# Final Progress Report for Research Projects Funded by Health Research Grants

Instructions: Please complete all of the items as instructed. Do not delete instructions. Do not leave any items blank; responses must be provided for all items. If your response to an item is "None", please specify "None" as your response. "Not applicable" is not an acceptable response for any of the items. There is no limit to the length of your response to any question. Responses should be single-spaced, no smaller than 12-point type. The report **must be completed using MS Word**. Submitted reports must be Word documents; they should not be converted to pdf format. Questions? Contact Health Research Program staff at 717-783-2548.

1. **Grantee Institution:** Drexel University

2. **Reporting Period (start and end date of grant award period):** 1/1/2009-12/31/2010

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** Anne Martella

4. **Grant Contact Person's Telephone Number:** (215) 895-6471

5. **Grant SAP Number:** 4100047631

6. **Project Number and Title of Research Project:** 3 - *Role of CTF18 in Female Germ Cell Development and Fertility*

7. **Start and End Date of Research Project:** 1/1/2009-12/31/2010

8. **Name of Principal Investigator for the Research Project:** Karen M. Berkowitz, MD

9. **Research Project Expenses**.

   9(A) Please provide the amount of health research grant funds spent on this project for the entire duration of the grant, including any interest earned that was spent:

   $ $112,500

   9(B) Provide the last names (include first initial if multiple individuals with the same last name are listed) of **all** persons who worked on this research project and were supported with health research funds. Include position titles (Principal Investigator, Graduate Assistant, Post-doctoral Fellow, etc.), percent of effort on project and total health research funds expended for the position. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

| Last Name | Position Title | % of Effort on Project | Cost |
|---|---|---|---|
| Berkowitz | Assistant Professor | 3% | $ 4,500 |
| Singh | Research Technician | 100% | $31,000 |

9(C) Provide the names of **all** persons who worked on this research project, but who *were not* supported with health research funds. Include position titles (Research Assistant, Administrative Assistant, etc.) and percent of effort on project. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

| Last Name | Position Title | % of Effort on Project |
|---|---|---|
| None | | |

9(D) Provide a list of **all** scientific equipment purchased as part of this research grant, a short description of the value (benefit) derived by the institution from this equipment, and the cost of the equipment.

| Type of Scientific Equipment | Value Derived | Cost |
|---|---|---|
| None | | |

**10. Co-funding of Research Project during Health Research Grant Award Period.** Did this research project receive funding from any other source during the project period when it was supported by the health research grant?

Yes_____ No____X_____

If yes, please indicate the source and amount of other funds:

**11. Leveraging of Additional Funds**

11(A) As a result of the health research funds provided for this research project, were you able to apply for and/or obtain funding from other sources to continue or expand the research?

Yes_____ No____X_____

If yes, please list the applications submitted (column A), the funding agency (National Institutes of Health—NIH, or other source in column B), the month and year when the application was submitted (column C), and the amount of funds requested (column D). If you have received a notice that the grant will be funded, please indicate the amount of funds to be awarded (column E). If the grant was not funded, insert "not funded" in column E.

Do not include funding from your own institution or from CURE (tobacco settlement funds). Do not include grants submitted prior to the start date of the grant as shown in Question 2. If you list grants submitted within 1-6 months of the start date of this grant, add a statement

below the table indicating how the data/results from this project were used to secure that grant.

| A. Title of research project on grant application | B. Funding agency (check those that apply) | C. Month and Year Submitted | D. Amount of funds requested: | E. Amount of funds to be awarded: |
|---|---|---|---|---|
| None | ☐NIH<br>☐ Other federal (specify:_____)<br>☐ Nonfederal source (specify: _____) | | $ | $ |

11(B) Are you planning to apply for additional funding in the future to continue or expand the research?

Yes____X_____ No_____

If yes, please describe your plans:

I will be applying for the NIH grant, PAS-10-226: Advancing Novel Science in Women's Health Research (ANSWHR) (R21) in October 2011

**12. Future of Research Project.** What are the future plans for this research project?

Our results indicate that *CTF18* (termed *Chtf18* in mice) plays important roles in germ cell development and ovarian folliculogenesis in mammals. In particular, our data demonstrate that homologous chromosomes of *Chtf18* -deficient oocytes separate prematurely during meiotic prophase I, suggesting a role for *CTF18* in maintaining the linkage of homologous chromosomes during meiosis. Our findings also suggest that *Chtf18* functions in meiotic progression and helps to maintain the early and developing ovarian follicle pools in female mice. These results generate a firm basis to investigate the mechanisms of premature separation of homologous chromosomes, delayed meiotic progression, and impaired ovarian follicular development in *Chtf18*-deficient female mice. We will also explore the role(s) of *Chtf18* in genome integrity during embryonic development with the use of our *in vitro* system for culturing mouse blastocysts that we developed during the time period of this project.

**13. New Investigator Training and Development**. Did students participate in project supported internships or graduate or post-graduate training for at least one semester or one summer?

Yes___X_____ No_____

If yes, how many students? Please specify in the tables below:

| | Undergraduate | Masters | Pre-doc | Post-doc |
|---|---|---|---|---|
| Male | | | | |
| Female | | | 1 (M.D.) | |
| Unknown | | | | |
| **Total** | | | **1** | |

| | Undergraduate | Masters | Pre-doc | Post-doc |
|---|---|---|---|---|
| Hispanic | | | | |
| Non-Hispanic | | | 1 | |
| Unknown | | | | |
| **Total** | | | **1** | |

| | Undergraduate | Masters | Pre-doc | Post-doc |
|---|---|---|---|---|
| White | | | 1 | |
| Black | | | | |
| Asian | | | | |
| Other | | | | |
| Unknown | | | | |
| **Total** | | | **1** | |

**14. Recruitment of Out-of–State Researchers**. Did you bring researchers into Pennsylvania to carry out this research project?

Yes_____ No____X_____

If yes, please list the name and degree of each researcher and his/her previous affiliation:

**15. Impact on Research Capacity and Quality**. Did the health research project enhance the quality and/or capacity of research at your institution?

Yes_____ No____X_____

If yes, describe how improvements in infrastructure, the addition of new investigators, and other resources have led to more and better research.

**16. Collaboration, business and community involvement.**

16(A) Did the health research funds lead to collaboration with research partners outside of your institution (e.g., entire university, entire hospital system)?

Yes_____ No____X_____

If yes, please describe the collaborations:

16(B) Did the research project result in commercial development of any research products?

Yes_____ No_____X_____

If yes, please describe commercial development activities that resulted from the research project:

16(C) Did the research lead to new involvement with the community?

Yes_____ No_____X_____

If yes, please describe involvement with community groups that resulted from the research project:

**17. Progress in Achieving Research Goals, Objectives and Aims.**

List the project goals, objectives and specific aims (as contained in the grant application's strategic plan). Summarize the progress made in achieving these goals, objectives and aims for the entire grant award period. Indicate whether or not each goal/objective/aim was achieved; if something was not achieved, note the reasons why. Describe the methods used. If changes were made to the research goals/objectives/aims, methods, design or timeline since the original grant application was submitted, please describe the changes. Provide detailed results of the project. Include evidence of the data that was generated and analyzed, and provide tables, graphs, and figures of the data. List published abstracts, poster presentations and scientific meeting presentations at the end of the summary of progress; peer-reviewed publications should be listed under item 20.

This response should be a DETAILED report of the methods and findings. It is not sufficient to state that the work was completed. Insufficient information may result in an unfavorable performance review, which may jeopardize future funding. If research findings are pending publication you must still include enough detail for the expert peer reviewers to evaluate the progress during the course of the project.

Health research grants funded under the Tobacco Settlement Act will be evaluated via a performance review by an expert panel of researchers and clinicians who will assess project work using this Final Progress Report, all project Annual Reports and the project's strategic plan. After the final performance review of each project is complete, approximately 12-16 months after the end of the grant, this Final Progress Report, as well as the Final Performance Review Report containing the comments of the expert review panel, and the grantee's written response to the Final Performance Review Report, will be posted on the CURE Web site.

**There is no limit to the length of your response. Responses must be single-spaced below, no smaller than 12-point type. If you cut and paste text from a publication, be sure symbols print properly, e.g., the Greek symbol for alpha (α) and beta (ß) should not print as boxes (□) and include the appropriate citation(s). DO NOT DELETE THESE INSTRUCTIONS.**

## Project Title and Purpose

*Role of CTF18 in Female Germ Cell Development and Fertility* - Infertility is a major medical concern that affects about 1 of every 10 individuals of childbearing age worldwide. Although a significant proportion of infertility is accounted for by identifiable causes, the molecular basis of these defects is often not known or well characterized. Ovulatory dysfunction and diminished ovarian function in infertile women and low sperm count in infertile men are examples. It is becoming increasingly clear that many more disorders in medicine, including infertility, have a genetic basis than was previously realized. It is our goal to advance the field so that a better understanding of the underlying defects of infertility disorders will lead to improved treatments.

### Project Overview

Better understanding of the role of *CTF18/Chtf18* in females broadens our knowledge of the underlying molecular aspects of oogenesis and folliculogenesis and sheds light on the underlying defects of infertility in women. Thus, the specific aims of this proposal were:

1. To determine the function of *Chtf18* in mouse oogenesis and folliculogenesis *in vivo*. *Hypothesis: Chtf18 is a critical mediator of oogenesis and folliculogenesis, and its absence causes follicle degeneration.* We assessed oocyte and follicle development in ovaries of *Chtf18*$^{-/-}$ and wild-type females using histological criteria and immunofluorescence microscopy. The underlying cause(s) of a decreased number and aberrant appearance of follicles seen in *Chtf18*-deficient ovaries were evaluated. Developmental competency of oocytes was assessed, and blood was collected in order to evaluate hormonal profiles.

2. To determine the cause of subfertility in *Chtf18*$^{-/-}$ females. *Hypothesis: Premature separation of homologous chromosomes in meiosis results in aneuploidy and embryonic death leading to significantly impaired fertility.* Oocytes were studied immunocytologically for chromosome segregation defects, and an *in vitro* system for culturing mouse embryos was developed for evaluating aneuploidy.

### Expected Research Outcomes and Benefits

We generated an experimental system to study *CTF18* (known as *Chtf18* in the mouse), an evolutionarily conserved gene that is crucial for fertility in the fruitfly and that is essential for the accurate segregation of chromosomes in yeast. We recently demonstrated that loss of *Chtf18* in the mouse leads to impaired fertility and chromosomal defects during meiosis. In addition, almost half of mice lacking *Chtf18* die during embryonic development. These data strongly suggest that *Chtf18* plays a significant role in normal segregation of chromosomes during development of germ cells (i.e., the cells destined to become eggs and sperm). Defects in these

processes are known to contribute greatly to causes of an abnormal chromosome number in offspring, and an abnormal chromosome number often leads to birth defects and/or pregnancy loss. Thus, a better understanding of *Chtf18* and its role in germ cell development and chromosomal segregation will shed light on one of the most frequent types of genetic defects that occur during reproduction. These studies will provide insight into human infertility and reproductive disorders, ultimately leading to better treatments.

**Summary of Research Completed**

Data collected from each of the following aims (described below) were presented at the following meetings during the reporting period: 1) Drexel University College of Medicine Discovery Day, Philadelphia, PA, October 7, 2009; 2) the 57th Annual Meeting of the Society of Gynecologic Investigation, Orlando, Florida, March 24-27, 2010 (oral presentation); 3) Drexel University College of Medicine Discovery Day, Philadelphia, PA, October 7, 2010 (first place for outstanding technician poster).

Specific Aim 1. To determine the function of *Chtf18* in mouse oogenesis and folliculogenesis *in vivo*.

To ascertain the role that *Chtf18* plays in both mouse oogenesis and folliculogenesis, we examined ovarian follicle development, developmental competency of oocytes, and hormonal function of *Chtf18*$^{-/-}$ females in three separate subaims:

1) Examination of follicle development.
We evaluated ovaries of *Chtf18*$^{-/-}$ females compared with wild-type controls from embryonic development through adulthood in order to determine the stage(s) at which the defects in follicle development occur. Our studies have allowed us to begin to determine the underlying cause(s) of the defects. *Chtf18*$^{-/-}$ ovaries are smaller, contain fewer follicles than wild-type ovaries, and are morphologically abnormal (Figure 1). We counted ovarian follicles to quantitate the differences observed at different stages of development and scored and categorized follicles as primordial, primary, secondary, and antral according to morphological criteria. Dissected ovaries were fixed in Bouin's solution or 4% paraformaldehyde solution at 4°C overnight, embedded in paraffin blocks, sectioned, and viewed by light microscopy. Ovarian sections used for histological evaluation were stained with hematoxylin and eosin. Ovaries from *Chtf18*$^{-/-}$and wild-type control females at postnatal days 7 and 21, and at 3 and 6 months of age were collected, processed, paraffin embedded, and counted. We found significantly decreased numbers of primordial follicles at postnatal days 7 and 21 (Figure 2) in *Chtf18*$^{-/-}$ compared with wild-type ovaries. We also found a significant reduction in the numbers of primary, secondary and antral follicles of *Chtf18*$^{-/-}$ ovaries at 3 and 6 months of age (Figure 3). Overall, our data from follicle counts in *Chtf18*$^{-/-}$ ovaries at postnatal days 7 and 21 and at 3 and 6 months of age were statistically significant or trended towards statistical significance (only antral follicle counts of *Chtf18*$^{-/-}$ at 3 months of age), and demonstrated a large size effect, suggesting that these results are biologically relevant. We speculate that antral follicle counts in *Chtf18*$^{-/-}$ ovaries trended toward statistical significance but were not significant because the number of antral follicles in both wild-type and *Chtf18*$^{-/-}$ ovaries were so low. We had proposed counting follicles in females at 9 months of adult age, but we found that follicle numbers were so low and degenerated even in wild-type females that data from this time-point are not meaningful. Our data strongly suggest

that *Chtf18* is necessary to maintain the early and developing ovarian follicle pools in female mice, and they indicate that *Chtf18* plays significant roles in mammalian folliculogenesis.

2) Assessment of the competency of oocytes.
Oocytes were cultured and allowed to grow and mature *in vitro* in order to assess the developmental competency of *Chtf18*$^{-/-}$ oocytes. In our original research plan, we proposed collecting antral follicles from ovaries of 6-week-old equine chorionic gonadotropin (eCG)-primed females. However, to improve the yield of antral follicles containing fully grown oocytes, we collected oocytes at 3 weeks of age (an accepted alternative age in the literature), which provided significantly greater numbers of oocytes to collect and culture (data not shown). Female mice were given a single injection of 5 units (U) of eCG (Calbiochem, La Jolla, CA) intraperitoneally and sacrificed 48 hours later. Ovaries were collected in medium and then punctured with 27G½ inch needles to release antral follicles. The oocyte-cumulus cell complexes were then collected and transferred to fresh medium. Cumulus cells were mechanically removed from oocytes by pipetting, and cumulus-free oocytes were then incubated in fresh medium at 37°C in 5% $CO_2$ and air. The total number of oocytes per genotype was recorded, and oocytes were scored for the following stages of meiotic progression (Figure 4): presence of a germinal vesicle, germinal vesicle breakdown, or extrusion of first polar body at collection time and after 16 hours in culture. Germinal vesicle breakdown signifies resumption of meiosis I, and extrusion of a polar body signifies progression to metaphase II. Progression is measured by calculating percentages of oocytes reaching each stage (i.e., number of oocytes for each stage divided by the total number of oocytes placed into culture for each genotype). Chromosomes and spindle structure of wild type oocytes were examined. Stages were assessed morphologically with brightfield microscopy using Nomarski optics. In order to evaluate the ability of *Chtf18*$^{-/-}$ oocytes to progress in meiosis, we developed an *in vitro* system of cultured mouse oocytes. Owing to genetic influences of our mouse strain (hybrid of C57BL/6; 129Sv), significant time was required for us to determine and optimize the conditions in which oocytes from our wild-type mice develop and progress. We utilized multiple collection and culture conditions, and we recently found that a significantly greater number of wild-type oocytes progress to meiosis I and II when they are collected in minimum essential medium (MEM alpha medium) and cultured in Chatot Ziomek Bavister (CZB) medium (Figure 5). After determining the optimal culture conditions, we collected data from *Chtf18*$^{-/-}$ female mice and compared it to data from wild-type female mice. We calculated the percentage progression of germinal vesicle–intact oocytes from prophase to metaphase II for *Chtf18*$^{-/-}$ and wild-type mice. Using a Chi square test we analyzed the percentage of GV-intact oocytes reaching metaphase II. We found that 57% of *Chtf18*$^{+/+}$ ooctyes reached metaphase II, whereas only 36% of *Chtf18*$^{-/-}$ ooctyes did so (Figure 5). Due to submendelian ratios our *n* (number of mice per experiment) for *Chtf18*$^{-/-}$ mice was small, so we have continued to collect more data to increase our *n* value.

3) Hormonal evaluation.
We successfully collected serum from *Chtf18*$^{-/-}$ and wild-type females. Evaluation of serum levels of steroid hormones (including estradiol and progesterone) and gonadotropins (including follicle-stimulating hormone and luteinizing hormone) is pending. As mentioned earlier, our efforts to collect substantial data from *Chtf18*$^{-/-}$ females were limited owing to submendelian ratios of offspring born from our heterozygous crosses. Serum and ovaries could not be collected from the same female mice, as the specific time points for collection were different for the two experiments. So we decided to focus on oocyte collection because impaired developmental

competency is a primary defect in *Chtf18*$^{-/-}$ mice and appears to be due to defective germ cell development and not to a somatic cell defect.

Specific Aim 2. To determine the cause of subfertility in *Chtf18*$^{-/-}$ females.

1) Chromosomal localization of CTF18 in meiosis.
As an initial step in determining the role of CTF18 protein in chromosome segregation and dynamics, we utilized embryonic ovaries at 17.5 dpc, when meiosis has progressed to the pachytene stage of meiosis, to prepare surface spread nuclei from oocytes. Each pair of ovaries was dissected from female embryos and placed into a 50-µL drop of hypotonic buffer for 40 minutes. Each pair of ovaries was then moved to a 20-µL drop of 100 mM sucrose, and the ovaries poked with a 27G½ inch needle to release oocytes. Another 40 µL was added to resuspend the cells and 25 µL each of the suspension was placed on each of two slides previously dipped in fresh 2% paraformaldehyde/0.15% Triton X-100. Slides were placed on a flat surface and allowed to air-dry. For immunofluorescence, surface spread nuclei were blocked (1X phosphate-buffered saline containing 10% donkey serum, 3% bovine serum albumin, 0.05% Triton X-100) for 1 hour at room temperature and then incubated with primary antibodies overnight at 4°C. We are utilizing rabbit antimouse CTF18 and guinea pig antimouse-SYCP2 (from P.J. Wang, University of Pennsylvania School of Veterinary Medicine), which stains the axial chromosomal cores of the synaptonemal complex. Slides are viewed with fluorescence microscopy.

2) Evaluation of chromosomes in meiosis.
We confirmed that homologous chromosome pairing and synaptonemal complex formation were normal by examining *Chtf18*$^{-/-}$ chromosomes in oocytes from embryonic ovaries at 17.5 dpc during prophase I (Figure 6). Immunofluorescence microscopy was performed (as described above) with rabbit antimouse SYCP1 (Abcam, Inc., Cambridge, UK) and guinea pig anti-mouse-SYCP2 antibodies. We also analyzed surface spread nuclei from oocytes of mice at 18.5 dpc (Figure 6) or postnatal day 0, when the majority of oocytes meiosis has arrested in the diplotene phase of meiosis I. In order to optimize the number of diplotene nuclei evaluated, we modified our protocol and hypotonic buffers. To increase numbers of diplotene nuclei, we also prepared surface spread nuclei from the oocytes of postnatal day 0 and postnatal day 1 pups. Immunofluorescence microscopy was performed (as described above) with rabbit antimouse SYCP3 (Abcam, Inc.), which stains the axial/lateral elements of the synaptonemal complex and human anti-CREST (ImmunoVision, Springdale, AZ), which stains centromeres. We found that the diplotene stage was normal in wild-type females, but we observed four or more univalent chromosomes in 65% (40 nuclei counted) of *Chtf18*$^{-/-}$ females (Figure 6). For studies of metaphase I and metaphase II, oocytes were obtained from the antral follicles of ovaries from 3-week-old eCG-primed females as described above (see "Assessment of the competency of oocytes"). Oocytes were fixed at 16 hours in culture following the first polar body extrusion in order to prepare chromosome spreads at metaphase II. We have begun to evaluate metaphase spreads for congression of chromosomes to the metaphase spindle plate and the distribution of chromosomes along the spindle fibers. We optimized the immunofluorescence and fixation protocols for staining of the meiotic spindle with mouse monoclonal anti-β-tubulin antibody (Sigma, St. Louis, MO) and To-pro-3-iodide, a counterstain to visualize the chromosomes with fluorescence microscopy (Figure 7). Assessment of the results obtained from staining is pending.

3) Evaluation of embryos for aneuploidy.
We hypothesized that subfertility observed in *Chtf18*$^{-/-}$ females leads to chromosome mis-segregation, aneuploidy, and ultimately embryonic death. Thus, it is necessary to determine the time point at which this occurs. Since we speculated that loss occurs much earlier than E17.5, we sacrificed *Chtf18*$^{+/-}$ females (mated with *Chtf18*$^{+/-}$ males) at E9.5 and collected and genotyped embryos present in order to determine when embryonic death might occur. We found that the ratio of observed/expected *Chtf18*$^{-/-}$ embryos was significantly less than those of *Chtf18*$^{+/-}$ or *Chtf18*$^{-/-}$ embryos (data not shown). Because we did not note obvious resorption at this embryonic age, we speculated that loss occurs at an earlier time point. In order to determine whether loss occurs prior to implantation, we bred eCG-primed *Chtf18*$^{+/-}$ females with *Chtf18*$^{+/-}$ males and collected and cultured blastocysts at E3.5 (Figure 8). We have optimized our *in vitro* system for culturing blastocysts and extraction of DNA from cultured blastocysts over the course of several months. Our initial PCR method for genotyping the blastocyst DNA did not work, so we designed new PCR primers and have employed nested PCR in order to achieve our goal. The genotyping and karyotyping of cultured blastocysts are pending.

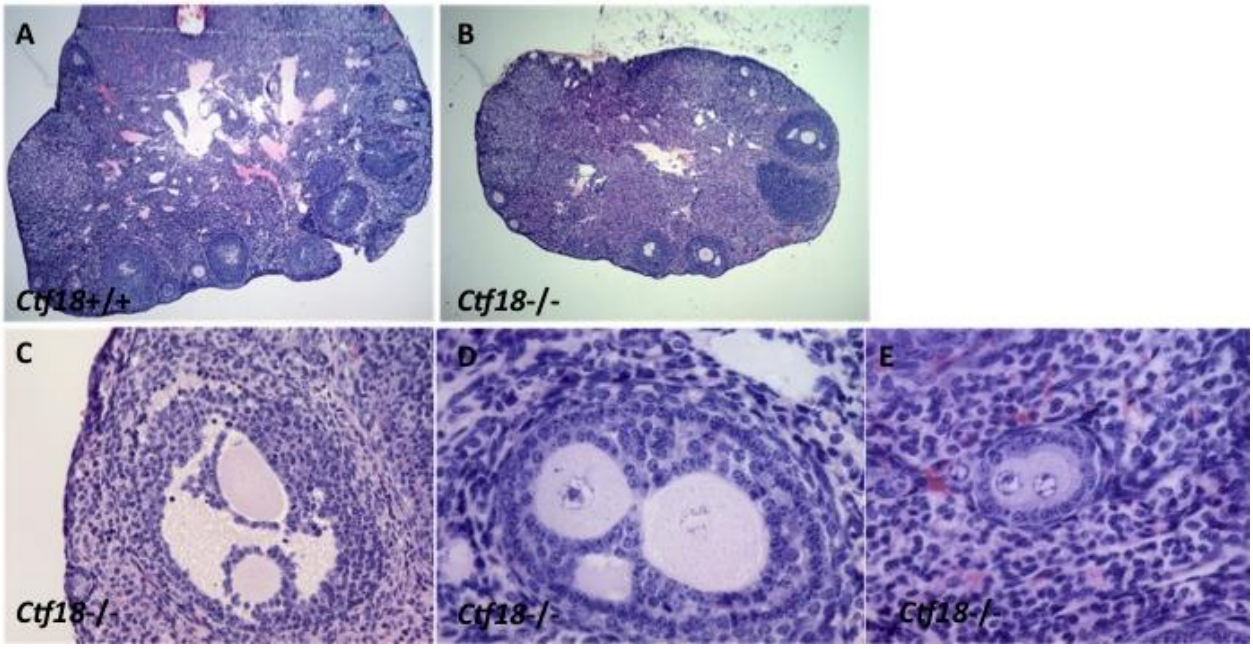


Figure 1. Ovaries from *Chtf18*$^{-/-}$ females were morphologically abnormal. Sections of ovaries stained with hematoxylin and eosin from wild-type (A) and *Chtf18*$^{-/-}$ (B-E) females are shown. Ovaries from *Chtf18*$^{-/-}$females (B) were smaller and contained fewer follicles than those of wild-type females (A). All stages of follicular development were seen, but many of the follicles appeared to be degenerating (C) while some appeared to be binucleate, consistent with premature release from meiotic arrest (C-E).

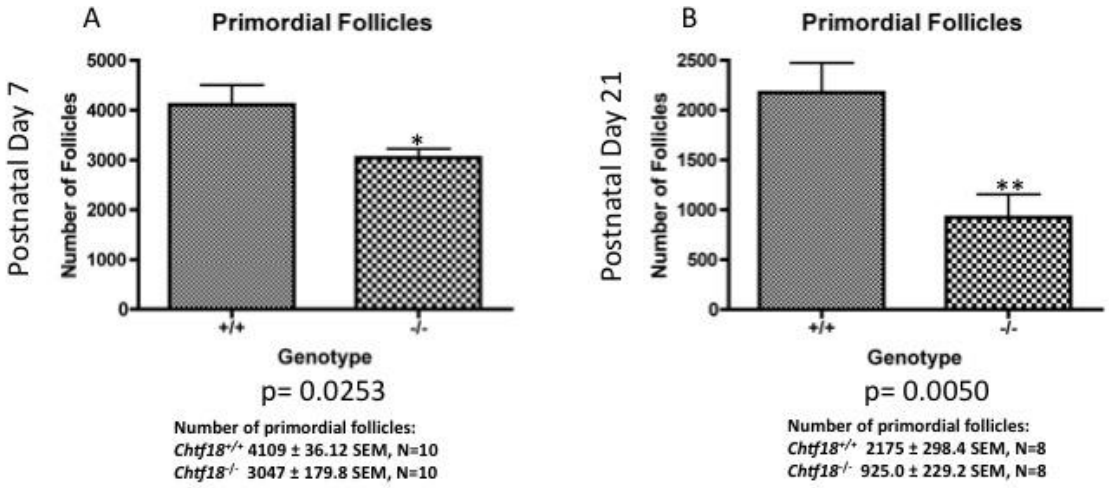


Figure 2. Numbers of primordial follicles were significantly decreased in *Chtf18*$^{-/-}$ ovaries at postnatal days 7 (A) and 21 (B).

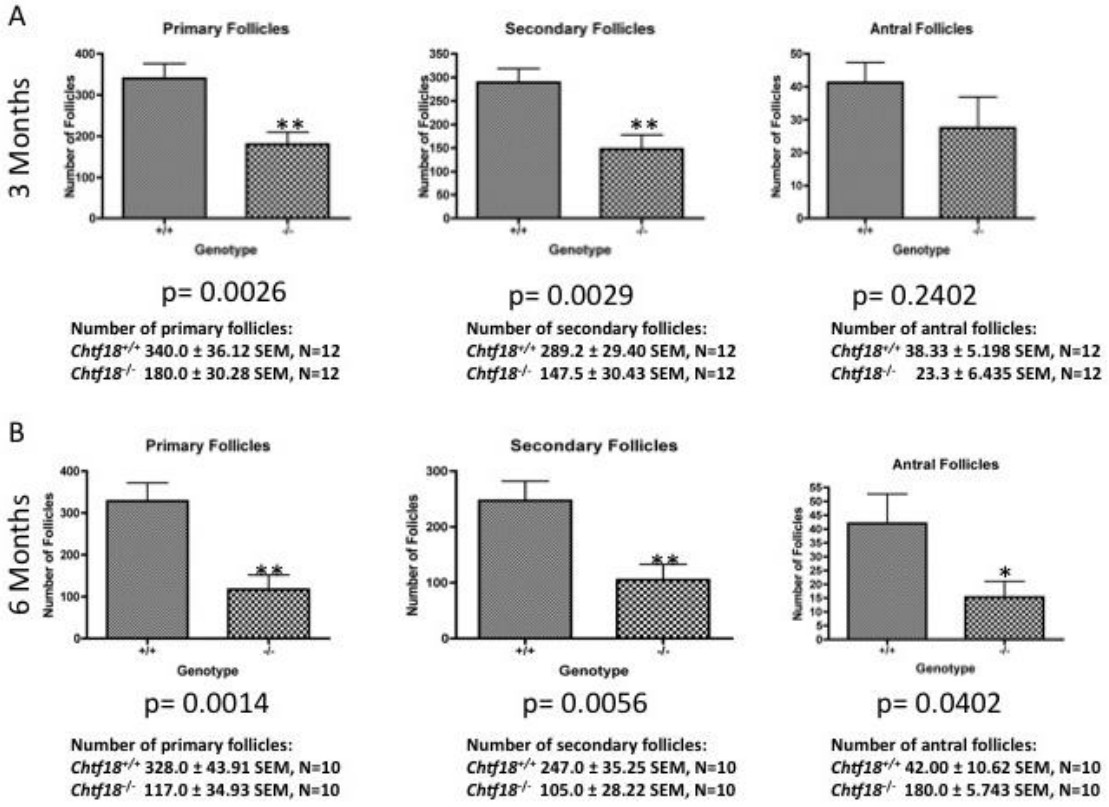


Figure 3. Numbers of primary, secondary, and antral follicles were significantly decreased in *Chtf18*$^{-/-}$ ovaries at 3 (A) and 6 (B) months of age.

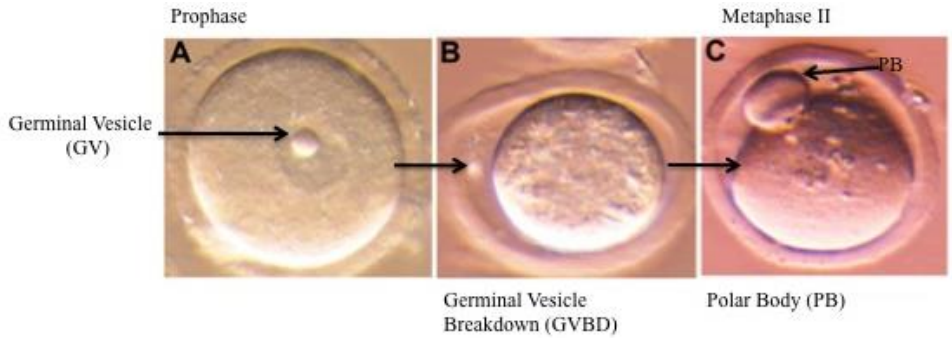


Figure 4. *In vitro* maturation of *Chtf18* wild-type oocytes. Oocytes cultured from *Chtf18* wild-type ovaries show the germinal vesicle stage (A), germinal vesicle breakdown indicating progression to metaphase I (B), and polar body extrusion indicating progression to metaphase II (C).

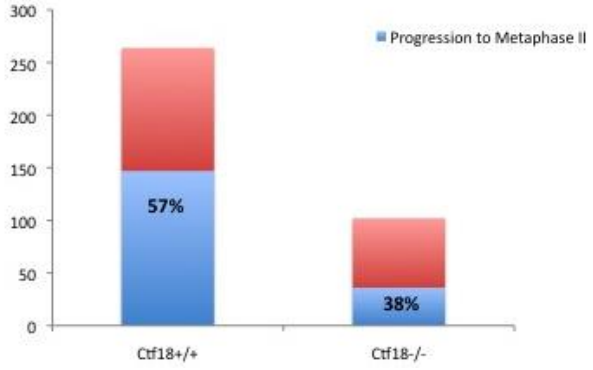


Figure 5. Meiotic progression was significantly impaired in *Chtf18*$^{-/-}$ ooctyes. Percent progression of GV intact oocytes from prophase to MII (metaphase II) was calculated for *Chtf18*$^{+/+}$ control mice and *Chtf18*$^{-/-}$ mice. Statistical comparison was done using Chi-square test to analyze the percentage of GV-intact oocytes reaching MII. Fifty-seven percent of *Chtf18*$^{+/+}$ oocytes reached MII, whereas only 36% of *Chtf18*$^{-/-}$ oocytes reached MII. Using Chi-square test we calculated $p = 0.001$ (**).

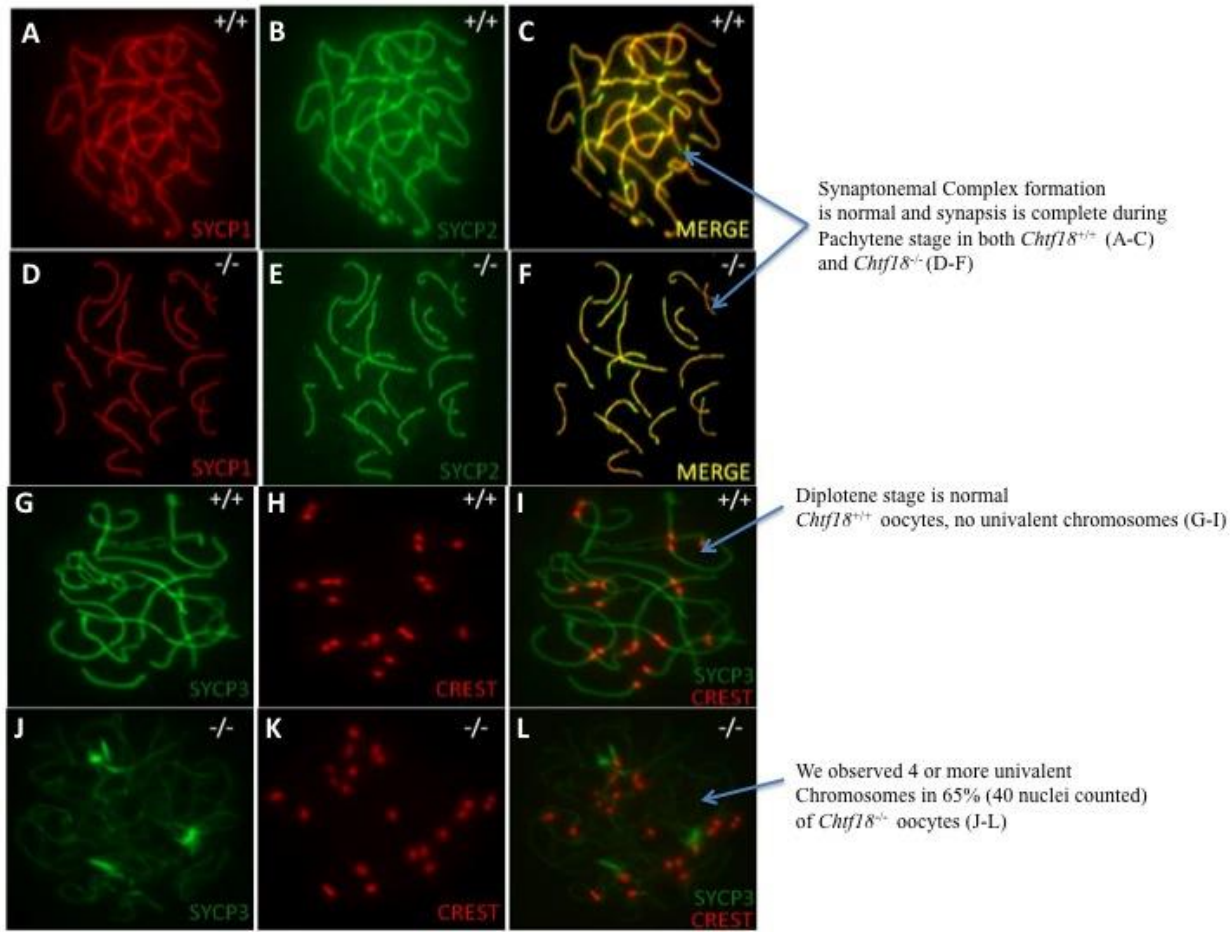


Figure 6. Surface spread nuclei from wild-type (+/+) and *Chtf18*$^{-/-}$ (-/-) oocytes were stained with antisera against SYCP1 (red) and SYCP2 (green) during the pachytene stage of meiotic prophase I (A-F). SYCP1 localizes to central elements of the synaptonemal complex and SYCP2 localizes to the axial/lateral elements of the synaptonemal complex. The merged images (C and F) demonstrate that formation of the synaptonemal complex occurs normally and that synapsis is complete during the pachytene stage in both *Chtf18* $^{+/+}$ (A-C) and Chtf18$^{-/-}$ (D-F) oocytes. Surface spread nuclei from *Chtf18*$^{+/+}$ (+/+) and *Chtf18*$^{-/-}$ (-/-) oocytes were stained with anti-SYCP3 (green), which stains axial/lateral elements of the synaptonemal complex, and anti-CREST (red), which stains centromeres (G-L) during the diplotene stage of meiotic prophase I. Diplotene stage is normal in *Chtf18*$^{+/+}$ oocytes; no univalent chromosomes were present (G-I) as demonstrated by the presence of 20 centromeres per cell. However, we observed four or more univalent chromosomes in 65% (40 nuclei counted) of *Chtf18*$^{-/-}$ oocytes (J-L) as demonstrated by the presence of more than 20 centromeres per cell. Our results suggest that *CTF18* functions to maintain linkage between homologous chromosomes, because synapsis occurs normally but homologues of *Chtf18*$^{-/-}$ oocytes separate prematurely. Using the Mann-Whitney test we calculated p = 0.001 (**)

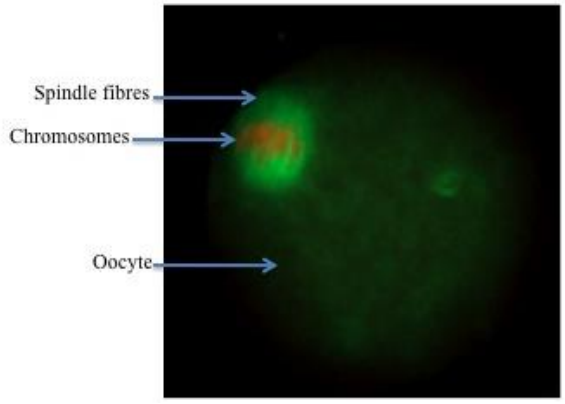


Figure 7. Immunofluorescence staining of a wild-type mouse oocyte with anti-beta-tubulin, which stains the spindle fibers (green), and To-pro-3-iodide, a counter stain which allows visualization of the chromosomes (red) and their alignment on the spindle plate.

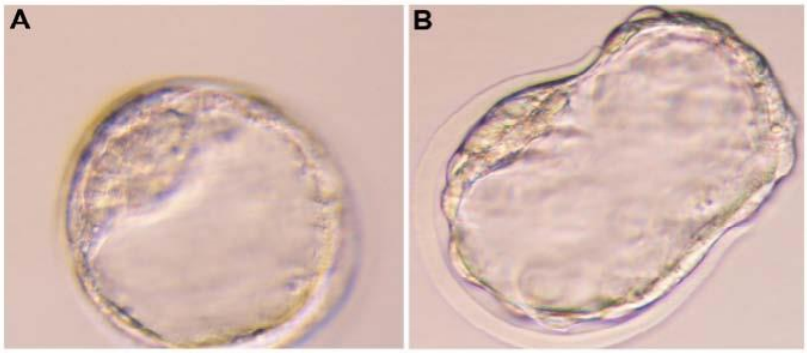


Figure 8. Blastocysts collected from the oviducts of *Chtf18* heterozygous female mice. Cultured blastocysts exhibited attachment (A) and hatching (B).

**18. Extent of Clinical Activities Initiated and Completed**. Items 18(A) and 18(B) should be completed for all research projects. If the project was restricted to secondary analysis of clinical data or data analysis of clinical research, then responses to 18(A) and 18(B) should be "No."

18(A) Did you initiate a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
___X__No

18(B) Did you complete a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
___X__No

**If "Yes" to either 18(A) or 18(B), items 18(C) – (F) must also be completed.** (Do NOT complete 18(C-F) if 18(A) and 18(B) are both "No.")

18(C) How many hospital and health care professionals were involved in the research project?

_____Number of hospital and health care professionals involved in the research project

18(D) How many subjects were included in the study compared to targeted goals?

_____Number of subjects originally targeted to be included in the study
_____Number of subjects enrolled in the study

18(E) How many subjects were enrolled in the study by gender, ethnicity and race?

Gender:
_____Males
_____Females
_____Unknown

Ethnicity:
_____Latinos or Hispanics
_____Not Latinos or Hispanics
_____Unknown

Race:
_____American Indian or Alaska Native
_____Asian
_____Blacks or African American
_____Native Hawaiian or Other Pacific Islander
_____White
_____Other, specify:_____
_____Unknown

18(F) Where was the research study conducted? (List the county where the research study was conducted. If the treatment, prevention and diagnostic tests were offered in more than one county, list all of the counties where the research study was conducted.)

**19. Human Embryonic Stem Cell Research.** Item 19(A) should be completed for all research projects. If the research project involved human embryonic stem cells, items 19(B) and 19(C) must also be completed.

19(A) Did this project involve, in any capacity, human embryonic stem cells?

_____ Yes
___X__ No

19(B) Were these stem cell lines NIH-approved lines that were derived outside of Pennsylvania?

_____Yes

_____ No

19(C) Please describe how this project involved human embryonic stem cells:

**20. Articles Submitted to Peer-Reviewed Publications**.

20(A) Identify all publications that resulted from the research performed during the funding period and that have been submitted to peer-reviewed publications. Do not list journal abstracts or presentations at professional meetings; abstract and meeting presentations should be listed at the end of item 17. **Include only those publications that acknowledge the Pennsylvania Department of Health as a funding source** (as required in the grant agreement). List the title of the journal article, the authors, the name of the peer-reviewed publication, the month and year when it was submitted, and the status of publication (submitted for publication, accepted for publication or published.). Submit an electronic copy of each publication, listed in the table, in a PDF version 5.0.5 format, 1,200 dpi. Filenames for each publication should include the number of the research project, the last name of the PI, the number of the publication and an abbreviated research project title. For example, if you submit two publications for PI Smith for the "Cognition and MRI in Older Adults" research project (Project 1), and two publications for PI Zhang for the "Lung Cancer" research project (Project 3), the filenames should be:

Project 1 – Smith – Publication 1 – Cognition and MRI
Project 1 – Smith – Publication 2 – Cognition and MRI
Project 3 – Zhang – Publication 1 – Lung Cancer
Project 3 – Zhang – Publication 2 – Lung Cancer

If the publication is not available electronically, provide 5 paper copies of the publication.

**Note:** The grant agreement requires that recipients acknowledge the Pennsylvania Department of Health funding in all publications. Please ensure that all publications listed acknowledge the Department of Health funding. If a publication does not acknowledge the funding from the Commonwealth, do not list the publication.

| Title of Journal Article: | Authors: | Name of Peer-reviewed Publication: | Month and Year Submitted: | Publication Status (check appropriate box below): |
|---|---|---|---|---|
| 1. None | | | | ☐Submitted<br>☐Accepted<br>☐Published |

20(B) Based on this project, are you planning to submit articles to peer-reviewed publications in the future?

Yes____X_____ No_____

If yes, please describe your plans:

A manuscript entitled "*CTF18* plays critical roles in female gametogenesis and ovarian folliculogenesis in mammals" is currently in preparation and will be submitted to a peer-reviewed journal for publication within the year. The results obtained from our future studies investigating the basis of underlying defects of impaired folliculogenesis and premature separation of homologous chromosomes will also be submitted to a peer-reviewed journal.

**21. Changes in Outcome, Impact and Effectiveness Attributable to the Research Project.** Describe the outcome, impact, and effectiveness of the research project by summarizing its impact on the incidence of disease, death from disease, stage of disease at time of diagnosis, or other relevant measures of outcome, impact or effectiveness of the research project. If there were no changes, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

None

**22. Major Discoveries, New Drugs, and New Approaches for Prevention Diagnosis and Treatment**. Describe major discoveries, new drugs, and new approaches for prevention, diagnosis and treatment that are attributable to the completed research project. If there were no major discoveries, drugs or approaches, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

Defects in chromosome segregation during meiosis are known to contribute greatly to causes of aneuploidy in offspring, and aneuploidy is one of the most frequent types of genetic defects that occur during reproduction. Aneuploidy due to mis-segregation of chromosomes is a leading cause of birth defects and pregnancy loss, yet our understanding of how these occur is largely lacking. Thus, there is a great need to extend our knowledge of the mechanisms of chromosome segregation and of the underlying defects that lead to mis-segregation. We have found that *CTF18* plays critical roles in both female germ cell development and fertility in mammals and that loss of function of *CTF18* leads to chromosomal defects during meiosis, and likely aneuploidy. Our findings also suggest that *CTF18* plays an important role in maintaining the early ovarian follicle pools in mammals, and it is well known that diminishing ovarian follicle numbers correlate with decreased fertility, premature menopause, and menopause in women. Thus, the current and future studies should contribute significantly to our understanding, and ultimately to evaluation and treatment of underlying causes of infertility and aneuploidy in women.

**23. Inventions, Patents and Commercial Development Opportunities.**

23(A) Were any inventions, which may be patentable or otherwise protectable under Title 35 of the United States Code, conceived or first actually reduced to practice in the performance of work under this health research grant? Yes_____ No____X_____

If "Yes" to 23(A), complete items a – g below for each invention. (Do NOT complete items a - g if 23(A) is "No.")

a. Title of Invention:

b. Name of Inventor(s):

c. Technical Description of Invention (describe nature, purpose, operation and physical, chemical, biological or electrical characteristics of the invention):

d. Was a patent filed for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____

If yes, indicate date patent was filed:

e. Was a patent issued for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____
If yes, indicate number of patent, title and date issued:
Patent number:
Title of patent:
Date issued:

f. Were any licenses granted for the patent obtained as a result of work performed under this health research grant? Yes_____ No____

If yes, how many licenses were granted?_____

g. Were any commercial development activities taken to develop the invention into a commercial product or service for manufacture or sale? Yes___ No____

If yes, describe the commercial development activities:

23(B) Based on the results of this project, are you planning to file for any licenses or patents, or undertake any commercial development opportunities in the future?

Yes_____ No____X_____

If yes, please describe your plans:

**24. Key Investigator Qualifications.** Briefly describe the education, research interests and experience and professional commitments of the Principal Investigator and all other key investigators. In place of narrative you may insert the NIH biosketch form here; however, please limit each biosketch to 1-2 pages.

Karen M. Berkowitz, M.D., is an obstetrician/gynecologist and clinician scientist who completed subspecialty training in reproductive endocrinology and infertility (REI) at the University of Pennsylvania in 2000. During her fellowship training she completed advanced didactic work in genetics and a research project to clone and characterize the human and mouse orthologues of *CTF18*, an evolutionarily conserved gene that is crucial for fertility in the fruitfly and that is essential for the accurate segregation of chromosomes in yeast. This project became the basis of her current work. Following the completion of her REI subspecialty training in obstetrics and gynecology, she stayed at Penn and applied for and was awarded an NIH K12 Career Development Award, the Women's Reproductive Health Research (WRHR) scholarship. When she entered the WRHR program her goal was to acquire the skills necessary to pursue molecular and cellular studies in reproduction. During her scholarship training period (which ended in 2005) she gained a wide variety of skills in molecular and cellular biology and genetics, as well as some independent grants to fund her research. She also independently developed the required reagents to generate a mouse model lacking *CTF18* (termed *Chtf18* in the mouse) function in order to determine the role of *CTF18* in mammalian germ cell development. Due to the complex nature of generating *Chtf18*-deficient (null and conditional) mice and the inherent long timeline, it took several years to successfully derive this mouse model. Following her WRHR scholarship, she continued her research full time at Penn in order to establish her research program in the genetic control of mammalian gametogenesis and to launch her career as a clinician scientist. On July 1, 2008 she accepted a tenure track position as an Assistant Professor at Drexel University College of Medicine in the Departments of Obstetrics and Gynecology and Biochemistry and Molecular Biology. She has her own independent research laboratory in the Department of Biochemistry and Molecular Biology, and at least 50% of her time is devoted to developing her research program. Over the past 2 years her research has come to fruition, and she has recently demonstrated important roles for *Chtf18* in mammalian gametogenesis and fertility in both male and female mice. Her work has now been presented at national and international meetings and invited talks and recently resulted in a publication entitled "Germline expression of mammalian CTF18, an evolutionarily conserved protein required for germ cell proliferation in the fly and sister chromatid cohesion in yeast" [Berkowitz KM, Kaestner KH, Jongens TA, *Mol Hum Reprod* 2008;14(3):143-150; cover], and a manuscript in revision (*PLoS Genetics*) entitled "Premature separation of homologous chromosomes during meiosis in *Chtf18*-deficient male mice." Another manuscript entitled "*CTF18* plays critical roles in female gametogenesis and ovarian folliculogenesis in mammals" is currently in preparation and will be submitted to a peer-reviewed journal for publication within the year. The results obtained from future studies investigating the basis of underlying defects of impaired folliculogenesis and premature separation of homologous chromosomes in female mice will also be submitted to a peer-reviewed journal. Data collected from her project funded by the CURE Award, entitled "Role of CTF18 in female

germ cell development and fertility," have already been presented at the following meetings during the reporting period: 1) Drexel University College of Medicine, Philadelphia, PA, Discovery Day, October 7, 2009 (first prize for best poster); 2) the 57th Annual Meeting of the Society of Gynecologic Investigation, Orlando, FL, March 24-27, 2010 (oral presentation); 3) Drexel University College of Medicine Discovery Day, Philadelphia, PA, October 7, 2010 (first place for outstanding technician poster).

In the short term, Dr. Berkowitz will continue to establish herself as an independent principal investigator, and she will continue to build her clinical practice, which will facilitate the interface between clinical medicine and translational research.

In the long term, Dr. Berkowitz's academic objective is to become tenured faculty and to establish a strong NIH-funded translational and clinical research program in the Department of Obstetrics and Gynecology at Drexel University College of Medicine that engages residents, students, and postdoctoral fellows in research. A major goal of this work is to elucidate the processes that regulate development of germ cells and reproductive potential. Her ultimate goal is to make a significant impact on disorders of human reproduction and infertility that have a genetic basis, and she aims to be instrumental in improving the diagnosis and treatment of many of these disorders.