<table>
<thead>
<tr>
<th>Date</th>
<th>Topic</th>
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<tbody>
<tr>
<td>Jan 23</td>
<td>(1) Exponential, Logistic, Prioritizing global challenges</td>
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<tr>
<td>Jan 30</td>
<td>(2) Sequencing &amp; understanding biosphere omes, 3D molecular design</td>
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<td>Feb 6</td>
<td>(3) Genome Edit/Write (beyond CRISPR), Codon Recoding</td>
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<td>Feb 13</td>
<td>(4) In situ Sequencing : Mammalian Cell Atlas &amp; BRAIN Initiative</td>
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<td>Feb 20</td>
<td>(5) Epigenetic programming, signaling pathways, SynEvoDevo</td>
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<td>Feb 27</td>
<td>(6) Microbiomes: therapeutics, diagnostics, nanopores</td>
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<td>Mar 6</td>
<td>(7) Synthetic Organs for VUS &amp; Transplantation</td>
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<td>Mar 20</td>
<td>(8) Aging Reversal</td>
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<td>Mar 27</td>
<td>(9) Global Warming</td>
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<td>Apr 3</td>
<td>(10) Germline editing &amp; <em>H. sapiens 2.0</em></td>
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Germline editing examples

Schaefer et al. (14-Jun-2017) Nature Methods. Unexpected mutations after CRISPR–Cas9 editing in vivo

If I had to predict the most consequential report on reproductive medicine in the second decade of the 21st century, this would be it.
CRISPR Repair Reveals Causative Mutation in a Preclinical Model of Retinitis Pigmentosa

Wu, Tsai, Justus, Lee, Zhang, Lin, Bassuk, Mahajan, Tsang (Aug. 2016) Molecular Therapy

A mixture of 3 ng/µl of sgRNA plasmid, 3 ng/µL of Cas9 protein (NEB) and 1 µM of ssODN (IDT) was injected into the pronuclei and cytoplasm of FVB/N inbred zygotes.

F03 & F05 incorporated donor template in 35.7% and 18.8% of somatic cells, respectively.
Unexpected mutations after CRISPR–Cas9 editing *in vivo*


DNA was isolated from two CRISPR-repaired mice (F03 and F05) and one uncorrected control... As additional controls, each of the variants was compared with the FVB/NJ genome in the mouse dbSNP database (v138), and ... with all 36 strains in the Mouse Genome Project (v3) .. which further confirmed that these WGS-identified SNVs were the result of CRISPR–Cas9 off targeting.

sequenced at an average depth of 50×, and the control at 30×. Variant calls were confirmed by at least 23× sequencing coverage ... 24 CRISPR-associated variants were selected, and all were confirmed by Sanger sequencing
Corrigendum & follow-up: Whole genome sequencing of multiple CRISPR edited mouse lines suggests no excess mutations

Biorxiv  2018/03/26/154450.full.pdf
Schaefer¹,², Darbro³, Colgan¹, Tsang⁴,⁵, Bassuk³,⁶, Mahajan¹,⁷

1 Omics Laboratory, Stanford University,
2 Interdisciplinary Graduate Program in Genetics, University of Iowa,
3 Department of Pediatrics, University of Iowa,
4 Jonas Children’s Vision Care, Columbia University, New York,
5 Edward S. Harkness Eye Institute, New York-Presbyterian Hospital,
6 Neurology, University of Iowa,
7 Byers Eye Institute, Stanford University

CRISPR off-targets: a reassessment
There was insufficient data to support the claim of unexpected off-target effects due to CRISPR in a paper published in Nature Methods. More work is needed to determine whether such events occur in vivo. Editorial: 30-Mar-2018 nature.com/articles/nmeth.4664.pdf

Unexpected mutations after CRISPR– Cas9 editing in vivo Schaefer, Wu, Colgan, Tsang, Bassuk, Mahajan. Nature Methods, Jun 2017
60% of SNVs & indels are shared between F03 & F05 and distinct from the FVB control ...unexpected for mutagenesis in independently created mice,

730 SNVs in the FVB control mouse homozygous for a genotype not matching the mm10 (C57BL6) reference. Of these, 578 SNVs (79%) appear as ‘complete switches’ for both the F03 and F05 mice back to the homozygous mm10 reference. Only 27 variants (4%) where both F03 and F05 mice have homozygous changes that do not match the mm10 reference. Expected: 67% to non-mm10 reference and 33% to the mm10 reference, yet here we see 4% and 96%, chi-squared P < 0.00001

C57BL/6J littermates showed 985 sites of variation (SNVs and indels) between individuals. While the number of segregating variants in C57BL/6 and FVB/N has not been independently compared, the 985 sites in C57BL/6J littermates is similar to that found in the authors’ FVB/NJ data set (696 SNVs and indels).
1. Wilson et al. Editas Medicine, Cambridge
Fulcrum Genomics, Somerville
Harvard Medical School, Boston

2. Lescarbeau et al. Intellia Therapeutics, Inc. Cambridge

3. Kim et al. Institute for Basic Science, Seoul, South Korea
Division of Theoretical Bioinformatics, Heidelberg, Germany
Department of Chemistry, Hanyang University, Seoul, South Korea

4. Lareau et al. Molecular Pathology Unit, MGH, Charlestown
Department of Biostatistics, HSPH, Boston
Broad Institute of MIT and Harvard
Pathology, Stem Cell & Regenerative Biology, Harvard
Biological Engineering, MIT, Cambridge

5. The Hospital for Sick Children, Toronto
Baylor College of Medicine, Houston
Mouse Biology Program, Univ. of California, Davis
The Jackson Laboratory, Bar Harbor, ME, Farmington CT
Mammalian Genetics Unit, Harwell Institute, Harwell, UK.
Do extraordinary claims require extraordinary evidence? –Deming 2016 (Hume 1748, Sagan 1979)

Schaefer et al. (14-Jun-2017) Nature Methods. Unexpected mutations after CRISPR–Cas9 editing in vivo

If I had to predict the most consequential report on reproductive medicine in the second decade of the 21st century, this would be it.
D. Schematic of intracytoplasmic sperm injection (ICSI) followed by progression through the first cell cycle during day 1 of development. The number of maternal and paternal genomes are indicated at each phase of the cell cycle.

E. Immunofluorescence of a mouse zygote at telophase of the second maternal meiotic division. Note that only the maternal genomes are attached to microtubules, while the paternal genome begins to form an interphase nuclear membrane to replace the sperm membrane. BF= bright field.

F. Progression of human zygotes through the first cell cycle from the two-pronuclear stage to prometaphase, when the two genomes can be removed from the egg by a needle. Note separation of the two genomes (arrows & dashed circles).

NEBD: pronuclear envelope breakdown.

G. Cell cycle progression during day 1 in fertilized mouse zygotes. Of 23 mouse eggs, none showed direct contact between maternal & paternal genomes.

Size bars = 10 μm.
Critiques of Ma et al.

Doubts raised about CRISPR gene-editing study in human embryos
Ewen Callaway (31-Aug-2017) Nature

“The critique levelled by Egli et al. offers no new results but instead relies on alternative explanations of our results based on pure speculation … We will respond to their critiques point by point in the form of a formal peer-reviewed response in a matter of weeks” – Mitalipov 31-Aug-2017

after fertilization, the genomes of the egg and sperm reside at opposite ends of the egg cell, and each is enshrouded in a membrane for several hours. --Perry at Reproductive biologist, Univ. of Bath, UK

“In my view Egli et al. convincingly provided a series of compelling arguments explaining that the correction of the deleterious mutation by self repair is unlikely to have occurred.” -- Burgio, geneticist at Australian National University, Canberra.
Genetic counseling
$1K vs gene therapy $1M

Tay Sachs: Deafness, blindness, seizures, dementia, loss of motor control
IVF-PGD & termination

The frequency of serious mendelian diseases is 5-10%

Of over 1000 single gene disorders that have been identified, the 7 most common for which PGD has been used: Cystic fibrosis, Tay-Sachs disease, Spinal muscular atrophy (SMA), Hemophilia, Sickle cell disease, Thalassemia, Duchenne’s muscular dystrophy (DMD)

advancedfertility.com/pgd-genetic-testing-embryos.htm
Gamete stem cell genome-editing could help parents carrying serious mutations to have genetically healthy babies. Edited stem cells can be checked for success without risk to embryos.

Prenatal testing & termination: 25%
In vitro fertilization screening: 80%
Sperm or egg editing: 0%
NAS report 14-Feb-2017

“Clinical trials using heritable germline genome editing should be permitted … absence of reasonable alternatives…preventing a serious disease … multigenerational follow-up that still respect personal autonomy…oversight mechanisms to prevent extension to uses other than preventing a serious disease or condition”

Vatican commission 2004: “Germ line genetic engineering with a therapeutic goal in man would in itself be acceptable … in the stem cells that produce a man’s sperm, whereby he can beget healthy offspring with his own seed by means of the conjugal act.”
Section 749 rider on the Consolidated Appropriations Act of 2016 “none of the funds made available by this Act may be used to notify a sponsor or otherwise acknowledge receipt of a submission for an exemption for investigational use of a drug or biological product … in research in which a human embryo is intentionally created or modified to include a heritable genetic modification.”

Are random germline mutations from cancer chemotherapy more acceptable than careful restoration of a verified normal state?

Is the current practice of abortions to prevent genetic diseases more acceptable than engineering sperm?

Church GM Compelling Reasons for Repairing Human Germlines NEJM 2017

Humans 2.0 Space Colony Challenges

Gravity  Osteoporosis  Neuro-behaviorial  Radiation  Microbiome  …
Rare protective alleles

<table>
<thead>
<tr>
<th>Gene</th>
<th>Allele/Phenotype</th>
<th>Function/Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>LRP5</td>
<td>G171V/+</td>
<td>Extra-strong bones</td>
</tr>
<tr>
<td>MSTN</td>
<td>-/-</td>
<td>Lean muscles &amp; low atherosclerosis</td>
</tr>
<tr>
<td>SCN9A</td>
<td>-/-</td>
<td>Insensitivity to pain</td>
</tr>
<tr>
<td>ABCC11</td>
<td>-/-</td>
<td>Low Odor production</td>
</tr>
<tr>
<td>CCR5</td>
<td>-/-</td>
<td>HIV resistance</td>
</tr>
<tr>
<td>FUT2</td>
<td>-/-</td>
<td>Norovirus resistance</td>
</tr>
<tr>
<td>PCSK9</td>
<td>-/-</td>
<td>Low coronary disease</td>
</tr>
<tr>
<td>APP</td>
<td>A673T/+</td>
<td>Low Alzheimer’s</td>
</tr>
<tr>
<td>APOE</td>
<td>E2/E2</td>
<td>Low Alzheimer’s (E2=R112C, R158C)</td>
</tr>
<tr>
<td>GHR, GH</td>
<td>-/-</td>
<td>Low cancer</td>
</tr>
<tr>
<td>SLC30A8</td>
<td>+/-</td>
<td>Low T2 Diabetes</td>
</tr>
<tr>
<td>IFIH1</td>
<td>E627X/+</td>
<td>Low T1 Diabetes</td>
</tr>
<tr>
<td>TERT</td>
<td>overprod.</td>
<td>Low Aging</td>
</tr>
<tr>
<td>CDKN2A</td>
<td>overprod.</td>
<td>Low cancer</td>
</tr>
<tr>
<td>TP53</td>
<td>overprod.</td>
<td>Low cancer</td>
</tr>
<tr>
<td>GRIN2B</td>
<td>overprod.</td>
<td>High learning &amp; memory</td>
</tr>
<tr>
<td>PDE4B</td>
<td>inhib.</td>
<td>Low anxiety, high problem solving (mice)</td>
</tr>
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Extraterrestrial surgery: Potential opportunities

Contagion. Germ free Chickens & goats since 1920s.
Inflammation.
Anesthesia. Conscious insensitivity to pain. SCN9A -/-
Problem Set #10  Germline editing & *H. sapiens* 2.0

10A. Examples of diseases which might be best handled by pre-dating counseling vs. somatic vs. germline therapies? (if any)

10B. What disease therapies might also result in enhancement as likely outcomes?

10C. What edits might be considered for new environments or new markets?

10D. Unintended consequences & ways to minimize those? Gain of diversity?