In case you missed it: The Prenatal Diagnosis editors bring you the most significant advances of 2018.

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Running title: review of the hot topics emerging in prenatal diagnosis during 2018

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INTRODUCTION

This year, the editors of *Prenatal Diagnosis* met on an October sunny day in Bethesda, MD, to make plans for the journal's next year (Figure 1). Following our tradition first established six years ago, we discussed what we felt were some of the exciting new advances in prenatal screening and diagnosis. We hope our readers will enjoy reading our overview on the hot topics of 2018, including expanded carrier screening, the role of preimplantation genetic screening prior to *in vitro* transfer, the value of prenatal exome sequencing in fetuses with structural abnormalities, as well as annual updates in fetal surgery, noninvasive prenatal testing (NIPT), and fetal gene therapy.

Non-Invasive Prenatal Testing

Seven years after its introduction into clinical prenatal care, over 10 million non-invasive prenatal testing (NIPT) have been completed globally.[1] Remarkably, 70% of the tests to date have been done on Chinese women. While the majority of cell-free DNA tests are performed for the common fetal autosomal and sex chromosome aneuploidies, the test menus have expanded to include microdeletions, copy number variations and single gene disorders.[2-9] In an extraordinary study published in October 2018, Liu and colleagues performed a large-scale population analysis using shallow sequencing data from 141,431 NIPTs performed at the Beijing Genome Institute in Shenzhen.[1] They analyzed these data to track the migration of different population sub-groups within China. Using genome-wide association studies they identified a novel gene, *NRG1*, that is associated with predisposition to twin pregnancies. They also demonstrated the presence of unique patterns of circulating viral DNA that were associated with a high prevalence of hepatitis B, human herpes viruses 5 and 6, human parvovirus B19, and other clinically important infections. Finally, they identified multiple genes that are associated with height and body mass index in the Chinese population, as well as fertility as a function of maternal age. In summary, noninvasive prenatal DNA sequencing test results can serve as a novel resource for understanding population genetic variation and to identify genotype-phenotype associations.

One of the main reasons that NIPT has become incorporated so quickly into prenatal care is that a negative test result has a >99% negative predictive value. For pregnant women who only want reassurance that their baby does not have one of the common autosomal trisomies (13, 18 or 21), a negative screening result is enough information, and they subsequently decline a diagnostic procedure. This has resulted in a 70% decrease worldwide in amniocenteses and chorionic villus samplings, providing proof of clinical utility for NIPT. A significantly decreased number of diagnostic tests has reduced health care costs, but it has also been assumed that integration of NIPT into care would also be *safer* for pregnant women and their fetuses because there would be fewer procedure-associated miscarriages. To test this hypothesis, a randomized clinical trial was conducted in 57 centers in France between April 2014 and April 2016 (ClinicalTrials.gov identifier: NCT02127515).[10] Two thousand fifty one pregnant women at high risk of having a fetus with trisomy 21 either received an immediate invasive diagnostic test or first received a cell-free (cf)-DNA test, with invasive testing only performed for a positive screening result. Interestingly, the cfDNA test had a detection rate of 100%. Over 97% of the enrolled subjects who had an initial test (n=1997) completed the trial. The primary outcome showed that the rate of miscarriage was not statistically

different between the two groups (both groups had miscarriage rates of 0.8%), although the authors suggested that the study may have been underpowered to find clinically meaningful differences in losses before 24 weeks of gestation. There were, however, 11 chromosomal abnormalities detected by karyotyping in the group that went directly to invasive testing. Three were apparently balanced translocations without clinical manifestations, one was confined placental mosaicism for trisomy 12, two were mosaic trisomy 13 cases, three were sex chromosome aneuploidies, and two were complex rearrangements expected to be associated with developmental disabilities. Some limitations of this study include the fact that 24% of women initially randomized to the invasive testing dropped out of the study, cfDNA testing was only performed for trisomy 21, and there was a 5.6% false positive rate for the cfDNA group, which led to more diagnostic procedures.

As cfDNA sequencing moves towards first tier testing for fetal aneuploidy, it is reasonable to assume that there will be more studies such as this one that will more deeply investigate its clinical utility and the benefits of testing beyond its superior positive predictive values to the current approach of serum screening and nuchal translucency measurement.

Expanded carrier screening

Expanded carrier screening (ECS) has seen quite an evolution in the last few years, and 2018 has not been an exception. Since its introduction 10 years ago, ECS has been driven mainly by commercial laboratories, which have selected the conditions to be included in the panels based on unclear criteria, often driven by competition to include as many disorders as possible. The most recent publication confirmed that the larger the number of disorders screened, the higher the likelihood of identifying a carrier (as high as 36% for a panel inclusive of 218 diseases).[11]

Professional organizations have published consensus-determined points to consider when ordering ECS, based on severity, age of onset, consistent phenotype, carrier frequency, and accuracy of the screening test, among other metrics.[12] However, when verified, only 27% of the conditions on commercially available ECS panels meet such criteria.[13] Since clinicians often do not have the time and genetic expertise to translate such points into customized list of conditions appropriate for prenatal screening, the American College of Obstetrics and Gynecology (ACOG) has suggested a list of 22 conditions that are considered reasonable for inclusion in an ECS panel based on the above-mentioned criteria.[14] Currently both ethnicity-based screening and ECS testing are considered acceptable strategies by ACOG.[14] However, the conclusions of an initial mathematical modeling, which suggested that ECS would detect more fetuses at risk for severe or profound conditions across all ethnic groups than screening based on ethnicity,[15] have partially been confirmed by a few studies, two of which were published in 2018.[11,16] One of such studies compared the screen positive rate of a "standard panel" of 23 conditions (inclusive of most of those suggested by ACOG) with that of a "global panel" of 218 conditions.[11] The screen positive rates were 13% and 36%, respectively. However, the 12 most commonly conditions detected by the global panel (i.e. those with a positive rate of 1% or greater) included those suggested by ACOG, in addition to 3 conditions mild or amenable to neonatal screening and treatment, and non-syndromic hearing loss related to mutations in *GJB2*. In the second study, nine of the 15 couples found to be at risk based on a commercial panel of 100 genetic diseases would have been identified through the ACOG-suggested panel;[16] the missed conditions were Pompe disease (glycogen storage disease Type II), familial Mediterranean fever, *GJB2*-related non syndromic hearing loss, and two mild conditions

(achromatopsia and familial Mediterranean fever). It appears that the optimal balance has not yet been achieved; couples are still are risk for undue anxiety and for spending time and money on follow-up testing for mild conditions or conditions with low screening performance.

Any benefits of ECS would be mediated by how parents use the information provided by the ECS panels. In 2018, initial data regarding reproductive outcomes of couples that undergo ECS has been published. A survey of 537 couples, in which both partners were identified as carriers for the same autosomal recessive condition, classified as profound, severe, or moderate based on published criteria, found that disease severity had a significant association with changes in decision making.[17] The study was potentially biased by the low response rate (12%). Pregnancy status is another important variable that predicts whether ECS translates into decision making. Studies originating from IVF clinics have found that 100% of couples in which both partners carry pathogenic variants of the same gene elect to screen embryos through pre-implantation genetic testing, independently from the severity of the condition.[16,18] In contrast, among those screened during pregnancy, only 37% elect prenatal diagnostic testing for that pregnancy.[19] A survey of pregnant women's perspectives on ECS confirms such findings: about 42% were unsure what they would do if they discovered their fetus had a genetic disorder, 34% would continue the pregnancy and prepare for the birth of an affected child, and only 24% would likely terminate their pregnancy.[20] The study also highlighted the deficit in knowledge of pregnant women. In the study, ECS was proposed in a standardized way with pretest genetic counseling. Among the 50% of women who declined ECS, the most quoted reason (selected by 77% of participants) was that they had no family history. Since ECS screens for monogenic recessive disorders, family history would not be expected to be informative; conversely, women with a positive family history would not be good candidates for ECS, as they would require genetic assessment of their own specific risk and appropriate testing.

What kind of information do patients receive related to ECS? Since many healthcare providers lack genetic knowledge to deliver appropriate pre-test counseling, are hindered by time constraints and workload, and are fearful of overloading prospective parents with too much information during a consultation, couples may go directly to the ECS websites to gather information on conditions tested, potential benefits and limitations of the screen. A study conducted a comprehensive online search to analyze the content of marketing materials on ECS providers' websites, most of which were commercial.[21] Making an informed decision about ECS would require providing neutral information about ECS, nondirective offers of testing, and informed decisions about disorders to be screened. The authors found that there are wide variations in the way ECS is offered, with some sites being quite directive. Most ECS providers offered complimentary genetic counseling to their consumers, although this was often optional, limited to the post-test context, and, in some cases, appeared to be available only to test-positive individuals. Limitations of ECS were usually downplayed, such as the uncertainties involved with many of the conditions screened in ECS panels, including the variable age of onset for some conditions, phenotypes that are not clearly defined, conditions that are not recommended for general population screening in current screening guidelines, and rare conditions for which risk after negative screening results is not known.

The above issues are being debated at the same time as innovations are being introduced in ECS, which is moving from targeted mutation screening to genome sequencing of gene-disorder pairs.[22,23] This new technology may improve the sensitivity of detecting clinically significant variants.

What to do with Mosaic Aneuploid Embryos? The Debate Continues

Since the initial publication in 2015 by Greco and Colleagues,[24] that demonstrated that transfer of mosaic aneuploid embryos at preimplantation genetic testing for aneuploidy (PGT-A) can lead to the birth of apparently healthy babies, there have been at least six other studies that reaffirm this finding.[25-30] The transfer of a mosaic embryo does not always lead to a live birth, and in fact carries with it a greater likelihood of miscarriage and lower birth rates compared to the transfer of non-aneuploid embryos.[25-30] The most recent study by Zhang and coworkers showed a live birth rate of 43.5% in the mosaic whole chromosome aneuploidy group compared to 59.1% in the euploid embryo group (P=0.02).[30] This rate is consistent with that reported in other studies.[26,28] Given that some mosaic embryos will result in a normal outcome and others will not, Spinella et al.[29] looked for additional predictors that would assist in identifying which mosaic embryos have the best reproductive potential. Their prospective study revealed significantly better clinical outcomes in embryos with lower mosaic aneuploidy proportions (i.e. with mosaicism in less than 50% of the biopsy cells) compared to those with a higher proportion of abnormal cells (>50%). Specifically, higher implantation (48.9% vs. 24.2%), clinical pregnancy rates/embryo transfer (40.9% vs. 15.2%), and live-birth rates (42.2% vs. 15.2%). Significantly poorer clinical outcomes across all categories were observed in embryo biopsies with >50% mosaic aneuploidy compared to normal euploid blastocysts. Specifically: lower clinical pregnancy rates/embryo transfer (15.2% vs. 46.4%), implantation rates (24.4% vs. 54.6%), and live-birth rates (15.2% vs. 46.6%). There were no significant differences observed in clinical outcomes when comparing the euploid embryo group to those with lower level mosaicism (<50%). This led the investigators to conclude that "mosaic embryos with low aneuploidy percentage have higher chances of resulting in the birth of healthy babies compared with embryos with higher mosaicism levels." More recently, a publication by Kushnir and colleagues directly contrasts with this conclusion.[31] The authors reanalyzed the dataset originally published by Munne and co-workers[28] and performed a corrected analysis of pregnancy outcomes following transfer of mosaic embryos. Their independent results supported the initial study findings with higher ongoing pregnancy rates (63.3% vs. 39.2%) and lower miscarriage rates (10.2% vs. 24.3%) observed in euploid embryos compared to mosaic embryos. In addition, Kushnir and colleagues found no significant differences in ongoing pregnancy or miscarriage rates among mosaic embryo transfers at any threshold of aneuploidy. They concluded that the degree of trophectoderm mosaicism was a poor predictor of ongoing pregnancy and miscarriage.[31]

It seems clear that simple assessment of the mosaic level in a trophectoderm biopsy does not provide a reliable predictor of the reproductive potential of a mosaic embryo. This is not surprising, as studies indicate that a random biopsy of five to ten cells may not accurately reflect the genomic picture in the remainder of the embryo when there is mosaicism.[32,33] Apart from sampling error, other factors can certainly impact reproductive potential. For example, a complex mosaic aneuploid embryo with multiple chromosomes involved may be far less viable at a lower mosaic threshold than a simple mosaic aneuploidy at a higher level. Indeed, the Munne study demonstrated the lowest viability in mosaic blastocysts with complex mosaic aneuploidies (10% ongoing pregnancy rates; P<0.001).[28] The specific chromosome involved certainly impacts clinical outcome as demonstrated by the prevalence of only certain chromosome aneuploidies in prenatal diagnosis and live births. Grati and colleagues extrapolated this logic to provide an evidence-based scoring system for prioritizing the transfer of mosaic aneuploid embryos.[34] Since trophectoderm cells are the precursors

to the cytotrophoblast cells found in chorionic villi, Grati et al. reasoned that follow-up data on mosaic CVS samples provides clinically useful information when dealing with mosaic embryos. They followed mosaic CVS samples and assessed how often a particular chromosomal mosaic aneuploidy was ultimately confirmed in the fetus at the time of amniocentesis. Certain chromosomes such as 1, 2 and 7 were almost never observed in the fetus while others such as 16 and 21 were confirmed with much greater frequencies. Since mosaicism is associated with an increased risk for uniparental disomy (UPD) via trisomy/monosomy rescue mechanisms, the investigators also assessed the incidence of clinically significant fetal UPD in cases with a mosaic aneuploidy detected at the time of CVS. UPD for chromosomes 14, 15 and 16 were observed with the highest frequencies (21.4%, 6.5%, and 16% respectively) while the other imprinted chromosomes were not seen at all in their cohort. In addition, they also evaluated the incidence of mosaic aneuploidies in products of conception. Taking all these factors into account plus the likelihood that a given aneuploidy would be observed in a liveborn, Grati and colleagues generated a composite score for each chromosome. The higher the score, the lower the priority for transfer. The Grati study does not provide support for the efficacy of PGT-A nor does it specifically recommend the use of PGT-A.[35] Rather, it offers an evidenced-based tool for decision making when counseling patients dealing with mosaic embryos.[35] Such a tool seems timely given the recent findings from a USA-based survey of 417 respondents that indicated that 42.1% of the IVF clinics (that answered the survey) have transferred mosaic embryos and the majority (63.2%) would opt to transfer mosaic embryos in the future.[36] The same survey revealed that the most common PGT-A technology utilized was next generation sequencing, which is regarded as the most sensitive technique for uncovering mosaicism. As such, it is likely to expect that the challenge of managing mosaic embryos will remain a constant issue for IVF centers. For patients encountering zero euploid embryos, the transfer of mosaic embryos offers some hope of achieving a pregnancy that will lead to a normal liveborn. However, the research to date shows that not all mosaic embryos are equal in their reproductive potential, and strategies to prioritize which embryos to transfer should be employed. Genetic counseling of patients in this situation is critical and until further research elucidates the precise factors that govern reproductive potential, in the meantime, the tool by Grati and colleagues may prove to be highly clinically useful.

Breakthroughs in molecular fetal therapy

Over the last decades, we have uncovered the molecular and genetic basis of many fetal disorders. This deeper understanding has allowed the identification of new potential therapeutic targets for some of these conditions and significant breakthroughs have been reported in this field in 2018, thereby increasing our therapeutic armamentarium.

Using a *drug-based molecular* approach, a group at John Hopkins (Baltimore, US) recently published how they successfully shrank a large fetal cardiac rhabdomyoma causing outflow tract obstruction and supraventricular tachycardia in a fetus with tuberous sclerosis by transplacental (maternal) administration of Sirolimus.[37] This drug inhibits the molecular mTOR (mammalian target of rapamycin) pathway, which is known to be upregulated in tuberous sclerosis and had previously been tried in infants with rhabdomyomas,[38] but was never applied prenatally before.

Using a *protein-based molecular approach*, Schneider et al. reported a treatment for the rare condition X-linked hypohydrotic ectodermal dysplasia.[39] Newborns with this condition lack normal sweat glands, which leads to life-threatening hyperthermia. Additionally, they have fewer

meibomian glands in the eyelids, a decreased number of teeth and abnormal salivary gland development. The condition is caused by a lack of EDA protein during critical phases of skin development, due to a loss of function of the EDA gene. Based on their understanding of the disease physiology, the research group developed a recombinant protein consisting of the constant domain of IgG1 and the receptor-binding portion of EDA, which activates the EDA receptor.[40] After extensive testing in animal models, the protein was administered in the amniotic fluid of three fetuses (including a set of twins) who had been shown to carry the gene mutation responsible for X-linked hypohydrotic ectodermal dysplasia. At birth, the neonates were shown to have a (near) normal number of sweat glands and more tooth germs and meibomian glands than their untreated older siblings.[39] In parallel, the investigators explored further how to diagnose this condition in utero and recently published in our Journal that tooth germ sonography can identify affected fetuses non-invasively, thereby facilitating clinical implementation of their discovery.[41]

Finally, different groups are exploring *gene therapy* for fetal disorders. One of the most commonly used approaches for integrating a therapeutic transgene into the fetal targets cells is through the use of adenoviral vectors.[42] Using this methodology, Massaro et al. recently showed that they could transfect the brains of fetal knock-out mice with the gene involved in neuronopathic Gaucher disease (GBA), thereby rescuing brain histology and preventing the disease phenotype.[43] Interestingly, but not surprisingly, fetal treatment led to better results than neonatal treatment as the intervention happened earlier in the disease process. This group also showed that they could achieve gene transfection in the fetal macaque brain,[43] which puts translation to the human setting within arms reach. At present, human applications of fetal gene therapy have not been published, but just before the submission of this editorial, a Chinese researcher claimed to have modified the genome of a set of twin embryos using the CRISPR/Cas9 approach.[44] Gene editing was one of the promising methodologic advances we discussed in this editorial two years ago.[45] Dr He attempted to induce resistance to the human immunodeficiency virus by removing the *CCR5* gene in the offspring of sero-discordant couples. The announcement in the media, which at the time of our manuscript submission had not yet been confirmed by a scientific publication, created an uproar within the ethics and gene therapy communities. Indeed, the safety of the CRISPR/Cas9 approach is at present insufficiently proven and many worry about 'off-target' effects.[46] Additionally, although most groups agree that gene therapy would be ethically acceptable to cure genetic disorders, the application described here leans more towards the development of 'designer babies', which poses significant ethical concerns.[47] It is unclear whether appropriate research ethics approvals and parental consent were available. We do hope that this reckless 'Wild West' behaviour will not put a brake on appropriately done gene therapy research, as this form of therapy may bring significant progress, especially in the field of fertility treatment, hematologic disorders (thalassemia and sickle cell disease) as well as for other genetic conditions, including cystic fibrosis.[48]

Prenatal Sequencing

At the beginning of 2018, *Prenatal Diagnosis* published a special topic issue on the prenatal diagnosis of monogenic disorders. In that issue we highlighted the advent of prenatal sequencing, including a review of several small series of cases describing the value of prenatal exome sequencing for the diagnosis of monogenic disorders in fetuses with structural abnormalities,[49] along with a detailed account of the laboratory[50] and ethical challenges[51] that this new approach brings. Most of the reports published prior to 2018 described research studies or small case series designed to return

results after the pregnancy ended. Early in 2018, the first publication describing delivery of results within the timeframe of a pregnancy reported an 81% diagnostic yield with a turnaround time of less than two weeks in fetuses suspected to have a skeletal dysplasia.[52] This speedy delivery of results was enabled by careful case selection that included review by a multidisciplinary team including clinical geneticists, fetal medicine specialists and clinical scientists and the use of a targeted clinical panel for sequence interpretation, thereby avoiding most of the ethical challenges raised by Horn and Parker.[51] A similarly high diagnostic yield was reported by a group from Shanghai who used proband only targeted exome sequencing to make a diagnosis in 10 or 12 fetuses with skeletal anomalies suggestive of a skeletal dysplasia.[53] As the year progressed, we saw the publication of a larger cohort from the Baylor team who reported a retrospective series of 146 consecutive prenatal exomes. These authors reported a turnaround time of 14 days for initial reporting and a diagnostic yield of 35% in the 62 cases where trio sequencing was performed because of the presence of at least one structural abnormality.[54]

Given the potential complexities associated with exome sequencing, particularly in the prenatal period, it is not surprising that researchers are exploring parental experiences to identify issues that will need to be addressed when implementing clinically. One study confirmed the range of challenges that existed, including the need for adequate counseling and informed consent, prenatal variant interpretation, inability to identify a genetic aetiology, and identifying secondary findings in the parents when offering trio testing.[55] The other study clearly showed that parents wanted as much information as possible, including uncertain results that might be related to the diagnosis.[56] These studies highlighted the need for clinicians to understand the power of sequencing, the ethical implications and parental perceptions, as well as showing the need for specialized genetic counseling and health professional education.

As 2018 closed we saw the back to back publication in the Lancet of two large series of unselected fetuses with abnormalities, one of 610 fetuses from the UK – the PAGE Study – and the other describing 234 cases from the USA.[57,58] Both prospective studies used trio exome sequencing (both parents and the fetus) for the diagnosis of monogenic disorders in fetuses with any abnormality and a normal karyotype and/or microarray but no selection by genetic or other multidisciplinary review. Both showed an added diagnostic yield overall of 8.5% and 10.3% respectively. Of note both studies showed a higher diagnostic yield in fetuses with multisystem anomalies (15.0% and 18.9%), and in fetuses with skeletal anomalies (15% and 24%), but reported differences in the yield when other systems were involved perhaps reflecting sample bias or that, even in these large studies, the sample size is small when considering the individual anatomical systems. These studies have further expanded our understanding of the prenatal phenotype of some conditions, reporting for the first time prenatal identification of mutations in several genes and highlighting the need to share these data, suitably anonymized, in databases to facilitate interpretation of variants as fetal exome sequencing is used more widely.

All of these publications conclude that exome sequencing is a valuable tool for diagnosing monogenic disorders in the dysmorphic fetus, with some showing that results can be delivered within the timeframe of a pregnancy, thereby potentially offering significant clinical utility. All emphasise the need for multidisciplinary teams working together, and highlighting the logistical issues that need to be addressed before widespread clinical implementation, concluding that, given the practical, diagnostic, and ethical challenges, prenatal genome sequencing for fetal structural

anomalies is best applied to selected subgroups, in particular those with multiple congenital anomalies or after clinical genetic review. These conclusions support the consensus guidelines published by the International Society of Prenatal Diagnosis, the Society of Maternal Fetal Medicine, and the Perinatal Quality Foundation early in 2018.[59] No doubt 2019 will see further clinical implementation of prenatal sequencing as the UK National Health Service plans to include fetal sequencing in the National Genomic Medicine Test Directory and on the other side of the Atlantic insurers will learn the value of this powerful technology and include it in insurance coverage.

Fetal surgical therapy

In the field of fetal therapy, the prenatal treatment of selected cases of spina bifida aperta keeps on drawing attention. Pioneering centers are now publishing large series and outcomes have improved with experience.[60] Whether all the centers will be able to match the results of the most experienced centers, remains to be seen.[61] This year, the full cohort results of the "Management Of Myelomeningocele Study" (MOMS) were published,[62] as were systematic meta-analyses of the published controlled studies.[63] Controlled studies demonstrate that for every two fetuses operated, there will be one not requiring a shunt, and for every five operated fetuses there will be one additional child able to walk. Conversely, there seems to be no measurable adverse impact on neurodevelopment caused by the increased prematurity risk associated with in-utero surgical repair. The long-term outcomes of MOMS-patients are eagerly awaited. For instance, will the initial improvement in bladder function be confirmed and become more prominent?[64] Non-randomized controlled data from Poland would suggest so.[65] Along the same lines, five- and 10-years' observations on fetal repairs done in the pre-MOMS era as compared to matched historical controls suggest sustained motor and ambulatory status improvement, a better than expected preschool functional independence, self-care independence, no increase in behavioral problems, impaired social interactions or restricted behavioral patterns.[66]

The focus of most fetal medicine specialists is whether prenatal spina bifida repair can be performed equally well via fetoscopy. Increasingly more fetoscopic experience is being published, but the neurosurgical results remain in question.[67,68] Endpoints in these reviews are the need for re-do surgery in the neonatal period. Other proxies of neurosurgical precision may be the occurrence of inclusion cysts (which may be more likely in prenatal surgery),[69] or the rate at which the hindbrain reverses to its normal position, which is considered an early marker for success.[62] In that respect, the bar is high as more recent studies report very high reversal rates, as evidenced on ultrasound or magnetic resonance imaging (MRI).[60,70,71] The pressure for moving to a minimally invasive solution is high as it reduces maternal invasiveness, certainly when done percutaneously. This is a concern, as maternal risks of the open procedure should not be underestimated.[72] Avoiding or reducing the size of the hysterotomy also reduces the risk of uterine dehiscence, which is common after open surgery. An endoscopic access even allows vaginal delivery.[67,68,73] This gain is relevant as cesarean delivery of fetuses with spina bifida does not seem to improve neurologic outcome.[74,75]

Fetoscopic repair requires creation of a comfortable intra-abdominal workspace, which is typically done by partial carbon dioxide insufflation (PACI). A vigorous debate remains on the clinical safety of PACI as well as on how this should be researched.[76] Recent articles in *Prenatal Diagnosis* have reviewed the physiological effects of PACI[77] and have shown experimental evidence that PACI induces acidosis, reduces uterine blood flow and increases inflammation of the fetal membranes at

higher (25 mmHg) pressures.[78] Fortunately these effects do not seem to occur in clinical human scenarios, as they seem to be alleviated by the humidification and temperature control used clinically.[79,80] There is also some initial evidence from venous blood gas data suggesting that clinical CO2 amnio-insufflation does not cause severe fetal acidemia.[81] Other modifications that have been suggested to minimize fetal risks are to avoid magnesium sulfate or indomethacin as tocolytics for their effect on cardiac function.[82,83] On the other hand, appropriate tocolysis seems to reduce the need for inhalational anesthetics.[84]

As for Congenital Diaphragmatic Hernia (CDH), it seems that in 2019 the TOTAL-trial ("Tracheal Occlusion To Accelerate Lung Growth" - www.totaltrial.eu) will be completed.[85,86] In this context *Prenatal Diagnosis* published this year a helpful tutorial on prenatal assessment of fetuses with CDH, including individualized risk assessment.[87] This is based on prenatal imaging, but also includes advanced genetic testing. The use of targeted sequencing identifies a genetic cause in 10% of isolated CDH; moreover it can identify new candidate genes.[88] Current research efforts in antenatal treatment of CDH focus on novel treatment modalities, including medical strategies.[86,89] Some of these, like sildenafil, are entering phase I trial.[90,91]

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Figure 1.

The *Prenatal Diagnosis* section editors at their annual meeting, held October 2018 in Bethesda, MD, USA. From left: Jan Deprest, Tim Van Mieghem, Alessandro Ghidini, Diana Bianchi, Lisa Hui, Lyn Chitty, Amanda McLean-Inglis (Wiley-Blackwell), Brynn Levy.