Questions for the Record for Dr. Kevin Donovan –

1. At the hearing, the claim was made that without fetal tissue, Zika virus research could not go forward. Only days later, the Washington Post cited a Cell Stem Cell research project in which induced pluripotent stem cells were engineered to study the characteristics of the virus's infectious potential in developing neural tissue. Since the breakthrough study produced vital information, why the insistence that fetal tissue is required to develop a vaccine or to study the infection's progress?

This does not get at the ***motivation*** for the insistence on fetal tissue, but gives you more background on the licit alternatives. The insistence seems to be motivated from a desire to continue the same research and cell sources (i.e., tradition, what's worked in the past), resistance to change (it might delay experiments), and likely an ideological undertone (failure to recognize the basis of the controversy, or to give any credence or consideration to alternative ethical viewpoints.)

While the earliest attempts at growing viruses did use cultures of unpurified human fetal tissue, most research, as well as vaccine production, quickly shifted to purified cell lines, which lent themselves to considerably less variability and provided large numbers of quality-controlled cells, providing reproducible results. In the 1960's and 1970's, cell culture work operated under an assumption that younger cells grew better, faster, and longer, so fetal cells obtained from abortion were sometimes used to create these cell lines (indicating they were developed as a lineage from a specific, original source of cells grown in the lab. A few human fetal cell lines (WI-38, MRC-5) are still in use for some vaccine production. However, <u>few vaccines are now produced using fetal cell lines, and none using fetal tissue</u>. Newer cell lines, e.g., A549 cells (adult human),[1] Sf9 cells (insect),[2] EB66 (duck),[3] and better culture techniques make reliance on fetal cells an antiquated science. In addition, the CDC and other leading medical authorities have noted since 2001 that "No new fetal tissue is needed to produce cell lines to make these vaccines, now or in the future."[4]

The example referenced in the question is another clear answer for the lack of need for freshly aborted fetal tissue in virus and vaccine studies. Scientists developed a successful model system to show that the Zika virus can infect and damage some developing brain cells.[5] The established experimental model, which the authors of the paper note can now be used for further investigations of developing brain as well as screening therapeutic compounds, was not developed using fetal tissue. The successful system uses human induced pluripotent stem cells (iPS cells), which are ethically created from skin or other

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[1] See e.g., Shabram P and Kolman JL, Evaluation of A549 as a New Vaccine Cell Substrate: Digging Deeper with Massively Parallel Sequencing, *PDA J Pharm Sci Technol* 68, 639, 2014

[2] See e.g., Glenn GM et al., Safety and immunogenicity of a Sf9 insect cell-derived respiratory syncytial virus fusion protein nanoparticle vaccine, *Vaccine* 31, 524, 2013; AND Khan AS, FDA Memo: Cell Substrate Review for STN 125285, January 14, 2013; accessed at:
http://www.fda.gov/downloads/BiologicsBloodVaccines/Vaccines/ApprovedProducts/UCM339125.pdf

[3] See e.g., Brown SW, Mehtali M, The Avian EB66(R) Cell Line, Application to Vaccines, and Therapeutic Protein Production, *PDA J Pharm Sci Technol*. 64, 419, 2010

[4] See, *e.g.*, "Vaccine Ingredients – Fetal Tissues," The Children's Hospital of Philadelphia, 2014; accessed July 21, 2015 at www.chop.edu/centers-programs/vaccine-education-center/vaccine-ingredients/fetal-tissues; CDC quote originally accessed July 2015 at: http://www.ascb.org/newsfiles/fetaltissue.pdf

[5] Tang H *et al*., Zika Virus Infects Human Cortical Neural Progenitors and Attenuates Their Growth, *Cell Stem Cell* 18, 2016; *in press*, doi: 10.1016/j.stem.2016.02.016

normal cell types; the development of iPS cells earned the 2012 Nobel Prize for Dr. Shinya Yamanaka of Japan.

Another recent study by a Brazilian group confirmed the susceptibility of developing human brain cells to Zika virus infection, with potential damage to infected brain cells. Again, the successful study did not use human fetal tissue, but rather human iPS cells.[6]

Human iPS cells have demonstrated excellent potential to model developing brain, producing what are termed "organoids" for detailed study of the various cell types, brain structures, and even abnormal development that can occur. In particular, one model system using human iPS cells to produce brain organoids has been shown also to be an accurate model to study Microcephaly, the brain development condition that seems to be associated with infection by Zika virus in the womb.[7] And a newly-published paper further validates the superior ability of human iPS cells to model brain development. While this new reference does discuss Zika in particular, it convincingly demonstrates that this model system for brain development – which does not use aborted human fetal tissue – can be used to model normal human brain development, the timing of brain development associated with production of various neuronal cell types, and even to compare human brain development versus that of monkeys.[8]

[6] Garcez PP *et al*., Zika virus impairs growth in human neurospheres and brain organoids, *PeerJ Preprints* 4:e1817v3; doi: 10.7287/peerj.preprints.1817v3

[7] Lancaster MA *et al.*, Cerebral organoids model human brain development and microcephaly, *Nature* 501, 373, 19 Sept 2013

[8] Otani T *et al*., 2D and 3D Stem Cell Models of Primate Cortical Development Identify Species-Specific Differences in Progenitor Behavior Contributing to Brain Size, *Cell Stem Cell* published online March 31, 2016, doi: 10.1016/j.stem.2016.03.003

2. You testified that "it does exist and it is more ethical" in response to a question about the existence of alternative sources of tissue to form fetal cell lines, "such as spontaneous miscarriages."
Please expand your testimony to include other alternative sources of tissue that you are aware of which may be used to form fetal cell lines and that you believe to be ethical.

Those opposed to using fetal tissue from miscarriages argue that an insufficient amount of suitable tissue would be available for certain studies, such as transplantation. Several papers published by Dr. Maria Michejda at Georgetown University School of Medicine outline very clearly that spontaneous miscarriages are a useful and ethical alternative source of fetal stem cells for hematopoietic cell transplantation (for review see Michejda, 2002, 2004), and other labs have agreed with her findings (Low et al, 1994; Wu et al., 1999). An additional report characterized 12 and 18 week old fetuses from spontaneous abortions and was able to study key cells involved in brain development (Virgintino et al., 1998). Another study was recently conducted using fetal tissues from both induced and spontaneous abortions, side-by-side (Kang et al., 2016).
Other arguments against the use of fetal tissue from miscarriages include the unknown time of death and possible genetic abnormalities. In regard to timing, numerous reports, together with Dr. Michejda's epidemiological studies, have indicated that over 15% of the 300,000 second-trimester miscarriages studied were suitable for transplantation (which has some of the most rigorous requirements for tissue viability), when collected and preserved properly (Michejda, 2002). In response to genetic concerns, a fetus can appear "normal" until an inherent genetic abnormality manifests itself after birth. In fact, birth defects are the leading cause of infant deaths, accounting for 20% of all infant deaths (Matthews et al., 2015). So like miscarriages, fetal tissue from induced abortions can also carry genetic abnormalities. In addition, the use of the abortion drug, mifepristone (RU-486), and prostaglandins for the medical termination of pregnancy may substantially reduce the availability of human fetal tissues from induced abortions (Branch et al., 1995). Furthermore, if there are concerns that tissues from miscarriages are not "normal", comprehensive screening tools are available and can be used to identify relevant genetic abnormalities.

Branch, D.W., et al. Suitability of fetal tissue from spontaneous abortions and from ectopic pregnancies for transplantation. JAMA, 273:66, 1995
Kang, X., et al., Granulocytic myeloid-derived suppressor cells maintain feto-maternal tolerance by inducing Foxp3 in CD4+CD25-T cells by activation of the TGF-b/b-catenin pathway. Mol Hum Reprod, 2016 [Epub ahead of print]
Low, W.C., et al., Human fetal tissue from spontaneous abortion as potential sources of donor tissue for cell transplantation therapies. Transplantation Proceedings, 26:1, 1994
Matthews, T.J., et al., Infant mortality statistics from the 2013 period linked birth/infant death data set. Center for Disease Control and Prevention, National Vital Statistics Reports, 64 (9):1, 2015.
Michejda, M., Spontaneous miscarriages as a source of fetal stem cells. The national catholic bioethics quarterly, 2:401, 2002
Michejda, M., Which stem cells should be used for transplantation? Fetal diagnosis and therapy, 19:2, 2004
Virgintino, D., et al., Astroglia-microvessel relationship in the developing human telencephalon. Int. J. Dev. Biol., 42:1165, 1998
Wu, A.G., et al. Analysis and characterization of hematopoietic progenitor cells from fetal bone marrow, adult bone marrow, peripheral blood, and cord blood. Pediatric Research, 46:163, 1999

3. **Why is it necessary to have different ethical guidelines governing consent to donate fetal tissue for minors as opposed to adults? Further, how might a minor be unduly influenced to donate fetal tissue if presented with the consent form, such as Exhibit A-3?**

Informed consent cannot take place unless the patient has decision-making capacity. This requires three distinct aspects:

A) Comprehension, or the ability to understand. This would include the ability to appreciate the impact and consequences of procedures donations etc.

B) The ability to evaluate or deliberate in accordance with one's own values. This presupposes the ability to compare risks and benefits of the options and to make rational choices that are consistent over time.

C) Communication, and absence of coercion.

The obvious problem is that minors have poorly developed decision-making capacity, due to immature value systems, poor appreciation of possible consequences, and particularly in this situation, undue influence of the situation and environment in which they find themselves. In fact, any negative responses might make them feel they are jeopardizing their chances of going forward with the planned abortive procedure, thus impairing the truly free exercise of their will, i.e. they feel "they have no choice". This is why minors are not free consent to medical and surgical procedures under normal circumstances, without parental permission.

Moreover, truly valid informed consent requires adequate and honest disclosure of information. To be presented with a document such as Exhibit A – 3 would be highly misleading. It states, "Research using the blood from pregnant women and tissue that has been aborted has been used to ***treat and find a cure*** (emphasis added) for such diseases as diabetes, Parkinson's disease, Alzheimer's disease, cancer, and AIDS." As any well-informed adult knows, there have been no successful cures developed for any of the stated diseases, with or without the use of aborted fetal tissue, and no treatments that are based on fetal body parts. This is a flagrant misrepresentation, creating an undue and therefore coercive incentive, and should not appear in any legally or ethically approved consent form.