PI: [redacted]	Title: [redacted]	
Received: [redacted]	FOA: [redacted]	Council: [redacted]
Competition ID: ADOBE-FORMS-B1	FOA Title: Mentored Clinical Scientist Research Career Development Award (Parent K08)	
[redacted]	Dual: DK	[redacted]
[redacted]	Organization: [redacted]	
Former Number:	Department: Grant &Contract Administration	
[redacted]	AIDS: N	Expedited: N
Subtotal Direct Costs (excludes consortium F&A) Year 1: 143,350 Year 2: 143,800 Year 3: 144,250 Year 4: 144,700 Year 5: 145,150	Animals: N Humans: Y Clinical Trial: N Current HS Code: 44 HESC: N	New Investigator: N Early Stage Investigator: N
Senior/Key Personnel:	*Organization:*	*Role Category:*
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]

Reference Letters

[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]

OMB Number: [redacted]

APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)

3. DATE RECEIVED BY STATE

State Application Identifier

1. * TYPE OF SUBMISSION

☐ Pre-application ☐ Application ☒ Changed/Corrected Application

2. DATE SUBMITTED

Applicant Identifier [redacted]

4. a. Federal Identifier [redacted]

b. Agency Routing Identifier

5. APPLICANT INFORMATION

*** Organizational DUNS:** [redacted]

* Legal Name: [redacted]

Department: [redacted] Division: [redacted]

* Street1: [redacted]

Street2: [redacted]

* City: [redacted] County / Parish:

* State: [redacted] Province:

* Country: [redacted] * ZIP / Postal Code: [redacted]

Person to be contacted on matters involving this application

Prefix: * First Name: [redacted] Middle Name:

* Last Name: [redacted] Suffix:

* Phone Number: [redacted] Fax Number: [redacted]

Email: [redacted]

6. * EMPLOYER IDENTIFICATION *(EIN) or (TIN):* [redacted]

7. * TYPE OF APPLICANT: [redacted]

Other (Specify):

Small Business Organization Type ☐ Women Owned ☐ Socially and Economically Disadvantaged

8. * TYPE OF APPLICATION:

☒ New ☐ Resubmission

☐ Renewal ☐ Continuation ☐ Revision

If Revision, mark appropriate box(es).

☐ A. Increase Award ☐ B. Decrease Award ☐ C. Increase Duration ☐ D. Decrease Duration

☐ E. Other (specify):

* Is this application being submitted to other agencies? Yes ☐ No ☒ What other Agencies?

9. * NAME OF FEDERAL AGENCY: [redacted]

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER:

TITLE:

11. * DESCRIPTIVE TITLE OF APPLICANT'S PROJECT: [redacted]

12. PROPOSED PROJECT:

* Start Date [redacted] * Ending Date [redacted]

*** 13. CONGRESSIONAL DISTRICT OF APPLICANT** [redacted]

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: [redacted] * First Name: [redacted] Middle Name: [redacted]

* Last Name: [redacted] Suffix: [redacted]

Position/Title: [redacted]

* Organization Name: [redacted]

Department: [redacted] Division: [redacted]

* Street1: [redacted]

Street2: [redacted]

* City: [redacted] County / Parish:

* State: [redacted] Province:

* Country: [redacted] * ZIP / Postal Code: [redacted]

* Phone Number: [redacted] Fax Number:

* Email: [redacted]

Tracking Number: [redacted]

15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested	778,950.00
b. Total Non-Federal Funds	0.00
c. Total Federal & Non-Federal Funds	778,950.00
d. Estimated Program Income	0.00

16. * IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS?

a. YES ☐ THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON:

DATE:

b. NO ☒ PROGRAM IS NOT COVERED BY E.O. 12372; OR

☐ PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

17. By signing this application, I certify (1) to the statements contained in the list of certifications* and (2) that the statements herein are true, complete and accurate to the best of my knowledge. I also provide the required assurances * and agree to comply with any resulting terms if I accept an award. I am aware that any false, fictitious. or fraudulent statements or claims may subject me to criminal, civil, or administrative penalities. (U.S. Code, Title 18, Section 1001)

☒ *** I agree**

**** The list of certifications and assurances, or an Internet site where you may obtain this list, is contained in the announcement or agency specific instructions.***

18. SFLLL or other Explanatory Documentation

Add Attachment | Delete Attachment | View Attachment

19. Authorized Representative

Prefix: * First Name: [redacted] Middle Name:

* Last Name: [redacted] Suffix:

* Position/Title: [redacted]

* Organization: [redacted]

Department: [redacted] Division: [redacted]

* Street1: [redacted]

Street2: [redacted]

* City: [redacted] County / Parish:

* State: [redacted] Province:

* Country: [redacted] * ZIP / Postal Code: [redacted]

* Phone Number: [redacted] Fax Number: [redacted]

* Email: [redacted]

*** Signature of Authorized Representative** [redacted]

*** Date Signed** [redacted]

20. Pre-application Add Attachment | Delete Attachment | View Attachment

424 R&R and PHS-398 Specific
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OMB Number: [redacted]
Expiration Date: [redacted]

Project/Performance Site Location(s)

Project/Performance Site Primary Location

☐ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name: [redacted]

DUNS Number: [redacted]

* Street1: [redacted]

Street2: [redacted]

* City: [redacted] County:

* State: [redacted]

Province:

* Country: [redacted]

* ZIP / Postal Code: [redacted] * Project/ Performance Site Congressional District: [redacted]

Project/Performance Site Location 1

☐ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name:

DUNS Number:

* Street1:

Street2:

* City: County:

* State:

Province:

* Country: [redacted]

* ZIP / Postal Code: * Project/ Performance Site Congressional District:

Additional Location(s) Add Attachment Delete Attachment View Attachment

RESEARCH & RELATED Other Project Information

1. * Are Human Subjects Involved? ☐ Yes ☒ No

1.a If YES to Human Subjects

Is the Project Exempt from Federal regulations? ☐ Yes ☐ No

If yes, check appropriate exemption number. ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6

If no, is the IRB review Pending? ☐ Yes ☐ No

IRB Approval Date:

Human Subject Assurance Number:

2. * Are Vertebrate Animals Used? ☐ Yes ☒ No

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ☐ Yes ☐ No

IACUC Approval Date:

Animal Welfare Assurance Number

3. * Is proprietary/privileged information included in the application? ☐ Yes ☒ No

4.a. * Does this project have an actual or potential impact on the environment? ☐ Yes ☒ No

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ☐ Yes ☐ No

4.d. If yes, please explain:

5. * Is the research performance site designated, or eligible to be designated, as a historic place? ☐ Yes ☒ No

5.a. If yes, please explain:

6. * Does this project involve activities outside of the United States or partnerships with international collaborators? ☐ Yes ☒ No

6.a. If yes, identify countries:

6.b. Optional Explanation:

7. * Project Summary/Abstract [redacted] Add Attachment Delete Attachment View Attachment

8. * Project Narrative [redacted] Add Attachment Delete Attachment View Attachment

9. Bibliography & References Cited [redacted] Add Attachment Delete Attachment View Attachment

10. Facilities & Other Resources [redacted] Add Attachment Delete Attachment View Attachment

11. Equipment Add Attachment Delete Attachment View Attachment

12. Other Attachments Add Attachments Delete Attachments View Attachments ☒

Project Summary/Abstract

[redacted] is a board-certified [redacted], who is pursuing a career in translational research with a specific focus on insulin resistance and reproductive pathology. Insulin resistance is an underlying pathology in obesity, the Polycystic Ovary Syndrome (PCOS), and Type 2 Diabetes (T2DM). [redacted] immediate research goal is to follow women with insulin resistance, and to determine whether their endometrial dysfunction, such as endometrial hyperplasia and infertility, is a consequence of the metabolic abnormalities occurring in insulin resistance. [redacted] is collaborating with [redacted] of [redacted] to examine a potential mechanism for how insulin may have a direct effect on endometrial tissue. [redacted] long-term goal is to contribute to a multi-disciplinary Women's Health research program at the [redacted].

Over the next five years, [redacted] will spend 85% of [redacted] time dedicated to developing research skills with mentorship from [redacted] and [redacted]. [redacted] will acquire more experience in basic molecular biology to apply to [redacted] project on the endometrium, as well as improving [redacted] knowledge in the design, conduct, and analysis of clinical research. [redacted] will benefit from the exposure to teaching faculty, association with a peer group, and didactic coursework that is provided through the [redacted] and the [redacted].

[redacted] proposes to study the effect of insulin on human endometrial cells, using techniques in molecular biology. Specifically, [redacted] will examine a potential mechanism for how insulin may augment estrogen action in the endometrium. Preliminary data shows that insulin activates its own signaling pathways, alters gene transcription, and promotes cellular proliferation of endometrial epithelial cells. It is hypothesized that insulin may induce a post-translational modification of the estrogen receptor, resulting in a change in gene transcription. This work will contribute to the understanding of insulin's role as a growth factor in the endometrium. Since the prevalence of obesity and Type 2 Diabetes is increasing in the United States, it is important to determine whether hyperinsulinemia contributes to endometrial pathology such as hyperplasia and impaired decidualization. The potential implications of this research may include recommendations for weight loss or the use of insulin sensitizers in the treatment of select endometrial pathology. Also, the treatment paradigm for women with Type 2 Diabetes may be changed to limit exposure to high doses of therapeutic insulin.

Project Narrative

Endometrial dysfunction commonly occurs in women with obesity, Polycystic Ovary Syndrome or Type 2 Diabetes Mellitus. These women have high levels of insulin, and insulin may contribute to irregular bleeding, implantation failure, miscarriage, endometrial hyperplasia, and endometrial carcinoma. This research looks at one way in which insulin may affect endometrial tissue.

Bibliography and References Cited

[redacted]

Facilities and Other Resources

The [redacted] has significant resources for the conduct of basic and clinical research. I will be able to perform the entirety of my project on campus. I have independent bench space within [redacted] fully equipped molecular biology laboratory of approximately 1200 square feet in the [redacted]. This is located in the [redacted], adjacent to the [redacted], which allows for the convenient, timely delivery of fresh tissue specimens. The lab space contains electrophoresis equipment, an RT-PCR machine, microscopes, multiple -4°, -20°, and -80° freezers, and a separate cell culture room with two hoods. I also have a desktop computer with internet access in an office within the [redacted].

For current studies, [redacted] has core laboratories for the processing of histological samples, cell sorting, and microarrays. The [redacted] provides genomic, proteomic, biostatistical, and high performance computing technologies. For future studies anticipated by the end of the five-year award period, [redacted] also has resources for both mouse *in vivo* studies and human clinical studies. The [redacted]-funded [redacted] [redacted] [redacted] [redacted] [redacted] has core resources in [redacted], Mouse Genetics, and Transgenic Mice. The [redacted] provides unique opportunities for studies in genetically altered mice. The [redacted] has a fully-equipped metabolic research unit with designated research nursing staff, where clinical studies can be run. The [redacted] also offers resources for biostatistics and bioinformatics, and a wide array of core resources (including imaging, genomics and proteomics, protein synthesis, high throughput chemical screening, metabolomics, etc.).

I am fortunate to be part of two departments, [redacted], which have established research programs and [redacted]-funded infrastructure for the training and education of junior research faculty. I will participate in training and coursework along with scholars of both the [redacted] and the [redacted]. These programs were designed to mentor junior faculty in their pursuit of a translational research career. I will benefit from the exposure to teaching faculty, association with a peer group, and didactic coursework that they provide. In addition, I will be required to demonstrate proficiency in the fundamentals of research, and meet specific bench-marks in preparing for an independent research career. I also have the opportunity to take additional classes in any of the [redacted] associated departments such as the [redacted]. [redacted] oversees best practices training. With the guarantee of protected research time from the [redacted], I will be immersing myself in supported research training over the next five years.

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

OMB Number: [redacted]
Expiration Date: [redacted]

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: [redacted] * First Name: [redacted] Middle Name: [redacted]
* Last Name: [redacted] Suffix: [redacted]
Position/Title: [redacted] Department: [redacted]
Organization Name: [redacted] Division: [redacted]
* Street1: [redacted]
Street2: [redacted]
* City: [redacted] County/ Parish:
* State: [redacted] Province:
* Country: [redacted] * Zip / Postal Code: [redacted]
* Phone Number: [redacted] Fax Number:
* E-Mail: [redacted]

Credential, e.g., agency login: [redacted]

*** Project Role:** [redacted] **Other Project Role Category:**

[redacted]

***Attach Biographical Sketch** [redacted] Add Attachment Delete Attachment View Attachment
Attach Current & Pending Support Add Attachment Delete Attachment View Attachment

[redacted]

[redacted]

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Senior/Key Person 2

Prefix: [redacted] * First Name: [redacted] Middle Name:

* Last Name: [redacted] Suffix:

Position/Title: [redacted] Department: [redacted]

Organization Name: [redacted] Division: [redacted]

* Street1: [redacted]

Street2: [redacted]

* City: [redacted] County/ Parish:

* State: [redacted] Province:

* Country: [redacted] * Zip / Postal Code: [redacted]

* Phone Number: [redacted] Fax Number: [redacted]

* E-Mail: [redacted]

Credential, e.g., agency login: [redacted]

*** Project Role:** Other (Specify) **Other Project Role Category:** [redacted]

Degree Type:

Degree Year:

***Attach Biographical Sketch** [redacted] Add Attachment Delete Attachment View Attachment

Attach Current & Pending Support [redacted] Add Attachment Delete Attachment View Attachment

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
[redacted]	[redacted]
eRA COMMONS USER NAME (credential, e.g., agency login)	
[redacted]	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]		[redacted]	[redacted]
[redacted]		[redacted]	[redacted]

A. Personal Statement

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

D. Research Support

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

Other Support

[redacted]

Active

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

Role on project: [redacted]

[redacted]

Role on Project: [redacted].

[redacted]

Role on Project: [redacted]

Principal Investigator/Program Director: [redacted]

For New and Competing Applications (PHS 398) – DO NOT SUBMIT UNLESS REQUESTED
For Non-competing Progress Reports (PHS 2590) – Submit only Active Support for Key Personnel

PHS 398/2590 OTHER SUPPORT

[redacted]

ACTIVE:

[redacted]

Principal Investigator/Program Director: [redacted]

[redacted]

[redacted]

[redacted]

OMB Number: [redacted]
Expiration Date: [redacted]

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 1

*** ORGANIZATIONAL DUNS:** [redacted]

*** Budget Type:** ☒ Project ☐ Subaward/Consortium

Enter name of Organization: [redacted]

Delete Entry *** Start Date:** [redacted] *** End Date:** [redacted] **Budget Period** 1

A. Senior/Key Person

	Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary ($)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
1.	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	92,700.00	1.65			90,000.00	28,350.00	118,350.00
2.													
3.													
4.													
5.													
6.													
7.													
8.													

9. **Total Funds requested for all Senior Key Persons in the attached file**

Total Senior/Key Person 118,350.00

Additional Senior Key Persons: Add Attachment Delete Attachment View Attachment

B. Other Personnel

* Number of Personnel	* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
	Total Number Other Personnel					**Total Other Personnel**	

Total Salary, Wages and Fringe Benefits (A+B) 118,350.00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 1

[redacted]

* Budget Type: ☒ [redacted] ☐ [redacted]

Enter name of Organization: [redacted]

Delete Entry * Start Date: [redacted] * End Date: [redacted] Budget Period 1

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

	Equipment item	* Funds Requested ($)
1.		
2.		
3.		
4.		
5.		
6.		
7.		
8.		
9.		
10.		
11.	**Total funds requested for all equipment listed in the attached file**	
	Total Equipment	

[redacted] Add Attachment Delete Attachment View Attachment

D. Travel		**Funds Requested ($)**
1.	Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	
2.	Foreign Travel Costs	
	Total Travel Cost	

E. Participant/Trainee Support Costs		**Funds Requested ($)**
1.	Tuition/Fees/Health Insurance	
2.	Stipends	
3.	Travel	
4.	Subsistence	
5.	Other	
	Number of Participants/Trainees **Total Participant/Trainee Support Costs**	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION F-K, BUDGET PERIOD 1

Next Period

*** ORGANIZATIONAL DUNS:** [redacted]

*** Budget Type:** ☒ Project ☐ Subaward/Consortium

Enter name of Organization: [redacted]

Delete Entry *** Start Date:** [redacted] *** End Date:** [redacted] **Budget Period 1**

F. Other Direct Costs	Funds Requested ($)
1. Materials and Supplies	25,000.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8.	
9.	
10.	
Total Other Direct Costs	25,000.00

G. Direct Costs	Funds Requested ($)
Total Direct Costs (A thru F)	143,350.00

H. Indirect Costs

	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1.	Modified Total Direct Cost (MTDC)	8.00	143,350.00	11,468.00
2.				
3.				
4.				
	Total Indirect Costs			11,468.00

Cognizant Federal Agency [redacted]

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	154,818.00

J. Fee	Funds Requested ($)

K. * Budget Justification 1240-CF_Budget_Justification.pdf

Add Attachment Delete Attachment View Attachment

(Only attach one file.)

Principal Investigator/Program Director (Last, first, middle): [redacted]

OMB Number: [redacted]
Expiration Date: 0[redacted]

Previous Period

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 2

*** ORGANIZATIONAL DUNS:** [redacted]

*** Budget Type:** ☒ Project ☐ Subaward/Consortium

Enter name of Organization: [redacted]

Delete Entry *** Start Date:** [redacted] *** End Date:** [redacted] **Budget Period** 2

A. Senior/Key Person

	Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary ($)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
1.	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	92,700.00	11.65			90,000.00	28,800.00	118,800.00
2.													
3.													
4.													
5.													
6.													
7.													
8.													

9. **Total Funds requested for all Senior Key Persons in the attached file**

Total Senior/Key Person 118,800.00

Additional Senior Key Persons: Add Attachment Delete Attachment View Attachment

B. Other Personnel

* Number of Personnel	* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
	Total Number Other Personnel					**Total Other Personnel**	

Total Salary, Wages and Fringe Benefits (A+B) 118,800.00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 2

*** ORGANIZATIONAL DUNS:** [redacted]

*** Budget Type:** ☒ Project ☐ Subaward/Consortium

Enter name of Organization: [redacted]

Delete Entry *** Start Date:** [redacted] *** End Date:** [redacted] **Budget Period 2**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

	Equipment item	* Funds Requested ($)
1.		
2.		
3.		
4.		
5.		
6.		
7.		
8.		
9.		
10.		
11.	**Total funds requested for all equipment listed in the attached file**	
	Total Equipment	

Additional Equipment: Add Attachment Delete Attachment View Attachment

D. Travel		**Funds Requested ($)**
1.	Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	
2.	Foreign Travel Costs	
	Total Travel Cost	

E. Participant/Trainee Support Costs		**Funds Requested ($)**
1.	Tuition/Fees/Health Insurance	
2.	Stipends	
3.	Travel	
4.	Subsistence	
5.	Other	
	Number of Participants/Trainees **Total Participant/Trainee Support Costs**	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION F-K, BUDGET PERIOD 2

Next Period

*** ORGANIZATIONAL DUNS:** [redacted]

*** Budget Type:** ☒ Project ☐ Subaward/Consortium

Enter name of Organization: [redacted]

Delete Entry *** Start Date:** [redacted] *** End Date:** 0[redacted] **Budget Period 2**

F. Other Direct Costs	Funds Requested ($)
1. Materials and Supplies	25,000.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8.	
9.	
10.	
Total Other Direct Costs	25,000.00

G. Direct Costs	Funds Requested ($)
Total Direct Costs (A thru F)	143,800.00

H. Indirect Costs

	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1.	Modified Total Direct Cost (MTDC)	8.00	143,800.00	11,504.00
2.				
3.				
4.				
	Total Indirect Costs			11,504.00

Cognizant Federal Agency [redacted]

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	155,304.00

J. Fee	Funds Requested ($)

K. * Budget Justification 1240-CF_Budget_Justification.pdf

Add Attachment Delete Attachment View Attachment

(Only attach one file.)

Previous Period

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 3

*** ORGANIZATIONAL DUNS:** [redacted]

*** Budget Type:** ☒ Project ☐ Subaward/Consortium

Enter name of Organization: [redacted]

Delete Entry *** Start Date:** [redacted] *** End Date:** [redacted] **Budget Period** 3

A. Senior/Key Person

	Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary ($)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
1.	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	92,700.00	11.65			90,000.00	29,250.00	119,250.00
2.													
3.													
4.													
5.													
6.													
7.													
8.													

9. **Total Funds requested for all Senior Key Persons in the attached file**

Total Senior/Key Person 119,250.00

Additional Senior Key Persons: Add Attachment Delete Attachment View Attachment

B. Other Personnel

* Number of Personnel	* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
	Total Number Other Personnel					**Total Other Personnel**	

Total Salary, Wages and Fringe Benefits (A+B) 119,250.00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 3

*** ORGANIZATIONAL DUNS:** [redacted]

*** Budget Type:** ☒ Project ☐ Subaward/Consortium

Enter name of Organization: [redacted]

Delete Entry *** Start Date:** [redacted] *** End Date:** [redacted] **Budget Period 3**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

	Equipment item	* Funds Requested ($)
1.		
2.		
3.		
4.		
5.		
6.		
7.		
8.		
9.		
10.		
11.	**Total funds requested for all equipment listed in the attached file**	
	Total Equipment	

Additional Equipment: Add Attachment Delete Attachment View Attachment

D. Travel		**Funds Requested ($)**
1.	Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	
2.	Foreign Travel Costs	
	Total Travel Cost	

E. Participant/Trainee Support Costs		**Funds Requested ($)**
1.	Tuition/Fees/Health Insurance	
2.	Stipends	
3.	Travel	
4.	Subsistence	
5.	Other	
	Number of Participants/Trainees **Total Participant/Trainee Support Costs**	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION F-K, BUDGET PERIOD 3

Next Period

[redacted]

* Budget Type: ☒ [redacted] ☐ [redacted]

Enter name of Organization: [redacted]

Delete Entry * Start Date: [redacted] * [redacted] [redacted]

F. Other Direct Costs	Funds Requested ($)
1. Materials and Supplies	25,000.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8.	
9.	
10.	
Total Other Direct Costs	25,000.00

G. Direct Costs	Funds Requested ($)
Total Direct Costs (A thru F)	144,250.00

H. Indirect Costs

	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1.	Modified Total Direct Cost (MTDC)	8.00	144,250.00	11,540.00
2.				
3.				
4.				
	Total Indirect Costs			11,540.00

Cognizant Federal Agency [redacted]

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	155,790.00

J. Fee	Funds Requested ($)

K. * Budget Justification 1240-CF_Budget_Justification.pdf

[redacted] Attachment | Delete Attachment | View Attachment

(Only attach one file.)

Principal Investigator/Program Director (Last, first, middle): [redacted]

OMB Number: [redacted]
Expiration Date: [redacted]

Previous Period

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 4

*** ORGANIZATIONAL DUNS:** [redacted]

*** Budget Type:** ☒ Project ☐ Subaward/Consortium

Enter name of Organization: [redacted]

Delete Entry *** Start Date:** [redacted] *** End Date:** [redacted] **Budget Period** 4

A. Senior/Key Person

	Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary ($)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
1.	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	92,700.00	11.65			90,000.00	29,700.00	119,700.00
2.													
3.													
4.													
5.													
6.													
7.													
8.													

9. **Total Funds requested for all Senior Key Persons in the attached file**

Total Senior/Key Person 119,700.00

Additional Senior Key Persons: Add Attachment Delete Attachment View Attachment

B. Other Personnel

* Number of Personnel	* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
	Total Number Other Personnel					**Total Other Personnel**	

Total Salary, Wages and Fringe Benefits (A+B) 119,700.00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 4

*** ORGANIZATIONAL DUNS:** [redacted]

*** Budget Type:** ☒ Project ☐ Subaward/Consortium

Enter name of Organization: [redacted]

Delete Entry *** Start Date** [redacted] *** End Date** [redacted] **Budget Period 4**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

	Equipment item	* Funds Requested ($)
1.		
2.		
3.		
4.		
5.		
6.		
7.		
8.		
9.		
10.		
11.	**Total funds requested for all equipment listed in the attached file**	
	Total Equipment	

Additional Equipment: Add Attachment Delete Attachment View Attachment

D. Travel		**Funds Requested ($)**
1.	Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	
2.	Foreign Travel Costs	
	Total Travel Cost	

E. Participant/Trainee Support Costs		**Funds Requested ($)**
1.	Tuition/Fees/Health Insurance	
2.	Stipends	
3.	Travel	
4.	Subsistence	
5.	Other	
	Number of Participants/Trainees **Total Participant/Trainee Support Costs**	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION F-K, BUDGET PERIOD 4

Next Period

*** ORGANIZATIONAL DUNS:** [redacted]

*** Budget Type:** ☒ Project ☐ Subaward/Consortium

Enter name of Organization: [redacted]

Delete Entry *** Start Date:** [redacted] *** End Date:** [redacted] **Budget Period 4**

F. Other Direct Costs	Funds Requested ($)
1. Materials and Supplies	25,000.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8.	
9.	
10.	
Total Other Direct Costs	25,000.00

G. Direct Costs	Funds Requested ($)
Total Direct Costs (A thru F)	144,700.00

H. Indirect Costs

	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1.	Modified Total Direct Cost (MTDC)	8.00	144,700.00	11,576.00
2.				
3.				
4.				
	Total Indirect Costs			11,576.00

Cognizant Federal Agency [redacted]

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	156,276.00

J. Fee	Funds Requested ($)

K. * Budget Justification 1240-CF_Budget_Justification.pdf

Add Attachment Delete Attachment View Attachment

(Only attach one file.)

Previous Period

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 5

*** ORGANIZATIONAL DUNS:** [redacted]

*** Budget Type:** ☒ Project ☐ Subaward/Consortium

Enter name of Organization: [redacted]

Delete Entry *** Start Date:** [redacted] *** End Date:** [redacted] **Budget Period** 5

A. Senior/Key Person

	Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary ($)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
1.	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	92,700.00	11.65			90,000.00	30,150.00	120,150.00
2.													
3.													
4.													
5.													
6.													
7.													
8.													

9. **Total Funds requested for all Senior Key Persons in the attached file**

Total Senior/Key Person 120,150.00

Additional Senior Key Persons: Add Attachment Delete Attachment View Attachment

B. Other Personnel

* Number of Personnel	* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
	Total Number Other Personnel					**Total Other Personnel**	

Total Salary, Wages and Fringe Benefits (A+B) 120,150.00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 5

*** ORGANIZATIONAL DUNS:** [redacted]

*** Budget Type:** ☒ Project ☐ Subaward/Consortium

Enter name of Organization: [redacted]

Delete Entry *** Start Date:** [redacted] *** End Date:** [redacted] **Budget Period 5**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

	Equipment item	* Funds Requested ($)
1.		
2.		
3.		
4.		
5.		
6.		
7.		
8.		
9.		
10.		
11.	**Total funds requested for all equipment listed in the attached file**	
	Total Equipment	

Additional Equipment: Add Attachment Delete Attachment View Attachment

D. Travel		**Funds Requested ($)**
1.	Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	
2.	Foreign Travel Costs	
	Total Travel Cost	

E. Participant/Trainee Support Costs		**Funds Requested ($)**
1.	Tuition/Fees/Health Insurance	
2.	Stipends	
3.	Travel	
4.	Subsistence	
5.	Other	
	Number of Participants/Trainees **Total Participant/Trainee Support Costs**	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION F-K, BUDGET PERIOD 5

*** ORGANIZATIONAL DUNS:** [redacted]

*** Budget Type:** ☒ Project ☐ Subaward/Consortium

Enter name of Organization: [redacted]

Delete Entry *** Start Date:** [redacted] *** End Date:** [redacted] **Budget Period 5**

F. Other Direct Costs	Funds Requested ($)
1. Materials and Supplies	25,000.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8.	
9.	
10.	
Total Other Direct Costs	25,000.00

G. Direct Costs	Funds Requested ($)
Total Direct Costs (A thru F)	145,150.00

H. Indirect Costs

	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1.	Modified Total Direct Cost (MTDC)	8.00	145,150.00	11,612.00
2.				
3.				
4.				
	Total Indirect Costs			11,612.00

Cognizant Federal Agency [redacted]

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	156,762.00

J. Fee	Funds Requested ($)

K. * Budget Justification 1240-CF_Budget_Justification.pdf

Add Attachment | Delete Attachment | View Attachment

(Only attach one file.)

Budget Justification:

Personnel:
[redacted], will oversee all direction and management of this project. 75 % percent effort will be required.

[redacted], assumes the roles of primary mentor and research advisory board member. [redacted] will contribute [redacted] experience and expertise in the field of molecular biology and reproductive pathology to the candidate's education and research efforts. Frequent meetings are going to verify training progress and adequate study design.

[redacted] will be a co-mentor and research advisory board member for [redacted]. [redacted]. [redacted] will lend [redacted] experience and exptertise in the field of diabetes research and metabolism. [redacted] will review and discuss experimental design and subsequent data evaluation with the candidate.

Lab Costs: Materials and supplies for the proposed experiments include general lab reagents, cell culture plates and media, electrophoresis gels, antibodies, cDNA reverse transcriptase, RT-PCR related reagents and primers, microarray chips and processing, luciferase assay and ChIP assay reagents.

RESEARCH & RELATED BUDGET - Cumulative Budget

		Totals ($)
Section A, Senior/Key Person		596,250.00
Section B, Other Personnel		
Total Number Other Personnel		
Total Salary, Wages and Fringe Benefits (A+B)		596,250.00
Section C, Equipment		
Section D, Travel		
1. Domestic		
2. Foreign		
Section E, Participant/Trainee Support Costs		
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other		
6. Number of Participants/Trainees		
Section F, Other Direct Costs		125,000.00
1. Materials and Supplies	125,000.00	
2. Publication Costs		
3. Consultant Services		
4. ADP/Computer Services		
5. Subawards/Consortium/Contractual Costs		
6. Equipment or Facility Rental/User Fees		
7. Alterations and Renovations		
8. Other 1		
9. Other 2		
10. Other 3		
Section G, Direct Costs (A thru F)		721,250.00
Section H, Indirect Costs		57,700.00
Section I, Total Direct and Indirect Costs (G + H)		778,950.00
Section J, Fee		

PHS 398 Cover Page Supplement

OMB Number: [redacted]

1. Project Director / Principal Investigator (PD/PI)

Prefix: [redacted]
* First Name: [redacted]
Middle Name: [redacted]
* Last Name: [redacted]
Suffix: [redacted]

2. Human Subjects

Clinical Trial? ☒ No ☐ Yes

* Agency-Defined Phase III Clinical Trial? ☐ No ☐ Yes

3. Applicant Organization Contact

Person to be contacted on matters involving this application

Prefix:
* First Name: [redacted]
Middle Name:
* Last Name: [redacted]
Suffix:

* Phone Number: [redacted]
Fax Number: [redacted]
Email: [redacted]

* Title: [redacted]

* Street1: [redacted]
Street2:
* City: [redacted]
County/Parish:
* State: [redacted]
Province:
* Country: [redacted]
* Zip / Postal Code: [redacted]

PHS 398 Cover Page Supplement

4. Human Embryonic Stem Cells

* Does the proposed project involve human embryonic stem cells? ☒ No ☐ Yes

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://stemcells.nih.gov/research/registry/. Or, if a specific stem cell line cannot be referenced at this time, please check the box indicating that one from the registry will be used:

Cell Line(s): ☐ Specific stem cell line cannot be referenced at this time. One from the registry will be used.

PHS 398 Checklist

OMB Number: [redacted]

1. Application Type:

From SF 424 (R&R) Cover Page. The responses provided on the R&R cover page are repeated here for your reference, as you answer the questions that are specific to the PHS398.

* Type of Application:

☒ New ☐ Resubmission ☐ Renewal ☐ Continuation ☐ Revision

Federal Identifier: [redacted]

2. Change of Investigator / Change of Institution Questions

☐ Change of principal investigator / program director

Name of former principal investigator / program director:

Prefix:
* First Name:
Middle Name:
* Last Name:
Suffix:

☐ Change of Grantee Institution

* Name of former institution:

3. Inventions and Patents (For renewal applications only)

* Inventions and Patents: Yes ☐ No ☐

If the answer is "Yes" then please answer the following:

* Previously Reported: Yes ☐ No ☐

4. * Program Income

Is program income anticipated during the periods for which the grant support is requested?

☐ Yes ☒ No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

*Budget Period	*Anticipated Amount ($)	*Source(s)

5. * Disclosure Permission Statement

If this application does not result in an award, is the Government permitted to disclose the title of your proposed project, and the name, address, telephone number and e-mail address of the official signing for the applicant organization, to organizations that may be interested in contacting you for further information (e.g., possible collaborations, investment)?

☒ Yes ☐ No

PHS 398 Career Development Award Supplemental Form

OMB Number: [redacted]

1. Application Type:

From SF424 (R&R) Cover Page. The response provided on that page, regarding the type of application being submitted, is repeated here for your reference, as you attach the sections that are appropriate for this Career Development Award.

☒ New ☐ Resubmission ☐ Renewal ☐ Continuation ☐ Revision

2. Career Development Award Attachments:

Please attach applicable sections, below.

Introduction (if applicable)				
1. Introduction to Application *(for RESUBMISSION applications only)*		Add Attachment	Delete Attachment	View Attachment
Candidate Information				
2. Candidate's Background	[redacted]	Add Attachment	Delete Attachment	View Attachment
3. Career Goals and Objectives	[redacted]	Add Attachment	Delete Attachment	View Attachment
4. Career Development/Training Activities During Award Period	[redacted]	Add Attachment	Delete Attachment	View Attachment
5. Training in the Responsible Conduct of Research	[redacted]	Add Attachment	Delete Attachment	View Attachment
6. Mentoring Plan *(when applicable)*		Add Attachment	Delete Attachment	View Attachment
Statements of Support				
7. Statements by Mentor, Co-Mentors, Consultants, Contributors *(as appropriate)*	[redacted]	Add Attachment	Delete Attachment	View Attachment
Environment and Institutional Commitment to Candidate				
8. Description of Institutional Environment	[redacted]	Add Attachment	Delete Attachment	View Attachment
9. Insitutional Commitment to Candidate's Research Career Development	[redacted]	Add Attachment	Delete Attachment	View Attachment
Research Plan				
10. Specific Aims	[redacted]	Add Attachment	Delete Attachment	View Attachment
11. * Research Strategy	[redacted]	Add Attachment	Delete Attachment	View Attachment
12. Inclusion Enrollment Report *(for RENEWAL applications only)*		Add Attachment	Delete Attachment	View Attachment
13. Progress Report Publication List *(for RENEWAL applications only)*		Add Attachment	Delete Attachment	View Attachment
Human Subject Sections				
14. Protection of Human Subjects		Add Attachment	Delete Attachment	View Attachment
15. Inclusion of Women and Minorities		Add Attachment	Delete Attachment	View Attachment
16. Targeted/Planned Enrollment		Add Attachment	Delete Attachment	View Attachment
17. Inclusion of Children		Add Attachment	Delete Attachment	View Attachment

PHS 398 Career Development Award Supplemental Form

2. Career Development Award Attachments (continued):

Other Research Plan Sections

18. Vertebrate Animals		Add Attachment	Delete Attachment	View Attachment
19. Select Agent Research		Add Attachment	Delete Attachment	View Attachment
20. Consortium/Contractual Arrangements		Add Attachment	Delete Attachment	View Attachment
21. Resource Sharing Plan(s)		Add Attachment	Delete Attachment	View Attachment

Appendix (if applicable)

22. Appendix Add Attachments Delete Attachments View Attachments

3. * Citizenship:

- [x] U.S. Citizen or noncitizen national
- [ ] Permanent Resident of U.S.
  (If a permanent resident of the U.S., a notarized statement must be provided by the time of award)
- [ ] Permanent Resident of U.S. Pending
- [ ] Non-U.S. Citizen with temporary U.S. visa

Specific Aims

Endometrial dysfunction commonly occurs in women with obesity, Polycystic Ovary Syndrome (PCOS) and Type 2 Diabetes Mellitus (T2DM). High levels of insulin are apparent in women with these conditions, and may contribute to endometrial pathology. We propose to study a potential mechanism for how insulin may augment estrogen action in the endometrium. Our preliminary data shows that insulin activates the AKT and MAPK pathways to induce a post-translational modification of estrogen receptor alpha (ERα) in primary endometrial epithelial cells. Insulin also causes upregulation of NCOA3, a nuclear co-activator for ERα, and $p90^{RSK1}$, a kinase that phosphorylates serine 167 on ERα. We hypothesize that insulin-induced phosphorylation of ERα potentiates ER mediated-gene transcription. The identification of hyperinsulinemia as a contributor to endometrial dysfunction suggests that weight loss or the use of insulin sensitizers may benefit the treatment of select endometrial pathology.

Hypothesis: Hyperinsulinemia promotes endometrial dysfunction in women with obesity, PCOS, and T2DM. In human endometrial cells, insulin activates its own receptor (IRβ) and signaling pathways to phosphorylate ERα, which causes potentiation of gene transcription in either a ligand independent or estradiol-dependent fashion.

Aim 1: Is ERα phosphorylation and ERα coactivation mediated by insulin signaling?

a) To verify preliminary data showing insulin stimulation of primary endometrial epithelial cells results in increased expression of $p90^{RSK1}$ and phosphorylation of ERα at serine 167.
b) To confirm whether insulin stimulation of primary endometrial epithelial cells results in increased expression of NCOA3, a nuclear co-activator for ERα.
c) To elucidate which insulin signaling pathway, PI3K/AKT, MAPK, or mTOR is responsible for ERα phosphorylation.

Aim 2: Does insulin promote potentiation of ERα induced gene transcription?

a) To determine if insulin induced ERα phosphorylation results in enhanced binding to target estrogen response elements (ERE) and potentiation of gene transcription in primary epithelial cells.
b) To ascertain whether this occurs in either a ligand independent or estradiol-dependent fashion.

Aim 3: Do the molecular findings in cell culture from Aims 1 & 2 translate to tissue differences between women of different metabolic phenotypes? Are there differences in baseline activation of ERα and IRβ or presence of ERα coactivators in endometrial tissue from women with obesity, PCOS, or T2DM compared to lean women without these conditions?

a) To determine whether endometrium from women with obesity, PCOS, or T2DM have higher levels of phospho-167 ERα and phospho-IRβ than lean women without PCOS or T2DM.
b) To determine whether NCOA3 is upregulated in endometrial tissue from women with obesity, PCOS, or T2DM relative to lean women without PCOS or T2DM.

Research Strategy

Significance

Endometrial dysfunction commonly occurs in women with obesity, Polycystic Ovary Syndrome (PCOS), and Type 2 Diabetes Mellitus (T2DM). This includes irregular bleeding, implantation failure, miscarriage, endometrial hyperplasia, and endometrial carcinoma ([redacted], 2006). Chronic anovulation with unopposed estrogen stimulation is considered the classical pathophysiological mechanism for irregular bleeding, endometrial hyperplasia, and endometrial carcinoma. However, even PCOS women with ovulatory cycles have defective decidualization with impairment of receptivity markers ([redacted]k, 2003), indicating that there is another factor involved in endometrial dysfunction besides unopposed estrogen. T2DM and obesity are independent risk factors for endometrial cancer ([redacted], 2001). Hyperinsulinemia is common to women with obesity, PCOS, and T2DM, and may play a role in their endometrial dysfunction. Metformin, which reduces levels of insulin, is currently used off-label for women with PCOS, and has been shown to decrease rates of miscarriage as well as treat progestin-resistant endometrial hyperplasia in select case studies ([redacted], 2002; [redacted], 2003; [redacted], 2003).

Insulin affects estrogen action indirectly by decreasing liver synthesis of sex hormone binding globulin and upregulating adipose tissue aromatase activity. The net result is an increased amount of circulating bioavailable estrogen. In the endometrium, insulin receptors are present and cyclically regulated with maximum expression during the secretory phase [redacted] 2006). Insulin has three post-receptor signaling pathways: phosphatidylinositide 3-kinase/ AKT (PI3K/AKT), mitogen-activated protein kinase/ extracellular signaling regulated kinase (MAPK/ERK), and mammalian target of rapamycin (mTOR). Each pathway is implicated in cellular proliferation. It is unknown whether insulin directly affects estrogen action in the endometrium.

This study proposes to examine for the first time a potential mechanism for how insulin may directly affect the endometrium through activation of the insulin receptor and its pathways to augment the action of estrogen. Preliminary data shows that insulin activates its own signaling pathways, alters gene transcription, and promotes cellular proliferation of endometrial epithelial cells. It is hypothesized that insulin may induce a post-translational modification of the estrogen receptor, resulting in a change in gene transcription. This work will contribute to the understanding of insulin's role as a growth factor in the endometrium. Since the prevalence of obesity and Type 2 Diabetes is increasing in the United States, it is important to determine whether hyperinsulinemia contributes to endometrial pathology such as hyperplasia and impaired decidualization. The potential implications of this research may include recommendations for weight loss or the use of insulin sensitizers in the treatment of select endometrial pathology. The treatment paradigm for women with Type 2 Diabetes may also be changed to limit exposure to high doses of therapeutic insulin.

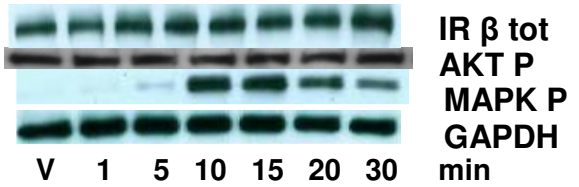
Innovation

This study examines endometrial cells for the first time using concepts initially applied to breast tissue, another estrogen-sensitive organ. In research examining mechanisms for tamoxifen-resistant estrogen receptor (ER) positive breast cancer, insulin has been shown to phosphorylate ERα. In MCF7 cells, a breast cancer cell line, insulin induces phosphorylation of ERα at serine residue 167, likely through the mTOR pathway ([redacted], 2010). These findings have not been repeated in primary breast cells or applied to endometrial cells. Phosphorylation of serine residues on ER may influence the recruitment of coactivators and alter ER-mediated transcription. One study used ERβ transfected COS-1 cells to demonstrate that phosphorylation of ERβ by MAPK leads to recruitment of steroid receptor coactivatior-1 (SRC-1) independent of steroid ligand binding ([redacted], 1999). While we are interested in the ERα subtype, this provides evidence that phosporylation of ER may result in estrogen independent activation of gene transcription. Hence, we propose to study the effect of insulin on ERα activation, coactivator gene expression, and ER-induced gene transcription in the endometrium.

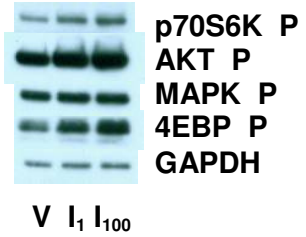
Approach

Aim 1: To determine whether ERα phosphorylation and ERα coactivation is mediated by insulin signaling.

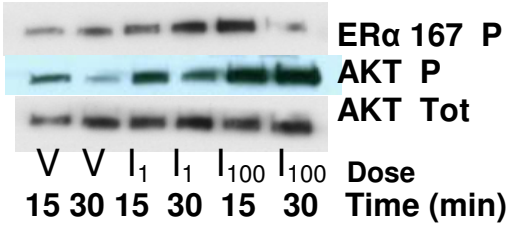
Preliminary Studies: Our preliminary results showed that insulin receptors are expressed in primary endometrial epithelial cells and Ishikawa cells, a well-characterized endometrial adenocarcinoma cell line. Insulin activated the AKT, MAPK, and mTOR pathways in a dose and time dependent fashion. Ishikawa cells have a PTEN deficiency and unregulated AKT activity, so insulin stimulation did not produce further AKT activation over baseline, but did activate MAPK (Figure 1). Furthermore, insulin induced phosphorylation of serine 167 on ERα, likely through the AKT and MAPK pathways since inhibiting PI3Kinase or MAPK signaling decreased ERα phosphorylation.



A.



B.



C.

Figure 1: Insulin stimulation of human endometrial cells. A) Time course of insulin 1nM stimulation of Ishikawa cells, showing presence of insulin receptors (IRβ) and activation of the MAPK pathway via phopshorylation (MAPK P). B) Primary endometrial epithelial cells treated with insulin 1 or 100nM for 30 minutes, demonstrating activation of the AKT and mTOR (p70S6K & 4-EBP) pathway. Note: MAPK activation is maximal at an earlier time point of 10-15 minutes. C) Time & dose course of insulin treated primary endometrial cells, showing phosphorylation of estrogen receptor α (ERα) at serine residue 167 and activation of AKT (AKT P).

Gene	Fold-change	p-value
p90RSK1	3.06	< 0.001
NCOA3	2.28	0.002
AKR1C3	2.54	< 0.001

Table 1: Microarray data of selected revelant genes upregulated at 24 hours by insulin 100nM in primary endometrial epithelial cells. Data confirmed by RT-PCR. These include 90kDa ribosomal S6 protein kinase 1 (p90RSK1), nuclear receptor coactivator 3 (NCOA3), aldo-keto reductase family 1, member C3 (AKR1C3).

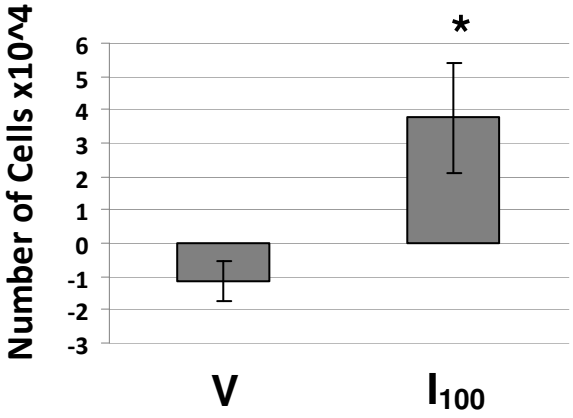


Figure 2: 24-hour Proliferation Study. Primary endometrial epithelial cells treated with insulin 100nM proliferated, 3.75 fold (* p=0.001) over baseline. Treatment with vehicle resulted in cell death from baseline.

We performed a microarray (Affymetrix Human Genome U133 Plus 2.0 Gene Chip) probing for 47,000 gene transcripts in primary endometrial epithelial cells treated with 24 hours of insulin 100nM. It showed significant upregulation of several genes related to ERα activation and local estrogen synthesis (Table 1). The 90kDa ribosomal S6 protein kinase 1 (p90RSK1) is one of two kinases that phosphorylate the serine 167 on ERα (Joel, 1998), and was upregulated by insulin. A nuclear co-activator for ERα, nuclear receptor coactivator 3 (NCOA3), was found to have increased gene expression with insulin treatment. This suggests that insulin may affect both ERα activation and gene transcription. Also, aldo-keto reductase family member C3 (AKR1C3) showed show increased gene expression, and is responsible for controlling local synthesis of estrogens. This indicates that insulin may increase local production of estradiol. Results were confirmed with RT-PCR. Finally, insulin 100nM stimulation for 24 hours promotes cellular proliferation over vehicle-treated and baseline indicating a functional role in the endometrium (Figure 2).

We plan to confirm these results in primary endometrial epithelial cell cultures, from control women without PCOS or T2DM. Then, we will determine whether insulin activation of ERα alters gene transcription in the presence of estradiol or induces ligand-independent gene transcription. Finally, we will compare differences in receptor activation and gene expression between women of different metabolic backgrounds.

Tissue Collection: Tissue for immunohistochemistry, RNA analysis, and primary cell culture is obtained during the proliferative phase of the menstrual cycle. The proliferative phase represents a time of predominant estrogen action, and offers better growth of epithelial cells. Immunohistochemistry and RNA analysis will be completed on tissue from a total of twenty women; five women for each of four categories: control, obese, PCOS, and T2DM. Tissue for cell culture and hormonal stimulation will be obtained from control women only since these are mechanistic studies. Control women are lean, defined by a body mass index (BMI) < 25 kg/m^2, without a history of endometrial pathology, PCOS, or T2DM. Obesity is defined as a BMI $\geq$ 30 kg/m^2. PCOS is defined using the 1990 NIH criteria, ie. menstrual irregularity and biochemical evidence of hyperandrogenemia. T2DM is defined by a history of therapy or biochemistry with an HbA1c $\geq$7, fasting glucose $\geq$120 mg/dl, or random glucose $\geq$200 mg/dl. The use of metformin or a triazolidinedione is a factor for exclusion. Women are identified from the clinical patient population who are undergoing surgery for reasons other than endometrial pathology. Excess tissue is collected as part of a larger ongoing study. We have HIC approval for receiving a portion of this tissue. Subject data and endometrial tissue specimens are identified only by serial numbers designated to each subject at the time of tissue collection. No identifying information is kept on any subject.

Hormone stimulation of primary epithelial endometrial cells and analysis of changes to protein activation and gene transcription: Primary endometrial epithelial cell cultures from lean women will be treated with insulin to determine whether insulin phosphorylates ERα and to elucidate which signaling pathway is responsible. Normal endometrial tissue will be minced and lysed with enzyme digest (Hank's Balanced Salt Solution, Collagenase B, and DNAase 1), then sequentially strained for separation of epithelial and stromal cell populations. Epithelial cells will be incubated at 37°C in low-glucose DMEM with 10% calf serum (CS) and 1% anti-bacterial/anti-mycotic. After one to two cell passages and a 24 hour period in serum-free, phenol-free media, the cells will undergo hormonal stimulation. For analysis of protein activation, primary cells will be treated with vehicle or regular human insulin in a dose range of 0.1nM to 100nM (Lilly, 100U/ml) for 15 and 30 minutes. Then the cells will be lysed and proteins from the cytoplasm and nucleus will be extracted separately in buffers containing protease and phosphatase inhibitors to protect the phosphorylated products.

To determine that insulin is acting through its own receptor, we will use siRNA to knockdown IRβ or IGF-1 receptor expression, then treat the primary cells with insulin. To determine which insulin pathway is responsible for ERα phosphorylation, the cells will be pre-treated with 60-90 minutes of the following inhibitors: Wortmannin (PI3K inhibitor), U0126 (MAPK inhibitor), or Rapamycin (mTOR

inhibitor). Each experiment will be done in triplicate, with use of ER positive MCF-7 cells, a breast cancer cell line, treated with insulin or estradiol as a positive control for western blot analysis. While Ishikawa cells are endometrial cells, they have a PTEN deficiency with unregulated activation of PI3K and AKT, making them unsuitable for these particular mechanistic studies.

Western blot analysis provides semi-quantitative estimates of proteins of interest in the endometrial cell cultures. Nuclear or cytoplasmic cell extracts will be separated by SDS-PAGE in a 4-12% gel, transferred to PVDF membrane, and probed with monoclonal antibodies. The following commercial target specific antibodies will be used for phosphorylated or total proteins: Akt, MAPK, mTOR downstream proteins ($p70^{S6K}$; 4E-BP1), IR, ERα, and $p90^{RSK1}$.

For analysis of whether insulin stimulation results in increased expression of essential kinases and co-activators, primary cells will be treated with vehicle or regular human insulin in a dose range of 0.1nM to 100nM for 24 hours. Then the cells will be lysed, messenger RNA extracted, and cDNA synthesized. Gene targets were determined from microarray screening. PCR analysis will be included for $p90^{RSK1}$, NCOA3, and receptors for insulin and estrogen. PCR primers will be designed through NCBI Primer-BLAST. Statistical analysis will be done as per [redacted], *et al.* (2001).

Expected Results: We expect to show that insulin stimulation of primary epithelial cells from control women leads to phosphorylation of ERα at serine 167 via the AKT and MAPK pathways. We also expect that insulin will consistently induce upregulation of $p90^{RSK1}$ and NCOA3.

Discussion, Pitfalls and Alternative Approaches: Challenges include the isolation and growth of pure epithelial cell cultures in sufficient quantity to run experiments. Ongoing optimization techniques include use of matrigel, selective trypsonization, and cell separation using epithelial markers. Also, once the lowest effective insulin dose is determined from initial studies, then the treatment variables will be reduced and require fewer cells. If results are inconclusive for insulin-induced phosphorylation of serine 167 on ERα, then we will quantify levels of the protein with ELISA. We may also examine other serine residues, 104/106 and 118, that are known to participate in activation of ER when phosphorylated.

Aim 2: To determine whether insulin promotes potentiation of ERα induced gene transcription.

Assessment of ERα binding to ERE and activation of gene transcription: Primary epithelial cells will be treated with vehicle, regular human insulin 1nM and 100nM and/or estradiol 10nM for a range of 60-120 minutes. Chromatin Immunoprecipitation (ChIP) will be used to determine whether insulin-induced phosphorylation of ERα promotes binding to the estrogen response element (ERE). This lab has significant experience with the HOXA10 ERE, so this will be used as well as the classic, consensus sequence ERE. The Luciferase Reporter Assay will be used to compare transcriptional activity of phosphorylated ERα in the presence and absence of estradiol, thereby determining whether insulin-induced ERα phosphorylation influences the transcriptional activity of ERα. The ChIP and Luciferase Reporter Assay experiments will be repeated in the presence of IR siRNA or inhibitors of AKT, MAPK, or mTOR pathways, as in Aim 1.

Expected Results: We expect to demonstrate that phosphorylation of ERα increases binding to the ERE's and leads to increased reporter gene expression.

Discussion, Pitfalls and Alternative Approaches: If the expected increase in binding does not occur, then we will examine for other ERE's in estrogen responsive genes.

Aim 3: To determine whether there are differences in baseline activation of ERα and IRβ or presence of ERα coactivators in endometrial tissue from women with obesity, PCOS, or T2DM.

Measurement of differences in receptor activation and gene expression in endometrial tissue from women with obesity, PCOS, & T2DM versus lean women without these conditions: Biopsy tissue will be immediately flash-frozen, placed in RNAlater to prevent RNA degradation, or formalin-fixed, paraffin-embedded, and cut for slides. After RNA is extracted from samples, PCR will be used to assess NCOA3, ERα and IRβ gene expression. From the tissue protein extracts, ERα and IRβ levels will be assessed by ELISA for comparative quantification between samples. In addition, immunohistochemical staining with phospho-167 ERα and phospho-IRβ antibodies will be analyzed for semi-quantitative results of activated status.

Expected Results: We expect to confirm our *in vitro* findings in women with obesity, PCOS, and T2DM. We expect they will have higher levels of phospho-167 ERα and phospho-IRβ proteins in their endometrial tissue relative to lean women without these conditions. In addition, NCOA3 expression is anticipated to be elevated.

Discussion, Pitfalls and Alternative Approaches: However, if no differences are found, then we will perform a microarray on RNA extracts to screen for changes in other genes that may explain impaired implantation and/or cellular hyperplasia.

Future Directions: Based on the outcomes of the above study, two studies in the immediate future are anticipated. First, a similar protocol will be used to assess the effect of metformin treatment on endometrial epithelial cell growth, insulin-induced ERα phosphorylation, and gene transcription. Other studies have indicated that metformin may have a direct effect on cell proliferation, and clinically it has been used to treat progestin-resistant endometrial cancer [redacted], 2003; [redacted], 2003). RNA expression for $p90^{RSK}$ and NCOA3 will also be measured in endometrial tissue from women on metformin to determine *in vivo* changes. The second study will examine the effect of insulin on primary endometrial stromal cells with regard to decidualization and implantation markers.

If a clear effect of insulin is demonstrated in endometrial cells, then the *in vitro* findings will be explored in an *in vivo* model toward the end of the award period. [redacted] lab has expertise in the design and implementation of studies to examine uterine pathology in mice. The [redacted] also has a full mouse metabolic typing center which can facilitate insulin clamp studies in metabolically abnormal mouse models, such as the ob/ob and db/db mice. We anticipate combining these resources to examine uterine changes as a result of hyperinsulinemia. The ultimate, long-term goal of this research pursuit is to determine whether high levels of circulating insulin in women with obesity, PCOS, or T2DM is associated with their endometrial dysfunction. The [redacted] has the resources and support to enable future dynamic metabolic clinical studies in women with endometrial pathology.

Literature Citations

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted].

Candidate's Background

During my research fellowship, I have focused on understanding the molecular pathogenesis of insulin resistance, and how it manifests in people prior to the development of full-blown Type 2 Diabetes (T2DM). I am currently involved in three ongoing studies: [redacted] These studies represent my transition from clinical medicine to clinical and translational research. I have acquired valuable skill sets through each of the studies, including how to design and execute clinical and molecular studies to examine the effects of hyperinsulinemia. After three years of work, each study is coming to fruition.

I have been an integral part of the [redacted] Polycystic Ovary Syndrome (PCOS) Program since its inception three years ago by [redacted] of [redacted] Endocrinology. The [redacted] is unique because it tracks young females who are already showing organ dysfunction as a result of insulin resistance. [redacted] We also hope to clarify the criteria for PCOS diagnosis in adolescents since many symptoms overlap with puberty and obesity can be a confounding factor. This [redacted] has given me first-hand experience in patient data collection and analysis, with attention to confidentiality and the most effective means of recording pertinent information. I audited the course [redacted] with the [redacted] Medicine Program to give me grounding in statistical analysis. From my analysis of this [redacted] I have given [redacted] presentations at annual meetings of the [redacted] and the [redacted] respectively. The analysis of [redacted] submitted for publication this month.

My clinical translational project with [redacted] and [redacted] examines the [redacted]. [redacted] I am responsible for each step of this clinical study including screening of subjects, leading thirty-six hour study protocols, processing serum samples for lipid evaluation, and analyzing the resulting data. This study is giving me experience in assessing [redacted] To date, we have completed over 30 studies and anticipate completion in this academic year. If the remaining studies confirm these findings, then we have demonstrated that [redacted]. In addition to working in [redacted] lab, I had prior experience looking at insulin signaling pathways in the lab of [redacted] at the [redacted], while I was a resident in Internal Medicine.

Given my clinical and research background in metabolism, I am in a unique position to pursue the role of insulin in reproductive pathophysiology. Two years ago, I sought out collaboration [redacted] to determine the [redacted] Based on initial histological data, I was awarded the [redacted] from the [redacted] to examine

[redacted] Last summer I presented these findings in a poster at the 92nd Endocrine Society Meeting. These results also provided preliminary data for a successful pilot [redacted] grant proposal, [redacted]. I am also examining the effect of [redacted] These results are being prepared for publication submission. Our study to demonstrate how insulin activates the estrogen receptor is the subject of this project proposal and detailed in the Research Strategy section. We are also presenting our [redacted].

My transition from being a clinician to a clinician-scientist is successful thus far given a great deal of mentoring and institutional resources. I am indebted to [redacted] for welcoming me to his lab, and providing the guidance and resources for my project. He is a clinician-scientist who realizes the bench to bedside approach in research, and I have a lot to learn from him. The Endocrinology faculty gave me the freedom and strong support to pursue research interests that are specific to my clinical observations and experience. I have been able to carve out a research niche that was not previously represented in the Endocrine section by collaborating with other departments. The [redacted] Endocrine section has a significant impact in research because the faculty focuses on mechanistic clinical and translational studies involving strong basic science and sophisticated techniques for dynamic metabolic testing. I hope to apply this model of research to investigation of reproductive pathology resulting from insulin resistance and diabetes. I extended my fellowship by two years to develop the necessary skill-sets in molecular biology, and look forward to furthering this development in science before incorporating a human study component.

Career Goals and Objectives

My goal is a career in translational research with a specific focus on insulin resistance and reproductive pathology. Insulin resistance is an underlying pathology in obesity, the Polycystic Ovary Syndrome (PCOS), and Type 2 Diabetes (T2DM). During my Endocrinology Fellowship at the [redacted] I have assembled the necessary support and resources to pursue my objectives in women's health research through collaboration with multiple departments, including [redacted] Endocrinology and [redacted] [redacted] With continued mentorship, I hope to develop as a physician-scientist who contributes to a multi-disciplinary Women's Health research program. My immediate research goal is to follow women with insulin resistance, and to determine whether their endometrial dysfunction, such as endometrial hyperplasia and infertility, is a consequence of the metabolic abnormalities occurring in insulin resistance.

During my research fellowship, I am working on three clinical and/or mechanistic studies examining organ dysfunction that occurs early in the pathogenesis of insulin resistance, prior to the development of T2DM. [redacted]. Since the clinical studies have intermittent time lines, the majority of my time is dedicated to the endometrial project. This project is inspired by my clinical experience, and enabled by [redacted] who lends his expertise, resources, and encouragement to expand my ideas. My immediate and long-term goals are the following.

<u>One Year Goals and Objectives</u>

- To further my knowledge and experience of molecular biology, incorporating more techniques and improved experimental design into my project
- To obtain a mentored career award and pilot research funding to continue my research project with [redacted]
- To present and publish my findings with peer review and critique

<u>Five Year Goals and Objectives</u>

- To operate a molecular biology laboratory with independent R01 funding
- To apply my *in vitro* experimental findings in endometrial tissue to the design and implementation of mechanism-based clinical studies that incorporate dynamic metabolic and hormonal evaluations
- To foster working collaborations between the departments of Endocrinology [redacted] at the [redacted]
- To maintain clinical exposure in the treatment of PCOS and Type 2 Diabetes to ensure my science trajectory follows the clinical need

<u>Ten Year Goals and Objectives</u>

- To initiate and lead an interdisciplinary research program with a specific focus on insulin resistance and reproductive pathology
- To provide evidence for changing the current therapeutic paradigm for treating endometrial pathology by addressing the underlying pathology of insulin resistance in women with obesity, PCOS, and Type 2 Diabetes.

Career Development and Training Activities during Award Period

I am fortunate to be part of two departments, Internal Medicine and Obstetrics and Gynecology, which have established research programs and NIH-funded infrastructure for the training and education of junior research faculty. I will participate in training and coursework along with scholars of both the [redacted] Program and the [redacted] These programs were designed to mentor junior faculty in their pursuit of a translational research career. I will benefit from the exposure to teaching faculty, association with a peer group, and didactic coursework that they provide. In addition, I will be required to demonstrate proficiency in the fundamentals of research, and meet specific bench-marks in preparing for an independent research career. I have not applied directly for the [redacted] Program since their funding institute does not allow simultaneous applications for other NIH sponsored career awards. Also, I am not eligible to be a [redacted] since my training is in Internal Medicine. With the guarantee of protected research time, I will be immersing myself in supported research training over the next five years.

Mentorship:
The most valuable part of my training remains the mentorship I receive from [redacted] as I undertake the experiments described in my proposal. We meet once a week to critically review recent results, and focus on appropriate design for upcoming studies. My research plan is further clarified by reading the published literature for new findings and methods, which we also discuss. In addition, I will meet with [redacted] every two weeks to discuss my progress. I meet three times a year with my Research Advisory Committee: Dr.'s [redacted] who give me formalized feedback on my project and career track. I am fortunate to have this mentorship since each physician runs his own [redacted] research lab.

Formal Didactic Learning:
My immediate objective in the next year is to acquire more knowledge and experience in basic molecular biology to apply to my project on the endometrium. While I have had a hands-on learning experience in the lab for two years, I need to expand my scope of possible experimental designs and analysis techniques to complete my project. As part of [redacted] I will take the six-month didactic [redacted]., faculty members in Ob/Gyn. It is a practical, small-group laboratory primer that I will take in my first year. I have already curb-sided both Dr.'s [redacted] in problem-solving specific experimental techniques, and look forward to being formally taught by them. Later, I may enroll in more advanced courses offered by the Departments [redacted] which have application for my project.

In the first two years of this award, I seek to improve my knowledge base in the design, conduct, and analysis of clinical research, given my long-term goals. Both the [redacted] and [redacted] programs overlap in the following required courses taught by the faculty of the [redacted] Medicine Program: [redacted]. I have the potential of earning a [redacted] through the [redacted] program with the completion of at least one more elective in [redacted].

The [redacted] Seminar Program is a monthly meeting of junior faculty research scholars with faculty mentors to present and critique ongoing original research. This serves as a valuable opportunity to connect with peers, and gain a broad perspective of potential research design and problem solving. My overall knowledge in the field of reproductive pathology and investigation will be enhanced by attending the weekly [redacted]), as organized by [redacted]. I will also continue to attend the weekly Endocrine

Grand Rounds, a two hour [redacted] conference. It is here that I have received most of my inspiration for examining real-world patient problems with dedicated scientific inquiry, and have myself given over [redacted] presentations as a fellow. I anticipate giving an update of my research findings every year at this conference or our [redacted] retreat.

Writing & Presentation Skills:
My writing has been mentored by Dr.'s [redacted], who are experienced editors. I am currently completing manuscripts for publication on my endometrial study and Polycystic Ovary Syndrome (PCOS) [redacted]. Over the next five years, I hope to write at least two papers per year of original research results that are suitable for publication in a peer-reviewed journal. Dr. [redacted] will also mentor me as I serve as a peer reviewer of papers. I will dedicate an increasing amount of time to writing grants with each year of the award, in anticipation of pilot grant applications to the [redacted] and Women's Health Research at [redacted] as well as an R01. I will continue to present my research findings at annual national conferences to prompt summation of my material and receive critical feedback.

Clinical and Teaching Responsibilities:
In order to maintain clinical experience in the evaluation and treatment of PCOS and Type 2 Diabetes, I will continue my one half day of clinic each week at the [redacted], where I have attended for the last 4.5 years. I will also continue the monthly half-day [redacted] PCOS clinic to contribute to the ongoing clinical registry. My teaching responsibilities include giving lectures and leading small-group modules for [redacted]. These are already limited to less than five percent of my time.

My time effort for each of the above activities per year of the award period is summarized in Table 1. With these activities and mentorship support, I anticipate developing the technical and theoretical foundation necessary to be a well-informed investigator.

Activity	**Year 1 – 2**	**Year 3 – 4**	**Year 5**
Research*	70 %	70 %	70 %
Publication & Grant writing	5 %	10 %	10 %
Coursework & Seminars	10 %	5 %	5 %
Clinical Responsibilities	12 %	12 %	12 %
Teaching	3 %	3 %	3 %

Table 1: Time effort (% of total) per year of Mentored Career Award (K08). *Details of Research effort are described in the Research Strategy section.

Training in the Responsible Conduct of Research

I have received both informal and formal training in the responsible conduct of research during my endocrine fellowship. In addition to receiving individual mentorship during ongoing clinical studies, I have fulfilled required courses at the [redacted] and attended seminars at national conferences for trainees. In the course of our metabolic study in the [redacted] has mentored me through initial and revised submissions of the study protocol to the ethics committee, appropriate subject consent during screening visits, and the documentation and protection of study data for each subject. I have also had mentored experience with [redacted] in ensuring that consent criteria is fulfilled during clinical visits and that the clinical registry database is protected.

Formal instruction has also been required by the [redacted] in an annual online training and assessment of specific topics needed for my area of research. These include HIPPA privacy and security training, human subject protection training, clinical research compliance, research registration process, sponsored projects administration training, and multiple safety training modules for contact with blood-borne pathogens, laboratory chemicals, and radiation. I have also attended the Clinical Investigator Trainee's Workshop ([redacted] and Endocrine Society Trainee Day ([redacted]), both sponsored by the Endocrine Society to provide mentorship in research pursuits. Seminar topics included conflict of interest, mentor/mentee responsibilities and relationships, collaborative research including collaborations with industry, publication submission process and peer review, and responsible authorship and publication.

Over the next five years, I will continue to meet the standards set forth by the [redacted] and [redacted]. In addition to annual on-line training modules, educational seminars and brown-bag luncheon sessions are held regarding critical regulatory issues and challenges. I plan to enroll in a semester long course offered by the [redacted] Medicine Program, [redacted]. This course covers ethics of clinical investigation, scientific fraud, technology transfer, and interfacing with the pharmaceutical industry. With regard to my proposed project using endometrial tissue, [redacted] addresses HIPPA regulations, the safe and responsible collection of human tissue, and the use of non-identifying nomenclature for each tissue sample during our regular group lab meetings.

February

Dear Committee Member:

I am writing in strong support of application for a K08.

joined my laboratory one year into her research fellowship in Endocrinology. She had already gained impressive clinical research experience in the lab of Dr.'s , examining the effect of In their lab, she was exposed to a successful model of translational diabetes research Given her interest in the Polycystic Ovary Syndrome (PCOS) and clinical experience in the PCOS Clinic, she wanted to pursue her own project looking at reproductive consequences of insulin resistance. She approached me about collaborating on a project examining the role of hyperinsulinemia in endometrial dysfunction. The connection between impaired glucose metabolism and endometrial dysfunction is understudied, if not largely neglected. The incidence of PCOS and T2DM is increasing, and therefore the effect of these conditions on fertility and is of great clinical significance. initiated a project within my lab, beginning with a histological examination of abnormal lipid metabolism within the endometrium of women with PCOS and T2DM. She then learned cell culture techniques and began analysis of insulin signaling in human endometrial cells. Her preliminary data on the effect of insulin on estrogen receptor activation is novel, and may have significant clinical implications. To date, has generated abstracts, manuscript in submission, and small grants in support of her work. has a vigilant work ethic in research, and is strongly motivated by her clinical experience. She has the potential to change the field of reproductive health by being the right person at the right time to address the effect of the obesity and diabetes epidemic.

I welcome the opportunity to enhance the expertise of our section by collaborating with someone as uniquely qualified as in abnormal glucose metabolism and insulin resistance. Our section of Endocrinology has faculty members with an interest in the intersection of metabolism and reproductive pathology. I am an established investigator in . We study the effect of . My research includes . We have generated over original research publications. My research has been funded by the NIH since the beginning of my career, and I am currently the PI on a well as an R01, both focused on . I have experience as a mentor, having trained over fellows, students and junior faculty. Specifically I have served as the primary mentor for faculty members in similar training programs to the one proposed here ( ). Currently these have faculty appointments at the

[redacted] My office and laboratory space are located in the newly renovated [redacted], where [redacted] has her own bench space and office with administrative support.

I am committed to ensuring that [redacted] acquires the valuable skills needed for the success of her current project as well as her future career. We meet every [redacted] morning for at least an hour, separate from group lab meetings. During this time, we have discussed scientific topics, the approach to a scientific problem as well as guided her practical lab experience in trouble-shooting and modification of experimental design. We also edit her writing together, in preparation for manuscript and grant submissions. We discuss the topic of establishing an independent research career. Over the last two years, her time has been invested in learning new techniques, designing and optimizing experiments, as well as grant writing. She has demonstrated excellence in organization and leadership within the lab, skills that are critical to running her own lab in the future. She is well-versed in how to present scientific material, both in oral presentations and writing. We have organized a training plan that ensures sufficient formal didactic learning while maintaining plenty of time for running the actual experiments.

In collaboration with the section of Endocrinology, the Ob/Gyn department will be responsible in directing [redacted] mentored training. She will have the infrastructure of two departments to create an individually tailored program for her training. This includes receiving formal teaching alongside [redacted] Each [redacted] program has a fully developed curriculum for the training of young investigators, providing a strong foundation in the design, conduct, and analysis of research. The goal of this award period is to provide Dr. [redacted] with a broad theoretical and technical base from which to design rigorous scientific experiments. Courses in [redacted] are anticipated for her first year. The [redacted] programs also have regular seminars for the presentation and exchange of research ideas with senior faculty and other young investigators. In addition, there are multiple conferences including the [redacted] journal club, [redacted] journal club, and the [redacted] grand rounds. [redacted] clinical and teaching responsibilities will reside in the section of Endocrinology, and account for 15% or less of her overall time. [redacted] has guaranteed her protected time, as her Co-Mentor and [redacted] Chief [redacted].

During fellowship, Dr. [redacted] has already been successful in fostering communication between departments to meet her research and professional goals. In addition to individual meetings with Dr. [redacted] and myself, she will continue to meet at least three times a year with her Research Advisory Committee to receive formalized feedback. The [redacted] and [redacted] also give guidance as to a recommended timeline of accomplishments to transition from mentored to independent status. During her time in my lab, Dr. [redacted] has been awarded [redacted] pilot grants which have partially funded her work. My research funding and well-resourced lab will continue to provide the foundation for her experiments until she has adequate funding for an independent lab. As faculty, she will be competitive for internal pilot grants, and her work will be appropriate for R01 funding within a three year time period.

In conclusion, Dr. [redacted] is an outstanding candidate and uniquely qualified to carry out translational research in the area of diabetes and reproductive health. She has the clinical expertise in insulin action and diabetes along with a strong commitment to women's health. We are fortunate to have attracted someone with [redacted] background and training to an area of women's health that is currently understudied. She has the insight and independence to bridge two departments in order to accomplish her goals. We are dedicated to her development as a physician-scientist, and an investment in her career will be supporting strong inter-disciplinary research. [redacted] is perhaps the top trainee that I have had in my laboratory; her future success is virtually guaranteed. I strongly support her application with the utmost of enthusiasm.

[redacted]

February

Committee,

I write to provide my strong support for the application of for a Career Development Award from the National Institutes of Health (K08). Dr was recruited to the Endocrine Section from the house staff-training program, based on her strong commitment to an academic career. She initially gained valuable clinical research training by working on a human-based translational project under the direction of my colleague that examined . However, after a relatively short period she recognized that her passion was in another area, namely the interface between reproductive health and insulin resistance. It is for this reason she pursued projects involving PCOS patients, and is currently leading two studies using an ongoing clinical In the course of multiple conversations, I encouraged her to continue her research interests by extending her fellowship to maintain over 80% research effort. She is currently funded , and she is ready to pursue independent research support.

Her next step was more ambitious and reflected a commitment of significant proportions, namely to extend her training to gain the skill-set needed in basic science to pursue her goal. For this purpose, she joined the laboratory of Dr. . Her plan is to develop a new program to assess the These innovative studies have driven new interdisciplinary collaborations and required an understanding of hormone signaling, cell biology, metabolism, and biochemistry, a unique combination of skills that would never have happened without driving the agenda. In short, Dr. is developing a research niche in metabolic dysregulation and reproductive health. I have closely followed her trajectory as a Fellow in Endocrinology for over four years, during which time she has demonstrated a strong commitment to becoming an academic clinician-scientist. As Dr. continues her research career, I want to underscore she has the mentoring and resource support of our section. On occasion, one has an opportunity to identify a young scientist who has a vision that is a valuable programmatic fit, and at the same time, has an ideal opportunity for growth and independence. I believe has such an opportunity.

I believe is an ideal place for to realize her potential. In the of Endocrinology we have a long history of cross-disciplinary collaborations with many departments at

including Ob/Gyn. We together with our colleagues in Ob/Gyn published [redacted] As a result, I am happy serve as [redacted] co-mentor for the project and her long-term career development. As a Co-Mentor on her project, my predominant role is to guide the research ideas, and lend my expertise to the design of future clinical studies based on her *in vitro* findings. I am currently on her Research Committee that meets three times a year to review her accomplishments and give strategic recommendations for her continuing career development.

My current research is directed at the effect of [redacted]. While this research is not directed toward Dr. [redacted] specific project I believe I have the experience to help guide her career and realize her potential. I have received continuous NIH support for [redacted] years [redacted]. Moreover, I have been the primary mentor for over [redacted] postdoctoral fellows and junior faculty ([redacted] MD, [redacted] PhD, and [redacted] MD-PhD), [redacted] of which have achieved the rank of Professor. My efforts will be complemented by a number of other senior [redacted] faculty members in our [redacted] that are available to deal with specific problems as they arise. This group has extensive NIH research funding with [redacted] laboratory skills of this varied group of scientists offer an important resource for Dr. [redacted] development. Dr. [redacted] will meet individually with me for at least one hour every other week to discuss her research progress. In addition, the [redacted] of Endocrinology sponsors an extensive array of conferences and journal clubs including a monthly seminar that provides junior faculty with an opportunity to present and discuss their findings with colleagues in the [redacted] as well as an annual scientific retreat. The laboratory facilities available to [redacted] in the [redacted] of Endocrinology include approximately 5000 sq ft of state-of-the-art space located in the [redacted] building. [redacted] will also have available to her the rich resources of our NIH funded research centers in the area of [redacted] including the [redacted] Center with its core resources in [redacted] and opportunities for pilot funding. The [redacted] will provide [redacted] with unique opportunities for studies in genetically altered mice.

Finally, [redacted] will benefit from the [redacted]), which was funded in [redacted] by a Clinical Translational Science Award (CTSA), [redacted] This major institutional resource will provide her with abundant educational and scientific opportunities for career development as a translational scientist. Perhaps most important, [redacted] will have an opportunity to participate in the [redacted] program which was developed to offer selected junior faculty the strongest possible training and mentorship in the performance of clinical and translational research. To accomplish this goal, we have created a [redacted], chosen based on the breadth, depth, and quality of their clinical and translational research, on their track records in mentoring young scientists, and on their shared commitment to develop an exciting intellectual environment for the Scholars. [redacted] The goal is

also to have an independent advisory group to guide the Scholar toward an independent career. We also offer courses in [redacted]. I believe that scientific infrastructure we have created will be particularly useful to [redacted] as she moves forward with her plan to translate the basic findings from her studies to the clinical setting in patients with endometrial hyperplasia. The capacity to move rapidly from the bench to the bedside is a major strength of our program.

To ensure that Dr. [redacted] will be able to take full advantage of this K08 Award, she will have minimal teaching responsibilities and limited clinical activities (less than 10-15%) and no administrative responsibilities. I believe this is essential to give her the time needed today to become an independent RO1 funded physician-scientist before this award is completed. This is a task to which I am committed. I believe Dr. [redacted] has the opportunity to be a catalyst for the development of a new interdisciplinary program and based on her work ethic, tenacity, and meticulous approach to research, that an investment in her future will pay handsome dividends. She is successfully collaborating with three departments to pursue her research interests, which exemplifies a true multi-disciplinary approach to problem solving. I can assure you that the Section of Endocrinology will provide her with the structure and support she needs to succeed.

Sincerely,

[redacted]

Description of Institutional Environment

The [redacted]) has an established history of training clinician-scientists, with the infrastructure to support mentorship, didactic education, and core research facilities. I am fortunate to have mentorship from multiple faculty members with significant research experience. Dr. [redacted], is my primary mentor and an established investigator in molecular biology with an expertise in [redacted] He has a fully equipped molecular lab where I will conduct my research. Dr. [redacted] is a co-mentor for my project. As the PI [redacted] he has expertise in the design of translational studies, in particular for the assessment of abnormal glucose metabolism. He is also enabling me to join the educational resources of the [redacted] Program. My Research Advisory Committee also includes Dr. [redacted] Career [redacted] Center, will oversee my progress and include me in the weekly [redacted]. The [redacted] brings together junior and senior faculty in the field of reproductive pathology [redacted] for presentation and critique of research findings. Other conferences include weekly [redacted] Grand Rounds, bi-weekly [redacted] Journal club, and the monthly [redacted] Seminar Program. I will also be able to take courses from the [redacted] Medicine Program, which are created for research physicians.

[redacted] has numerous core facilities that will assist me in my current project and help me to incorporate new methods as my project develops. [redacted] has core laboratories for the processing of histological samples, cell sorting, and microarrays. The [redacted] Laboratory provides [redacted] technologies. [redacted] the goal of promoting research in diabetes and related metabolic and endocrine disorders at the university. The [redacted] brings together a multidisciplinary group of nearly [redacted] independent member scientists as well as professional supporting staff, new investigators and research trainees. The [redacted] Core facilitates metabolic research in patients, whereas the [redacted] that comprise the [redacted] offer investigators the tools to create and test novel animal models starting from the molecule and ending with biological outcomes. The [redacted] provides an infrastructure [redacted]

As a faculty member, I will have opportunities for pilot funding through the [redacted], and [redacted] at [redacted] The mission statements for each of these centers commit support for new investigators in interdisciplinary research.

February

Dear Committee Members,

It gives me great pleasure to confirm support of KOS application. Her work in understanding the overlap of insulin resistance and reproductive pathology represents a model of cross-departmental collaboration that we strongly support at the .

Dr. upon recruitment to our Endocrinology fellowship program, joined the lab of , one of our studies, using *in vivo* techniques for assessing metabolic dysregulation. Thus far, publications in have been generated, and a third has been submitted. With the strong support of the Endocrine faculty, Dr. sought to develop her own interests in understanding why insulin resistance in women results in reproductive organ dysfunction, specifically endometrial hyperplasia. In the laboratory of Dr. in she is examining she has successfully competed for grants, including pilot grants to pursue her research ideas involving the endometrium. She was also awarded .

We are committed to supporting Dr. career at She completes her research fellowship in and will then join our faculty. This appointment is independent of her receipt of a K award. Dr. currently has 80% protected time for research without administrative duties. She has minimal teaching duties within her own section and the medical school. As described in Dr. mentor statement, Dr. will have ample laboratory and office space to complete the proposed research. She will also have access to all the core research facilities and equipment required to complete her aims, and will benefit from the full array of scientific and educational resources that the medical school offers. Lastly, she will benefit from working with two highly respected mentors, Drs. , both of whom have mentored a significant number of clinician-scientists and have made substantial contributions in their fields of research.

In summary, it is always a pleasure to learn of a physician who actively pursues a clinical observation into the laboratory. We are committed to Dr. successful development as an independent physician scientist. An investment in her career at this point will go a long way in supporting her ultimate goal of achieving clinically meaningful cross-disciplinary collaborations. I recommend her to you with the highest possible level of enthusiasm. Please let me know if any additional information is required. Best wishes.

Sincerely,