CIVIL STATUS AND VARIOUS ASPECTS OF TRUTH : SCIENTIFIC TRUTH AND USE OF SCIENCE FOR IDENTIFICATION PURPOSES

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1. Introduction in biology

1.1. Organization of genetic information in humans

1.1.1. Deoxyribonucleic acid and genes

The genetic instructions used in the development and function of all known living organisms and of some viruses are contained in the deoxyribonucleic acid (DNA) (**Figure 1**). DNA is made of nucleotides. Each nucleotide is formed by a chemical base, a sugar, and a phosphate. There are four types of chemical bases found in DNA: adenine (A), guanine (G), cytosine (C), and thymine (T). DNA nucleotides are arranged in two long strands forming a spiral called a double helix. As a result, the structure of the double helix resembles that of a ladder with the sugar and the phosphate molecules forming the vertical sidepieces of the ladder and the DNA bases pairing up with each other (A with T and C with G) to form the ladder's rungs.

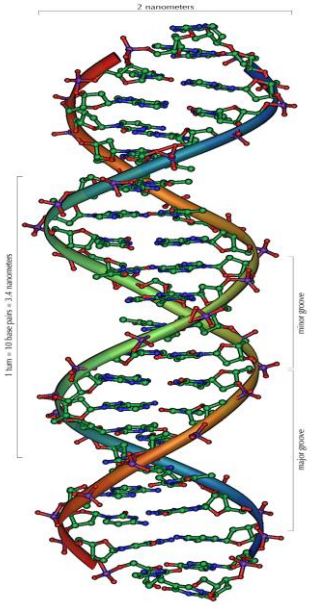

Figure 1. Deoxyribonucleic acid

Genes usually act as templates, or indirect patterns, for the production of: a) polypeptides (chains of amino acids that are the major, if not the sole, constituents of the proteins), or b) other nucleotide chains as various forms of RNA (another type of nucleic acid) that, are involved in the assembly of the polypeptide chains. In either case, the ultimate function of the gene is, to govern the synthesis of proteins (structural proteins, enzymes or proteins that act as regulators of gene activity). The vast majority of human DNA is not organized in genes and has no genetic expression or visible function (non-coding DNA, "junk" DNA), but contains genetic markers important for human identification. On the other hand, all genes have regulatory regions (i.e. promoter, enhancer) and regions that code for a polypeptide or RNA (known as exons), while they are often fragmented by non-coding regions which are not translated into polypeptides (known as introns). The expression of genes begins by transcribing the gene into RNA, which in turn in the case of polypeptide coding genes is translated into a polypeptide. Genes that encode polypeptides are composed of a series of three-nucleotide sequences called codons, which serve as the words in the genetic language and specify the sequence of the amino acids within polypeptides. As a result, the genetic code specifies the correspondence during polypeptide translation between codons and amino acids. The physical development and phenotype of organisms is a product of genes interacting with each other and with the environment.

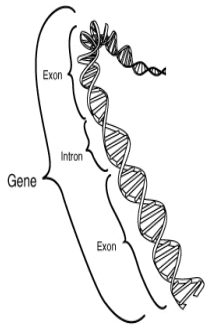

Figure 2. Gene

1.1.2. Chromosomes

DNA is wrapped around structural proteins (histones), which serve to package the DNA and control its functions. Depending on the stage of the cell cycle (the series of events that take place in a cell leading to its division), DNA can be found either in an uncondensed form called chromatin or a condensed form called chromosome (**Figure 3**). Two types of chromosomes are found in humans: sex-chromosomes (X and Y) and non-sex chromosomes (autosomes: ordinarily paired type of chromosomes that are the same in both sexes of a species). Autosomes, like other chromosomes display polymorphisms such as single nucleotide polymorphisms (SNP: DNA sequence variation observed in the coding region or the non-coding regions of DNA, which occurs when a single nucleotide in the genome or other shared sequence differs between member of a species or between paired chromosomes of an individual) and short tandem repeats (STR, a class of microsatellites: polymorphisms observed usually in the non-coding regions of DNA, consisting of tandemly repeated sequences of 2 to 6 base pair length monomers).

Each eukaryotic cell (eukaryotes: organisms whose cells are organized into complex structures enclosed within membranes; main structure of eukaryotic cells is the nucleus) contains in its nucleus a specific number of homologous sets of chromosomes, known as ploidy. In addition, each cell contains in the mitochondria (membrane-enclosed organelles located in the cytoplasm of most eukaryotic cells, supplying most of the cell's energy and involved in a range of other cellular processes) hundreds of copies of the mitochondrial genome. The ploidy of cells can vary within an organism. In humans, most cells are diploid containing 46 chromosomes in total (two sets of 23 chromosomes with each set being inherited from each parent: 22 different types of autosomes and one pair of sex chromosomes X and Y). Sex cells (sperm and oocyte) in humans are haploid containing 23 chromosomes in total (22 different types of autosomes and one sex chromosome X or Y). As a result, each individual has two alleles for each trait, one from each parent that may or may not contain the same information. If the two alleles are identical the individual is called homozygous for the trait, while if the two alleles are different, the individual is called heterozygous for the trait. In heterozygous individuals the only allele that is expressed is the dominant, while the recessive allele is present but its expression is hidden.

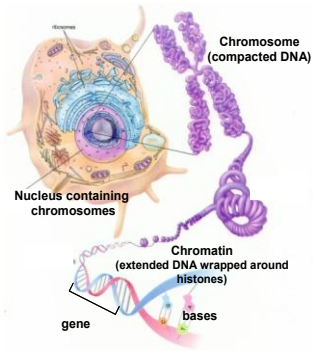

Figure 3. From DNA to chromosomes

1.2. DNA transfer across cells and organisms

1.2.1 DNA replication

DNA replication is one of the most important properties of DNA and the basis for biological inheritance allowing transfer of DNA to the cells of each organism and to the next generations. During DNA replication each strand of the original double-stranded DNA molecule serves as template for the reproduction of the complementary strand. At the end of the process two identical DNA molecules are produced from a single double-stranded DNA molecule, while near perfect fidelity for DNA replication is ensured by cellular proofreading and error-checking mechanisms (**Figure 4**). DNA replication occurs during the interphase of the cell cycle [the cell cycle can be divided into interphase (during which the cell grows, accumulates nutrients

needed for cell division and replicates its DNA), and the mitotic (M) phase, during which the cell divides] (**Figure 5**).

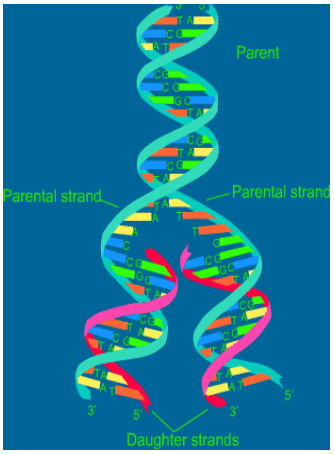

Figure 4. DNA replication

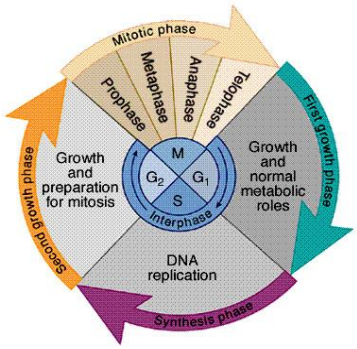

Figure 5. Cell cycle

Two types of cell division have been described in eukaryotes: mitosis and meiosis. In mitosis, one cell divides to produce two genetically-identical cells, that are able to divide again and in which both the DNA and the unique genetic code of the original cell are preserved. Meiosis on the other hand, which is required in sexual reproduction, is a process of reductional division during which the number of chromosomes per cell is cut in half. More precisely, during meiosis, one diploid cell undergoes recombination of each pair of parental chromosomes, and then two stages of cell division. As a result, four haploid cells (gametes) are generated, containing half the amount of DNA as the original cell, not able to divide again, and with genetic codes differing between each other and with the original (**Figure 6**).

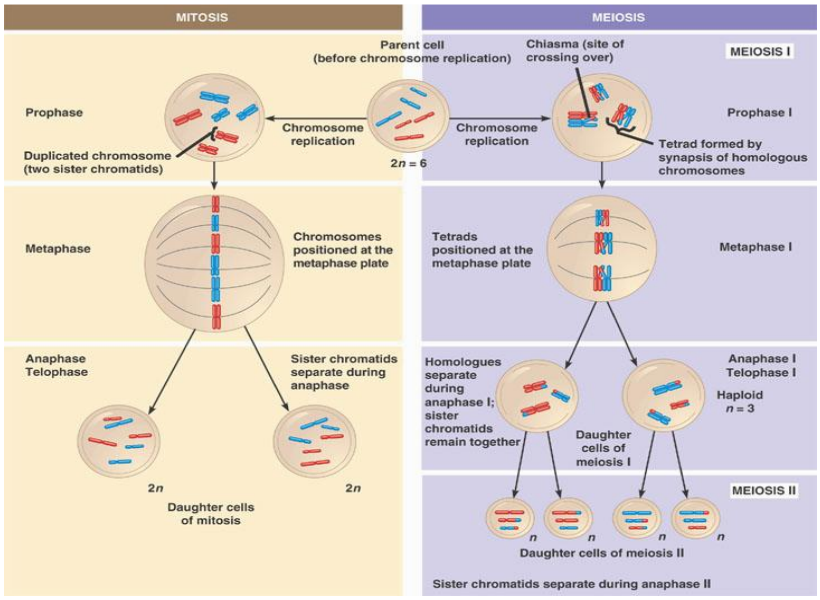

Figure 6. Mitosis and Meiosis

1.2.2. Human reproduction

While mitosis allows transfer of the parent DNA to the daughter cells, transfer of DNA to the next generations is achieved by reproduction. Reproduction is the biological process by which new individual organisms are produced. Sexual reproduction requires the involvement of two sexually mature individuals, typically one of each sex (male and female) able to produce gametes (sperm, oocyte). Reproduction starts by fertilization of the oocyte by the sperm resulting in the formation of the zygote. Both the oocyte nucleus and the sperm nucleus contribute equally to the genetic makeup of the zygote nucleus. During reproduction, a male can give either an X sperm or a Y sperm, while a female can only give an X oocyte.

Both males and females retain one of their mother's X chromosomes. In females the second X chromosome is inherited from their father. Since the father retains his X chromosome from his mother, a human female has one X chromosome from her paternal grandmother, and one X chromosome from her mother. In contrast, the Y chromosome is passed exclusively from father to son and as a result the information contained in the Y

chromosome is of particular interest. Mitochondrial DNA is also of particular interest, since it is inherited only from the mother.

Heredity of genes during reproduction follows the Mendel's Laws: a) Mendel's Law of Segregation: When gametes form, the paired alleles separate randomly so that each gamete receives a copy of one of the two alleles and b) Mendel's Law of Independent assortment: Two or more pairs of alleles segregate independently of one another during gamete formation.

1.2.3. Embryo development

During reproduction, males provide the sperm and females provide the oocyte and the necessary environment for the growth of the embryo and fetus. The fertilized egg divides inside the female's uterus to become an embryo (period starting at fertilization and ending at the end of the 10th week of gestation), which over a period of 38 weeks of gestation becomes a human fetus (period starting at end of 10th week of gestation and ending at birth). After a period of thirty-eight weeks, the fully-grown fetus is birthed and breathes independently as an infant for the first time (**Figure 7**). In most modern cultures the baby is recognized as a person entitled to the full protection of the law, while some jurisdictions extend personhood earlier to human fetuses and during their presence in the uterus.

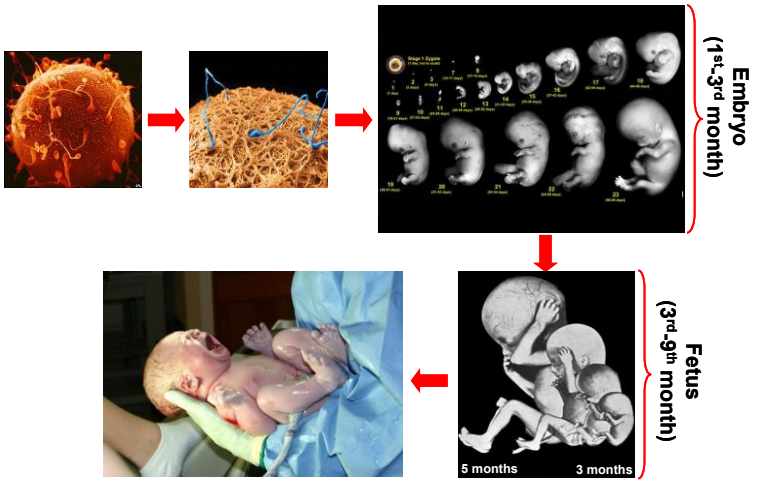

Figure 7. Human reproduction and embryo development

During the first trimester the developing embryo is susceptible to toxic exposures (alcohol, drugs, toxins, infections, radiation and nutritional deficiencies). In contrast to embryo which is sensitive to damage from environmental exposures, fetus is not as sensitive - since all the precursors of the organs are already formed - yet toxic exposures can often cause physiological abnormalities or minor congenital malformation. Interestingly, during pregnancy there is a continuous flow of cells and DNA between mother and fetus, which remain in their body for the rest of their lives (fetal and maternal microchimerism for mother and child respectively).

2. Determination of identity, sex and parenthood in humans

2.1. Human identification

The four main features of biological identity are sex, age, stature and ethnic background. In several cases there is need for human identification, such as in crime cases where there is need to link a suspect to a crime scene or victim, in cases where there is need to identify human remains, or in cases where paternity has to be identified.

DNA profiling (DNA typing or DNA fingerprinting) has been an extremely powerful tool in all the above cases allowing the identification of individuals on the basis of their respective DNA profiles, by usage of small amount of samples. Both nuclear and mitochondrial DNA can be used for DNA profiling. Nuclear DNA evidence can usually be recovered from several sources such buccal swab, blood, semen, saliva, skin cells, hair as well as from other appropriate fluid or tissue from personal items (e.g. toothbrush, razor, etc), stored samples (e.g. banked sperm or biopsy tissue), fetus after abortion or even from chorionic or coelomic cells during pregnancy (in cases of prenatal parental testing). In addition, mitochondrial DNA can be recovered from both bone and teeth dating back thousands of years. The DNA profile of the individual is then compared against another sample to determine whether there is a genetic match. Samples obtained from blood relatives (biological relative) can also provide an indication of an individual's profile, as could human remains which had been previously profiled.

DNA profiling is based on DNA polymorphism. Most precisely, polymorphisms that are highly similar between very closely related humans, but variable enough so that it is extremely unlikely for unrelated humans to have the same alleles, are used for DNA profiling such as i.e. highly variable repeat sequences (variable tandem repeats, VNTR), single nucleotide polymorphisms (SNP) and short tandem repeats (STR). Over the years a number of techniques have been developed and used for DNA profiling, such as restriction fragment length polymorphism (RFLP: digestion with a restriction enzyme, followed by southern blot analysis), polymerase chain reaction (PCR: amplification of a specific region of DNA, using oligonucleotide primers and a thermostable DNA polymerase, followed by electrophoresis), amplified length polymorphism (AmpFLP: uses PCR to amplify DNA samples), Y-chromosome analysis (targeting polymorphic regions on the Y-chromosome, Y-STR), and mitochondrial analysis (amplification of regions of the mtDNA, sequencing of each region and comparison of single nucleotide differences to a reference).

In addition to DNA profiling, other tools can also be used for human identification. In particular, in cases where human remains need to be identified, several characteristics of the skeletal remains such as measurements, dental characteristic and structural clues in the bones can also be used, in order to determine sex, age, ethnic background, and stature. Finally, in cases where the identity of the genetic parents is not certain, parental testing (maternity or paternity test) can be conducted by using several tools. More precisely, over the years several tools such as congenital traits, serotyping of blood cells [erythrocyte antigens and the highly polymorphic human leukocyte antigens (HLA)] and allotyping of proteins (red blood cell enzymes and serum protein polymorphisms) have been used and served as indicators of non-parenthood. DNA profiling though, which is based on the fact that the child inherits half of its DNA pattern from the mother and half from the father according to Mendel's laws of inheritance, can provide probability of paternity of 99.99% or higher corresponding to paternity "practically proven" and thus can indicate that the alleged father is the biological father.

2.2. Sex Determination in Humans and intersex

Sexual differentiation is the process of development of the differences between the two sexes (males and females) from an undifferentiated fertilized oocyte (zygote). As male and female individuals develop from zygotes into fetuses, infants, children, adolescents, and eventually into adults, sex and gender differences develop at the level of genes, chromosomes, gonads, hormones, anatomy, psyche, and social behavior.

In humans sex chromosomes ultimately define sexual identity. More precisely, sex is defined by the XY sex-determination system in which females have the same kind of sex chromosome (XX), while males have two distinct sex chromosomes (XY). In addition, male have a gene SRY (sex determining region Y) on the Y chromosome that determines maleness. Subsequent differentiation of the gonad along the female or male pathway is induced by specific genes. Gonadal development in turn, dictates the development of the secondary sex organs and leads to hormonal differences, which in turn cause anatomic differences, leading eventually to psychological and behavioral differences. Due to the lack of anatomic or hormonal differences between male and female human fetuses until approximately six weeks of pregnancy, fetuses are essentially indistinguishable until this stage and thus sex determination during this period is only possible by karyotype analysis (karyotype: characteristic chromosome complement of a eukaryote species) (**Figure 8**).

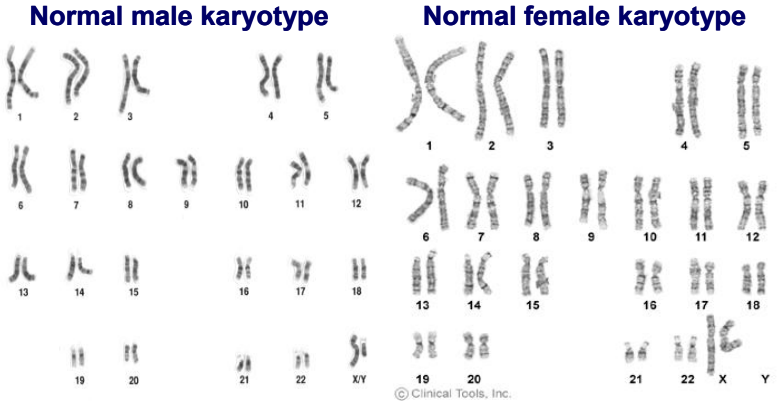

Figure 8. Normal male and female karyotypes

Sex differences are not always absolute in humans. Physical conditions in which the chromosomal sex is inconsistent with gonadal and phenotypic characteristics, or in which the phenotype can not be clearly classified as either male or female are defined as “intersex conditions”. In 2005, a consensus group introduced a new nomenclature, in which “intersex conditions” are subsumed under the umbrella term “disorders of sex development, (DSD)” and are now more broadly defined as congenital conditions in which development of chromosomal, gonadal, or anatomical sex is atypical. Intersex conditions may result from structural or numerical chromosomal defects, as well as from developmental defects (sex differentiation, hormonal, gonadal, or end-organ defects). Karyotype display of a tissue sample as well as body and genitalia inspection can be used in order to determine which of the causes of intersex is prevalent in each case.

Briefly, chromosomal defects can result in a non-traditional male genotype, male phenotype (47,XXY Klinefelter’s syndrome and mosaicism; 48,XXXY; 49,XXXXY; 50,XXXXXY; 48,XXYY; 47,XYY; 45,X/46,XY mosaicism) or in a non-traditional female genotype, female phenotype (45,X Turner Syndrome and variations; polysomy of X: 47,XXX, 48,XXXX or 49,XXXXX). Defects in sex determination can result in male genotype (XY), female phenotype [as a result of mutations of SRY, haploinsufficiency of SOX9 (SRY-box 9), duplication of the Xp21 region encompassing the NROB1 gene, large deletion upstream of NROB1 or duplication of the region of chromosome 1 encompassing the RSPO1 or WNT4 genes) or in female genotype (XX), male phenotype (due to translocations of SRY usually to the X chromosome during parental meiosis, activating mutations of SOX9 or loss of RSPO1). Finally, hormonal, gonadal, or end-organ defects can result in male genotype XY, female phenotype (absent or incomplete virilization, male pseudohermaphrodism due to defects in steroid hormone biosynthesis and signaling pathway, defects in the AMH signaling pathway, or due to a spectrum of X-linked disorders leading to androgen insensitivity syndrome) or in female genotype (XX), male phenotype (masculinization due to abnormally high levels of virilizing hormones leading to congenital adrenal hyperplasia).

2.3. Parenthood in the era of assisted procreation

2.3.1. Traditional definition of parenthood

A parent (mother or father) is one who sires or gives birth to and/or nurtures and raises an offspring. As a result, a parent can be defined by his/hers biological and/or his/hers social/legal relationship with the offsping. Biological (genetic) parent is a parent who is the biological (genetic) mother or father of an individual. More precisely, biological mother is the female who gestates her fertilized ovum in her uterus from conception until the fetus is sufficiently developed to be born, goes into labor, gives birth and produces milk during lactation to feed the child, while biological father is the male whose sperm fertilizes the ovum.

2.3.2. Assisted procreation

Assisted reproductive technology (ART) is a general term encompassing a range of methods devised to help infertile couples to become pregnant and to provide the opportunity for singles and same-sex couples to have children outside the confines of heterosexual intercourse. Several methods have been used to achieve pregnancy by artificial or partially artificial means (assisted procreation) such as: a) fertility medications that stimulate the development of follicles in the ovary, b) artificial insemination (AI) in which case sperm is placed into a female's uterus or cervix using artificial means rather than by natural copulation and c) in vitro fertilization (IVF) during which oocytes are fertilized by sperm outside of the womb, in vitro.

Since the birth of the first so-called “test tube baby” in 1978, IVF has become the most widely used ART, with over one million children born worldwide through the assistance of IVF. The traditional IVF scenario, in which a husband and a wife supply their own sperm and oocytes, involves hormonal control of the ovulatory process, removal of ova (oocytes) from the wife’s ovaries, fertilization of oocytes by the husband’s sperm in a fluid medium and transfer of the fertilized oocyte (zygote) to the wife’s uterus with the intent to establish a successful pregnancy. The IVF process requires sperm, oocytes and uterus: any of these requirements can be provided by a third person in order to achieve pregnancy (third party reproduction). As a result, IVF has evolved in several ways: sperm and oocytes may be supplied by third-party donors or vendors, fertilized embryos may be screened based on genetic testing before implantation, and may be carried to term by a woman other than the biological mother, surplus embryos may be frozen and stored for later use, donated to other couples, or used for biomedical research ranging from stem cell cultivation to cloning. Several techniques are involved in the IVF process such as cryopreservation of gametes, reproductive tissue (i.e. ovarian tissue) or embryos, gamete donation, surrogacy and preimplantation genetic diagnosis/preimplantation genetic haplotyping (PGD/PGH, performed on embryos prior to the embryo transfer).

Regarding gamete donation, a man or a woman can provide semen or oocytes respectively, for purposes of assisted reproduction or biomedical research. In the case of sperm donation, the donor can provide its semen directly to the recipient or at a sperm bank, can be anonymous or not, does not have sexual relations with the recipient of the sperm, and is the biological parent of each offspring produced by his sperm. Sperm donation assists couples unable to produce children because of 'male' fertility problems, single women and lesbians. In the case of oocyte donation, oocytes are retrieved from a donor's ovaries, fertilized in the laboratory with the sperm from the recipient's partner, and the resulting healthy embryos are returned to the recipient's uterus. Oocyte donation is a resource for women with no oocytes, poor oocyte quality, previously unsuccessful IVF cycles or advanced maternal age. Surrogacy on the other hand, is a method of reproduction whereby a woman agrees to become pregnant and deliver a child for a contracted party. The surrogate mother may be the child's genetic mother, or she may, as a gestational carrier, carry the pregnancy to delivery after having been implanted with an embryo. The option of gestational carrier is used when a patient's medical condition prevents a safe pregnancy, when a patient has ovaries but no uterus, and where a patient has no ovaries and is also unable to carry a pregnancy to full term.

Several variations/improvements of IVF have been developed such as: a) the Intracytoplasmic Sperm Injection (ICSI) which is applied when the sperm is defective and has difficulties penetrating the oocyte or when sperm numbers are very low and during which the sperm (sometimes directly collected from epididymis or testes in men with no sperm in their semen) is directly injected into the oocyte, b) the Zygote Intrafallopian Transfer (ZIFT) during which the oocytes are removed from the woman, fertilized and then placed in the woman's fallopian tubes rather than the uterus, and c) the Gamete Intra-Fallopian Transfer (GIFT) during which the oocytes are removed from the woman, and placed in one of the fallopian tubes, along with the man's sperm, allowing fertilization to take place inside the woman's body (in vivo and not in vitro fertilization).

There is no uniform regulation of IVF and research. Britain is a pioneer in IVF research and regulation, with nationally uniform and enforceable guidelines applicable to research and ART developed at early stages of IVF. In addition, a body has been developed [Human Fertilization and Embryology Authority (HFEA)] with flexibility to make decisions on a case-by-case basis, while an early development of databases of donors and recipients of IVF allows follow up of ongoing health of these children and the success of IVF. Finally, a bill has been recently implemented which bans most cases of sex selection but gives legal sanction to: a) mixed animal-human embryos: insertion of human DNA in an animal egg in order to address the shortage of human eggs for research and investigation of serious diseases, but with embryos not allowed to develop for longer than 14 days, b) preimplantation genetic diagnosis for health reasons and c) saviour siblings: selection of an embryo that would, if it became a baby, provide a sick older sibling with matching tissue. In Australia there has been an early regulation of reproductive technologies, while in Israel and Belgium where embryology research is very advanced, there are disjointed laws on fertility treatments (reproductive tourism in Belgium, unlimited IVF attempts in Israel). In Canada and China implementation of permissive national guidelines has just started, while in Italy embryo freezing and research are banned while IVF is restricted to heterosexual couples. In Greece, legal provisions established recently build the respective legal frame on assisted procreation. Finally in USA, ART is nearly an unregulated black market with no databases of donors and treatments, no uniform standards, no control on the sale and advertising of gametes and lack of any regulation of sex or traits selection. In addition, researchers are hampered by strict laws, inability to use federal funds to create new embryonic stem-cell lines and lack of nationwide prohibitions on keeping embryos for more than 14 days.

2.3.3. Modern definition of parenthood

In most cases the same person is both he biological parent and the person who raises the offspring social/legal parent: adoptive parent, foster parent or stepparent). In modern families, where parenthood can be often achieved via traditional reproduction, adoption, or assisted procreation, the difference among the genetic parents, the person who gave birth and the social/legal parent is becoming increasingly frequent. More precisely, in modern era the definition of parenthood is becoming more complex, since there can be an explicit difference among the person from whom an individual inherited half of their gene (genetic or biological parent), the person who gave birth, the person who nurture and the person who raised the offspring. As a result, in the modern era the function of biological motherhood can be split between the genetic mother (who provides the ovum) and the gestational mother (who carries the pregnancy), and in theory neither might be the social mother (the one who brings up the child). On the other hand, the function of biological fatherhood can be split between the genetic father (who provides the sperm) and the social father (the one who brings up the child).

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