The term zygosity is used to indicate whether twins result from one or two fertilized ova (zygotes). Two types are generally recognized: monozygotic (MZ) and dizygotic (DZ). This terminology is preferable to identical and non-identical (fraternal). In reality, truly identical twins do not exist, because phenotypic differences of varying degrees can always be found with MZ pairs. These differences may be significant and lead to a misdiagnosis of DZ. Misclassification may also occur from genetic discordance within MZ pairs.

The methods of zygosity assessment have changed frequently with advancements in science. Extant methods, including gene products of the time, i.e. blood groups, were reviewed in 1961 by Gedda [1]. Subsequently, the independent publication by Derom et al [2] and Hill and Jeffreys [3] on the use of DNA for zygosity testing was perceived by many as heralding the dawn of a new age in definitive zygosity testing, especially in cases of like sex twins with two placentas. In reality, this was not the case. The following case vignette illustrates problems in the interpretation of zygosity in one twin pair over a sixty-year time span.

Case Vignette: On April 24th, 1935 a set of naturally-conceived male twins was delivered to a 37-year old nullipara. The boys were assessed DZ based on the presence of two placentas. Phenotypic similarities during childhood were so consistent that the twins thought per chance they were MZ, but were dissuaded from this by the false assumption that their individual placentas left no other diagnosis but DZ. At the same time, slight differences in skin pigmentation, location of moles and handedness were thought by many to lie outside the possibilities of monozygosity. At age 41, gene products, (blood) and fingerprint samples were assessed as MZ with a probability of 0.996. At age 61, samples of buccal cells were analyzed using 7 individual DNA markers, one of which was discordant. Based on this observation, zygosity was reassessed as DZ. The twins subsequently submitted samples of blood to two additional laboratories for traditional “bar code” (low stringency RFLP) DNA analyses. Both laboratories assigned the pair MZ status. It became clear that the difference in these diagnoses was based upon the fact that the buccal examinations consisted of a small number of markers with high stringency whereas the blood samples were examined using low stringency tests of many RFLP VNTR sites. This disparity highlighted the absence of and the need for generally accepted criteria for zygosity diagnosis by DNA analysis.

Comments
The importance of accurate zygosity assignment is considerable. In the cited case, zygosity assignment changed three times within a 60-year span depending on the technology used. From a medical point of view, misdiagnosis of zygosity within a twin pair contemplating organ donation may have serious consequences [4],[5]. From a social point of view, accurate understanding of zygosity is part of the twin’s birth right. And finally, from a scientific point of view a more complete understanding of zygosity and all of its genetic implications may force a reexamination of older medical literature in which zygosity testing used less than robust methodology and thus incorporated the potential for incorrect interpretation. One classic analysis of the genetic contribution to disease is the study of schizophrenia and manic-depressive disorder by Torrey et al [6]. Zygosity was assessed using a combination of photos, questionnaires, and nineteen red-blood cell antigens. Years later, aliquots of the original blood samples were examined in the laboratories of Cassandra Smith at Boston University. In this reanalysis, based on hundreds of targeted data points, allegedly MZ pairs discordant for schizophrenia had levels of DNA similarity comparable to the range found in unselected sibling pairs. All others had very high or intermediate similarity levels. One interpretation of these data is that the twin pairs discordant for schizophrenia were actually DZ, despite the fact that competing gene product zygosity assessments were MZ. An alternative interpretation is equally if not more likely, this is, that within the genetic component of schizophrenia, MZ twins exhibit a range of post-zygotic genomic discordance that has not yet completed been characterized. This later explanation is particularly attractive because it addresses the hitherto unanswerable question of why MZ twins are not completely alike. The four presently accepted post-zygotic mechanisms by which MZ twins have been shown to be genetically discordant are as follows: 1) chromosomal differences; 2) X-inactivation difference in females; 3) differential gene imprinting; and 4) post-zygotic mutations. Further details can be found in reference 5.

At present no gold standard exists for zygosity assignment using DNA products. Simply stated, there is no agreement as to which biologic sample is preferable (hair, skin, saliva, blood) or whether it is preferable to use tests of low stringency (classic bar code) or tests of high stringency (individual markers). Moreover, if tests of high stringency are used, their number and exact location has yet to be decided upon. In the final analysis, the answer to the question to the number of genetic markers may hinge upon the concept of statistical strength and interpretation, Just as was the case using the older, standard method of testing, e.g. blood groups, one must test enough loci to achieve statistical validity which, in turn would depend on the relative frequency of the allele(s) in the general population. Most post-zygotic mutations do not occur in coding DNA and thus the detection of discordant mutation in non-coding DNA does not immediately render a diagnosis of DZ. It is important to acknowledge that the exact number of mutational differences that lie within the range an acceptable assignment of monozygosity is presently unknown.

The issues discussed above are of sufficient importance as to demand a reexamination of the attitudes of contemporary obstetricians to using the placenta for zygosity assignment. Since the advent of ultrasound and the use of vaginal probes early in pregnancy, this modality has been adopted worldwide and is advocated in recent texts and monographs as a means of providing better prenatal care and potentially avoiding some of the complications of monochorionic placentation, such as twin-to-twin transfusion syndrome. The singular advantage of using placentaion is that it is a gold-standard in which, for all practical purposes, monochorionic placentation is equivalent to monozygosity.
In summary, the simultaneous recognition of the existence of post-zygotic mutations and the awareness that such mutations may result in a number of differences within MZ pairs the need for suggested a new paradigm [7]. We now propose a four-tier classification system, respecting the classical definition of zygosity and addressing contemporary medical scientific issues most likely to cause confusion:

Level I – assessment by fetal membrane status (monochorionic = MZ) — dichorionic like sex does not mandate DZ state

Level II – phenotypic characteristics – MZ, discordant for schizophrenia, diabetes, cancer, etc.

Level III – genotypic characteristics – possible discordant for coding (single gene disease) or non-coding DNA region, for example

Level IV – genomic characteristics – 93% similar using Targeted Genomic Differential Display (TGDD), 99% similar using bar code, for example.

In the final analysis, obstetricians have an obligation to play active and informed roles in the process of assigning zygosity by ensuring that early and accurate assessments of membranes and placenta(s) are obtained by high quality ultrasound early in pregnancy [5]. After birth, pathologic confirmation of antenatally diagnosed chorion states should be placed in the chart with a copy given to the parents, remembering always that MC=MZ [5]. We advise that all DC like sex pairs have DNA analysis to discern the remaining MZ pairs.

References