PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 153, 138-142 (1976)

# Effects of Hypoxic Exposure on Embryonic Implantation in Mice[1] (39496)

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A number of studies describe the effects of hypoxia (exposure to low oxygen in gaseous mixtures, reduced oxygen tension in hypobaric chambers, and natural high altitude) on pregnancy during the postimplantation phase of gestation (1-7). Little is known of the effects of varying degrees of hypoxia on reproductive function prior to placentation. Recently, Rattner and Ramm (8) demonstrated that exposure of gravid mice to a 7% oxygen environment (equivalent to the partial pressure of oxygen at 24,000 ft) resulted in death of embryos during the preimplantation period or shortly thereafter, indicating that gestation may be interrupted prior to placentation.

The present investigation was conducted to determine the level of oxygen which impairs blastocyst implantation and to follow the effects of progressively more severe degrees of hypoxia. Morphologic changes in the ovaries, uteri, and embryos were used to elucidate a possible mechanism by which exposure to hypoxia alters gestation.

*Materials and Methods.* Ten-week-old virgin female mice (strain CD-1), weighing 25-30 g, were mated monogamously. Mating was confirmed by visual inspection for vaginal plugs prior to 1000 hr on the following day. The day on which a vaginal plug was first observed was designated as Day 0 of pregnancy. Gravid mice were assigned to one of five groups and provided with food and water *ad libitum*.

The various oxygen environment exposures were performed in a Labconco fiberglass glovebox (Labconco Co., Kansas City, Mo.) to which ultrahigh purity nitrogen was added to reduce the partial pressure of oxygen ($pO_2$). A Beckman Model D2 Oxygen Analyzer (Beckman Instruments, Inc., Fullerton, Ca.) was used to monitor $pO_2$, and carbon dioxide tension ($pCO_2$) was measured with a Fyrite Carbon Dioxide Analyzer (Bacharach Industrial Instrument Co., Pittsburgh, Pa.). The $pCO_2$ within the chamber was reduced by intermittently circulating the atmosphere through a 6 *M* KOH bubbling flask. Relative humidity was held between 40-70% by means of a $CaSO_4$ drying train. Chamber temperature ranged from 22-25°C and a 14-hr light:10-hr dark photoperiod was employed.

Group (Gp) 1 included mice maintained at 21 ± 0.5% oxygen (approximate $pO_2$, 159 mm Hg and equivalent to sea level) with the carbon dioxide concentration less than 0.5 ± 0.5%. Animals in Gps 2 and 3 were exposed to 14 ± 0.5% oxygen (approximate $pO_2$, 131 mm Hg and equivalent to 10,000 ft) and 12 ± 0.5% oxygen (approximate $pO_2$, 91 mm Hg and equivalent to 14,000 ft), respectively, with the carbon dioxide concentration less than 1.5 ± 0.5% of total atmospheric constituents. Group 4 consisted of mice maintained in 10 ± 0.5% oxygen (approximate $pO_2$, 76 mm Hg and equivalent to 18,000 ft) with the carbon dioxide concentration $\leq$1.5 ± 0.5% and, lastly, Gp 5 at 8 ± 0.5% oxygen (approximate $pO_2$, 60 mm Hg and equivalent to 22,000 ft) with the carbon dioxide concentration $\leq$2.0 ± 0.5%. Following insemination, animals were placed into the glovebox and exposed continuously to the various oxygen environments from 1000 hr on Days 0 through 6 of pregnancy. Mice were maintained in an animal care facility (21 ± 0.5% oxygen, $\leq$0.5 ± 0.5% carbon dioxide) until Day 8, at which time they were sacrificed by cervical dislocation.

Body weights were measured before and immediately after chamber exposure and on Day 8 of pregnancy. From the suborbital sinus, 50 μl of blood was drawn for microhematocrit determination on Days 6 and 8.

[1] This research was supported by the Public Health Service (RRO-742-09) and the Computer Science Center of the University of Maryland.

Weights were obtained on uteri and paired ovaries and adrenal glands. Generally, the two largest corpora lutea (CL) were dissected with a needle from each ovary, blotted on filter paper, and rapidly weighed to a 10 μg sensitivity on a Cahn Model RTL Electrobalance (Cahn Instruments, Cerritos, Ca.). If no implantation sites were present the contents of one uterine horn were flushed into a watch glass with 0.5 ml of 0.9% NaCl and examined under a dissecting microscope for preimplantation embryos and cellular debris. Tissues were fixed in neutral formalin, sectioned serially at 10 μm, and stained with hematoxylin and eosin for light microscopic analysis.

The number of mice with implantation sites in each treatment group was compared using serial contingency analysis. The data for each variable were tested for homogeneity to ensure that utilization of analysis of variance or the Student's *t* test was appropriate. The Newman-Keuls multiple range test was employed to determine significant differences between means.

*Results. Macroscopic observations.* The percentage of mice with implantation sites declined with the reduction in oxygen concentrations (Table I). Implantation was not impaired at 21 or 14% oxygen concentrations (Gps 1 and 2). At 12 and 10% oxygen (Gps 3 and 4, respectively) an intermediate response was apparent with less than 50% of inseminated mice having nidatory sites. In 8% oxygen blastocyst implantation was blocked. Uterine flushings of mice without implantation sites in Gps 3-5 contained cellular debris and occasionally blastocyst embryos. The flushed uterine horns of mice without implantation sites contained one to three blastocysts in three of ten mice in Gp 3, two of ten mice in Gp 4, and four of nineteen mice in Gp 5. There was no difference in the number of embryonic swellings per uterus in any group when implantation sites were present at Day 8 of pregnancy.

Uterine and ovarian weights declined significantly as oxygen concentration was reduced (Table II). Mean CL weights in Gps 3 and 5 were significantly less than the 21% oxygen control. In Gp 5, only seven small CL could be dissected and weighed due to extensive luteal regression.

Responses symptomatic of hypoxia (adypsia, anorexia, hyperpnea, and polycythemia) were observed in Gps 2-5. Mean body weight of mice in Gp 1 increased by 6.3% during the exposure period, whereas Gp 2 mice maintained their body weight, and Gps 3-5 mice lost 10-20% of their initial weight (Table II). Between Days 6 and 8, mice in Gps 3-5 demonstrated a significant gain in body weight with the resumption of growth when compared to mice in the 21 and 14% oxygen groups. The mean hematocrit value of the groups at Day 6 of exposure was inversely related to the oxygen concentration to which mice were exposed, ranging from (mean ± SE) 42.8 ± 0.52 at 21% oxygen to 59.1 ± 0.72 at 8% oxygen. The mean hematocrits at Day 8 in all groups were significantly less than the corresponding Day 6 means. The mean adrenal gland weight of the groups did not vary significantly.

*Histologic observations.* Hyperemic corpora lutea of pregnancy of Gp 1 mice were composed of irregular columns of homogeneous lutein cells. Individual lutein cells were composed of a large band of cytoplasm

TABLE I. IMPLANTATION SITES AT DAY 8 OF GESTATION

| Group | Oxygen concentration[a] (%) | Total number of animals | Animals with implantation sites | | Mean number of embryonic swellings per uterus of mice with implanted embryos ± SE[b] |
|---|---|---|---|---|---|
| | | | *n* | %[b] | |
| 1 | 21 (sea level) | 68 | 48 | 70.5* | 13.2 ± 0.34* |
| 2 | 14 (10,000 ft) | 27 | 18 | 66.6*,Δ | 11.7 ± 0.44* |
| 3 | 12 (14,000 ft) | 17 | 7 | 41.2Δ | 12.7 ± 0.51* |
| 4 | 10 (18,000 ft) | 17 | 7 | 41.2Δ | 13.7 ± 0.36* |
| 5 | 8 (22,000 ft) | 20 | 1 | 5.0† | 12.0[c] |

[a] Approximate altitude in parentheses corresponding to the oxygen concentration.
[b] Groups with different superscripts were significantly ($P < 0.05$) different.
[c] This value is within the range of the means of Groups 1-4.

TABLE II. ORGAN AND BODY WEIGHTS

| Group | Oxygen concentration[a] (%) | Uterine weight (mg ± SE)[b,c] | Mean ovarian weight (pair) (mg ± SE)[b,c] | Mean corpora lutea weight (μg ± SE)[b,c] | Body weight change between Days 0–6 (g ± SE)[b,c] | Body weight change between Days 6–8 (g ± SE)[b,c] |
|---|---|---|---|---|---|---|
| 1 | 21 (sea level) | 468.9 ± 37.65 (21)* | 17.8 ± 1.18 (21)* | 252.1 ± 15.12 (76)* | +1.26 ± 0.246 (26)* | +0.65 ± 0.252 (26)* |
| 2 | 14 (10,000 ft) | 402.8 ± 57.34 (11)* | 13.7 ± 1.17 (11)* | 263.1 ± 16.79 (32)* | +0.01 ± 0.457 (10)Δ | +0.67 ± 0.351 (10)* |
| 3 | 12 (14,000 ft) | 309.5 ± 44.30 (16)Δ | 11.2 ± 0.65 (16)Δ | 196.9 ± 17.63 (35)Δ | −2.34 ± 0.326 (16)† | +1.96 ± 0.286 (16)Δ |
| 4 | 10 (18,000 ft) | 209.0 ± 31.45 (17)Δ | 14.7 ± 0.74 (17)† | 245.8 ± 17.87 (38)* | −3.38 ± 0.248 (17)** | +2.19 ± 0.234 (17)Δ |
| 5 | 8 (22,000 ft) | 143.1 ± 16.01 (19)† | 8.5 ± 0.59 (20)** | 125.7 ± 24.68 (7)Δ | −4.22 ± 0.250 (20)†† | +2.37 ± 0.228 (20)Δ |

[a] Approximate altitude in parentheses corresonding to the oxygen concentration.
[b] Value in parentheses refers to number of observations.
[c] Group means with different superscripts were significantly ($P < 0.05$) different.

surrounding a vesicular nucleus. Corpora lutea of mice in Gps 2–4 were less hyperemic and had a smaller ratio of nuclear:cytoplasmic diameter when compared to Gp 1. In Gps 2–4 CL were incompletely luteinized, being composed of a heterogeneous cell population of vacuolated, pale-staining, lutein cells and small, intensely staining, eosinophilic cells. In Gp 5, lutein cells were highly vacuolated and the cytoplasmic:nuclear ratio was further reduced over Gp 1. These CL were just below the surface of the ovary, often infiltrated with connective tissue, and appeared to be retreating toward the medulla of the ovary.

Ovarian interstitial development was incomplete in mice exposed to oxygen concentrations of less than 12% when compared to Gp 1 controls. Small antral follicles were present in ovaries of mice in Gps 1–4. Numerous secondary and tertiary follicles undergoing atresia were present in the cortex of mice in Gp 5. The membrane granulosa of these follicles was only a few cell layers thick, or absent, and the antral cavity was frequently filled with blood.

In Gp 1, and usually in Gps 2–4, embryonic development proceeded to neurulation by Day 8. Embryonic resorption sites were more frequently observed in Gps 2–4. Pyknotic nuclei were present in embryonic and extraembryonic structures, and the uterine lumen contained an abundance of leucocytes. Uterine stromal hyperplasia and epithelial gland development were reduced in Gp 5 when compared to all other groups. In mice exposed to 8% oxygen an occasional free blastocyst was found in the cervix or adjacent to a crypt in the uterine horn.

*Discussion.* These experiments demonstrate that pregnancy may be terminated prior to implantation in the nonacclimated mouse exposed to oxygen concentrations of 12%. Embryonic implantation during hypoxic exposure appears to be an "all or none" response. Although the percentage of inseminated animals with implantation sites decreases as oxygen tension is reduced, those animals in which nidation occurs have a constant number of implantation sites regardless of the $pO_2$ treatment level. This constant number of implantation sites is governed by total ovulations and the normal

nidatory failure of a small number of blastocysts.

Fecundity in rodents exposed to reduced $pO_2$ is "graded" during the *post*implantation period. This response may range from the resorption or abortion of an individual fetus to loss of an entire litter (2, 4). The number of viable offspring is dependent upon the degree of maternal hypoxia and the extent of acclimatization attained during exposure (2, 4).

Weights and morphology of the ovaries and their CL indicate that secretion and/or delivery of luteotropic hormones [FSH and prolactin (9); LH and prolactin, (10)] to the ovary is depressed (Gps 2–4) or blocked altogether (Gp 5). Follicular development in mice exposed to as little as 10% oxygen (approximate $pO_2$ equivalent at 18,000 ft) was comparable to control ovaries, indicating maintenance of FSH secretion. Thus, inhibition of prolactin (PRL) secretion may be the primary means by which luteal function is impaired. Regressing CL and numerous hemorrhagic follicles undergoing atresia in mated mice exposed to 8% oxygen may indicate that cyclic follicular development continued after conception, but ovulatory quantities of LH were not available to the ovary. A similar selective inhibition of hypophyseal PRL and LH secretion has been reported in the cycling hamster exposed to 23,000 ft of simulated altitude [oxygen concentration approximately 8.6%, (6)].

The depression of uterine weight produced by hypoxia is associated with nidatory failure and reduced progestational and estrogenic support of pregnancy. A precise sequence of progesterone in combination with estrogen is essential for sensitizing the uterine endometrium for decidualization and implantation (11). The presence of free blastocyst embryos, reduced progestational growth, and regressing luteal tissue in mice exposed to 8% oxygen indicate that preparation of the uterus for implantation was incomplete. A nutritive environment incapable of maintaining viable embryos is implicated because uterine flushings contained cellular debris, leucocytes, and only a few blastocysts. In the nonprogestational uterus, clutches of preimplantation embryos are lost through the cervix on the day of ovulation of subsequent cycles (12). In rare instances, blastocysts which appeared to be normal were observed in uterine crypts. Such embryos may be viable, since a 2-week delay of implantation in prepuberal mice (hormonally induced to ovulate and mate) may be followed by successful nidation upon progesterone administration (12).

Baird and Cook (4), using acclimated mice exposed to a simulated altitude of 14,000–25,000 ft, reported that resorption of fetuses was initiated when crown–rump length approached 7 mm [Day 12 of gestation (13)]. In the present study, exposure of the nonacclimated mouse to hypoxia resulted in the initiation of resorption before Day 8 of pregnancy when crown–rump length is less than 2 mm. This difference in susceptibility of the fetus in the nonacclimated mouse may be due to the altered tropic and endocrine support of gestation and to the direct effects of hypoxia on the conceptus prior to maternal acclimatization.

The progressively greater loss in body weight of pregnant mice produced by increasing severity of hypoxia may be due to several factors including reduced food intake, excessive loss of body water, and the direct effect of hypoxia on tissues. Recently, it was found that most of the reduction in weight of mice exposed to hypoxia was primarily loss of body water (14–16). It is difficult to establish unequivocally that hypoxia, per se, directly affects the ovary, uterus, or implantation. Severe nutritional deprivation has also been demonstrated to block implantation in the mouse (17). It has been shown that increased prenatal mortality occurs in rats exposed to 18,000 ft of simulated altitude (oxygen concentration approximately 10%) in the absence of any alteration in the normal gain in body weight (2). This suggests that loss in body weight during exposure to hypoxia (12 and 10% oxygen) may not be the primary cause of alterations in the reproductive system.

*Summary.* Inseminated mice were exposed to different degrees of hypoxia ranging from 8–14% during the first 6 days of gestation. The frequency of animals with embryonic implantation sites was reduced by 42% after exposure to either 12 or 10% oxygen and by 93% at 8% oxygen. When

implantation occurred, the number of nidatory sites per uterus did not differ significantly, which suggests an all or none response for blastocyst implantation. The failure of embryos to implant was associated with histologic evidence of luteal regression. An increased incidence of fetal resorption was apparent following implantation in mice exposed to 14, 12, or 10% oxygen. The reproductive dysfunction noted during hypoxic exposure is attributed to a substantial loss in body weight and to altered tropic and endocrine support of pregnancy.

The authors are deeply indebted to Ms. Marcia M. Pargament and Mr. Milton G. Parker for their assistance in this study.

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Received April 6, 1976. P.S.E.B.M. 1976, Vol. 153.