A. For the overall grant, briefly describe your grant oversight process. How will you ensure that future health research grants and projects are completed and required reports (Annual Reports, Final Progress Reports, Audit Reports, etc.) are submitted to the Department in accordance with Grant Agreements? If any of the research projects contained in the grant received an “unfavorable” rating, please describe how you will ensure the Principal Investigator is more closely monitored (or not funded) when conducting future formula funded health research.

We are cognizant of the impact that the Formula Research Funds have had upon our research at Magee-Womens Research Institute, and are truly grateful for this support. In 2011, program funds supported four projects that centered on diverse issues in reproductive biology and women’s health, ranging from spermatogenesis to placental development, preeclampsia, and cancer. We provide the following comments regarding our management of the program award to MWRI.

Over the past six years, MWRI has established a system for monitoring and supervision, designed to enhance scientific success, efficiency, and ensure the responsible conduct of research with the Formula Research Funds. This system begins with careful identification of projects that, while ambitious, challenging, and in their early stages of development, have real potential to launch impactful research pursuits. Each investigator’s proposal is discussed with the Director for scientific merit, and refined as needed. Post award, the Director conducts progress meetings with all investigators prior to the six-month and one-year marks. These are conducted in addition to standard scientific interactions and reporting mechanisms. Special attention is given to junior investigators, who meet regularly with mentorship teams to monitor all aspects of research and professional development. Additionally, we have instituted a routine mechanism for internal review of all NIH-level grant applications, as well as grant-in-progress (GRIP) sessions in which established investigators provide key feedback on proposals in development. Administratively, Ms. Cheryl Richards, MWRI’s Director of Grants and Contracts, provides oversight of our compliance with the program. She assists investigators with all aspects of budget preparation, monitors regulatory compliance in the responsible conduct of research, and ensures that all reports are completed in a timely and thorough manner. Furthermore, Ms. Richards maintains detailed records of all investigators and projects funded during the life of the program, and assists the Director in identifying candidates for funding. The success of these policies is evident that, in the past six years, projects have been enthusiastically received and only one of the 31 projects received an “unfavorable” score.

We are grateful to the program reviewers for their thorough evaluation of the 2011 projects. Each investigator has provided a detailed response to the reviewers’ critiques and values the reviewer’s input in the research process. We highlight that, given the “seeding” focus of the one-
year awards and the germination time of new lines of biomedical research, reporting measurable outcomes of success at the conclusion of the one-year term is very difficult. Indeed, some of these projects have continued in the subsequent year, and will be summarized in the 2012 report.

We thank the Pennsylvania Department of Health for its support of the Health Research Award program, which has been integral to MWRI’s status as the top funded research institute in the United States in the field of reproductive sciences and women’s health. The program has afforded our investigators the opportunity to creatively address key health issues and promote cutting edge-scientific discovery under a mantle of conscientious oversight. Interestingly, recent review of the program from its inception revealed that, for every one dollar of program funds received, MWRI has been able to leverage almost four dollars of extended research funding.

We look forward to the opportunity to partner with the Department of Health in future endeavors, and furthering our research to improve the health of women and their infants, in Pennsylvania and beyond.
B. To ensure that feedback provided in the Final Performance Summary Report is utilized to improve ongoing and future research efforts, briefly describe your plans to address each specific weakness and recommendation as noted in Section B of the Final Performance Summary Report and listed below. If no weaknesses are listed below, no response is required.

(As you prepare your response please be aware that the Final Performance Summary Report, this Response Form, and the Final Progress Report will be made publicly available on the CURE Program’s Web site.)

SPECIFIC WEAKNESSES AND RECOMMENDATIONS

Reviewer 1:
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.

Response: Our in silico study indicates that a large number of predicted target genes are conserved across the different species and, hence, might be silenced in both organisms. The identification of a small number of transcripts targeted in mouse placentas could bring valuable information even if it does not recapitulate the complete spectrum of actions mediated by the C19MC miRNAs in human trophoblasts. Another point is that the C19MC miRNAs are produced in the placenta but are also released into the maternal circulation, where they could affect remote targets. As in pregnant women, we detected circulating C19MC miRNAs in the blood of pregnant transgenic mice. We showed recently that secreted C19MC miRNAs enhance the resistance of non-trophoblast target cells to infection by viruses. Investigation to determine whether transgenic pregnant mice experience higher resistance to viral infections is currently underway. Therefore, while a mouse transgenic model is probably not an “ideal” model, we believe it can help to answer some specific questions regarding the biology of this unique family of miRNAs, which cannot be ethically and effectively addressed in humans.

2. 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?

Response: We agree with the reviewer and have considered this possibility. We performed preliminary experiments using LNA miRNA inhibitors but low transfection efficiency in primary trophoblasts did not allow us to significantly antagonize the abundant miRNAs. A promising alternative was to use miRNA sponges that can be efficiently delivered to hard-to-
transfect cells using a lentivirus. Another interesting feature of miRNA sponges is that they can target multiple miRNAs from the same family and sharing the same seed sequence. However, preliminary experiments using this approach indicate a rather modest inhibition of abundant miRNAs and no effect on the phenotype of cells. We recently modified the design of the sponge and opted for a new strategy called “tough decoy” or TuD inhibitors that were recently found to be more potent inhibitors. We have obtained promising results but we have not yet applied this method to the inhibition of C19MC 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.

Response: We agree that study of the expression of the C19MC miRNAs at different time points could be of particular interest with regard to better characterization of the timing and dynamics of C19MC expression. These studies are underway in our lab. However, we did not perceive this as a priority during that funding period since we have not detected any anomalies in the placental development of transgenic embryos, clearly indicating that expression of the transgene did not negatively impact the placenta. After all, the delivery of normal and healthy pups is the ultimate reporter assay for the functionality of the placenta.

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.

Response: The RNA-FISH method is particularly efficient for the detection of transcription at the C19MC locus in cultured cells but is more difficult to apply to tissue sections. Instead, we used laser capture micro-dissection coupled to qPCR for the sampling of placental sub-regions with C19MC expression. Using this method, we found that different subpopulations of trophoblasts express the C19MC miRNAs at different levels. For example, we found C19MC levels were significantly reduced in invasive extravillous trophoblasts. These findings are reported along with other results in a paper that is currently in its final preparation steps.

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.

Response: The funding provided in 2011 supported the infrastructure that was necessary to embark with this type of innovative research. The research done in the context of this work represents only a small part of a larger project on the C19MC miRNAs in which we employed techniques such as RNA-seq that produce very large data sets requiring most
extensive bioinformatics skills and substantial computational resources. Some of these data will be presented in the paper mentioned above, and in subsequent work that is currently underway. Moreover, several research assistants whose work was acknowledged in the report did not directly participate to the experiments described in the report but were nonetheless essential to the project. For example, one of them (Hilko) was a student in charge of identifying and approaching pregnant women at Magee-Womens hospital to enroll them in the study for placenta collection. Trophoblast isolation and culture is a lengthy process that is performed by a skilled technician (Ziegler) in our laboratory in order to ensure consistency in the quality of the primary human trophoblasts preparations. Another technician (Sun) is responsible for managing the mouse colony, including (but not limited to) the transgenic lines that were produced during this project.

Reviewer 3:
1. Morphometric analyses of the placentae of the C19MC transgenics should be attempted.

Response: We agree, and a detailed morphological analysis of transgenic placentas was performed by examining numerous histological sections of placentas, embryos, and various organs. We have not observed any difference between the transgenic and WT animals. The lack of phenotypic difference is not surprising considering that the transgenic mice develop normally and live a normal life span. However, we cannot rule out the possibility that differences may appear under very specific conditions. For example, we have shown that expression of C19MC miRNAs in human trophoblasts is associated with a higher resistance to viral infection. We have initiated a collaboration with a virologist at the University of Pittsburgh (Dr. Klimstra) in order to determine whether these transgenic miRNAs could have an effect on resistance to viral infection.

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.

Response: We analyzed global gene expression patterns using gene expression microarrays and assessed expression levels of several potential targets by real-time PCR but did not find any significant differences. Again, this negative result is not surprising since the transgenic mice appear completely normal when exposed to normal breeding conditions. We are now in the process of using RNA precipitation to address specific targets in humans and mice.

3. Laser capture micro-dissection coupled qPCR is needed to verify expression patterns of the C19MC cluster in the mouse placentae.

Response: Laser capture microdissection coupled to qPCR has been performed, and the results will appear in a paper currently in preparation. Briefly, these studies show that, in transgenic mouse placentas, C19MC expression is highest in the labyrinth layer and lower in the spongium. We also detected a low level of expression in the decidua at the site of implantation, reflecting the penetration of invasive trophoblasts into the decidua.
C. If the research project received an “unfavorable” rating, please indicate the steps that you intend to take to address the criteria that the project failed to meet and to modify research project oversight so that future projects will not receive “unfavorable” ratings.

Response: This project received a “Favorable” rating.

D. Additional comments in response to the Final Performance Review Report (OPTIONAL):

Response: This is a very exciting line of research that clearly exceeds the one year timeframe to attain full results. We are in process of submitting manuscripts that reflect our success.
B. To ensure that feedback provided in the Final Performance Summary Report is utilized to improve ongoing and future research efforts, briefly describe your plans to address each specific weakness and recommendation as noted in Section B of the Final Performance Summary Report and listed below. If no weaknesses are listed below, no response is required.

(As you prepare your response please be aware that the Final Performance Summary Report, this Response Form, and the Final Progress Report will be made publicly available on the CURE Program’s Web site.)

SPECIFIC WEAKNESSES AND RECOMMENDATIONS

Reviewer 1:
1. Establish SDC-1 expression in MVM (microvillous membrane fractions).

   **Response:** This suggestion is much appreciated. Dr. Hubel will confer with Dr. Sadovsky regarding optimal approaches. There is opportunity for using the laser capture microdissection equipment in the Magee-Womens Research Institute histology core.

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.

   **Response:** Dr. Hubel’s recently submitted R03 application to NIH-NICHD (“Glycocalyx Syndecan-1 in Trophoblast Lipid Transport”) centered upon SDC-1 and triglyceride-rich lipoprotein transport. The role of oxygen and oxidative stress (and cytokines) on SDC-1 expression in villous explants and primary isolated cells is envisaged as part of an upcoming grant proposal from Dr. Hubel dealing with SDC-1 regulation of placental microparticle secretion and the bioavailability of soluble vascular endothelial growth factor receptor-1 (sFlt1; an anti-angiogenic soluble receptor implicated in pathogenesis of preeclampsia).

3. Include rationale for models examined.

   **Response:** The inclusion of human uterine microvascular endothelial cells (HUtMVEC) cells was primarily to compare them with trophoblast cells. It is hypothesized that SDC-1, on both maternal endothelial cells and fetoplacental trophoblast cells, is the major heparan sulfate proteoglycan (HSPG) that binds (sequesters) sFlt1 to the cell surface. Further, investigation of the effects of endothelial-derived and trophoblast-derived soluble SDC-1 on sFlt1 dynamics is envisaged. Dr. Hubel hopes to test whether cellular uptake of sFlt1 occurs through SDC-1. In regard to potential binding and uptake of both sFlt1 and triglyceride-rich lipoproteins by SDC-1, the degree of sulfation of SDC-1 and other HSPGs may be an important determinant of SDC-1 action. The degree of sulfation of HSPGs can vary significantly by cell type and can be measured.
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.

Response: Considerable effort has focused on this aspect during 2013-2014. We have increased the placenta sample size from n=12 normal pregnant/n=10 preeclamptic to n=16 per group. Neither beta actin nor glyceraldehyde 3-phosphate dehydrogenase (GAPDH) is adequate loading controls for normalization. They do not accurately reflect differences in protein concentration for western blot of placental homogenates, and this is supported by recent publications by other research groups. A literature search led us to consider use of total protein stains as better loading controls. We have now validated and used Amido Black for loading control. We performed densitometry on all 16 preeclampsia cases and the 16 control samples and found significantly lower band intensity (SDC-1 protein mass) in the preeclampsia group, supporting the immunohistochemistry visual scores [western blot densitometry: Mann-Whitney U Statistic P = 0.048]. A next objective, as suggested by Reviewer 1, will be to compare microvillus fractions. Given that SDC-1 may exist in the cell nucleus, microvillus fractions may show bigger differences.

Regional differences could be important; we will search for an appropriate biobank.

2. Future efforts need to increase the number of clinical samples analyzed so the robustness of these preliminary conclusions can be verified.

Response: We increased our placenta biopsy sample size as noted above. We have extended our line of investigation to interrogate glycocalyx status, 1 year postpartum, in women with a history of preeclampsia or normal pregnancy. As a result we will also be able to measure plasma-soluble glycocalyx components in an additional nine prior normal pregnancy and 6 prior preeclampsia patients. By sidestream dark field imaging of sublingual microvessels in these postpartum women, we find that vascular glycocalyx depth (thickness) is significantly reduced in women with prior preeclampsia compared to women with prior normal pregnancy (P=0.01). The work was supported, as a logical extension, by the most recently completed PA Department of Health Formula Grant (Hubel, Principal Investigator).

3. Aim 2 needs to be verified using primary term trophoblast which might produce much more compelling data.

Response: We agree. This objective was built into Dr. Hubel’s R03 submission to NIH (initial submission, currently under review).
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.

Response: We now have preliminary data showing successful knockdown of SDC-1 in trophoblast cell lines. The suggested objectives and siRNA preliminary data are in the submitted R03 now under review.

5. Aim 3: definitive experiments using primary trophoblast for this aim need to be completed.

Response: This aspect has been/will be written into funding proposals.

6. Aim 3: knockdown of SDC-1 to investigate its role in the lipid trafficking aspects needs to be completed.

Response: We now have preliminary data showing successful knockdown of SDC-1 in trophoblast cell lines. The suggested objectives and siRNA preliminary data are in the submitted R03 under review.

C. If the research project received an “unfavorable” rating, please indicate the steps that you intend to take to address the criteria that the project failed to meet and to modify research project oversight so that future projects will not receive “unfavorable” ratings.

Response: This project received an “Outstanding” rating.

D. Additional comments in response to the Final Performance Review Report (OPTIONAL):

Response: None.
B. To ensure that feedback provided in the Final Performance Summary Report is utilized to improve ongoing and future research efforts, briefly describe your plans to address each specific weakness and recommendation as noted in Section B of the Final Performance Summary Report and listed below. If no weaknesses are listed below, no response is required.

(As you prepare your response please be aware that the Final Performance Summary Report, this Response Form, and the Final Progress Report will be made publicly available on the CURE Program’s Web site.)

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.

Response: We appreciate the reviewer’s insight. Within the confines of a single-year project and limited budget, we felt it prudent to limit the scope of our project to defining the scientific issue of centrosome (de)clustering by drug interference combined with time-lapse confocal imaging.

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.

Response: We agree. We designed feasible experiments to perform, given the single-year length of the project and budgetary constraints. The project was never intended to encompass molecular, genetic, or other advanced experimental techniques or to be all-inclusive. We chose to perform localization and drug interference protocols in combination with TLVM as those are the existing strengths in our laboratory that could be rapidly utilized within the grant’s timeframe to define the broader issues of the study. With those background experiments, additional funding can now be sought to delve deeper into the mechanistic issues regarding centrosome clustering by way of molecular motors.

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.
Response: The reviewer is correct that we employed sophisticated, yet now routine, techniques for this study, as it was not our goal to address every Aim in-depth for a single-year project. Conversely, we believe the necessary preliminary background studies have been identified, linking molecular motors and centrosome behavior in cancer cells. Among our observations, we found that (i) the cancer cells investigated are deficient in dynein heavy chain protein at their spindle poles and demonstrate slower microtubule dynamics than a control fibroblast line; (ii) cdk-1 inhibition by RO3306/verapamil initiates a significant increase in spindle multipolarity, centriole amplification, and DNA misalignment in cancer cells, with a minority of these cancer cells handling hyperamplification of centrioles by clustering at spindle poles prior to cell division. Additionally, we showed that knockdown of endogenous NuMA by the lentiviral shRNA NuMA at amounts sufficient to ensure cell survival was probably insufficient for dropping NuMA below critical thresholds to impact microtubule minus-end organization in spindle poles; (iii) RO3306i impacted DNA replication onset, with cells arresting earlier in the cell cycle than the predicted G2/S phase as reported for Chinese hamster ovary (CHO) cells. This could not have been anticipated from the reported effects of RO3306i in the literature. And (iv) TLVM data showed that cancer cells have a number of methods for assembling bipolar spindles and completing cytokinesis in the presence of hyperamplified centrioles, including centriole spindle dissociation after spindle assembly and daughter cell fusion at the end of cytokinesis. Overall, the mechanism of “clustering” extra centrioles might be a very rare event, leading to speculations about whether rarified cells that do cluster extra centrioles are “specialized” progenitor cells that might convey unique advantages for propagating tumors. These observations provide us with a broad framework to design future specific investigations on the connection between molecular motors, cell cycle inhibitors, and centrosome clustering to avoid initiating the cell death machinery in cancer cells.

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.

Response: We could not agree more with the reviewer. As this was a single-year project with limited budget, it was not possible to increase the cell lines beyond the three employed in the study. Using our selection of two cancer lines and one control fibroblast line gave us the ability to perform the necessary preliminary experiments in the specific aims while staying within the budgetary constraints of the award. We did try other control cell lines but found them either difficult to maintain consistently in the laboratory or requiring other medium constituents that were controlled substances requiring licenses not under our laboratory purview.

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.
**Response:** We understand the reviewer’s concern with replicate numbers and statistical analysis. For this single-year study, we found it necessary, in some cases, to report only preliminary scientific trends without sufficient replicates to validate statistical documentation. Once additional funding has been secured, experimental validation with appropriate statistical tests will be performed prior to publishing scientific findings from this study.

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.

**Response:** We agree, and are aware that adding additional cell lines for analysis with RO3306 and PARP inhibitors would be necessary to validate and extend our preliminary observations beyond the cell lines employed in this study. We also found it somewhat surprising that different control and cancer cell lines behave differently in the presence of the employed inhibitors from those in the published literature. However, budgetary constraints prohibited expansion of the number of cell lines beyond those employed. Once confirmed by additional experimentation, it will be important and fascinating to extend and report on variations in cancer cell line sensitivities to PARP inhibition.

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.

**Response:** We agree with the reviewer regarding the expansion of these studies to other documented CDK1 inhibitors. Although budgetary constraints in this 1-year study did not permit us to include other drugs for testing, it will be intriguing to investigate, once additional funding is secured, whether different cancer cells show varying responses to cdk1 inhibition.

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.

**Response:** Yes, we agree with the reviewer’s comment concerning the role of HSET, a member of KAR3 kinesin minus-end molecular motors, in centrosome clustering. We have already secured HSET antibody from a research colleague and can perform the necessary experiments to test HSET’s role in centrosome clustering in cancer cells once additional funding has been secured.
Reviewer 3:
None.

C. If the research project received an “unfavorable” rating, please indicate the steps that you intend to take to address the criteria that the project failed to meet and to modify research project oversight so that future projects will not receive “unfavorable” ratings.

Response: This project received a “Favorable” rating.

D. Additional comments in response to the Final Performance Review Report (OPTIONAL):

Response: None.
**Project Number:** 1166804  
**Project Title:** Regulation of Spermatogenesis by Classical and Non-classical Testosterone Signaling  
**Investigator:** William H. Walker

**B. To ensure that feedback provided in the Final Performance Summary Report is utilized to improve ongoing and future research efforts, briefly describe your plans to address each specific weakness and recommendation as noted in Section B of the Final Performance Summary Report and listed below. If no weaknesses are listed below, no response is required.**

(As you prepare your response please be aware that the Final Performance Summary Report, this Response Form, and the Final Progress Report will be made publicly available on the CURE Program’s Web site.)

**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.

   **Response:** Three NIH grant proposals were submitted related to the work performed for this project. None of the proposals were funded. Details of the grant proposals are listed below.

   1. 2R01HD043143-08A1, Nongenomic Androgen Signaling in Sertoli Cells submitted 2/29/12, Impact score 43, Percentile 36.
   2. 2U54HD008610-36 Project 4 Non-classical Androgen Receptor Signaling in Spermatogenesis, submitted 6/22/12. Impact score 36, Percentile not available.

   The R03 proposal will be revised and submitted in October of 2014. A new version of the R01 proposal will be submitted in February 2015.

   The results of this project were submitted to the Journal of Molecular Endocrinology on 3/21/14 and are being revised for resubmission.

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.

   **Response:** The PI thanks the reviewer for this advice. The PI will continue his commitment to 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 tfm model system could rescue spermatogenesis.

Response: We do not yet have data that determines whether spermatogenesis can be rescued by AR mutants that selectively activate either classical or non-classical testosterone signaling. Based on the results provided in our report (see Figures 6, 8 and 11), we suspect that the two pathways act in a complimentary fashion and that neither pathway alone will be sufficient to totally restore spermatogenesis. We are using transgenic mice to address this question. Thus far, we have created mice in which Sertoli cells normally lack expression of AR, but after insertion of a bacterial artificial chromosome containing the AR gene locus and flanking regions, AR expression is restored in Sertoli cells in a stage-specific manner. We plan to repeat the creation of these mice using bacterial artificial chromosomes in which the AR gene has been mutated such that classical or non-classical testosterone signaling is selectively permitted in Sertoli cells. The testes of the mice will be evaluated to determine the extent to which spermatogenesis can be completed.

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 Sertoli cells to the germ cells for spermatogenesis.

Response: The PI thanks the reviewer for this suggestion. In the future, we plan to identify mechanisms regulated by both pathways that are required to maintain meiosis, the attachment of germ cells to Sertoli cells, and the release of mature sperm.

C. If the research project received an “unfavorable” rating, please indicate the steps that you intend to take to address the criteria that the project failed to meet and to modify research project oversight so that future projects will not receive “unfavorable” ratings.

Response: This project received a “Favorable” rating.

D. Additional comments in response to the Final Performance Review Report (OPTIONAL):

Response: None.