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Decreased Fecundity and Sperm DNA Methylation Patterns

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Abstract

Objective—To evaluate the relationship between epigenetic patterns in sperm and fecundity.

Design—Prospective study of couples trying to conceive, utilizing semen samples collected through the HOPE study, at the University of Utah.

Setting—Academic Andrology and IVF Laboratory

Patients—DNA methylation alterations associated with fecundity were analyzed in 124 semen samples. 27 semen samples from couples who conceived within 2 months of attempting a pregnancy and a total of 29 semen samples from couples who were unable to achieve a pregnancy within 12 months were analyzed to identify regions of interest.

Interventions—None.

Main Outcome Measures—Genome-wide assessment of differential sperm DNA methylation and standard semen analysis.

Results—No differences in sperm count, sperm morphology, or semen volume were observed between the patients achieving a pregnancy within 2 months of study time and those not obtaining a pregnancy within 12 months. However, using data from the Human Methylation 450k array

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DATA AVAILABILITY

Data is available for any interested party through GEO. The GEO accession number is

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analysis we did identify 2 genomic regions with significantly decreased (FDR <0.01) methylation and 3 genomic regions with significantly increased methylation in the “failure-to-conceive” group. Interestingly, the only two sites where decreased methylation was associated with reduced fecundity are at closely related genes known to be expressed in sperm, HSPA1L and HSPA1B.

Conclusions—Our data suggest that there are genomic loci where DNA methylation alterations are associated with decreased fecundity. We have thus identified candidate loci for future study to verify these results and investigate the causative or contributory relationship between altered sperm methylation and decreased fecundity.

Keywords

Male infertility; Sperm; epigenetics; DNA methylation; HSP

INTRODUCTION

Decreased fecundity is a complex disease with many subtle associations with genetic, biologic, and lifestyle factors that contribute to the disease (^{1–6}). This complexity is further compounded by the fact that this disease ultimately affects a couple, such that the etiology of reproductive dysfunction can be found in the female or male alone, or potentially in both partners. The frequency of infertility is varied worldwide (peaking at approximately 30% of couples in some regions), but is thought to impact an average of 8 to 12% of all reproductive-aged couples globally (^{7–10}). While there are many known causes of infertility or subfertility, often the etiology of individual cases cannot be identified.

A common source of subfertility or infertility in human couples is male subfertility or decreased fecundity. It is believed that male reproductive dysfunction is an independent cause of reduced fecundity in approximately 30% of subfertile couples (¹¹). In another 20% of couples, abnormal male reproductive function contributes to a couple's inability to conceive but is not independently responsible (¹¹). What constitutes male subfertility is difficult to elucidate, as there are many fertility related diagnoses assigned to men based on abnormalities in the semen analysis as defined by the World Health Organization standards for semen analysis. These diagnoses appear to poorly predict an inability or decreased capacity to conceive a child (^{12–14}).

The most common diagnostic test used to screen for male factor infertility is the basic semen analysis. This test varies slightly from lab to lab, but in general the test analyzes basic measures including semen volume, semen pH, sperm concentration and count, sperm viability (measured with an eosin/nigrosin stain or with a hypoosmolarity test), sperm morphology, and sperm motility. These basic measures have long been the conventional approach to determine if an individual should be considered “normozoospermic”. If a normozoospermic individual is still incapable of fathering children, further analysis of DNA damage, sperm function assays, aneuploidy screening, strict morphology, or the presence of anti-sperm antibodies may be measured to attempt to determine the etiology of the otherwise idiopathic disorder (^{15, 16}). Despite the use of the most cutting edge technologies and assays, male infertility cases often remain idiopathic in nature. In fact, for approximately 15–30% of cases a couple's infertility is defined as unexplained, meaning that no major

abnormality in semen measures was identified in a male who is unable to conceive with a partner who likewise presents with a normal reproductive profile (¹⁷).

Because of the difficulty in diagnosing male factor infertility and the poor predictive value of currently available assays, there is a great need to identify additional sperm markers with prognostic value. While there may be many potential biological, genetic, and epigenetic alterations that are associated with, or predictive of, fecundity, sperm DNA methylation is a particularly interesting candidate because DNA methylation is plastic in the adult, and environmental insults, toxin exposures, aging, diet and lifestyle factors can alter the methylation profile of sperm (^{18–21}). In theory, these alterations can occur in sperm without impacting sperm phenotype as identified by any basic measure of sperm quality. For example, we have recently identified consistent age-associated alterations in sperm DNA methylation while finding that the measures of semen analysis (sperm viability, count, motility, etc.) in the young and aged groups did not differ (¹⁹). Thus, some of these epigenetic perturbations may effectively be invisible to conventional diagnostic techniques. These hidden alterations could potentially impact independently or, more likely, in concert with other perturbations, a couple's potential to conceive and for the embryo to undergo normal development.

In the present study, we have utilized a unique sample set from a study at the University of Utah, which has tracked 165 young couples who are attempting to achieve a pregnancy, with no known history of subfertility. Using semen samples from the male partner, we tested for sperm epigenetic differences between couples who rapidly achieved a pregnancy and those couples that were unable to achieve a pregnancy within 12 months. We focused our analysis on identifying new targets, in the form of genomic regions, with DNA methylation profiles that are associated with reduced fecundity. Increasing our understanding of such alterations may aid in the development of new diagnostic tools that could potentially improve our ability to describe the etiology of some cases of idiopathic male factor infertility.

METHODS

Patient population and collection procedures

All participants in this work were recruited via the Home Observation of Periconceptional Exposures (HOPE) study at the University of Utah under an institutional review board approved protocol. Briefly, this parent study was designed to observe the time to pregnancy of the general population and to associate these with sperm/semen parameters and various common chemical exposures. In order to comprehensively study time to pregnancy in the context of environmental exposure, semen quality parameters must also be examined. This paper addresses one analysis of the semen quality parameters and associations with time to pregnancy examined in this cohort. We categorized samples collected for this purpose based on their time to pregnancy and analyzed changes in methylation patterns in those who conceived early in pregnancy (we have defined this as 'within 2 months of attempting a pregnancy') and those that did not conceive in an effort to observe some of the most different test groups. The basic inclusion criteria for this study included females aged 18–35 with male partners aged 18–40 years old who were planning to conceive. Both the male and female partner were required to live within a 1-hour drive of Salt Lake City and able to

respond to study questionnaires and instructions in English. Exclusion criteria for the female included: fewer than 9 menstrual flows in the previous 12 months (unless due to breastfeeding or use of an IUD with a subsequent return to normal menses); use of injectable or implantable hormonal contraception in the previous 2 months (or use of Depo-Provera in the last 12 months); and previously diagnosed with infertility, sub-fertility, or a condition that might affect their fertility such as having undergone cervical or infertility treatment or having been diagnosed with PCOS or endometriosis. Exclusion criteria for males in this analysis included any men who had known infertility diagnoses or who had no sperm present in their ejaculate upon entering the study. An additional exclusion criteria by either member of the couple includes having ever been unsuccessful in conceiving a child after a year or more of regular, unprotected sexual intercourse. Semen samples were collected at the time of onset of menses for the first or second menstrual cycle under observation in the study.

A total of 124 semen samples collected between 2012 and 2014 were used in this analysis. Only couples having either achieved a pregnancy or reached study completion without achieving a pregnancy were considered in our analysis. For our initial analysis, only samples from individuals that fell into two distinct groups were considered: group 1, couples that achieved a pregnancy within 2 months of attempting to conceive (n=27); and group 2, couples who were unable to achieve a pregnancy during the study time frame (n=29). These study groups are referred to in this study as the “early pregnant” and “not pregnant” groups respectively. It is important to note that time points for these groups were selected prior to analysis. Epigenetic analyses are best performed between the most extreme groups, which is what we have utilized here while still maintaining adequate sample sizes. Subsequent analyses of sites with demonstrated differential methylation between the early pregnancy and not pregnant groups included all couples, separated by time to pregnancy.

Sample processing

Patients collected samples through intercourse into a sterile, condom (Apex Medical Technologies, San Diego CA). After collection the samples were immediately frozen at -20°C , then the sample was then transported to the University of Utah where it was stored until further analysis. While not the ideal storage conditions for these samples (as the immediate storage was out of technician's hands), care was taken to ensure that patients were aware of the importance of storing the sample immediately. Some data do suggest that temperature induced methylation changes can occur over time in some tissue types^(22, 23), though sperm have never been assessed. It is likely that, due to the highly compact and quiescent nature of the sperm nucleus, temperature induced methylation alterations would be far less frequent and/or severe than in other screened tissues. Further, there is no data that suggest that one of our test groups would be more or less effective at storing their samples in comparison to others, so any potential issues will not confound our results. Frozen samples were transported to a central lab at the University of Utah where they were held until all samples were thawed and an aliquot in a single batch for volume, count, and morphological assessment based on WHO IV criteria⁽²⁴⁾.

Sperm were then subjected to DNA extraction for methylation analysis at the University of Utah Andrology and IVF labs. Prior to DNA isolation semen samples were subjected to a stringent somatic cell lysis protocol to ensure the absence of potential contamination. Briefly, samples were run through a 40um cell strainer to remove any large contaminants. The strained sample was washed in 14 ml of PBS followed by two washes in 14 ml of distilled water. The sample was then centrifuged and the resulting pellet was incubated for a minimum of 60 minutes at 4° C in 14 ml of a somatic cell lysis buffer (0.1% SDS, 0.5% Triton X-100 in DEPC H₂O). Following this lysis step the sperm DNA was extracted using a sperm specific modification commonly used in our lab to a column based extraction protocol using the DNeasy DNA isolation kit (Qiagen, Valencia CA)^(18, 19).

DNA and array processing

Extracted sperm DNA was bisulfite converted with EZ-96 DNA Methylation-Gold kit (Zymo Research, Irvine CA) according to manufacturer's recommendations. Converted DNA was then hybridized to the Infinium Human Methylation 450 Bead Chip micro-array (Illumina, San Diego CA) and analyzed according to Illumina protocols. Hybridization array analysis was performed at the University of Utah genomics core facility. Once scanned for methylation levels at each CpG a β -value was generated by applying the average methylated and unmethylated intensities at each CpG using the calculation: β -value = methylated / (methylated + unmethylated). The resultant β -value ranges from 0 to 1 and indicates the relative levels of methylation at each CpG with highly methylated sites scoring close to 1 and unmethylated sites scoring close to 0.

Data processing and basic epigenetic analyses

Raw intensity values used to generate β -values were subjected to SWAN normalization using the methylation module in Partek (St. Louis, MO). The resulting β -values were used for basic descriptive analysis including global methylation, non-CpG context methylation, and methylation variability analysis as has been previously performed in our lab ⁽²⁵⁾.

DNA methylation window analysis

Data from each couple were subjected to a 1000 base pair sliding window analysis where regions of altered methylation associated with fecundity were identified using a t-test of average methylation patterns within the window of interest. This analysis is performed through an application named Methylation Array Scanner, which is part of the publically available USeq package of bioinformatics applications. To prevent the influence of outliers in the data set, methylation for a specific window was reported as a pseudo-median and differences between the two categories are reported as log₂ ratios. Two thresholds were applied to identify windows with significant differential methylation. A Benjamini Hochberg corrected t-test FDR (false discovery rate) of 0.01 and an absolute log₂ ratio 0.2 were considered significant findings. Raw FDR values were transformed for visualization in figures and reference in this text with the use of the following formula: $(-10 \log_{10} (q\text{-value FDR}))$, such that a transformed FDR value of 13 = 0.05, 20 = 0.01, 25 = 0.003, 30 = 0.001, and 40 = 0.0001, etc. This approach has been frequently used in our laboratory ^(19, 25).

Statistics

Statistical analyses were performed using multiple software tools. SWAN normalization of raw array intensity values and subsequent β -value calculation was performed on Partek (St. Louis, MO). Basic t-test and Chi squared analyses were performed using STATA 11 (College Station, TX). Window analysis and associated tests of significance were run through applications in the Useq platform of bioinformatics tools. GO, Pathway and associated analyses were performed with the web based Genomic Regions Enrichment of Annotations Tool (Great) from the Bejerano lab at Stanford University (²⁶).

RESULTS

Semen parameters

A basic analysis of semen parameters (sperm count, concentration, and morphology) revealed no statistically significant relationship between any one parameter and achievement of pregnancy (Table 1).

Patient characteristics

Patient characteristics observed include male and female age, as well as male and female BMI. Female age and male BMI were, on average, statistically similar between the early pregnant and not pregnant group ($p = 0.129$ and 0.783 respectively). However, male age was statistically significantly different, with the non-pregnant group having a lower average age (27.55 ± 0.71) than the early pregnant group (29.74 ± 0.71 ; $p = 0.034$). Female BMI was significantly higher on average in the not pregnant group compared to the early pregnant group ($p = 0.0016$). The details of patient characteristics data can be viewed in Table 2.

Global methylation alterations

This array platform allows us to analyze over 450,000 probes of potentially methylated cytosines in the human genome. We compared various aspects of β -values between the early pregnant and not pregnant groups with no major differences identified. Specifically, no statistically significant change in coefficient of variance (measuring the variability of methylation patterns between individuals within the groups of interest) was identified between the pregnant and not pregnant groups (p -value = 0.5198 ; Sup figure 1A). Similarly, no statistically significant difference in global methylation between these two groups was detected (p -value = 0.7608 ; Sup figure 1B). Lastly, no significant changes in methylation at non-CpG context cytosines were identified globally (p -value = 0.4331 ; Supplemental figure 1C).

Regional methylation analysis

Regional analysis of methylation alterations identified multiple interesting sites of significance. Specifically, we identified two sites where hypomethylation appears to be associated with decreased pregnancy rates. One of these alterations, HSPA1L, demonstrated a high level of significance (corrected FDR of < 0.00000001 ; Figure 1). The only other significant region where hypomethylation was associated with pregnancy outcome is a closely related protein, HSPA1B. Three regions were identified where hypermethylation was

found to be associated with pregnancy outcome, although these regions were less striking in terms of significance levels ($FDR < 0.01$). The genes USP6NL, SPON2, and PTPRN2 were found to be near these hypermethylated regions (Supplemental Table 2).

Time to pregnancy analysis of HSPA1L and HSPA1B

In an effort to determine the true association with fecundity at these hypomethylated sites identified in our stringent initial analysis, these sites were analyzed based on their time to pregnancy. Specifically, we identified the frequency of two distinct epigenetic profiles at these loci; intermediate methylation (average beta value of between 0.2 and 0.8) and strong hypomethylation (average beta value of < 0.1). We analyzed the frequency of these profiles in 4 distinct time to pregnancy categories: 0–3.99 months of attempted conception, 4–7.99 months of attempted conception, 8–14 months of attempted conception, and no pregnancy achieved during within the study timeframe. It should be noted that we extended the last category of individuals (8–14 months) who achieved a pregnancy to 14 months as some who did achieve a pregnancy did so just after the 12-month screening period. We identified similar profiles in the HSPA1L and HSPA1B groups (figure 2A–B). Specifically, the percent of samples with strongly hypomethylated regions of interest appeared to increase with decreasing fecundity (figure 2B). Conversely, the percent of samples with hemimethylated profiles at loci of interest progressively decreased with decreasing fecundity at HSPA1B and, though the identical profile was not observed in both groups, in the no pregnancy group the percent of samples with intermediate methylation profiles sharply declined to zero just above (Figure 2B). We additionally investigated whether methylation status at one of the sites (HSPA1L/B) correlated with the other within each individual via linear regression analysis, and we found a strong correlation between the methylation patterns at each site suggesting that when one of the sites in an individual is strongly hypomethylated, in general the other site would be also be strongly hypomethylated (Figure 2C; $p < 0.0001$, $R^2 = 0.6113$). We attempted to analyze the other sites of interest (hypermethylated sites) with a similar approach, however the distinct epigenetic profiles seen at the HSP regions were not seen in the other sites of interest. Instead they displayed slight hypermethylation in virtually all samples resulting in generally increased methylation patterns. Such a profile made these sites unfit for a similar analysis to what was performed for the HSP regions.

GO term and Pathway analysis

Analysis of the regions altered in our set of data revealed multiple GO terms, Pathways, and embryonic expression patterns that were significantly enriched (Supplemental Table 1). Findings were considered significant at a corrected FDR of < 0.05 . Pathway analysis revealed that apoptotic pathways may be affected, while GO analysis suggested the potential that protein folding and chaperone protein systems may be affected between the two groups. This is largely driven by the presence of the two HSPs mentioned previously. Further, analogous genes in mouse were used to provide us some idea of embryonic expression patterns that revealed enrichment in the yolk sac and trophectoderm.

DISCUSSION

Idiopathic infertility is a complex disease that affects many couples. While much is known regarding the basic biology of gametes and the process of fertilization and embryogenesis, the majority of measures used in the current semen analysis are poor predictors, independently or collectively, of achievement of pregnancy⁽¹⁴⁾. Recent studies have highlighted the potential role of the sperm epigenome in male fertility⁽¹⁾. In an effort to better understand the links between pregnancy and gamete quality we have analyzed a critical portion of the sperm epigenome, sperm DNA methylation. This is an interesting mark for study as it is malleable over time as a result of various exposures or lifestyle choices, and yet alterations to the epigenetic profile of sperm do not necessarily result in an abnormal sperm phenotype based on semen analysis measures.

Consistent with prior work, we found a lack of association between basic semen parameters and the pregnancy outcomes of couples screened. Age of the female, male BMI, sperm count, sperm morphology, and sperm concentration all appear to have no link to achievement of pregnancy. These data suggest that the regular semen analysis, while useful for many reasons, is a poor predictor of pregnancy. In further support of this idea, 5 of the 29 (approximately 17.2%) individuals screened and not pregnant within 12 months would be considered oligozoospermic, while 5 of the 27 (approximately 18.5%) individuals who achieved a pregnancy within two months of attempting to conceive would have the same diagnosis. We note that a large portion (20%) of individuals with proven fertility would meet the technical definition of oligozoospermia and that this frequency is approximately the same in individuals who are unable to achieve a pregnancy. Due to the design of the study with immediate freezing of semen samples after collection at home, sperm motility data were not available for comparison with fecundity.

While no semen analysis measures were found to be different between our two groups (early pregnancy group and the no pregnancy group), an important potential confounder was identified. Specifically, female BMI was significantly higher in the not pregnant group when compared to the early pregnant group. This is very important to note as it obviously has potential to affect fecundity. Despite this, female BMI is insufficient to independently explain all the pregnancy outcome differences that were observed. Further, female BMI is unrelated to our finding of methylation alterations, and thus our findings cannot be discounted.

Importantly, despite the lack of association between semen quality measures and fecundity we did identify multiple genomic loci of interest where DNA methylation perturbations appear to be associated with decreased fecundity. Specifically, the finding that decreased methylation at HSPA1L was strongly associated with decreased pregnancy rates was striking. This protein is considered to be a “testes-specific” heat shock protein and is expressed strongly in the sperm. Further, some studies have shown that this specific protein can result in antibody accumulation from previous infections and can effectively block fertilization⁽²⁷⁾. We discovered that, on average, fraction methylation was decreased at the HSPA1L promoter, which suggests that this protein may be upregulated, potentially making these HSPA1L enriched sperm more susceptible to such deleterious interactions.

Interestingly, the only other location where hypomethylation was associated with pregnancy outcomes was a closely related protein, HSPA1B.

While much is still required to fully understand the nature of this association, our findings suggest that there are sperm epigenetic associations with fecundity of couples. It should be noted that while our study has focused on healthy young couples, our group of “subfertile” couples have only attempted pregnancy for a minimum of 12 months, meaning that a portion of these couples, up to 50% based on previous data, may achieve a pregnancy within the next 12 months of attempting conception (28, 29). This may explain, in part, the higher than expected number of couples who did not achieve a pregnancy among those who participated in this portion of the HOPE study (approximately 23%). Though these perturbations are not likely to independently cause infertility or subfertility, as some of the same methylation patterns were present in the fertile group, our results suggest that this pattern is seen frequently in the population with reproductive dysfunction and that, when coupled with other issues such as possibly previous infections in the male or their partner, such a signature may contribute to subfertility. The idea that these methylation patterns are not independently predictive of fecundity are further supported by the fact that only approximately 54% of individuals we screened who had strong hypomethylation profiles at both HSP genes of interest (the profile more frequently seen in those that achieved no pregnancies) actually failed to conceive. Despite the lack of independent causation of decreased fecundity, the frequency of these marks in a population with subfertility is intriguing and supports the idea that these marks may be influential (though reliant on other alterations) to result in decreased fecundity. If these marks are found to be influential in many cases of decreased pregnancy success, they may warrant consideration as one of many potential targets for diagnostic testing in couples who present with idiopathic infertility, though much still needs to be elucidated regarding the exact role of this protein in the process. Ultimately, therapeutics may be identified which modify methylation at these sites, leading to improved fertility though a great degree of work is still required to determine if this is even a possibility.

CONCLUSION

These data suggest that there are genomic loci where DNA methylation alterations are associated with fecundity in a patient population where no associations between basic semen analysis measures can be similarly linked. This intriguing finding offers a potential target for future studies to analyze the biologic effect of these alterations. Further, this basic understanding demonstrates that there are new potential targets that can be studied for use as diagnostic markers to help define male factor infertility where conventional measures of male fertility are insufficient, though a great amount of work remains to determine how viable this approach may be.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

The most significant regional methylation alteration associated with pregnancy is shown in the Integrated Genome Browser at HSPA1L (A). Average absolute methylation change across all CpGs (B) analyzed and at the most highly affected CpGs (C) can be seen.

Hypomethylation at this site is associated with our group of couples who did not achieve a pregnancy with a transformed corrected FDR of 105.5.

Figure 2.

We analyzed the frequency of two methylation profiles at HSP loci of interest. We calculated the frequency of samples that displayed strong (A) hypomethylation (beta value <0.1), and (B) Intermediate methylation (beta value between 0.2 and 0.8) at two sites of interest, HSPA1L and HSPA1B in 4 distinct time to pregnancy categories: 0–4 months of attempted conception, 4–8 months of attempted conception, 8–14 months of attempted conception, and no pregnancy achieved during within the study timeframe. We additionally performed linear regression analysis (C) on fraction methylation values at HSPA1L and HSPA1B and identified a strong correlation between the two values within individuals ($p < 0.0001$).

Table 1

Semen Analysis Measures

Measures	Early Pregnancy ($\pm$ SE) n=27	No Pregnancy ($\pm$ SE) n=29	P value
<i>Volume</i>	2.61 ($\pm$ 0.32)	2.56 ($\pm$ 0.3)	0.90
<i>Total Count</i>	211.04 ($\pm$ 36.05)	156.91 ($\pm$ 37.12)	0.29
<i>Concentration</i>	79.01 ($\pm$ 9.88)	65.98 ($\pm$ 13.41)	0.43
<i>Normal Heads</i>	31.33 ($\pm$ 2.25)	26.39 ($\pm$ 2.66)	0.16
<i>Large Heads</i>	1.48 ($\pm$ 0.37)	1.17 ($\pm$ 0.5)	0.62
<i>Small Heads</i>	1.29 ($\pm$ 0.31)	1.07 ($\pm$ 0.27)	0.58
<i>Tapered Heads</i>	35.37 ($\pm$ 4.08)	38.03 ($\pm$ 4.16)	0.64
<i>Immature Heads</i>	3.96 ($\pm$ 0.77)	5.69 ($\pm$ 1.11)	0.21
<i>Duplicate Heads</i>	0.41 ($\pm$ 0.15)	0.79 ($\pm$ 0.4)	0.37
<i>Amorphous heads</i>	26.89 ($\pm$ 3.59)	25.82 ($\pm$ 3.68)	0.83
<i>Normal tails</i>	70.89 ($\pm$ 2.65)	70.44 ($\pm$ 2.77)	0.91

Table 2

Patient Characteristics

Characteristics	Early Pregnancy ($\pm$ SE) n=27	No Pregnancy ($\pm$ SE) n=29	P value
<i>Male BMI</i>	27.61 ($\pm$ 1.3)	27.17 ($\pm$ 1.03)	0.783
<i>Male Age</i>	29.74 ($\pm$ 0.71)	27.55 ($\pm$ 0.71)	0.034
<i>Female BMI</i>	22.96 ($\pm$ 0.72)	27.2 ($\pm$ 1.06)	0.0016
<i>Female Age</i>	28.04 ($\pm$ 0.62)	26.55 ($\pm$ 0.75)	0.129

Table 3

GO Term, Pathway, MGI Expression Results

Ontology	ID	Description	Q Value
GO Biological Process	GO:0090084	negative regulation of inclusion body assembly	3.10E-03
GO Biological Process	GO:0042026	protein refolding	4.52E-03
GO Biological Process	GO:0090083	regulation of inclusion body assembly	5.81E-03
GO Biological Process	GO:0006986	response to unfolded protein	2.15E-02
GO Biological Process	GO:0035966	response to topologically incorrect protein	1.82E-02
GO Biological Process	GO:0006457	protein folding	4.71E-02
GO Molecular Function	GO:0044183	protein binding involved in protein folding	2.19E-03
PANTHER Pathway	P00006	Apoptosis signaling pathway	4.74E-02
MGI Expression: Detected	14241	TS23_yolk sac mesenchyme	3.01E-05
MGI Expression: Detected	14240	TS23_yolk sac endoderm	7.38E-04
MGI Expression: Detected	3991	TS19_extraembryonic component	1.89E-02
MGI Expression: Detected	391	TS12_ectoplacental cone	4.22E-02
MGI Expression: Detected	387	TS12_trophectoderm	4.21E-02