hypobaric Chamber**

Experimental conditions	Estimated O <sub>2</sub> concentrations at sea level	P <sub>IO<sub>2</sub></sub> (Torr)	P <sub>B</sub> (Torr)	P <sub>B</sub> (inches of Hg)	Differential Pressure (inches of Hg)	Simulated elevation (meters)
Sea level	21	149	760	29.9	—	0
Kansas City, KS	21	143	730	28.7	—	300
Chamber-10	10	71	385	15.2	14.7	5150
Chamber-11	11	78	418	16.5	13.4	4540
Chamber-12	12	86	454	17.9	12.0	3990
Chamber-13	13	93	490	19.3	10.6	3470
Chamber-14	14	100	523	20.6	9.3	2990
Chamber-15	15	107	556	21.9	8.0	2560

### 3.3. Exposure of Pregnant Rodents to Hypobaric Hypoxia

1. Prior to exposure, the rat or mouse is weighed and then placed in the hypobaric chamber. Food is measured and allocated. Each animal is caged separately to monitor food intake (*see Note 4*).
2. The chamber is sealed and the vacuum is activated. Different settings are used for the pregnant Holtzman rat vs the pregnant CD-1 mouse (*see Note 5*).
  - a. For the Holtzman rat we routinely use conditions where air is circulated at a barometric pressure of approx 386 Torr, which results in an inspired PO<sub>2</sub> of approx 71 Torr, equivalent to breathing 10% O<sub>2</sub> at sea level (*see Table 1*).
  - b. For the CD-1 mouse, we routinely use conditions in which air is circulated at a barometric pressure of approx 420 Torr, which results in an inspired PO<sub>2</sub> of approx 78 Torr, equivalent to breathing 11% O<sub>2</sub> at sea level (*see Table 1*).
3. On a daily basis, the vacuum is released and the chamber opened for 15–20 min in order that the cages may be cleaned, the animals and their food weighed, and food and water replenished (*see Note 6*).
4. At the termination of the experiment, the animals are removed from the chamber and analyzed, as dictated by the experimental design.
5. Two types of controls are used: (1) *ad libitum*-fed animals and (2) pair-fed animals. *Ad libitum*-fed controls are weighed and food intake monitored daily. Pair-fed controls are weighed daily and are fed the amount of food that the hypoxic animals ate on that day of gestation (*see Note 7*).

### 3.4. Phenotypic Assessment

1. Systemic assessment: blood samples are collected by cardiac puncture of anesthetized animals in Pediatric BD Vacutainer Tubes containing spray-dried K<sub>2</sub>-EDTA and stored at 4°C until analyzed. Hematological parameters are measured using an Act10 Hematological Analysis System. This provides a complete hematological analysis, including: total leukocytes, total erythrocytes, hemoglobin, hematocrit, mean-corpuscular hemoglobin, mean cell hemoglobin concentration, total platelets, and mean platelet volume (*see Note 8*).
2. Strategies for assessing the maternal–fetal compartments of animals exposed to hypoxia and their respective controls have been presented in Chapters 20, 21, and 26 of Vol. 1 (15–17) (*see Note 9*).

## 4. Notes

1. Additional information about the hypobaric chamber design can be obtained by contacting Alt Manufacturing (Phone: 913-588-5690). The vacuum pump we have selected is relatively quiet, but optimally it should be housed in an adjoining room or utility closet, and the vacuum hose run through the wall thus minimizing the noise. If this is not possible, then it is essential that control animals be exposed to the same level of noise.
2. There are several alternatives to using a hypobaric chamber that can be used to create a hypoxic environment. Most utilize gas dilution. One approach is to use

an oxygen controller (e.g., Pro-Oxy Controller, Biospherix, Syracuse, NY). The controller monitors the oxygen concentration in a sealed box and regulates the rate of infusion of nitrogen gas. The oxygen controller releases nitrogen into the box, diluting the concentration of oxygen down to a set-point. The initial cost of such a controller is approximately twice that of fabricating a hypobaric chamber. Unlike the hypobaric system, the gas dilution system continues to require expenditures for nitrogen gas adding to the operational costs.

3. All animal experimentation should be approved by the institutional animal care and use committee.
4. Animals usually reduce their appetite the first few days and exhibit decreased physical activity. Overtime they adapt and regain some of their food intake; however, their physical activity remains limited during the course of exposure. The reduced food intake affects body weight and necessitates additional controls (see below).
5. Responses to maternal hypoxia are affected by species, strain, and gestational stage. In general, mice are more sensitive than rats and at least some inbred strains are more sensitive than outbred strains. Furthermore, the period of gestational exposure affects responses. Both rats and mice appear to be able to more effectively adapt to hypoxia during the interval immediately after implantation to midgestation. Adaptive responses to hypoxia during the last week of gestation are more variable, especially in the mouse. We have focused on two time intervals in the Holtzman rat: (1) d 6 to 12 of gestation and (2) d 13 to 20 of gestation. In the CD-1 mouse, we have focused on a time interval between d 6 and 12 of gestation.
6. A few issues are relevant regarding the opening of the chamber during the course of an experiment. First, the rate of pressure release from the chamber must be slow. We typically shut off the vacuum pump and let the chamber passively equilibrate with the ambient environment. Opening the chamber daily provides a convenient means for tracking responses of the animals during the course of an experiment and maintaining their food and water supply. This brief daily exposure to ambient pressure has also proved to be essential for maintaining viable pregnancies during the last week of gestation.
7. *Ad libitum* fed and pair-fed controls are required. These two control groups are important because hypobaric hypoxia influences food intake. Body weight gain in pregnant females exposed to hypobaric hypoxia is less than pregnant animals housed under ambient conditions. Restricting food intake of pregnant females housed under ambient conditions matches the maternal body weight changes in hypobaric hypoxia-exposed females. Pair-feeding is an essential control because maternal food restriction independently impacts placental and fetal growth.
8. Typical systemic responses to maternal hypoxia include increases in hemoglobin and hematocrit. The magnitude of the response appears to be more evident during the first half of gestation in comparison to responses observed during the last week of gestation (Ho-Chen, J. and Soares, M. J., unpublished results).

9. The most reproducible responses in pregnant rats or mice exposed to hypoxia are changes that occur in the uterine mesometrial compartment (also called the metrial gland). Maternal hypoxia increases vascularity and the diameter of uterine mesometrial blood vessels (14, Ho-Chen, J., Ain, R., and Soares, M. J., unpublished results). Additional responses are also noted in metrial gland gene expression profiles (Ain, R. and Soares, M. J., unpublished results). For the latter experiments, it is important to appreciate that some commonly used housekeeping genes are affected by hypoxia (e.g., glyceraldehyde-3-phosphate dehydrogenase).

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