

Pennsylvania Department of Health Final Performance Summary Report Formula Grants

Overview of the Health Research Project Performance Review Process and Criteria

An applicant that receives a health research grant under Tobacco Settlement Act / Act 77 of 2001, Chapter 9, is subject to a performance review by the Department of Health upon completion of the research project. The performance review is based on requirements specified by Act 77 and criteria developed by the Department in consultation with the Health Research Advisory Committee.

As part of the performance review process, each research project contained in a grant is reviewed by at least three experts who are physicians, scientists or researchers. Reviewers are from the same or similar discipline as the research grant/project under review and are not from Pennsylvania. Reviewers use the applicant's proposed research plan (strategic plan), the annual progress report and final progress reports to conduct the review. A grant that receives an unfavorable performance review by the Department may be subject to a reduction in funding or become ineligible for health research funding in the future. The overall grant evaluation rating is based on the ratings for the individual research projects contained in the grant.

This performance review report contains the outcome of the review for the grant as a whole (outstanding, favorable, or unfavorable), strengths and weaknesses of each research project, as well as recommendations for future improvement.

The following criteria were applied to information submitted by research grant recipients:

- **Criterion 1 - How well did the project meet its stated objectives? If objectives were not completely met, was reasonable progress made?**
 - Did the project meet the stated objectives?
 - Were the research design and methods adequate in light of the project objectives?
 - Consider these questions about data and empirical results: Were the data developed sufficiently to answer the research questions posed? Were the data developed in line with the original research protocol?
 - If changes were made to the research protocol, was an explanation given, and, if so, is it reasonable?
 - Consider (only for clinical research projects) the extent of laboratory and clinical activities initiated and completed and the number of subjects relative to the target goal.
 - Were sufficient data and information provided to indicate or support the fact that the project met its objectives or made acceptable progress?
 - Were the data and information provided applicable to the project objectives listed in the strategic research plan?

- **Criterion 2 - What is the likely beneficial impact of this project? If the likely beneficial impact is small, is it judged reasonable in light of the dollars budgeted?**
 - What is the significance of this project for improving health?
 - Consider the value of the research completed towards eventual improvement in health outcomes.
 - Consider any changes in risk factors, services provided, incidence of disease, death from disease, stage of disease at time of diagnosis, or other relevant measures of impact and effectiveness of the research being conducted.
 - Consider any major discoveries, new drugs and new approaches for prevention, diagnosis and treatment, which are attributable to the completed research project.
 - What are the future plans for this research project?

- **Criterion 3 - Did the project leverage additional funds or were any additional grant applications submitted as a result of this project?**
 - If leveraging of funds were expected, did these materialize?
 - Are the researchers planning to apply for additional funding in the future to continue or expand the research?

- **Criterion 4 - Did the project result in any peer-reviewed publications, licenses, patents, or commercial development opportunities? Were any of these submitted/filed?**
 - If any of the above listed were expected, did these materialize?
 - Are the researchers planning to submit articles to peer-reviewed publications, file for any licenses, or patents or begin any commercial development opportunities in the future?
 - Consider the number/quality of each.

- **Criterion 5 - Did the project enhance the quality and capacity for research at the grantee's institution?**
 - Were there improvements made to infrastructure?
 - Were any new investigators added or were any researchers brought into the institution to help carry out this research?
 - Were funds used to pay for research performed by pre- or post-doctoral students?

- **Criterion 6 - Did the project lead to collaboration with research partners outside the institution, or new involvement with the community?**
 - Are the researchers planning to begin any collaborations as a result of the research?
 - For clinical research only: consider the number of hospitals and health care professionals involved and the extent of penetration of the studies throughout the region or the Commonwealth.

Overall Evaluation Rating

An overall evaluation rating is assigned to each research project. The rating reflects the overall progress the project attained in meeting the stated goals and objectives. The rating is based on a scale of 1–3, with 1 being the highest. An average rating is obtained from all the reviews (minimum of 3) of each project and is the basis for the determination of the final overall rating for each project as follows:

1.00 – 1.33 = *Outstanding*

1.34 – 2.66 = *Favorable*

2.67 – 3.00 = *Unfavorable*

The grant level rating is an average rating from all projects as above. The numerical rating appears in parentheses for the grant and each project in the ***Overall Grant Performance Review Rating*** section of the report.

Overall Grant Performance Review Rating

Grant Rating: Favorable (1.75)

Project Rating:

Project	Title	Average Score
1166801	In Vivo Analysis of Human C19MC MicroRNAs in a Transgenic Mouse Model	Favorable (2.00)
1166802	Glycocalyx Syndecan-1 and Preeclampsia Pathogenesis	Outstanding (1.33)
1166803	Targeting Women's Cancer Cells with Novel Cell Cycle Inhibitors Blocking Centrosome Clustering	Favorable (2.00)
1166804	Regulation of Spermatogenesis by Classical and Non-classical Testosterone Signaling	Favorable (1.67)

Project Number: 1166801
Project Title: In Vivo Analysis of Human C19MC MicroRNAs in a
Transgenic Mouse Model
Investigator: Jean-Francois Mouill, PhD

Section A. Project Evaluation Criteria

Criterion 1 - How well did the project meet its stated objectives? If objectives were not completely met, was reasonable progress made?

STRENGTHS AND WEAKNESSES

Reviewer 1:

This project has two specific aims. The first is to analyze the ectopic expression of the human C19MC miRNAs in mouse organs and to delineate the resulting phenotype. The second is to examine gene expression profiles in transgenic organs and their wild-type counterparts using microarrays and determine the cellular pathways impacted by these miRNAs.

These aims are very ambitious and technically challenging, and unfortunately most of the objectives were essentially negative. High C19MC miRNA expression was achieved in transgenic placentas, but phenotypic abnormalities were not detected. Expression was detected in testes, and miRNAs from the cluster were detected in plasma. However, there was no success in determining the special expression of the transgenic miRNAs in mouse tissues using *in situ* hybridization. In addition, ectopic expression of the C19MC miRNAs did not have a significant effect on gene expression in the mouse placenta. Nevertheless, the project was very worthwhile since miRNAs have been shown to be critically important in differentiation, and very little is known about the roles of miRNAs in trophoblast development. The research design and methods used in the studies were state-of-the-art, and the investigator was aware of potential pitfalls. Some alternative strategies are planned, such as an RNA FISH approach to determine miRNA localization.

Reviewer 2:

The project appeared to meet its stated objectives. The initial research design seemed appropriate with some modest exceptions outlined below. Given the largely negative data set, it appeared that the data from specific studies were sufficiently analyzed. The data were developed in line with the original research proposal.

The strength here is analyses of the target C19MC miRNA cluster, thought to be specifically expressed in the human placenta, and the PI proposed and carried out logical experiments to evaluate this important miRNA cluster in an in vivo setting using mouse models. A potential weakness in the stated objectives is the apparent analysis of only a single gestation age (e18.5) in the mouse model. It is entirely plausible that expression profiling of the miRNA cluster and its

potential effects could be determined at earlier gestational ages which may have effects that are currently undetected due to this limitation.

Reviewer 3:

The overall goal of this project was to evaluate the production of a human-specific cluster of miRNA's (C19MC) in mouse transgenics and to determine phenotypic abnormalities and gene expression profiles impacted by expression of this miRNA's cluster of genes.

The PI was successful in producing 4 independent transgenic lines and analyses showed that the C19MC miRNA cluster was predominantly expressed in the placentae of transgenic, but not wild type, mice. Expression of the C19MC cluster products was detected in the plasma of females bearing transgene pups and limited expression levels were detected in testis and brain of pups. Breeding experiments showed that the C19MC expression in the placentae was driven predominantly by the paternal origin of the transgene. Studies using in situ hybridization approaches have not been successful in determining the cellular expression patterns of the C19MC miRNA's in the transgenic mice placentae. The PI is planning to use an RNA-fluorescent hybridization approach and presents a figure of its initial use in a cell line. This figure is confusing in that it appears that a single locus is highlighted within the nuclei – this looks very much like regular FISH detecting genomic target. The PI also proposes to use laser capture micro-dissection coupled qPCR to determine the expression patterns of the C19MC cluster. This seems like a much more definitive approach. These basic expression results in the mice are in line with expression patterns of C19MC in humans.

Despite expression of the C19MC cluster in the transgenic mice, so far, no overt phenotype has been detected. Birth rates, birth numbers, embryo weights and placenta weights, are similar between wild type and transgenic mice. These results are disappointing. It is not clear if morphometric analyses of the placentae of the transgenics have been attempted. Although major alterations are probably not likely, perhaps more subtle changes in cellular composition, vascularity or structure might be observed.

Aim 2 of the grant sought to identify altered gene expression patterns in the transgenic mice placentae by micro array analyses. Again here, the results were inconclusive in defining altered gene expression patterns in the mice. The PI reports a high degree of variability and background noise with the micro array data. Clearly, this could be affecting the ability to detect significant gene expression changes in the mice. An approach to help with this would be to use qRT-PCR for a handful of mouse genes that are homologous to known human genes regulated by C19MC miRNA and contain significantly conserved “seed” sites, for more specific analyses.

Criterion 2 - What is the likely beneficial impact of this project? If the likely beneficial impact is small, is it judged reasonable in light of the dollars budgeted?

STRENGTHS AND WEAKNESSES

Reviewer 1:

This project's objectives addressed issues directly relevant to improving health. Several pathologic conditions of pregnancy, including preeclampsia and fetal growth restriction, are

characterized by abnormal placental development. A better understanding of the molecular mechanism(s) involved in trophoblast differentiation, including the roles for miRNAs, is very likely to contribute to the development of new and novel therapeutic strategies to prevent or treat these disorders, which are among the leading diseases contributing to fetal mortality and morbidity.

Much can be learned from negative results. The negative findings of the project clearly indicate that alternative strategies will be necessary to address the specific aims. Some new approaches are planned by the investigator.

Reviewer 2:

The significance of this project could be quite high and it is clear from the original research plan that the PI and colleagues had very high hopes that the C19MC miRNA cluster would have remarkable impact on placental development and potential function in this mouse model. Based upon the outcomes presented here, the benefits of these studies are likely to be marginal to our comprehensive understanding of human or mouse placental development and function (with the potential caveat that earlier gestational time points could reveal important mechanistic information). Given the scale of the funding level (>\$340k) to the outcome of the studies, it is disappointing that there were no major findings or evidence of impact here. Some of this was due to technical hurdles that could not be overcome in the time frame of the grant. There appeared to be a remarkable number of “research assistants” participating in this project. This reviewer would have anticipated much more research progress given the sum of the manpower. The impact of the studies would have been improved if the analysis of the mouse placentas was carried out over a range of gestational ages. A single time point analysis is clearly a weakness of the studies.

It is unclear how “fetal plasma samples” were recovered. Perhaps I missed this in the text but I suspect this is challenging.

It is unclear why RNA-FISH was not carried out on sections of mouse placenta from wild type and Tg animals. This may have added impact to the studies.

Reviewer 3:

This is a truly exploratory project and clinical value awaits further testing. Although successful in creating the transgenic models, there has not been an obvious phenotypic consequence detected in the embryos or adults. As C19MC is sometimes associated with tumor development, longevity experiments may be needed to see an effect. The PI mentions that C19MC is associated with protecting human trophoblast from viral infections (unpublished data). Similar studies are planned to determine if ectopic expression of the human C19MC complex in murine placentae has a similar effect. This could provide interesting data.

Criterion 3 - Did the project leverage additional funds or were any additional grant applications submitted as a result of this project?

STRENGTHS AND WEAKNESSES

Reviewer 1:

No additional funds were leveraged, and no additional grant applications pertaining to the project were submitted.

Reviewer 2:

Leveraging additional funds to support this line of research is a clearly stated goal of the PI; however, there is no evidence that this has or will occur. This is an important weakness. The PI suggests that additional funding will be sought; however, if the current data were used as preliminary evidence for a new proposal, success of another proposal would likely be limited.

Reviewer 3:

No additional extramural funds were secured for this project. The PI plans to apply for additional funding to dissect the molecular components needed to restrict C19MC expression to trophoblast and to determine the paternal epigenetic regulation of this cluster. It is not stated, but given the lack of an observable phenotype in the mice, these follow up studies should be carried out in human cells. This would be an interesting extension, if the viral resistance effects are verified.

Criterion 4 - Did the project result in any peer-reviewed publications, licenses, patents, or commercial development opportunities? Were any of these submitted / filed?

STRENGTHS AND WEAKNESSES

Reviewer 1:

No publications, licenses, patents or commercial development opportunities were submitted or filed.

Reviewer 2:

This project did not result in any peer-reviewed publications to date.

Reviewer 3:

No publications or inventions have resulted from the project yet. This is not unexpected given the short (1yr) reporting time for the project. The PI does anticipate gathering further data to generate a publication.

Criterion 5 - Did the project enhance the quality and capacity for research at the grantee's institution?

STRENGTHS AND WEAKNESSES

Reviewer 1:

No improvements were made to infrastructure, and no new investigators were added or brought into the institution to perform the research. However, the techniques developed in the Investigator's laboratory have been made available to other investigators. A predoctoral student participated in the studies.

Reviewer 2:

This project clearly enhanced the quality and capacity for research at the grantee's institution. Based upon the reported distribution of resources from the proposal, 4.8 FTEs were supported during the funding period. This included the PI, coI, a programmer, a bioinformatician (near 100%) and four research assistants. The role and distribution of effort for the research assistants is unclear. The pay scale for these individuals varying greatly where in some cases, 100% effort reflects only \$3420.00. Thus it is unclear how these effort distributions are calculated or used. There does not appear to be any improvements in infrastructure with the exception of the creation of the humanized mouse model.

Reviewer 3:

Funds were used to support one pre-doctoral student in addition to the PI, co-I and five other support personnel on the project. No out-of-state researchers were recruited. It is not clear how the project enhanced research capacity/quality. The techniques used to produce the transgenic mice, the mating combinations to investigate epigenetic regulation and the transgene detection assays are all very standard and quite routine at most facilities.

Criterion 6 - Did the project lead to collaboration with research partners outside of the institution or new involvement with the community?

STRENGTHS AND WEAKNESSES

Reviewer 1:

The progress report indicates that no collaboration was performed with research investigators outside of the institution. However a new collaboration was begun with William Klimstra at the University of Pittsburg to study a possible role for C19MC miRNAs in enhancing viral resistance.

Reviewer 2:

The PI reports a new collaboration with William B. Klimstra at the Center for Vaccine research at the University of Pittsburgh examining the role of C19MC on viral resistance in human cells; however, there is not a clear connection between this proposed collaboration and the funded studies on mouse and human placental development and function.

Reviewer 3:

The PI reports that C19MC miRNA may function in human cells to increase resistance to viral infections (data not shown). Thus, although no overt phenotype is evident in the transgenic mice expressing the C19MC cluster with regards to reproductive performances, it could be that their embryos are more resistant to viral infections. A collaborative effort with Dr. Klimstra, Center for Vaccine Research, University of Pittsburgh is planned. The details of this collaboration and their anticipated project are not defined.

Section B. Recommendations

SPECIFIC WEAKNESSES AND RECOMMENDATIONS

Reviewer 1:

It is possible that the mouse is not a good model to study the C19MC miRNA cluster. Although there are many similarities between the human and mouse placentas, there are also some striking differences in the anatomy of the placentas. Consequently the regulation of trophoblast lineages in the two species may be quite different.

Success may be greater using individual miRNAs from the cluster in the transgenic studies. Has the Investigator considered studies using primary human trophoblast cells in culture? Would it not be possible to use lentiviruses to block individual miRNAs?

Reviewer 2:

1. It would be prudent to examine placental expression of the C19MC miRNA cluster at more than one time point during mouse gestation. This is viewed as an important missed opportunity. Without a more comprehensive analysis, it is unclear if this mouse will be informative regarding the role of C19MC on placental development or function.
2. It is disappointing that the RNA-FISH analysis was not used on sections from mouse placenta (only presented for human trophoblasts). This approach would likely provide important evidence for the C19MC expression domains and compartments within the mouse placenta.
3. The rationale/justification for such a large research group supported by the grant is unclear. Given the project supported 95% of a bioinformatician with such limited bioinformatics data shown is a clear weakness. There is a large % effort from research assistants ; however, the pay scale is variable and unclear. When added together, the project supported 4.8 FTEs which appears to be unjustified given the scope of the project and the data that emerged.

Reviewer 3:

1. Morphometric analyses of the placentae of the C19MC transgenics should be attempted.
2. An approach to verify homologous function of the human C19MC complex in the mice is needed. Use of qRT-PCR for a selection of mouse genes that are homologous to known human genes regulated by C19MC miRNA and that contain significantly conserved “seed” sites needs to be attempted.
3. Laser capture micro-dissection coupled qPCR is needed to verify expression patterns of the C19MC cluster in the mouse placentae.

Project Number: 1166802
Project Title: Glycocalyx Syndecan-1 and Preeclampsia Pathogenesis
Investigator: Carl Hubel, PhD

Section A. Project Evaluation Criteria

Criterion 1 - How well did the project meet its stated objectives? If objectives were not completely met, was reasonable progress made?

STRENGTHS AND WEAKNESSES

Reviewer 1:

In the first Aim the applicant proposed to examine a) the concentration/expression levels of specific proteoglycans resident in the glycocalyx, namely Syndecan-1 (SDC-1) and Glypican-1 (GPC-1), and their soluble forms, in the plasma archived and collected at mid-pregnancy, pre-delivery and post-partum from women with uncomplicated pregnancies and from women exhibiting clinical signs of preeclampsia; b) to investigate in parallel the expression of SDC-1 and GPC-1 in placental tissue from control and preeclamptic pregnancy with the goal of establishing a correlation with the variables tested in the plasma;c) to ascertain the impact of changes in oxygen tension on soluble SDC-1 and sGPC-1 in human villous explants.

Strengths: Overall, the applicant provided a compelling argument for conducting this first set of studies. The progress made in this first aim is impressive, and the proposed aims were for the most part achieved. The cross-sectional third trimester maternal studies were appropriately completed. The clinical parameters included for this part of the study were excellent. The methodology used (i.e ELISA) as well as the performed analysis for cross sectional and longitudinal case-control data and their relationship with variables examined were found to be appropriate. The data obtained from mid-pregnancy on decreased levels of SDC-1 in women who later developed preeclampsia but not gestational hypertension are quite relevant and have the potential to advance the clinical practice by using changes in sSDC-1 as an early predictor of preeclampsia. Overall, the obtained data have been greatly developed to examine all possible variables (i.e. pre-gestational hypertension, BMI, SGA). As stated by the applicant himself, a weakness of this set of studies relates to the use of term cases as controls rather than age-matched normotensive cases. This is important as the progress made by the applicant indicates a unique gestational SDC-1 profile. However, the applicant stresses this point and proposes to carry on the investigation including this control group.

Strengths: Substantial progress has also been made with respect to the investigation of SDC-1 and GPC-1 protein and mRNA levels in placental tissues from pathological and normotensive pregnancies.

Weaknesses: However, although obvious, analysis on their relationship to the plasma levels is not reported. The applicant should examine the expression of SDC-1 in isolated apical

microvillous fractions rather than in total lysates as the expression of this proteoglycan is primarily restricted to the syncytiotrophoblast layers. Also, the applicant needs to examine the expression of house-keeping protein for final normalization of Western blots.

Strengths: The experiments proposed in aim 1c on the effect of varying oxygen on SDC-1 release in villous explants have been carried out appropriately. The applicant states that they require completion. A weakness relates to their immediate interpretation as SDC-1 levels in the explants have not been carried out.

In the second Aim the applicant proposed to establish the contribution of trophoblastic SDC-1 in sequestering/binding sFlt-1, an important molecule involved in the pathogenesis of preeclampsia.

Strengths: Remarkable progress has been made on this aim. The applicant provides the first evidence that blood cells or plasma components are not involved in the heparin-induced release of sFlt-1. In addition, they found that a potential direct binding of sFlt-1 to HSPGs in vascular endothelial and HRT8 cells exist. The data have been compiled in a manuscript that was submitted last spring for publication.

In the original application, it was proposed to use both HRT8 cells and primary isolated trophoblast cells. I find the latter to be more appropriate to test the original hypothesis; however, in the progress report they shift to HUtMVEC cells. The rationale for doing so is not clearly stated.

In the third Aim the applicant proposed to examine the involvement of SDC-1 in lipid trafficking in trophoblast cells.

Strengths: The assays to measure SDC-1 binding and internalization of VLDL were established. Exciting preliminary results on binding of labeled VLDL to heparin and their uptake by cells were provided. This aim requires additional work to be completed.

Reviewer 2:

The project progressed very well in terms of execution and supportive data collected for the hypothesis as originally planned. No major changes were made against the plan.

Reviewer 3:

The overall goal of this project was to investigate the role of syndecan-1 (SDC-1), a prominent component of heparin sulfate proteoglycans in the glycocalyx of most cells, in contributing to and/or mediating changes of gene product expression at the maternal-fetal interface. Three Specific Aims were originally proposed.

The first used clinical samples to investigate the peripheral blood levels of syndecan-1 during normal and compromised (PE, SGA, GHTN) pregnancies. Although numbers of patients are rather small in the various categories, the clinical parameters and separation of patients are very well defined and discreet. Soluble SDC-1 levels were found to be ~2.5 fold lower in the PE patients and the majority of this decrease was clustered in those PE patients that also delivered SGA infants. Levels of soluble SDC-1 were not significantly different from normal in

pregnancies complicated with GHTN alone. In a separate cohort of patients, soluble SDC-1 was significantly lower at mid-gestation (20 weeks) in women that later developed PE versus those who progressed with uncomplicated pregnancies or those that developed GHTN alone.

Histochemical analyses of placenta samples suggest a positive correlation between maternal serum levels of soluble SDC-1 with syncytiotrophoblast staining of SDC-1. Verification of the histochemical data via immunoblot seems to support that normal placenta express higher levels of SDC-1 protein than PE placenta. Additional samples and proper loading controls are needed for this to be more definitive, and it is not clear if samples from different regions of the placenta are to be measured as well. Interestingly, SDC-1 mRNA expression levels did not seem to match differences in protein findings, suggesting a post-transcriptional regulation mechanism(s).

Overall, the objectives of this aim were generally met. Future efforts would be to increase the number of samples analyzed so the robustness of the conclusions can be increased.

Aim 2 sought to determine the ability of SDC-1 to mediate retention/release of sFlt-1 from trophoblast cell surfaces. Some preliminary data was provided suggesting that heparin addition to a cultured trophoblast cell line can increase release of sFlt-1 into media in an acute fashion (~20 min). Unfortunately, no data was provided using primary term trophoblast for these studies. These data might be much more compelling in that primary term trophoblast are known to express higher levels of sFlt-1 than trophoblast cell lines. Proposed experiments to use heparanase to confirm the specificity of the release data (Experiment 2) and specifically to SDC-1 (Experiment 3) do not seem to have been initiated yet. These are very logical and definitive next steps. Given the expertise of the lab, there are no concerns that these can be accomplished very quickly. The last aim proposes that SDC-1 may mediate lipid trafficking in trophoblast. Data was presented confirming the general assays for this aim, that uptake of their marker lipid is consistent with receptor-mediated mechanisms, and uptake can be inhibited by pre-treatment with agents that disrupt heparin sulfate proteoglycan functions. As with the previous aim, definitive experiments using primary trophoblast, or siRNA-mediated knockdown of SDC-1 do not seem to have been initiated yet. In total, the clinical aspects (Aim 1) of this project were well done. The mechanistic aspects (Aim 2 & 3) show adequate progress that, although not definitive yet, support the overarching goal of the project.

Criterion 2 - What is the likely beneficial impact of this project? If the likely beneficial impact is small, is it judged reasonable in light of the dollars budgeted?

STRENGTHS AND WEAKNESSES

Reviewer 1:

Undoubtedly, the current proposal is very significant, as preeclampsia remains one of the leading causes of maternal and fetal morbidity during pregnancy and later on in life can lead to detrimental consequences in both the mother and her child. Increasing our understanding on the mechanisms that regulate the contribution of glyocalix in trophoblast cell function in normal pregnancy and their dysregulation in pre-eclampsia, without a doubt, will increase our knowledge of the events, which are disarranged in this disorder of pregnancy. As stated above, the outcome of the proposed application may potentially lead to the development of a diagnostic

test to predict preeclampsia early on in pregnancy. The applicant discusses the importance of conducting future work aimed at examining GCX dysfunction in the genesis of preeclampsia and later-life CVD.

Reviewer 2:

This was a basic science project studying the pathogenesis of preeclampsia. Given the fact that there are no effective treatments or ideal diagnostic tools for the disease, this research is worthy of the funds invested.

A major strength is that data generated have helped the team to propose a renewal application of a PO1 grant.

Reviewer 3:

The most beneficial impact of this project may be its application to clinical diseases of pregnancy with regard to glycocalyx function and, in particular, lipid trafficking. Collectively, these are areas that are not well understood in pregnancy, normal or otherwise.

Criterion 3 - Did the project leverage additional funds or were any additional grant applications submitted as a result of this project?

STRENGTHS AND WEAKNESSES

Reviewer 1:

The applicant is currently seeking additional follow-up funds from NIH.

Reviewer 2:

No funds were leveraged. However, the PI's project contributed the samples to be analyzed, which would have cost more than what was invested.

Reviewer 3:

The PI has been active in attempting to solicit additional extramural funds to continue and extend this project. Data generated from this project was included in a PO1 NIH application submitted May 2012 which received a very respectable 12 percentile score. A revised PO1 application was submitted in January 2013, and is awaiting review/funding decision.

Data from the Formula Fund has been included in an NIH R21 application which received a 34 percentile score in February 2013. An additional R21 (Hubel, PI) was planned for June/October 2013 and an NIH RO1 for October 2013.

Criterion 4 - Did the project result in any peer-reviewed publications, licenses, patents, or commercial development opportunities? Were any of these submitted / filed?

STRENGTHS AND WEAKNESSES

Reviewer 1:

As stated in the progress report, a publication was submitted in the spring. If successful, the data obtained from the proposed studies will result in 2 additional papers.

Reviewer 2:

No peer-reviewed publications, licenses, patents, or commercial development as yet. However, three manuscripts are in preparation, which are in good hands.

Reviewer 3:

No publications have resulted from the project at its close. However, the PI lists three manuscripts in preparation that incorporate results from the funding of this Formula grant. This would constitute excellent production from the grant.

Criterion 5 - Did the project enhance the quality and capacity for research at the grantee's institution?

STRENGTHS AND WEAKNESSES

Reviewer 1:

A new assay has been developed. In addition, an undergraduate student and a postdoctoral fellow were involved in the study.

Reviewer 2:

The project did not enhance the quality and capacity for research at the grantee's institution.

Reviewer 3:

Funds from this Formula grant were used to support the training of one undergraduate student and one postdoctoral fellow. No outside researchers were recruited, and no significant improvements of research capacity or quality were reported.

Criterion 6 - Did the project lead to collaboration with research partners outside of the institution or new involvement with the community?

STRENGTHS AND WEAKNESSES

Reviewer 1:

Collaborations have been established with Dr. Rajakumar at Harvard University and Dr. Weissgerber at the Mayo Clinic, Rochester.

Reviewer 2:

The project did not lead to collaboration with research partners outside of the institution or to new involvement with the community.

Reviewer 3:

There seems to be limited collaborations outside the MWRI in this project. Continued involvement of a former postdoctoral fellow at the MWRI, now currently at the Mayo Clinic, was utilized for the data analyses and writing of a manuscript. A colleague from Harvard

provided assistance with monitoring syncan-1 protein expression, but it is not clear if this has been developed into something more substantial.

SECTION B. RECOMMENDATIONS

SPECIFIC WEAKNESSES AND RECOMMENDATIONS

Reviewer 1:

1. Establish SDC-1 expression in MVM (microvillous membrane fractions).
2. Expand on the role of oxygen and oxidative stress (possibly mediated by cytokines) on SDC-1 expression in villous explants and primary isolated cells.
3. Include rationale for models examined.

Reviewer 2:

None.

Reviewer 3:

1. Differences in SDC1 protein expression between normal and PE placentae must be verified with additional samples, proper loading controls, and evidence that there is not regional expression differences within individual placenta to be definitive.
2. Future efforts need to increase the number of clinical samples analyzed so the robustness of these preliminary conclusions can be verified.
3. Aim 2 needs to be verified using primary term trophoblast which might produce much more compelling data.
4. Aim 2 - proposed experiments to use heparanase to confirm the specificity of the sFlt1 release data (Experiment 2) and knockdown of SDC-1 (Experiment 3) to determine its role in this process must be completed.
5. Aim 3: definitive experiments using primary trophoblast for this aim need to be completed.
6. Aim 3: knockdown of SDC-1 to investigate its role in the lipid trafficking aspects needs to be completed.

Generic Recommendations for the Magee-Womens Research Institute and Foundation

Reviewer 1:

Overall, an original and exciting proposal from an experienced investigator who has a track record in vascular biology and preeclampsia. Future development of this research project aimed at establishing the role of placental GCX dysfunction in preeclampsia and its impact on CVD later on in life, will definitely advance the scientific knowledge and may potentially advance the clinical practice.

Project Number: 1166803
Project Title: Targeting Women's Cancer Cells with
Novel Cell Cycle Inhibitors Blocking Centrosome Clustering
Investigator: Calvin Simerly, PhD

Section A. Project Evaluation Criteria

Criterion 1 - How well did the project meet its stated objectives? If objectives were not completely met, was reasonable progress made?

STRENGTHS AND WEAKNESSES

Reviewer 1:

The project sought to determine if centrosome clustering and its underlying mechanism may play a role in the oncogenicity of a cancer cell. The strength is that the project met all its stated objectives. Unfortunately, the outcome did not reach any conclusive end, and it appears cancer cells have alternative mechanisms to undergo successful centrosome separation, making this mechanism an unlikely target for pharmacological intervention.

Overall the research design was properly designed to address the questions posed, but it did not provide sufficient alternative plans in the event that the outcome led to inconclusive results. This problem was quite evident in this case. While the experiments and data generated were relevant to the stated goals, they did not provide enough mechanistic insights, but rather depended heavily on phenomenological observations.

Reviewer 2:

The research adhered quite closely to the stated aims. No major changes were implemented, although other approaches that were mentioned in the strategic plan were not employed. One example is the characterization of HSET, a protein known to play a role in centrosome clustering. Although it would have been important to test the expression and role of this protein in normal and cancer cells, this was not pursued, and no explanation for this omission was provided. Another example is that additional normal cell lines should have been investigated, including MCF10A as proposed in the strategic research plan. The logic behind the choice of normal and cancer cell lines, as well as the number of such lines, was not articulated.

The PI could have selected one type of cancer cell (such as breast or lung cancer) and examined a large panel of such lines, along with normal controls, but this was not the approach taken. In addition, it is difficult to assess the overall impact of many results since the majority of experiments are presented graphically without error bars or statistics. Were these experiments performed multiple times, and if so, how much variability was encountered? Insufficient data were presented with normal cell lines to make conclusions regarding the impact of drugs on cancer cells versus normal cells.

Reviewer 3:

Strengths: The main objective of this project of "understanding the dynamics and molecular composition of the cell's spindle poles and centrosomes in normal and cancerous cells" was achieved by completing all 4 specific aims of this study.

The research design was very well developed and appropriate to achieve the goals of this study. It included logical step by step questions to achieve the objective: 1) Is centrosome clustering dependent on the expression patterns of molecular motors (dynein, HSET, dynactin) and NuMA at the spindle poles in cancerous and noncancerous cancer cells? ; 2) How does the Cdk1 inhibitor RO3306 affect cell cycle progression and centrosome duplication in cancerous versus noncancerous cells and expression of microtubule minus end molecular motors?; 3) Using dynamic confocal imaging with a living marker for centrioles or microtubules, does Cdk1 inhibitor RO3306 prevent centrosome clustering and/or increase spindle multipolarity at metaphase in cancer cells following drug rescue?; and 4) Does the PARP inhibitor PJ-34 block cell cycle progression and centrosome clustering at the metaphase spindle poles with or without RO3306 exposure in cancer versus noncancerous cells?

Weaknesses: None

Criterion 2 - What is the likely beneficial impact of this project? If the likely beneficial impact is small, is it judged reasonable in light of the dollars budgeted?

STRENGTHS AND WEAKNESSES

Reviewer 1:

This is a basic cell biological project aimed to unravel the dynamics and molecular composition of the cell's spindle poles and centrosomes in normal and cancerous cells. The ultimate goal is to pinpoint new targets for designing chemotherapeutic strategies in oncology. While the overall long-term objective carries potential, the overall experimental design was not aimed at finding drug targets, but more to understand the fundamental molecular mechanisms in centrosome stability. Only one drug was used throughout the experiment and it was used to trigger a centrosome malfunction for further studies.

Reviewer 2:

No major discoveries or new drugs or approaches were suggested by the research project. Because of the limitations enumerated in Criterion 1 (Section A), including the narrow scope and lack of key controls, the overall significance for improving health is not high. The future plans for this research project were not clearly delineated.

Reviewer 3:

Strengths:

There is potentially high impact of this project as it may afford a much better understanding of the link between spindle multipolarity, aneuploidy, and malignancy transformation in humans and, perhaps, give insights into innovative approaches for personalized treatment of cancers, and

thus may result in improving treatment options and therapeutic outcomes for patients suffering from a variety of malignant disorders.

Weaknesses: None

Criterion 3 - Did the project leverage additional funds or were any additional grant applications submitted as a result of this project?

STRENGTHS AND WEAKNESSES

Reviewer 1:

No additional funding has been leveraged, although the investigators plan to apply for additional funding.

While the current NIH funding situation is challenging, the quality and impact of the completed work are not likely to lead to a realistic chance of NIH funding unless further progress is made.

Reviewer 2:

No additional leveraging of funds is mentioned. The researchers plan to apply for NIH funding.

Reviewer 3:

Strength: There was no additional funds obtained during this research project. The PI plans to submit a proposal to NIH to expand this research direction.

Criterion 4 - Did the project result in any peer-reviewed publications, licenses, patents, or commercial development opportunities? Were any of these submitted / filed?

STRENGTHS AND WEAKNESSES

Reviewer 1:

The project did not lead to any peer-reviewed publications, licenses, patents or other commercial development opportunities. The investigators plan to submit articles to peer-reviewed journals.

Overall, the productivity of this funded project is deemed low. Besides developing a few time-lapse imaging techniques for the lab, as yet there is no high impact data generated that is currently suitable for publication.

Reviewer 2:

The project did not result in any publications, licenses, patents, or commercial development opportunities. The researchers plan to submit their findings as articles for peer-review.

Reviewer 3:

Strength: This project has not resulted in a peer-reviewed publication, yet. However, the results of this study are expected to be submitted to a prominent cell and cancer biology journal.

Criterion 5 - Did the project enhance the quality and capacity for research at the grantee's institution?

STRENGTHS AND WEAKNESSES

Reviewer 1:

The project did not require or introduce any major improvement to the infrastructure. There were no new investigators added. The project also did not support any pre- or post-doctoral students. The support was basically leveraged to build up the lab's ability to perform live cell imaging. In this regard, this is considered a weakness.

Reviewer 2:

The project did not enhance the infrastructure at the grantee's institution, nor were investigators added or brought into the institution to carry out the research. The Investigator states that funds were "instrumental in recruiting new National Cancer Institute resources to programs here. Furthermore, this sponsorship has been leveraged to recruit new collaborations to the Commonwealth and we are optimistic that further dividends will accrue." However, it is not clear which collaborators were recruited, nor were the "new NCI resources" delineated. No funds were used to pay pre-doctoral students or post-doctoral fellows.

Reviewer 3:

Strength: The development of new sophisticated dynamic approaches for investigating microtubule-mediated motility in living cancer cells using time-lapse video microscopy that resulted from this project benefited variety a of investigators in the grantee's institution.

Criterion 6 - Did the project lead to collaboration with research partners outside of the institution or new involvement with the community?

STRENGTHS AND WEAKNESSES

Reviewer 1:

The project did not lead to any collaboration with research partners outside of the institution. The final progress report argued that the development of live cell microscopy to enable centrosome imaging will enhance the recruitment of researchers. This is overly simplistic and optimistic considering time-lapse imaging of molecular motor in live cells is now a rather standard technique for most cell biology labs and is offered in most microscopy core facilities.

Reviewer 2:

The project did not lead to collaborations with research partners outside the grantee's institution or with the community. No plans are stated that include new collaborations as a result of the research.

Reviewer 3:

No new collaborations outside the institution was established.

Section B. Recommendations

SPECIFIC WEAKNESSES AND RECOMMENDATIONS

Reviewer 1:

1. The most significant weakness is in the experimental design. The experiments relied very heavily on qualitative observation. This weakness has severely limited the scope of the project, because if the outcome does not fit their hypothesis (and it did not), the investigators had not provided sufficient alternative plans to delve into the problem further.
2. The experiments did not provide significant mechanistic insights into the biological problem. Even though they tried to link molecular motors to the event of centrosome separation in cancerous vs. non-cancerous cells, they did so strictly by localization studies.
3. The invested funds did not lead to any significant improvement or enhancement of anything. The techniques 'developed' in this project are rather routine in most cell biology labs and even more so in established imaging core facilities.

Reviewer 2:

1. Choice of cell lines: the researchers should justify their choice of cell lines and include additional normal controls besides WI-38. The authors could have picked additional normal primary cells, MCF10A normal mammary cells, other normal lung fibroblasts, etc. The number of normal and cancer cell lines should be vastly expanded to increase the significance of the findings.
2. Reproducibility and statistics: It is not clear how many times each experiment was independently repeated. Biological replicates should be presented graphically, with all statistical testing. Sufficient numbers of cells should be imaged to attain statistical significance.
3. Include additional positive control cell lines in the study: Previous studies have shown that PARP inhibitors lead to centrosome declustering in certain cancer cell lines. It would have been advantageous for the investigators to test cell lines from the previous study in parallel as positive controls. This would have provided valuable confirmatory information. On the other hand, it is possible that previously published results may not be duplicated in other laboratories, and this would have also provided important new information.
4. Test other inhibitors: since the anticipated outcome for the CDK1 inhibitor was not observed (G1/S block was observed rather than a G2/M block), additional inhibitors that are expected to block cells at the G2/M transition could be tested on normal and cancer cell lines.
5. Test additional clustering mechanisms and proteins: the HSET protein has been established to play an important and potent role in centrosome clustering. The expression and role of this protein in normal and cancer cells should be tested using the approaches outlined in the research proposal and by ablating HSET in both types of cells. This may be very informative.

Reviewer 3:

None.

Generic Recommendations for Magee -Womens Research Institute and Foundation

Reviewer 3:

This is an outstanding proposal that achieved all its goals within a short period of funding, and has potentially high impact for the future development of novel anti-cancer therapeutic strategies.

ADDITIONAL COMMENTS

Reviewer 2:

The overall goal of this project--comparing the ability of normal and cancer cells to respond to cell cycle inhibitors and drugs that block centrosome clustering--is highly commendable, and significant progress was made toward achieving the stated goals of the strategic plan. However, overall, it is difficult to assess the significance of the research project given several limitations. First, the selection of cell lines in the study is too narrow. Additional cell lines should be examined and compared with adequate normal controls. Only one normal cell line was investigated (WI38), and in several cases, experiments with this cell line failed to lead to conclusive results, or an insufficient number of events were observed. Several results were surprising. These include the lack of response (G2/M phase block) of some cancer cell lines to the CDK1 inhibitor and the response to the PARP inhibitor, which differed from previously published work. A major caveat to the studies concerned the statistical treatment of data: many graphs appeared to have resulted from a single experiment. Were these experiments repeated, what was the variability, and why were no statistics presented?

Project Number: 1166804
Project Title: Regulation of Spermatogenesis by Classical and
Non-classical Testosterone Signaling
Investigator: William H. Walker, PhD

Section A. Project Evaluation Criteria

Criterion 1 - How well did the project meet its stated objectives? If objectives were not completely met, was reasonable progress made?

STRENGTHS AND WEAKNESSES

Reviewer 1:

Strengths:

1. The project meets the stated objectives well and got positive results in all 3 Aims.
2. The research design and methods were adequate in light of the project objectives.
3. The PI showed that both non-classical T signaling inhibitors S1 and D2 can block spermatogenesis.
4. The Adeno-virus delivery system worked quite well in vivo.

Weaknesses:

1. Although the PI claimed the non-classical T signaling can block sperm release, there is no statistical analysis in Fig. 1.
2. In Fig. 1B the S1-T lane seems not obviously decreased if normonized by the total ERK.
3. Also in Fig.1B, the S1s control treated with T the P-ERK increased obviously, why?
4. It would be better to consistently compare S1 and D2 effects in all the figures that apply.

Reviewer 2:

Strengths:

1. This project focuses on understanding the role of testosterone in the regulation of spermatogenesis and hence male fertility—an important aspect of human health which is understudied, particularly with regard to potential male contraceptives.
2. The three aims are clearly delineated and significant progress was made on each aim, particularly Aim 2.

3. The focus of the project on the non-classical function of testosterone in its interaction with the androgen receptor in activating Src kinase in Sertoli cells is novel and of potential importance in the regulation of spermatogenesis.

Weaknesses:

1. While overall, the data presented support the conclusions drawn, the experimental detail was a bit superficial in several experiments, for example, the sperm release assay presented in Figure 1C and the transepithelial resistance assay in Figure 1D.
2. Again, while the overall progress was excellent to outstanding, the PI did not always make an effort to explain why certain experiments were NOT done, for example, determining the efficiency of inhibitor uptake by Sertoli cells in the cultured fragments of testicular tubules.
3. Perhaps this reviewer missed something, but there was no information presented on the S1 peptidomimetic inhibitor designated D1 (nor on the apparently negative peptidomimetic D2). It was also not completely clear as to the choice of molecules for the localization studies in Aim 2.

Reviewer 3:

Most of the stated objectives for the project were met. Specifically, the investigators accomplished 65%, 90% and 70% of the proposed research for Specific Aims 1, 2 and 3, respectively. For those objectives that were not completely met, the investigators either made efforts without success due to uncontrolled factors or re-designed better experimental strategies to achieve the similar scientific knowledge.

Proposed work for Aim 1: In Aim 1, the investigators proposed to determine whether inhibition of non-classical T signaling blocks sperm release. It was planned to use the S1 peptide of AR that interacts with Src to block Src from interacting with AR. It was also planned to use D1 peptidomimetic that binds to AR to block AR to interact with Src. In the proposed experiments, seminiferous tubules from rats were dissected to isolate fragments containing only seminiferous stages VII-VIII. The isolated fragments were incubated for 20 h with T, T+S1 peptide, T+D1 peptidomimetic, T+Flutamide (positive control) or T+scrambled peptide (negative control). Sperm released into the medium and the number of elongated spermatids retained in the tubule fragment will be determined for each treatment. Inhibitor uptake by Sertoli cells could be determined by tracing the fluorescent tags on the inhibitors.

Accomplished work for Aim 1: Seminiferous tubule fragments containing fully developed sperm that are poised to be released were isolated from rats, treated with T, T+S1 peptide and T+scrambled peptide. S1 peptide treatment reduced sperm release by 50% compared to scrambled control peptide. T+S1 peptide treatment also decreased tight junction integrity by 52% compared to T stimulation alone. These results suggest that non-classical T signaling contributes to sperm release.

Evaluation: Overall, the investigators have accomplished 65% of their proposed work in Aim 1.

Proposed work for Aim 2: Determine whether inhibition of classical and non-classical T signaling blocks spermatogenesis in testis explants.

To examine whether T activates non-classical T signaling in the testis explant from rat by treating the explants with flutamide followed by T treatment. P-Src, p-ERK and p-CREB will be examined.

To determine whether the presence of the non-classical inhibitors block T-mediated protein phosphorylation, the investigators will treat testis explants with flutamide as above. S1 and D1 inhibitors will be added with T to assess how these inhibitors affect the levels of P-Src, p-ERK and p-CREB.

To determine the effects of blocking classical or non-classical T signaling in testis explants, the investigators will express S1 to inhibit non-classical pathway or HDAC-KRAB-AR122 to inhibit classical pathway. Explants will be cultured on agar and treated with T. mRNA levels of AR target genes Rhox5, Claudin 3, N-cadherin, Espin, Jam 3, Eppin and Tsx in the Sertoli cells will be measured. P-Src, p-ERK, and p-CREB will be examined. Germ cell loss will be checked. TUNEL assays will be performed to examine cell apoptosis. BTB integrity will be examined by staining occluding and ZO-1.

Accomplished work for Aim 2: The investigators have improved a 7-day testis explant culture model. In this model, functional cell-to-cell connection and interactions are maintained. This model is useful to study non-classical T effects on spermatogenesis.

Inhibition of either the classical or non-classical pathway disrupts spermatogenesis by different mechanisms in testis explants. The investigators found that treatment of the testis explants in culture with D1 peptidomimetic inhibitor reduced Src activation (phosphorylation) in the areas near basal lateral regions where the D1 subcellular locations were detected. The treatment also decreased CREB phosphorylation/activation. These results indicate inhibition of the non-classical T signaling could block T activation of CREB. Furthermore, adenoviral expression of the S1 peptide resulted in the loss of germ cells in the seminiferous tubules. Treatment with HDAC-KRAB-AR122, an inhibitor of classical T signaling, caused premature release of germ cells. These results indicate that inhibition of either the classical or non-classical pathways disrupts spermatogenesis in testis explants.

Pathway-specific activators can induce gene expression in the testis explants.

The investigators isolated testis explants from AR-defective testicular feminized (tfm) rats and expressed AR or AR mutants AdAR-delta372-385 and AdARC562 specific to classical or non-classical pathways in Sertoli cells by adenoviral approach. As evaluated by Rhox5 induction by T, the classical pathway was activated in tfm testis explants expressing wild type AR or AdAR-delta372-385, but not activated in tfm testis explants expressing AdARC562.

In testis explants isolated from wild type rats expression of either classic or non-classic inhibitor reduced the expression of Rhox5, a target gene of classic T signaling. The investigator thought this might be an indirect effect through alteration of other regulatory pathways in Sertoli cells.

Expression of either wild type AR or classic pathway AR mutant in Sertoli cells effectively increased PLZF and c-kit gene expression in germ cells. Expression of non-classic pathway AR mutant also increased the expression of these genes, although at a lesser degree. These results suggest that the classical and non-classical pathways both contribute to providing the factors that are required for the progression of spermatogenesis.

Evaluation: although the investigators did not exactly perform the experiments as proposed, they have designed and performed new experiments to address the similar scientific questions. Overall, the investigators completed 90% of the research proposed in Aim 2.

Proposed work for Aim 3: The investigators planned to determine whether the classical and/or non-classical pathway is required to maintain spermatogenesis in vivo. AdS1, AdHDAC-KRAB-AR122 or AdS1s will be injected into testis retes to express S1, Scrambled peptide or H-K-AR122 peptide, which inhibit non-classical or classical pathway, respectively, or serve as a control.

AR target expression and p-Src, p-ERK and p-CREB levels in Sertoli cells will be examined to assess classical and non-classical T signaling.

BTB integrity will be examined by injecting EZ-Link Sulfo-NHS-LC-Biotin dye into the lumen of seminiferous tubules.

Spermatid attachment will be checked.

Sperm release will be examined.

Accomplished work for Aim 3: Deletion of AR exon 3 blocks classical and non-classical signaling, suggesting that the previous studies with deletion of AR E3 model could involve the disruption of both classical and non-classical pathways.

Cetroreliz treatment reduces T to 16% and then, TP+TE treatment increases T to 137% of control levels in 1 h, providing an in vivo model for rapid T manipulation. P-ERK is rapidly increased in 1h after TP+TE treatment, suggesting it is a consequence of non-classical pathway.

The investigators also showed that injection of S1 adenoviruses into the rete testis caused vacuoles in the seminiferous epithelium due to the loss of spermatocytes, while expression of AdH-K-AR122 that inhibits classical pathway caused immature germ cell release. Inhibition of both pathways had an additive effect. These results indicate that both pathways are required for normal spermatogenesis. These results are similar to those obtained from testis explant models.

Biotin tracer assay demonstrated that inhibition of the non-classical pathway damaged the blood testis barrier.

Evaluation: this reviewer agrees with the investigators' estimation that Aim 3 was 70% completed. The remaining 30% of the proposed work includes the assays of actin expression and localization, as well as p-ERK and p-CREB in tissue sections from testes infected with adenovirus expressing pathway-specific inhibitors. In addition, because the extensive disruption of the seminiferous epithelium did not allow for identification of the stages, the investigators were unable to determine whether inhibition of the classical or non-classical pathways blocked sperm release in vivo.

The research design and methods were adequate in light of the project objectives.

The data developed addressed the research questions posed. The investigators hypothesized that both classical and non-classical T signaling pathways are required for normal spermatogenesis. The results obtained from this project proved this hypothesis. The data developed were precisely in line with the original research protocol.

Some but not a lot of changes were made to the research protocol. These changes were well justified and actually worked better than those initially proposed.

Sufficient data and information were provided to support that the project met its objectives. Please refer to the detailed description under Question 1.

The data and information obtained were applicable to the project objectives listed in the strategic research plan.

Strengths: In Aim 1, the investigators observed the expected results from the S1 peptide treated seminiferous tubule fragments, and showed that the non-classical T signaling pathway is required for full release of sperm. The investigators also found that inhibition of the non-classical T signaling pathway affected the tight junction between Sertoli-Sertoli cells, which might result in the integrity of the blood testis barrier. In Aim 2, the investigators developed the tm testis explant culture system in combination with adenoviral expression of wild type AR and AR mutants, which is a powerful system to define the specific function of classical and non-classical T-signaling pathways. In Aim 3, the model used to manipulate the T levels in rat is a better model to study the response of non-classical T signaling in terms of ERA phosphorylation compared with the initially proposed model with flutamide treatment followed by T treatment.

Weaknesses: In Aim 1, the efficiency of inhibitor uptake by Sertoli cells in the cultured tubule fragments was not examined. The proposed D1 peptidomimetic was not tested in this in vitro model system, making it unclear whether the inhibitory role of S1 peptide is attributed to its inhibition of Src interaction with /TAR or its binding and inhibition of normal Src kinase. However, this question was addressed in experiments performed in Aims 2 and 3. In Aim 2, it was not described whether expression of wild type AR or the non-classic AR mutant in the AR defective tm testis explants rescue spermatogenesis?

Criterion 2 - What is the likely beneficial impact of this project? If the likely beneficial impact is small, is it judged reasonable in light of the dollars budgeted?

STRENGTHS AND WEAKNESSES

Reviewer 1:

Strengths:

1. The beneficial impact could be the identification of T signaling control defects in infertility patients that can be targeted for therapies.
2. Another beneficial impact could be the development of male contraceptive drugs that would block early steps of spermatogenesis by both non-classical and classical T signaling inhibitors.
3. Because the PI used Adeno-virus which only infect Sertoli cells in the testis, the effect on other T-dependent processes is minimal.

Weaknesses:

1. The inhibition of sperm release by S1 inhibitor is only 50%. It is questionable for the contraceptive usage compared to the current available drugs.
2. The PI did not consistently compare S1 vs. D2 efficiency in each experiment.
3. Since both classical and non-classical T signalings are androgen receptor dependent (AR), how about targeting AR to get the better effect?

Reviewer 2:

Strengths: As noted above, the field of male fertility is important but poorly developed, especially with regard to possible new male contraceptives. The current study addressing various aspects of testosterone function in spermatogenesis, particularly with regard to the non-classical testosterone is thus novel and the research will clearly contribute to improving reproductive health in men.

The studies also contribute to our overall understanding of the regulation of spermatogenesis at the cellular and molecular levels.

Weaknesses: While the PI argues convincingly with regard to the significance of the research in understanding infertility, the relevance to male contraception is less well-developed. This is particularly true given the profound effects of the S1 peptide and its peptidomimetics on the blood testis barrier, a cellular process which would ideally remain intact in any male contraceptive approach.

The reviewer apologizes in advance if the information on future plans for the research was overlooked, but a clear discussion of the “next steps” was not presented (beyond applying for grants noted on page 3).

Comment: It should not be construed as either a strength or a weakness, but most of the considerations listed under Criterion 2 (for example, “consider any major discoveries, new drugs and new approaches for prevention, diagnosis and treatment that are attributable to the completed research project”) are not really relevant to this project, as it is very basic science focused.

Reviewer 3:

This study demonstrated that both classical and non-classical T signaling pathways are required for normal sperm release, Sertoli cell-mediated regulation of gene expression in germ cells, maintenance of BTB, and maturation of spermatids. These findings suggest that in addition to the classical pathway, the non-classical pathway can be also targeted for developing male contraceptives. In addition, defective signaling in the non-classical pathway may also count for some infertile cases.

Understanding the detailed mechanisms responsible for T signaling-regulated spermatogenesis may identify new therapeutic targets for developing male contraceptives and for treating infertile men.

There were no major discoveries, new drugs or new approaches for prevention, diagnosis and treatment that are attributable to the completed research project.

In future studies, the investigators will develop transgenic mouse models expressing only the classical or only the non-classical pathway to identify the spermatogenesis processes that are regulated by each pathway.

Strengths: The data obtained from this study clearly showed the non-classical T signaling pathway contributes to normal spermatogenesis. This finding suggests that further identification of specific signaling components in the testis could reveal novel targets for developing male contraceptives. This study also has some potential leading to identification of some unknown defective mutations responsible for male infertility.

Weaknesses: The study did not propose any experiments to understand the exact mechanisms responsible for mediating the T/AR signaling in Sertoli cells in relation to the control of proliferation and differentiation of the germ cells. However, this is not a weakness of the project performance since it was not proposed in the approved application.

Criterion 3 - Did the project leverage additional funds or were any additional grant applications submitted as a result of this project?

STRENGTHS AND WEAKNESSES

Reviewer 1:

The researchers applied for additional funds, and are planning to apply for additional funding in the future to continue the research.

Reviewer 2:

Strengths: The PI has applied for NIH funding three times for projects that would appear, based on the titles of the proposals, to be based on work supported at least in part by funds from this project.

Weaknesses: Although 3 proposals were submitted to the NIH and an R01 was to have been submitted to the NIH in June of 2013, it does not appear that any of the applications resulted in funding.

In addition, the PI did not specify as requested as to how the proposals in 2/2012 and 6/2012 incorporated data/results from this project.

Reviewer 3:

The researchers have submitted three NIH grant applications, with two of them unsuccessful and one of them pending. Given the current very low funding rate of NIH grants, the negative outcome does not mean the application was not of high quality.

The researchers plan to submit a R01 NIH application in June of 2013.

Strengths: The data obtained from this study can serve as a solid base to apply for a NIH R01 grant.

Weaknesses: The researchers may like to include some molecular analysis in depth to uncover the exact mechanisms responsible for the paracrine/intercellular events between Sertoli cells and the germ cells.

Criterion 4 - Did the project result in any peer-reviewed publications, licenses, patents, or commercial development opportunities? Were any of these submitted / filed?

STRENGTHS AND WEAKNESSES

Reviewer 1:

The project did not result in any peer-reviewed publications, licenses, patents, or commercial development opportunities as yet.

Reviewer 2:

Strengths: The PI states that a manuscript will be submitted to Molecular Endocrinology, based on the data obtained from this project.

Weaknesses: It is unclear when this final report was submitted, making it very difficult for the reviewer to comment as to whether it would be realistic or not to have expected a publication.

Reviewer 3:

No publication, licenses, patents or commercial development opportunities were listed in the final report. Publication is expected based on the basic research feature of this study.

The researchers indicated that a manuscript summarizing their results for the funding period will be submitted to Molecular Endocrinology within 3 months after the project was completed.

Criterion 5 - Did the project enhance the quality and capacity for research at the grantee's institution?

STRENGTHS AND WEAKNESSES

Reviewer 1:

The project allowed a strengthening of a collaboration with Dr. Pancharatnam Jeyasuria at the University of Pittsburgh, which provided needed preliminary data for a collaborative NIH RO1 proposal submitted in February 2012.

Reviewer 2:

Strengths: The PI enlisted the help of 2 undergraduate students, one pre-doctoral student, and one post-doctoral fellow on this project.

The project support permitted the retention of a technician whose services would have been lost without the grant.

Weaknesses: Although the PI claims that the project allowed a strengthening of a collaboration with Dr. Jeyasuria that resulted in a joint grant application, that grant was submitted one month after this project began. It is difficult to believe that there would have been significant contributions in less than one month.

Reviewer 3:

The project has maintained a technician's job. The project also helped the researchers to develop/improve some of their research model systems.

No new investigators or researchers were brought into the institution to help carry out the research. This would have been hard to do with such a short grant funding period (1 year).

Grant funds were used to pay for research performed by multiple pre- and postdoctoral trainees that were listed in the final report.

Strengths: The project has enhanced the research capacity by promoting collaboration, keeping a technician and training pre- and post-doctoral students.

Weaknesses: no obvious weakness was noticed.

Criterion 6 - Did the project lead to collaboration with research partners outside of the institution or new involvement with the community?

STRENGTHS AND WEAKNESSES

Reviewer 1:

The project did not lead to collaboration with research partners outside of the institution.

Reviewer 2:

No collaborations outside the University.

Reviewer 3:

The project has enhanced a collaboration between the principle investigator and another faculty member at the University of Pittsburgh.

No particular strength or weakness was noticed.

Section B. Recommendations

SPECIFIC WEAKNESSES AND RECOMMENDATIONS

Reviewer 1:

None

Reviewer 2:

1. The PI should update the agency with regard to both ability to secure funding for the project and as to whether the results have been submitted for publication or are in press.
2. The PI might wish to reconsider whether molecules that disrupt the blood testis barrier are really ideal candidates for contraception and rather, focus the research on male infertility.

Reviewer 3:

1. It is recommended that the researchers describe in the report whether restoration of wild type AR or AR mutants specific to classical or non-classical T pathways in the tem model system could rescue spermatogenesis.
2. It is suggested that the researchers propose some experiments in their future studies to assess the molecular mechanisms responsible for mediating the effect of T signaling in Serologic cells to the germ cells for spermatogenesis.