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TITLE: Novel Models to Study Effect of High-Altitude Hypoxic Exposure and Placental Insufficiency on Fetal Oxygen Metabolism and Congenital Heart Defects

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14. ABSTRACT

The idea for this proposal is that acute high altitude hypoxic exposure early in pregnancy during a critical period of organogenesis may critically reduce O2 transport to the fetus thereby causing Congenital Heart Defects in at-risk pregnancies. The first objective was to use a novel reporter of O2 concentrations, the ODDLuc mouse, to determine the dose-response relationship between hypoxia and reduced O2 delivery to the fetus. The second objective was to develop a novel mouse model of placental insufficiency to examine how maternal-fetal and gene-environment interactions may influence the effect of hypoxia on the developing heart.

15. SUBJECT TERMS

Pregnancy, high altitude, hypoxia, congenital heart defects, placenta, Hypoxia-inducible factor, mouse models

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1. INTRODUCTION:

The idea for this proposal is that acute high altitude hypoxic exposure early in pregnancy during a critical period of organogenesis may critically reduce O2 transport to the fetus thereby causing Congenital Heart Defects in at-risk pregnancies. The first objective was to use a novel reporter of O2 concentrations, the ODDLuc mouse, to determine the dose-response relationship between hypoxia and reduced O2 delivery to the fetus. The second objective was to develop a novel mouse model of placental insufficiency to examine how maternal-fetal and gene-environment interactions may influence the effect of hypoxia on the developing heart.

2. KEYWORDS:

Pregnancy, high altitude, hypoxia, congenital heart defects, placenta, Hypoxia-inducible factor, mouse models

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Experiment 1: O_2 deprivation modeling High Altitude (HA) hypoxia is examined in fetal heart vs other tissues. Pregnant ODD-Luc dams age 3-6 months will inhale O_2 concentrations ranging from 8-21% O_2 x 4 h, equivalent to O_2 in air at altitudes from 25,000-4,000 ft elevation. ODDluc activity is measures in the fetal tissues as an index of hypoxic stress. **Experiment 2A:** Will test the effect of conditional inactivation of HIF-1α in the mother on the development of the placenta and the fetus. HIF1a +/+,BACTCre+ (Control) and HIF1a f/f BACTCre+ females (n=3 each) will be mated with ODDLuc/Luc males and injected with Tamoxifen 3mg/40g body IP to inactivate HIF1a in the maternal tissues, in particular the placenta. On E15 mice are euthanized and placenta and embryos examined for morphology and histology. **Experiment 2B:** To test the hypothesis that Placental insufficiency compromises O_2 reserve in the placenta and O_2 delivery to the fetus, HIF1a +/+,BACTCre+ (Control) and HIF1α f/f, BACTCre+ females will be crossed with ODD-Luc/Luc males, pregnant females treated with 3 mg/40 g body weight tamoxifen and exposed to acute hypoxia (8 or12 or 16% O_2 for 4 hrs) during different stages of pregnancy (e11, e15). Placental tissues are harvested, homogenized in lysis buffer and used for the measurement of luciferase activity and gene expression.

What was accomplished under these goals?

For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

This is the final report. The major goals of the proposal were accomplished. The significant results and conclusions are as follows:

1) The sensitivity of the embryo to reductions in the mother's supply of O2 declines from Embryonic Day 9-15 of mouse development (of a 20 day gestation). The relationship between reductions in inspired O2 and fetal organ oxygenation is linear between 8-16% inspired O2. This corresponds to elevations of 25,000-7000 feet. The hypoxic stress placed on the embryo organs (heart, liver, brain) in a normal pregnancy by breathing 12-16% O2 (equivalent to 14K-7K feet) is relatively modest (~1.5-fold increase).
2) Placental insufficiency, in these experiments induced by knock-out of Hypoxia-Inducible factor 1a (HIF1a) in the pregnant dam, increases sensitivity to O2 deprivation specifically at mid-gestation (E13-15). In this instance, when maternal inspired O2 is reduce to 8 or 12% (elevation of 25K or 14K, respectively), there is a doubling of the induction of the hypoxia reporter as compared to what happens in a normal pregnancy. This suggests that in the setting of early onset placental insufficiency the embryo is particularly vulnerable to reductions in the supply of O2 coming from the mother.
3) The combined stress of placental insufficiency plus reduction in inspired O2 did not result in congenital heart defects. It did appear to have some generalized adverse effect on the embryo including delayed growth and even some non-viable embryos. However specific heart defects modeling human congenital heart defects were not observed.

What opportunities for training and professional development has the project provided?

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Dr Doreswamy Kenchegowda was supported by this project and received significant training and professional development under the mentorship of the PI, Dr. Steven A. Fisher. H learned about placental-fetal interactions during normal development, the role of tissue hypoxia in normal pregnancy and hypoxic stress in placental insufficiency and congenital heart defects. He executed these experiments, presented the data at the annual Weinstein Conference on heart development (2015), and was the 1st author on a paper that described a portion of the data from this project (*Developmental Biology* 2017).

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

The data was presented at the annual Weinstein Conference on heart development (2015), the premier meeting in this field. A portion of the data was also recently published in the journal *Developmental Biology* (2017).

What do you plan to do during the next reporting period to accomplish the goals?

Nothing to report.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report." Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

First, our studies have a novel application of a tool for monitoring oxygen (O2) levels during the development of the embryo in utero. This is the use of a "hypoxia reporter" mouse, described in shorthand as the ODDLuc mouse. In this hypoxia reporter mouse light generated from a modified firefly luciferase protein is used as an indirect indicator of tissue O2 concentrations. Measuring tissue O2 in vivo and its sufficiency is highly problematic. This technology fills that void.
Second, our studies define novel mechanisms of feto-placental coupling that impact the development of the fetus. IN this case we showed that relative lack of O2 (tissue hypoxia) provides information required for the morphogenesis of both the maternal placenta and fetus. The two are thus linked in this way. Defective response to the tissue hypoxia can thus have adverse effect on placental development, and thus the pregnancy, and the fetus.

What was the impact on other disciplines?

Other disciplines are likely going to want to use the ODDLuc mouse to report on tissue O2 concentrations, e.g. in cancer biology, brain and other traumatic injuries, and others.

What was the impact on technology transfer?

If there is nothing significant to report during this reporting period, state "Nothing to Report." Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to report

What was the impact on society beyond science and technology?

If there is nothing significant to report during this reporting period, state "Nothing to Report." Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

These studies suggest that women should not be exposed to high altitude hypoxia (above 7000 ft) in early-to-mid pregnancy. According to these studies in mouse models this could have adverse effects on the pregnancy and fetal development. Further studies of humans are warranted.

5. **CHANGES/PROBLEMS:** The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:

Nothing to report

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

Changes that had a significant impact on expenditures
Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee

(or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

Significant changes in use of biohazards and/or select agents

6. PRODUCTS: List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

1. Kenchegowda D, Natale B, Lemus MA, Natale DR and **Fisher SA** Inactivation of maternal HIF-1α at mid-pregnancy causes placental defects and deficits in oxygen delivery to the fetal organs under hypoxic stress. *Developmental Biology*, 2017:422: 171-185 PMID: 27940158 PMCID: PMC5303635 Support from Dept of Defense and NIH were acknowledged.

Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Weinstein Conference 2015 Durham, NC Poster presentation.

- **Website(s) or other Internet site(s)**
  List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to report.

- **Other Products**
  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:
  - *data or databases;*
  - *physical collections;*
  - *audio or video products;*
  - *software;*
  - *models;*
  - *educational aids or curricula;*
  - *instruments or equipment;*
  - *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
  - *clinical interventions;*
  - *new business creation; and*
  - *other.*

Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Name:	*David Natale, PhD*
Project Role:	*Co-Investigator*
Researcher Identifier:	orcid.org/0000-0001-8365-455X
Nearest person month worked:	*2*
Contribution to Project:	*Assays of placental histology, interpretation/collation of data*
Funding Support:	Canadian Institutes of Health Research Operating Grant: 201203MOP-275374-CIA-CBBA-109624
Name:	*Bryony Natale, BSc*
Project Role:	*Research Associate*
Researcher Identifier:	
Nearest person month worked:	*2*
Contribution to Project:	*Assays of placental histology, interpretation/collation of data*
Funding Support:	Canadian Institutes of Health Research Operating Grant: 201203MOP-275374-CIA-CBBA-109624
Name:	*Maria Lemus, BSc*
Project Role:	*Undergraduate student*
Researcher Identifier:	
Nearest person month worked:	*4*
Contribution to Project:	*Assays of placental histology*
Funding Support:	Canadian Institutes of Health Research Operating Grant: 201203MOP-275374-CIA-CBBA-109624

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Nothing to report

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:
<u>Organization Name:</u>
<u>Location of Organization: (if foreign location list country)</u>
<u>Partner's contribution to the project</u> (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

Organization Name: University of California San Diego
Location of Organization: San Diego
Partner's contribution to the project : Collaboration

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: N/A

QUAD CHARTS: N/A

9. APPENDICES: N/A