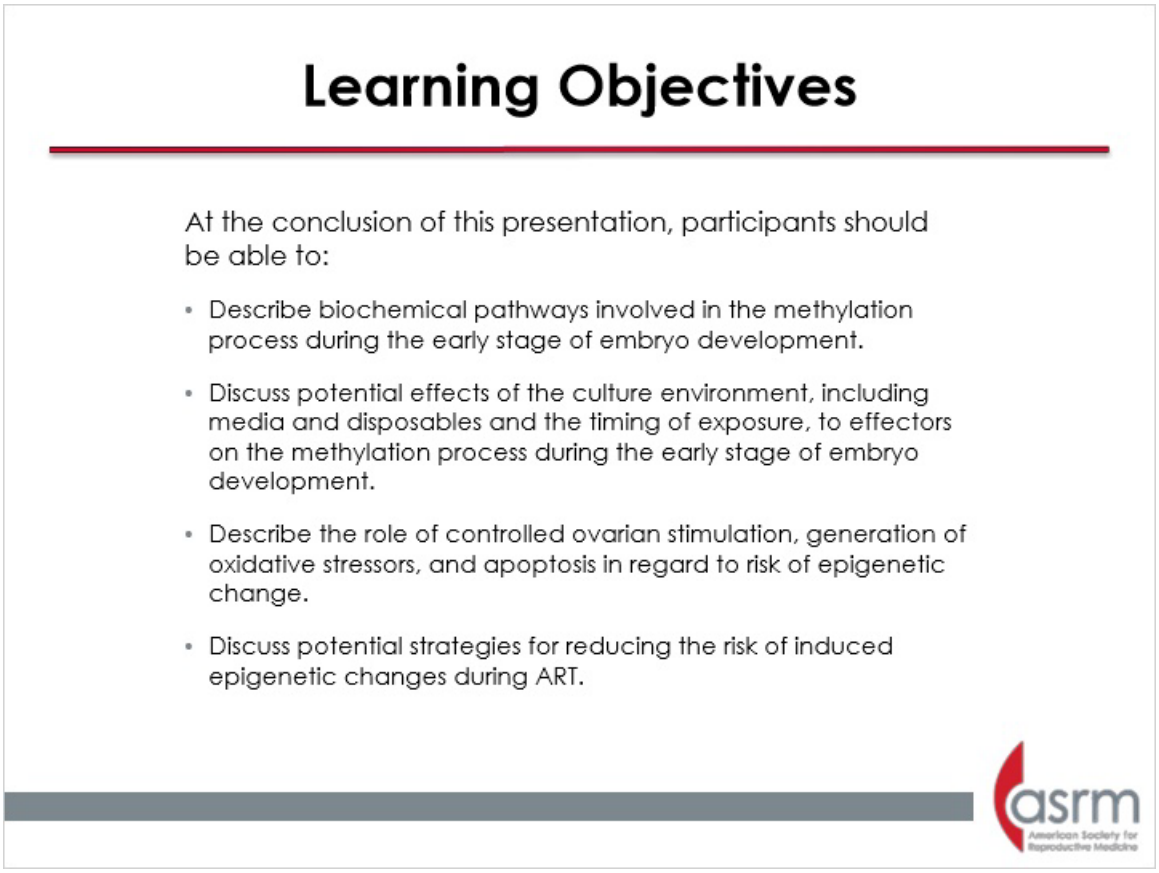
LABCC100 Lesson 10

1.1 Epigenetics:

Notes:

Welcome to the American Society for Reproductive Medicine's eLearning modules. This subject of this presentation is Epigenetics: Imprinting in the ART Environment.

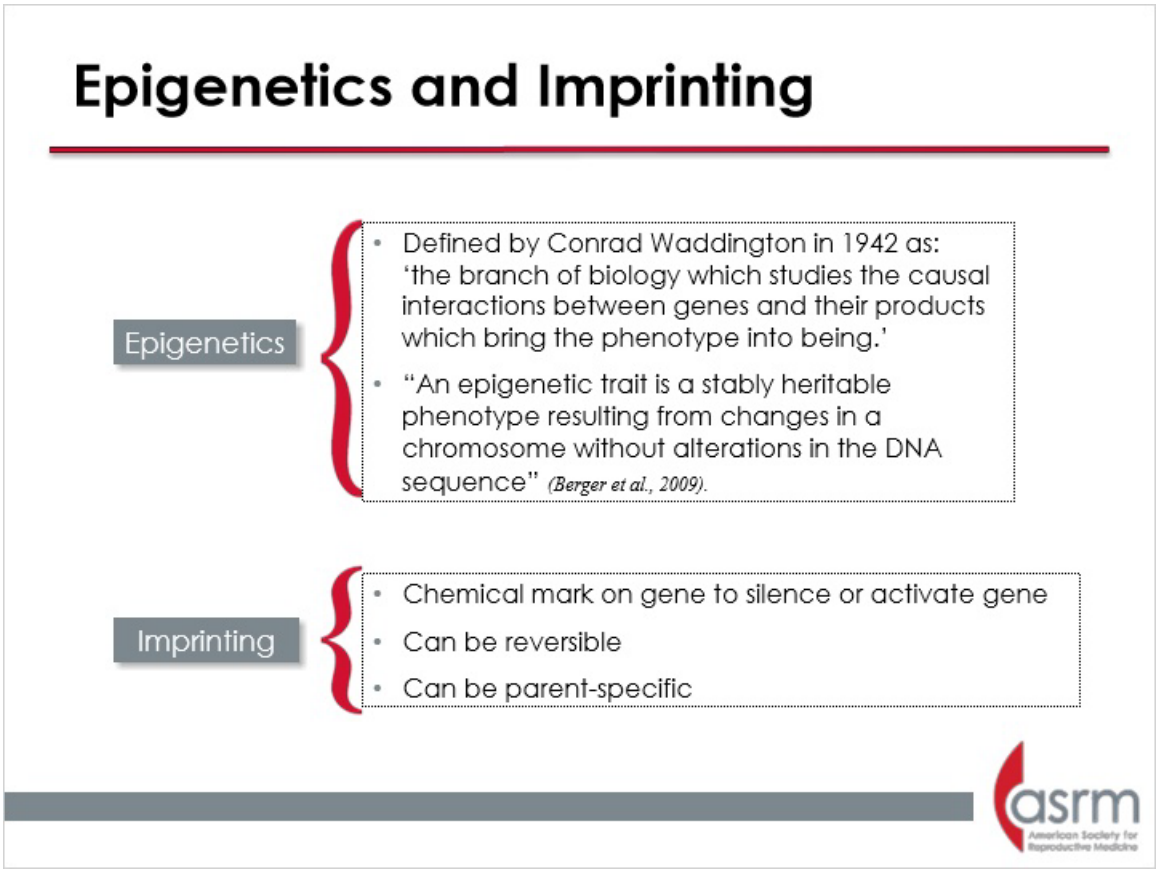
1.2 Learning Objectives



Notes:

At the conclusion of this presentation, participants should be able to:
Describe biochemical pathways involved in the methylation process during the early stage of embryo development.
Discuss potential effects of the laboratory culture environment, including media and disposables and the timing of exposure, to effectors on the methylation process during the early stage of embryo development.
Describe the role of controlled ovarian stimulation, generation of oxidative stressors, and apoptosis in regard to risk of epigenetic change.
Discuss potential strategies for reducing the risk of induced epigenetic changes during ART.

1.3 Epigenetics and Imprinting

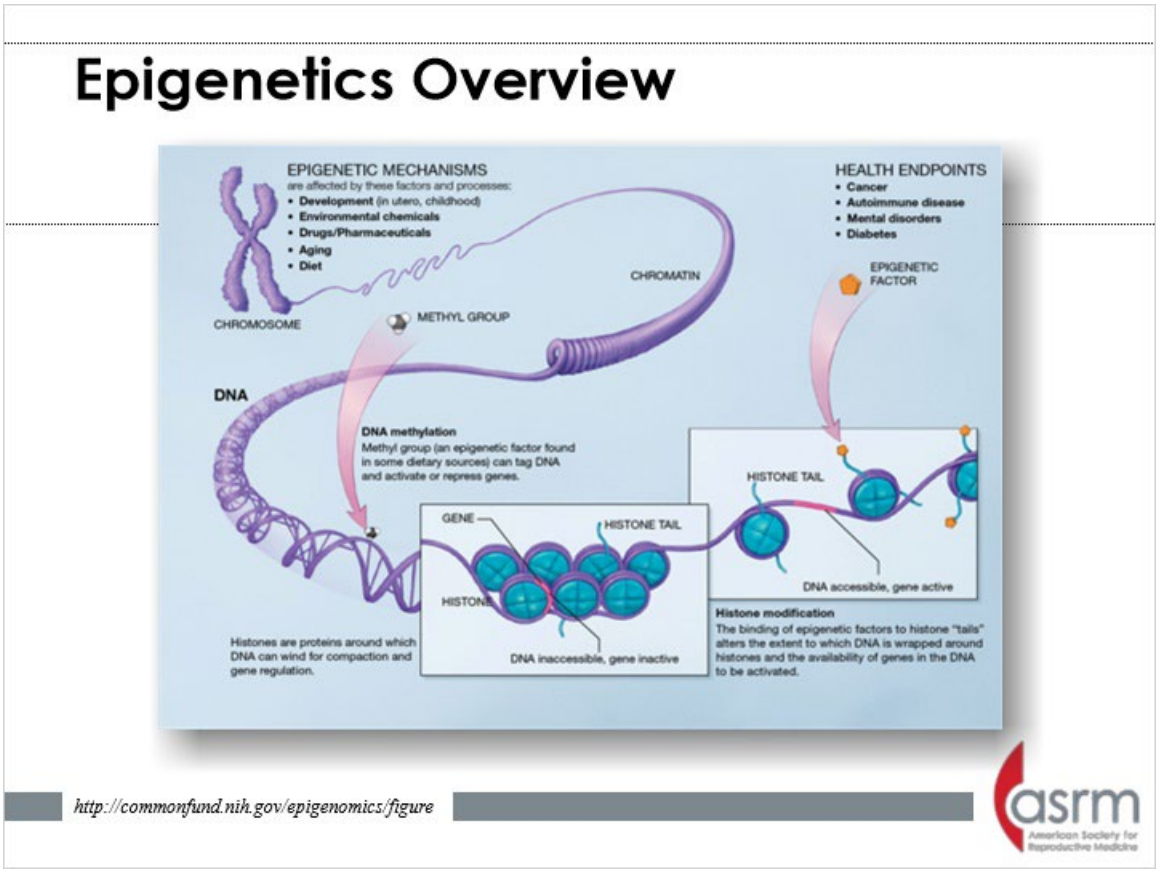


Notes:

If all of our cells have the same DNA, then why do the cells behave differently? What makes a neuron different from a muscle cell if both have the same DNA? This is the science of epigenetics. In 1942, developmental biologist Conrad Waddington defined epigenetics as ‘‘the branch of biology which studies the causal interactions between genes and their products which bring the phenotype into being.” More recently, the term ‘epigenetic trait’ was defined as “a stably heritable phenotype resulting from changes in a chromosome without alterations in the DNA sequence.” In other words, epigenetics is the study of modifications made to gene expression that occurs without changes in the DNA code. It is the set of instructions to genes to subsequently turn on or turn off gene expression, like a light switch.

Imprinting is the actual chemical mark or ‘stamp’ on the gene that designates those instructions to either silence or activate the gene. Imprinting can be reversible. Imprinting can also be specifically from the father or the mother as this module will later discuss.

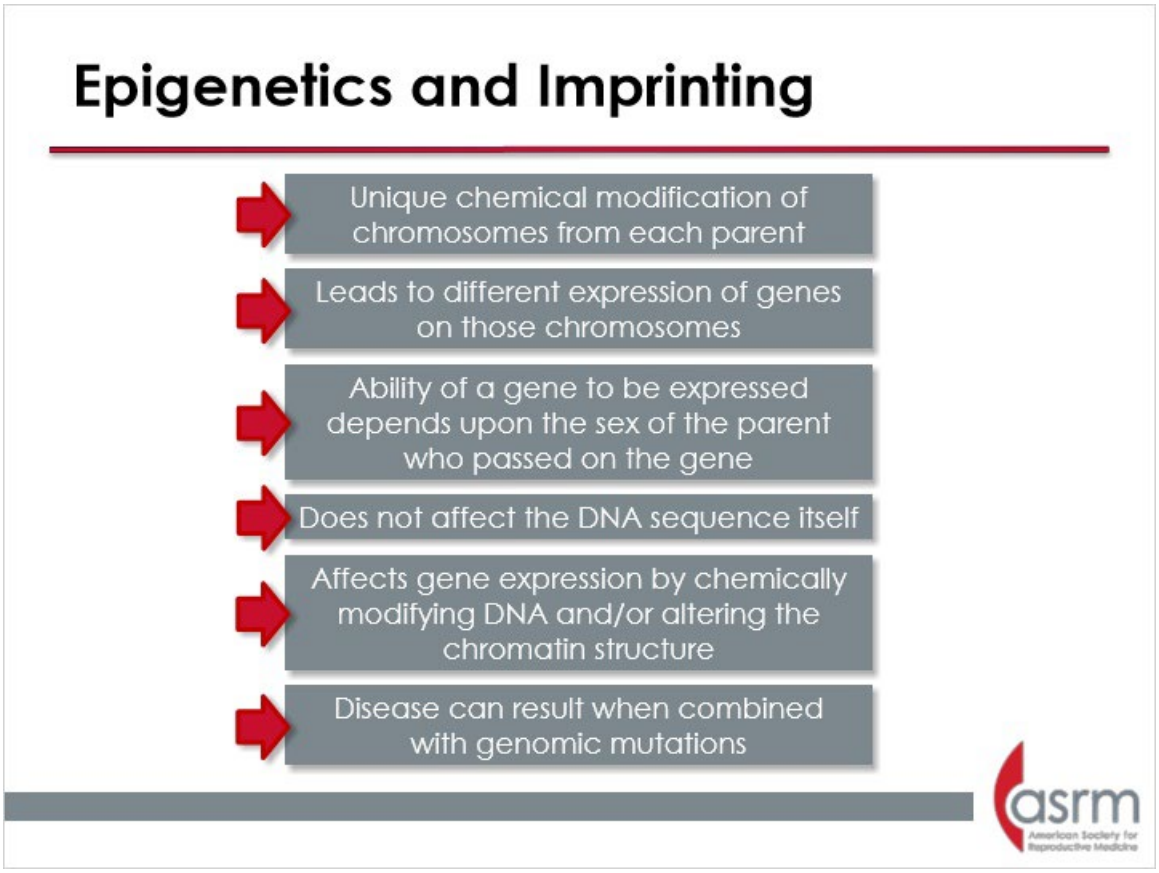
1.4 Epigenetics Overview



Notes:

Epigenetics is the study of heritable changes in gene expression caused by the activation and deactivation of genes without any change in the underlying DNA sequence of the organism. Epigenetic mechanisms are affected by several factors and processes including developmental changes in utero and in childhood, environmental chemicals, drugs and pharmaceuticals, aging, and diet. These mechanisms occur through DNA methylation, a process in which methyl groups tag DNA and influence gene expression, and histone modification. Histones are proteins around which DNA can wind for compaction and gene regulation. Histone modification occurs when the binding of epigenetic factors to histone "tails" alters the extent to which DNA is wrapped around histones and the availability of genes in the DNA to be activated. All of these factors and processes can influence risk of a number of conditions including cancer, autoimmune disease, mental disorders, and diabetes.

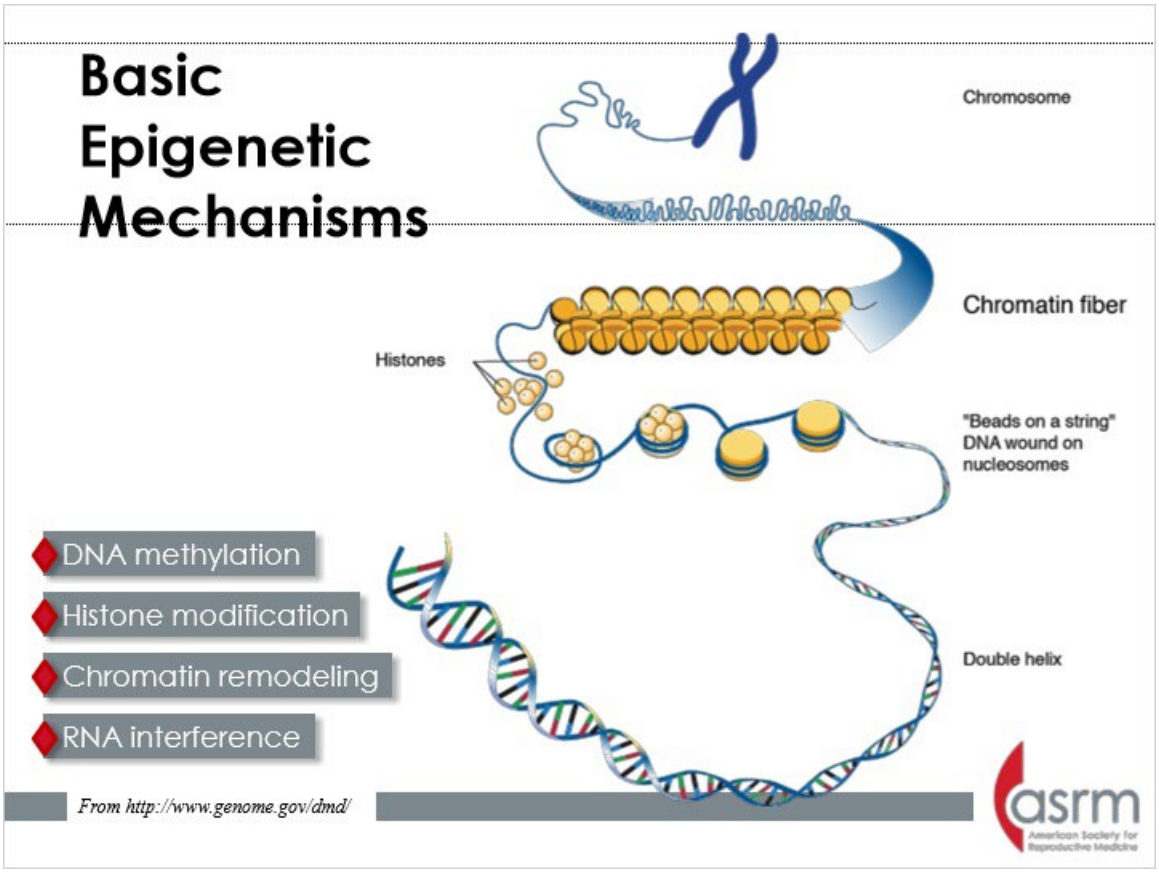
1.5 Epigenetics and Imprinting



Notes:

Imprinting is a process by which chromosomes derived from each parent are uniquely chemically modified. This modification leads to different expression of a gene or genes on those chromosomes depending on their parental origin. In genomic imprinting the ability of a gene to be expressed depends upon the sex of the parent who passed on the gene. In some cases imprinted genes are expressed when they are inherited from the mother. In other cases they are expressed when inherited from the father. Unlike genomic mutations that can affect the ability of inherited genes to be expressed, genomic imprinting does not affect the DNA sequence itself. Genomic imprinting affects gene expression by chemically modifying DNA and/or altering the chromatin structure. Often, genomic imprinting results in a gene being expressed only in the chromosome inherited from one or the other parent. While this is a normal process, when combined with genomic mutations, disease can result.

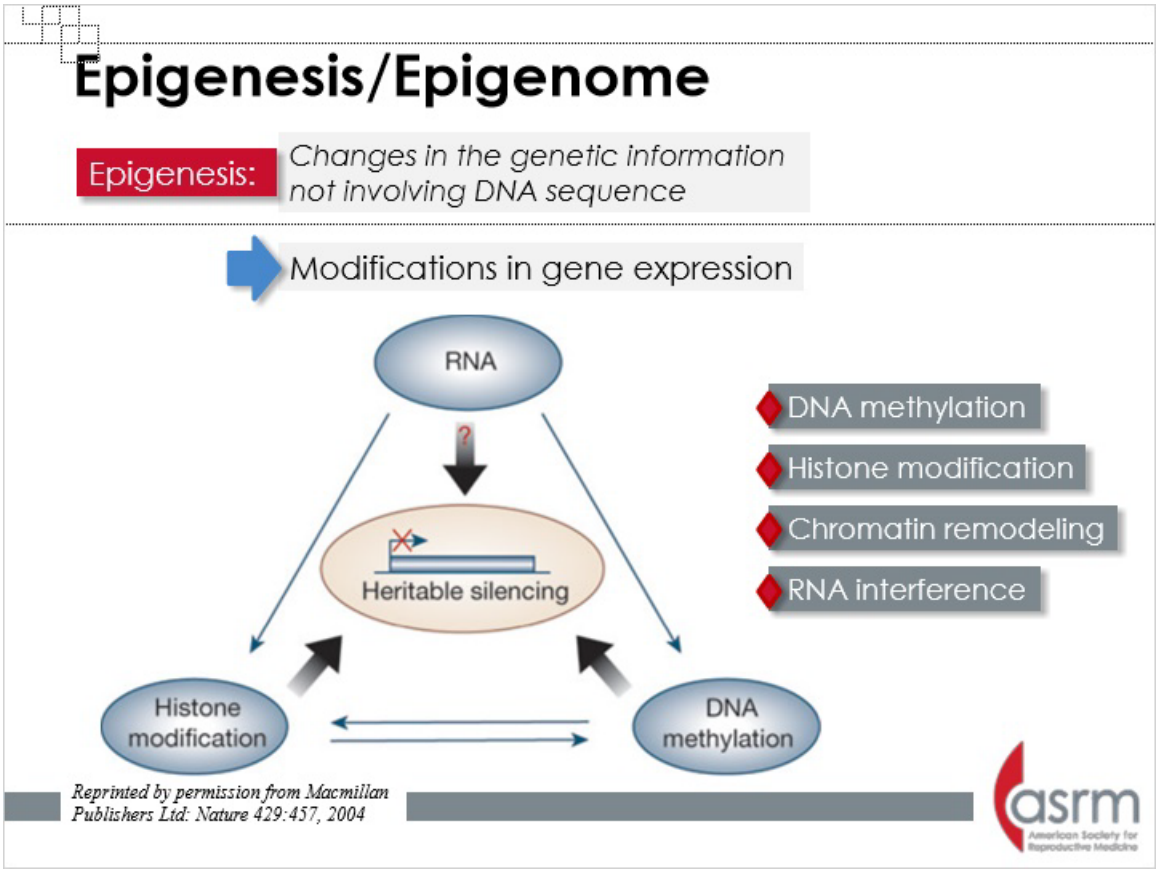
1.6 Basic Epigenetic Mechanisms



Notes:

The basic epigenetic mechanisms of imprinting are DNA methylation, histone modification, chromatin remodeling, and RNA interference.
Chromatin is a substance within a chromosome consisting of DNA and protein. The DNA carries the cell's genetic instructions. The major proteins in chromatin are histones, which help package the DNA in a compact form that fits in the cell nucleus. Changes in chromatin structure are associated with DNA replication and gene expression. Methylation is a chemical reaction that attaches small molecules called methyl groups to certain segments of DNA. In genes that undergo genomic imprinting, methylation is one way that a gene's parent of origin is marked during the formation of egg and sperm cells. Histone is a basic protein found in nucleosomes. The structure of chromatin can be modified by attachment of acetyl groups to core histones. Some small RNAs have been found to be involved in regulating gene expression.

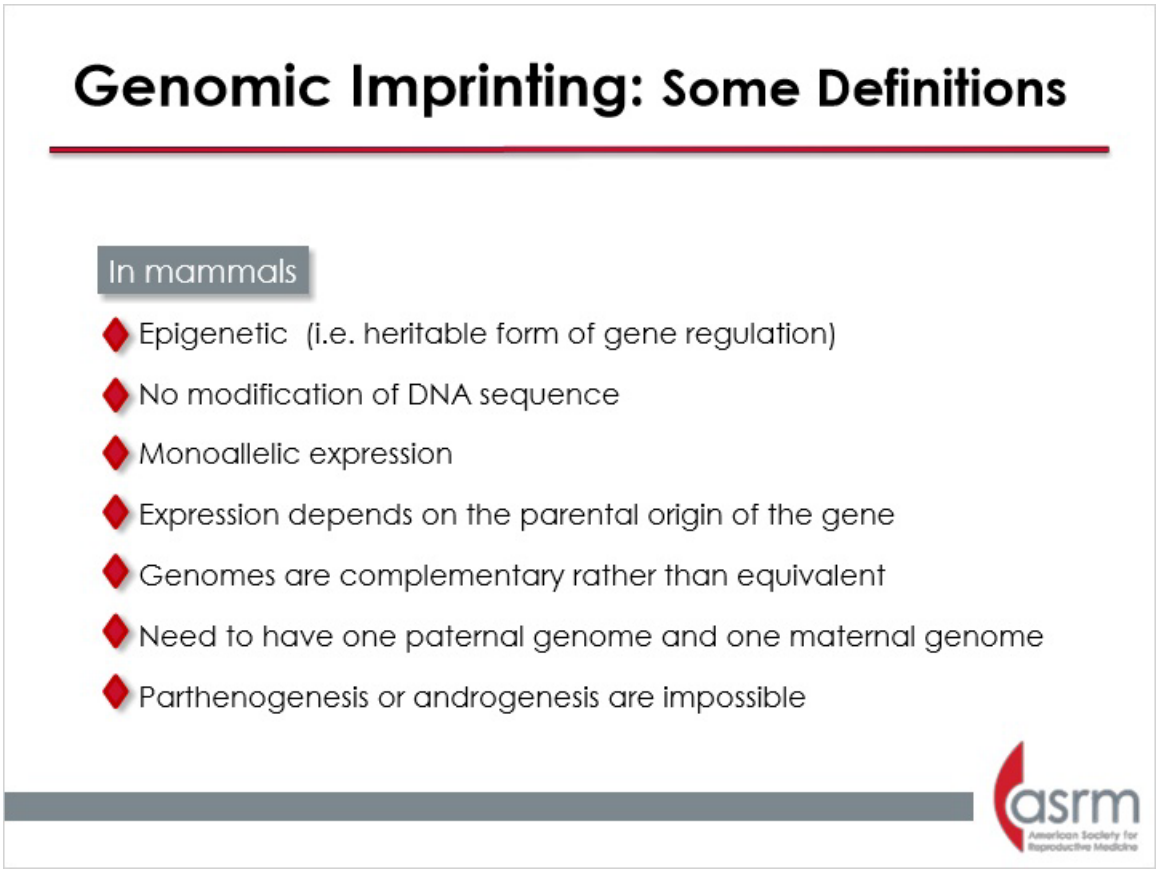
1.7 Epigenesis/Epigenome



Notes:

This diagram illustrates the changes in the epigenome leading to modifications in gene expression. The epigenome consists of chemical compounds that modify, or mark, the genome in a way that tells it what to do, where to do it, and when to do it. Different cells have different epigenetic marks. These epigenetic marks, which are not part of the DNA itself, can be passed on from cell to cell as cells divide, and from one generation to the next. Changes in histone through deacetylation and other modifications cause chromatin condensation and block the initiation of transcription. These histone changes can also attract DNA methyltransferases to start cytosine methylation. These in turn can reinforce histone modification patterns conducive to silencing. Experiments in yeast and plants have clearly shown the involvement of RNA interference in the establishment of heterochromatic states and silencing. RNA triggering of heritable quiescence might therefore also be involved in higher organisms.

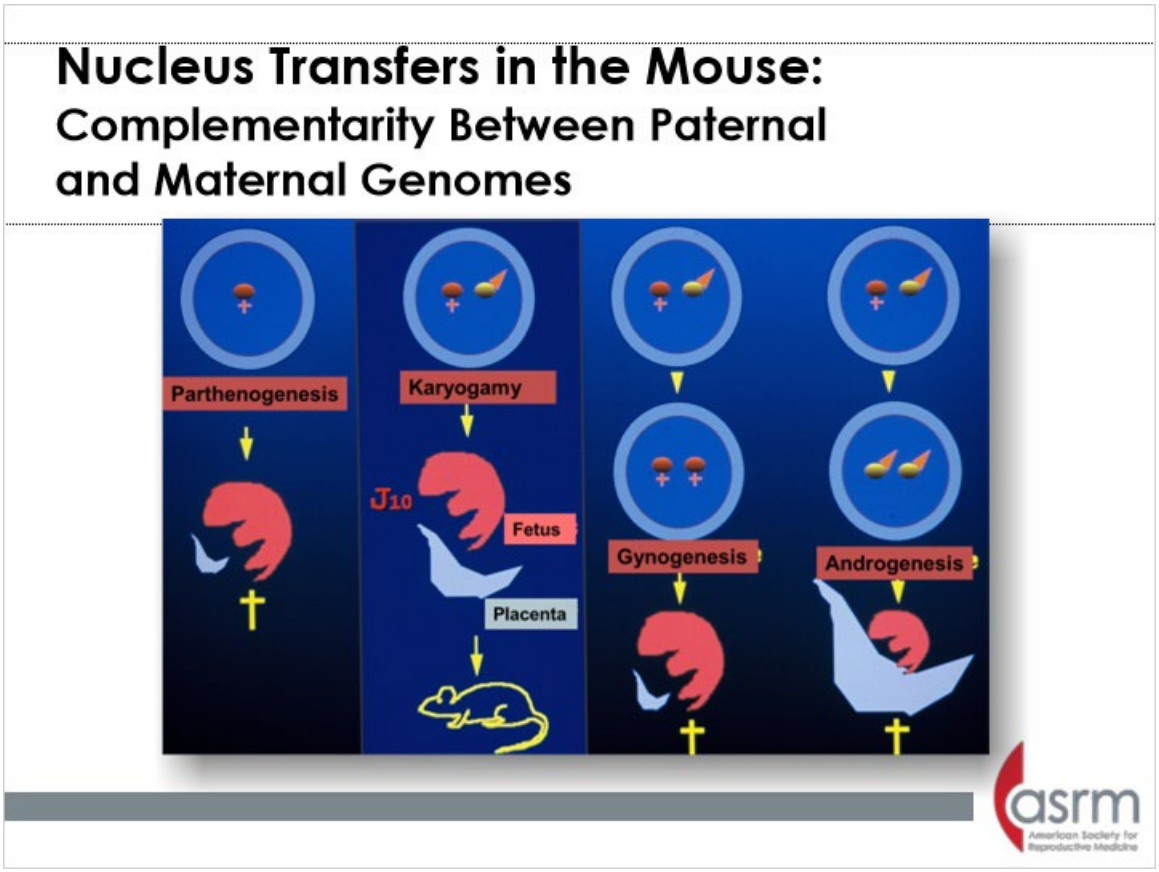
1.8 Genomic Imprinting: Some Definitions



Notes:

Genomic imprinting in mammals involves epigenetic changes such as a heritable form of gene regulation. There is no modification of DNA sequence, there is monoallelic expression, and expression depends on the parental origin of the gene. Genomes are complementary rather than equivalent. It is necessary to have one paternal genome and one maternal genome and parthenogenesis or androgenesis are impossible.

1.9 Nucleus Transfers in the Mouse: Complementarity Between Paternal and Maternal Genomes



Notes:

This chart illustrates the complementarity of the genome using a mouse model. At the pronucleus stage, the size difference between male and female pronucleus is easily seen. A pronucleus can be removed, and then the remaining DNA can be duplicated. In this way you can have the following:

Androgenotes: union of two paternal pronuclei to form the embryo

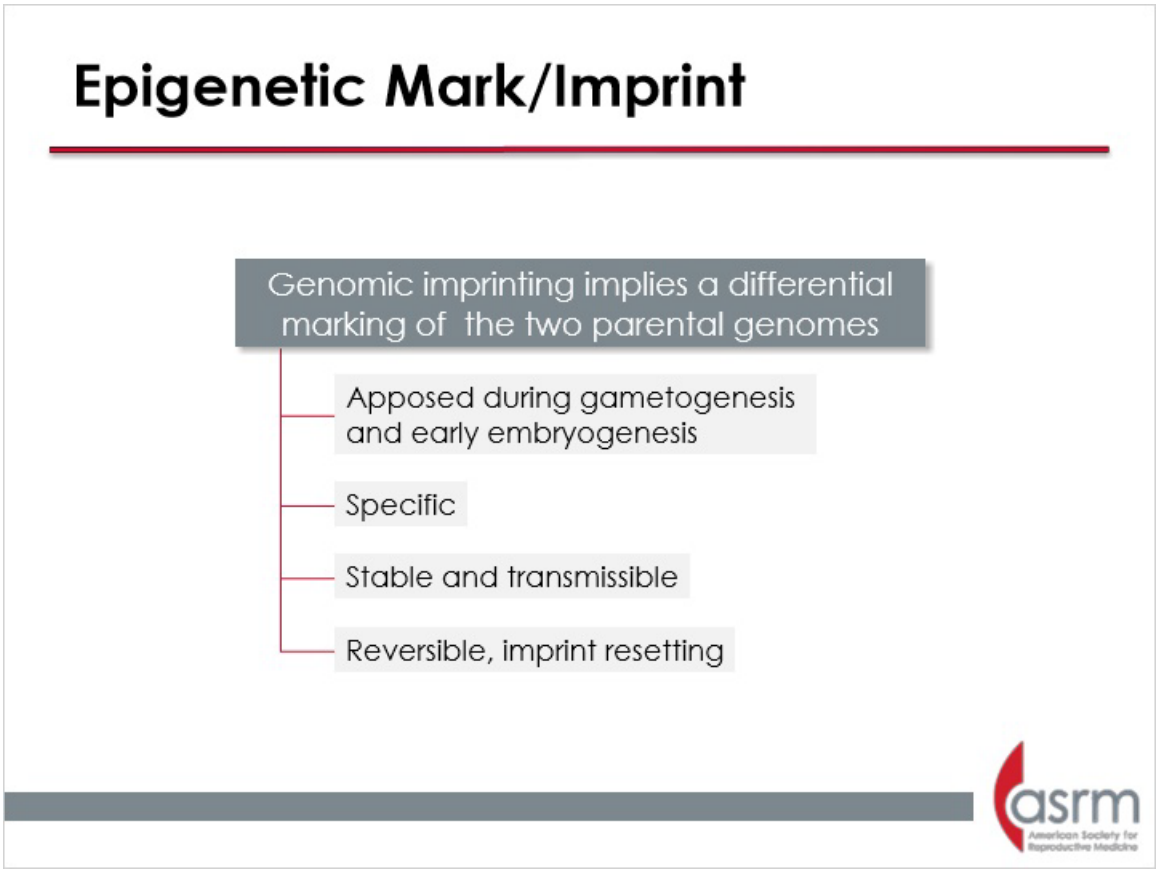
Parthenogenotes: union of two maternal pronuclei to form the embryo

Androgenotes lead to hypertrophy of the placenta and death.

Parthenogenotes/gynogenotes lead to hypotrophy of the placenta and extended growth of the embryo.

Parthenogenotes and androgenotes can implant but they do not lead to living pups. Thus there is a need for paternal and maternal genomes.

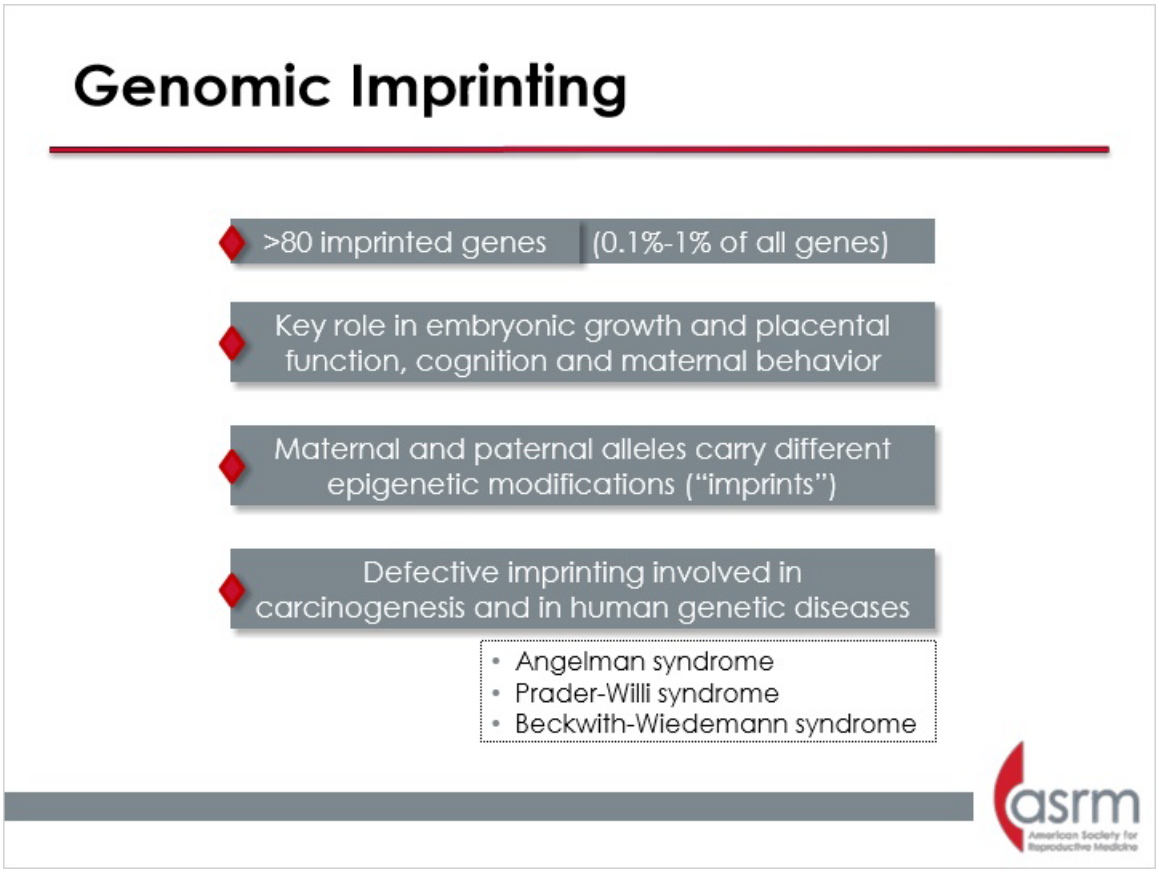
1.10 Epigenetic Mark/Imprint



Notes:

Imprint/epigenetic marks have specificity and distinct properties. They are apposed during gametogenesis and early embryogenesis; they are specific, stable and transmissible, and reversible with imprint resetting.

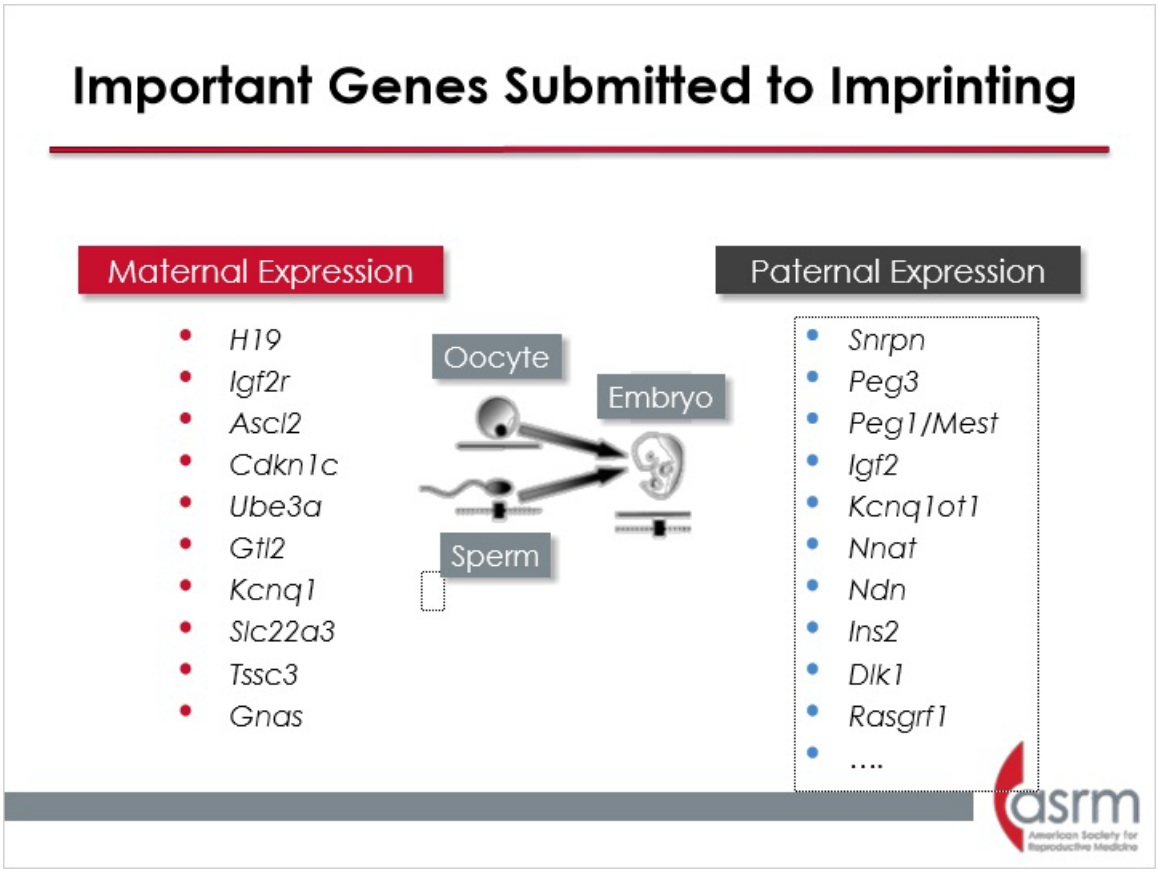
1.11 Genomic Imprinting



Notes:

There are more than 80 imprinted genes (0.1%-1% of all genes). Genomic imprinting plays a key role in embryonic growth and placental function, cognition, and maternal behavior. Maternal and paternal alleles carry different epigenetic modifications ("imprints"). Defective imprinting is involved in carcinogenesis and in human genetic diseases. For example, Prader-Willi syndrome and Angelman syndrome are two distinct diseases caused by a deletion in the same part of chromosome 15. When this deletion occurs on the chromosome 15 that came from the father, the child will have Prader-Willi syndrome. However, when the deletion occurs on the chromosome 15 that came from the mother, the child will develop Angelman syndrome. This occurs because genes located in this region undergo genomic imprinting. Abnormalities involving genes on chromosome 11 that undergo genomic imprinting are responsible for most cases of Beckwith-Wiedemann syndrome.

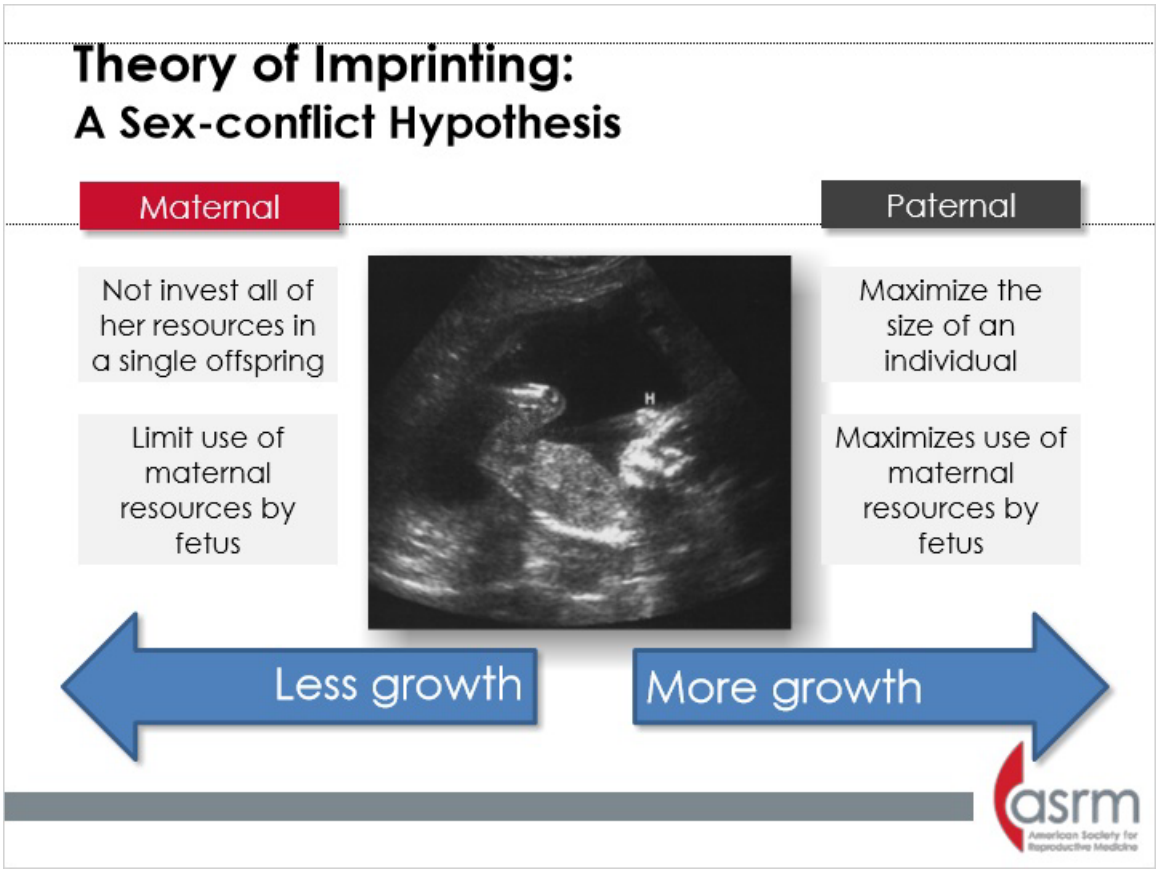
1.12 Important Genes Submitted to Imprinting



Notes:

Both male and female genes are subject to imprinting.

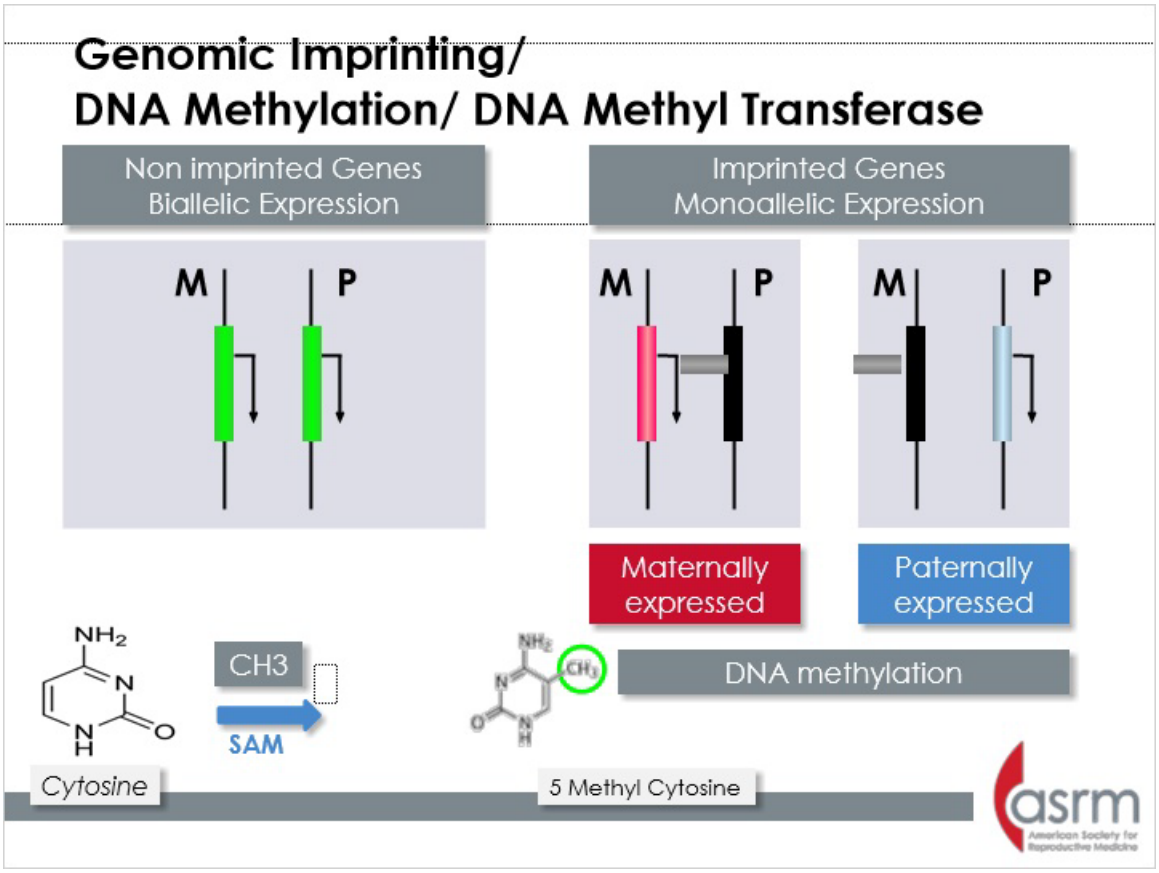
1.13 Theory of Imprinting:



Notes:

One philosophical and physiological theory for imprinting is the parental- or sex-conflict hypothesis. This theory holds that the variances in parental genomes with imprinting result from the different interests for each parent. The female does not have to invest all of her resources in a single offspring and must spare maternal reserves for further pregnancies. Thus, maternally expressed genes tend to be growth limiting. This is not the case for the male whose paternally expressed genes tend to be growth promoting. However, much of the control over imprinting of genes in the zygote is by the maternal genome during pregnancy. This makes the theory of paternal-derived gene dominance difficult to explain.

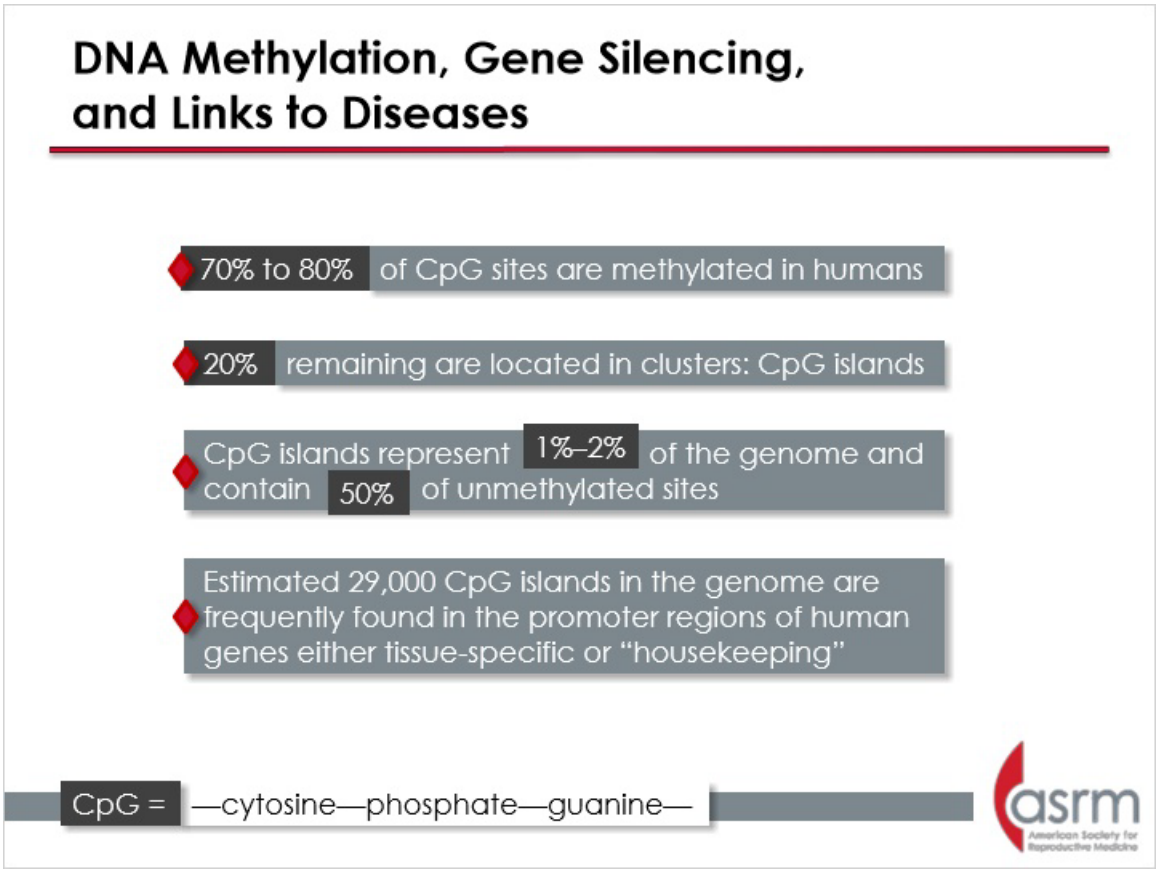
1.14 Genomic Imprinting/



Notes:

Methylation on cytosine forms the biochemical basis of imprinting marks. Essentially, when methylation is set, the gene is no longer expressed. These mechanisms will be explained later. The differential methylation between the paternal and maternal gene will lead to a difference in expression. Usually, methylation prevents binding of the transcription factors to the promoter and thus shuts down the expression of the gene.
Silencing through methylation of DNA violates the usual rule of inheritance that both alleles are equally expressed.

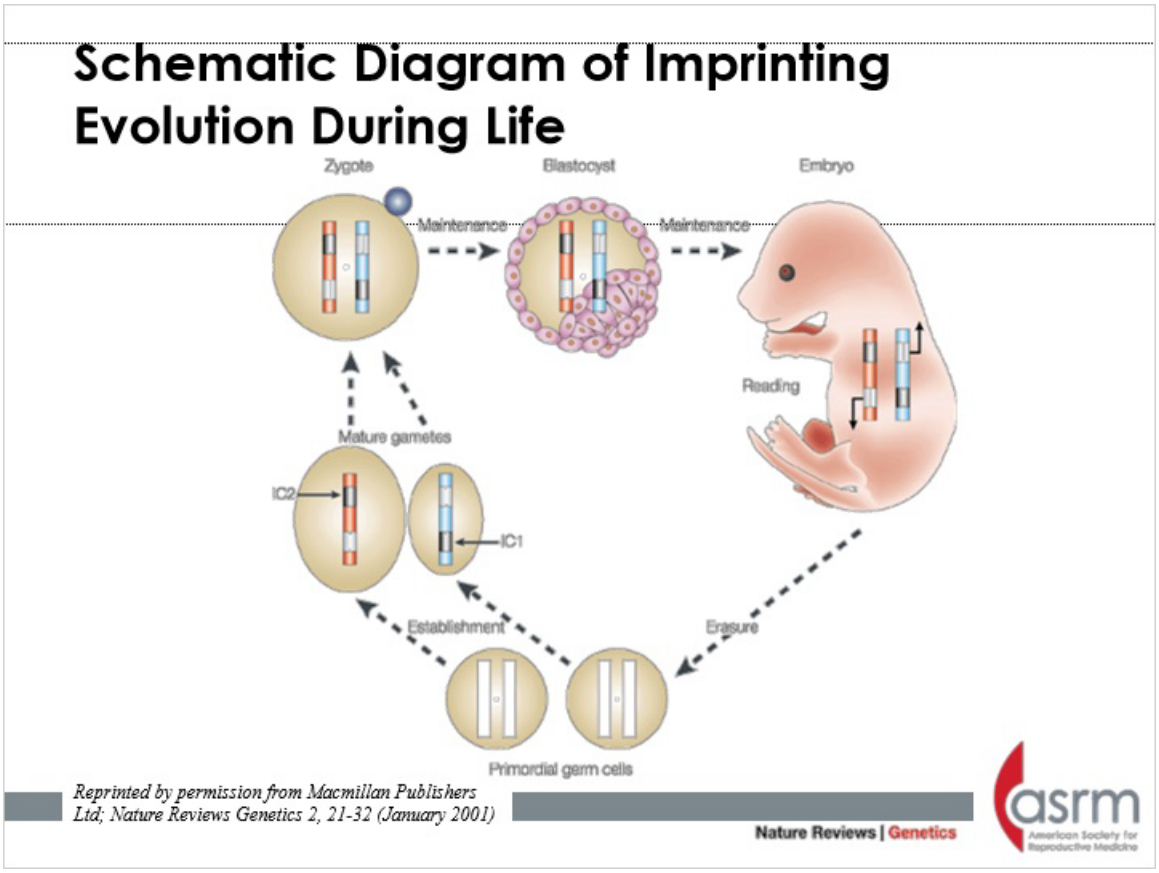
1.15 DNA Methylation, Gene Silencing,



Notes:

It is important to understand where methylation occurs. Between 70% and 80% of cytosine—phosphate—guanine (CpG) sites are methylated in humans. The 20% remaining are located in clusters called CpG islands. These CpG islands represent 1% to 2% of the genome and contain 50% of the unmethylated sites. The estimated 29,000 CpG islands in the genome are frequently found in the promoter regions of human genes and are either tissue specific or "housekeeping."

1.16 Schematic Diagram of Imprinting Evolution During Life



Notes:

Imprinting evolution occurs across the lifespan. The establishment of imprinting begins in the gametes. Imprints are maintained at fertilization and immediately after. A few de novo methylations are likely. Then there is a decrease in methylation (erasure for totipotency of embryonic cells) followed by the first differentiation into the inner cell mass and trophoblast. Then, the global genome is remethylated.

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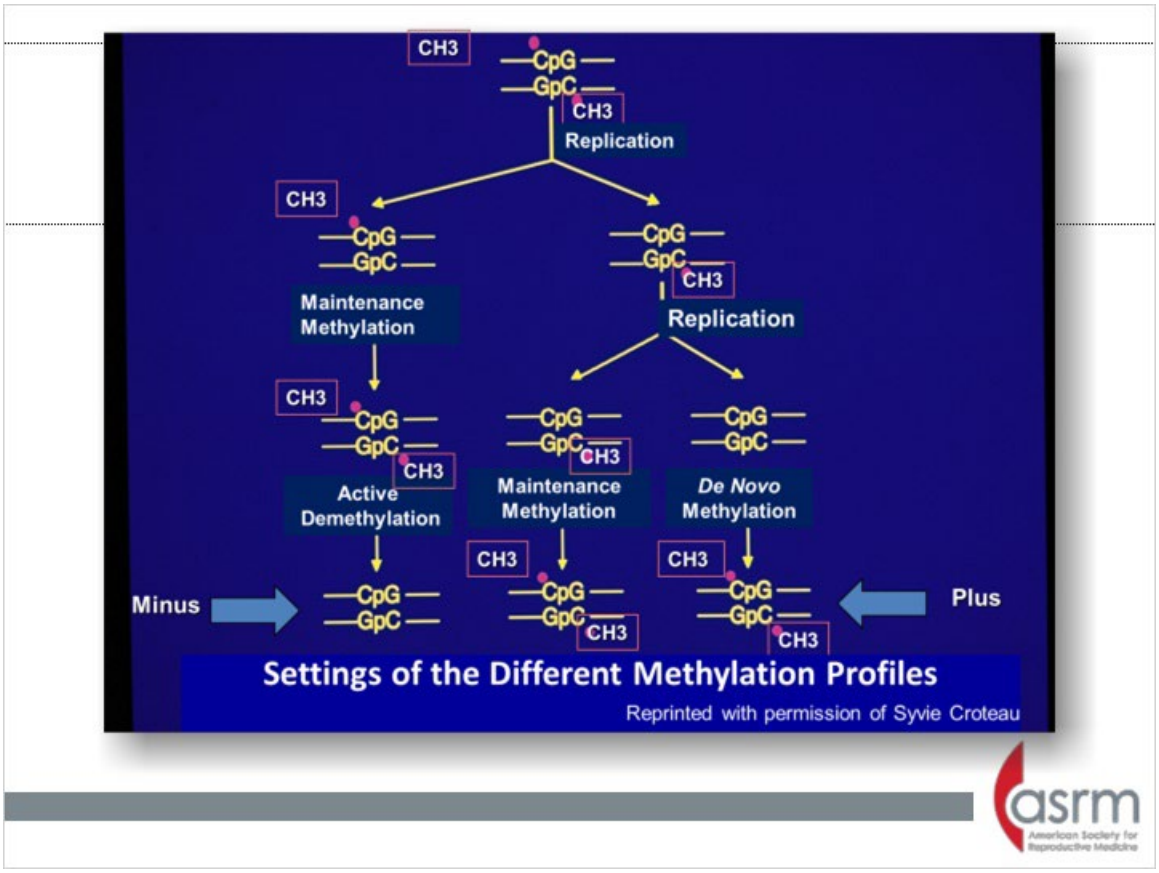
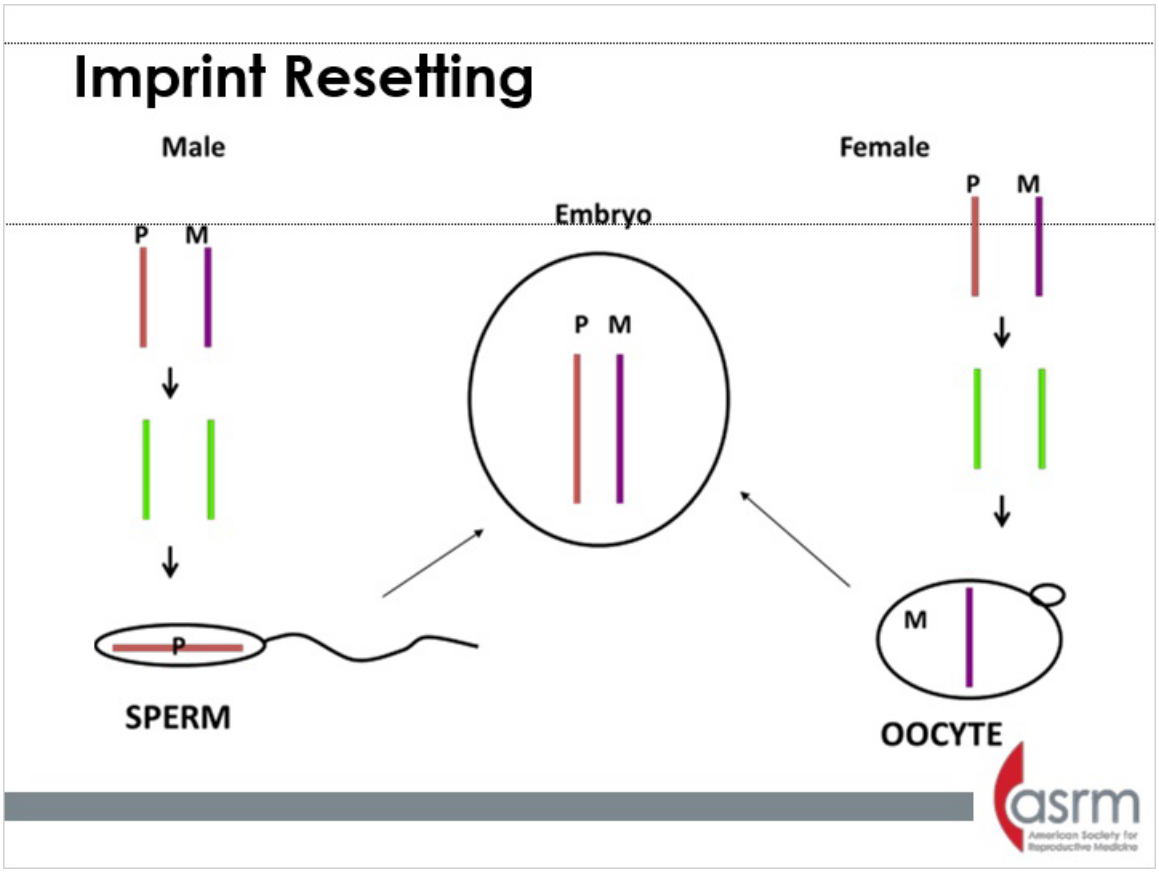


Notes:

The different methylation processes are shown here.

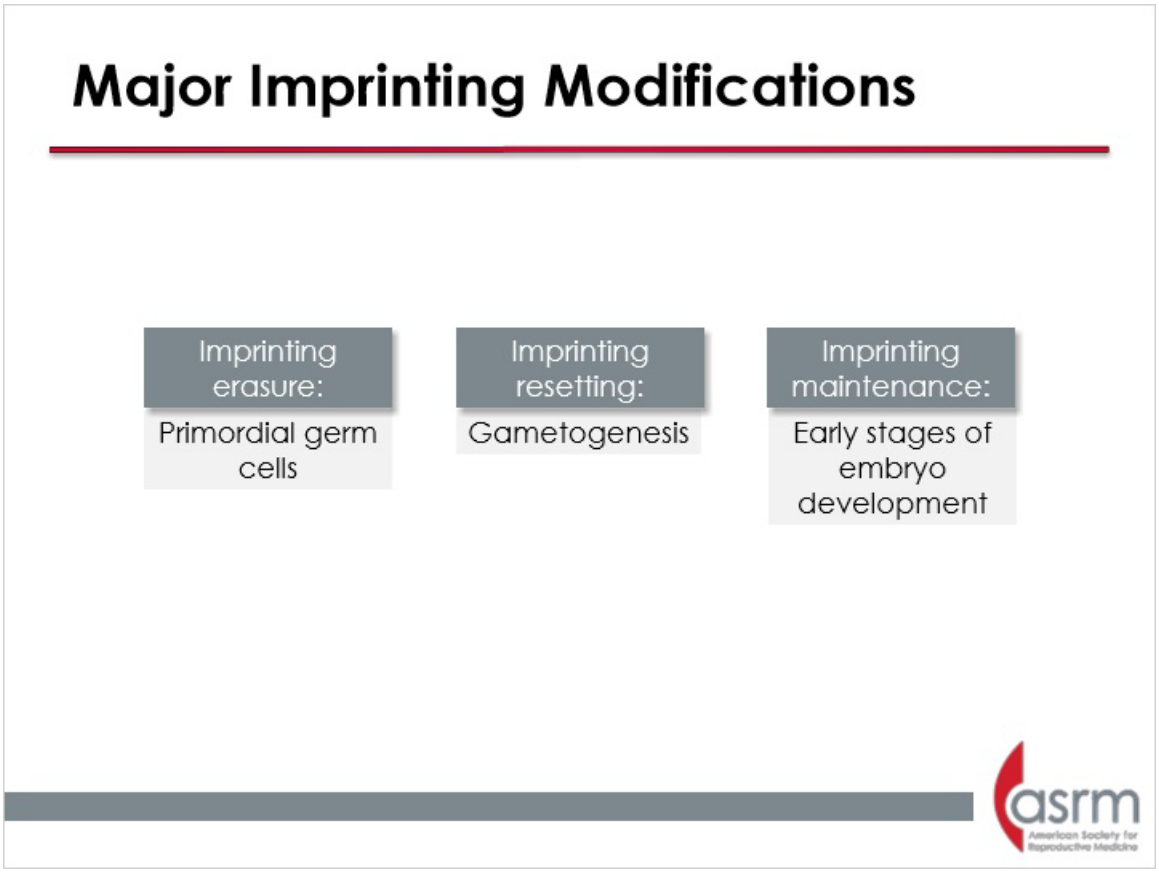
1.18 Imprint Resetting



Notes:

This is a simplified scheme of imprint resetting. DNA methylation patterns that are established during gametogenesis are largely erased during early embryogenesis and reestablished after implantation. However, specific DNA/histone methylation occurs at fertilization.

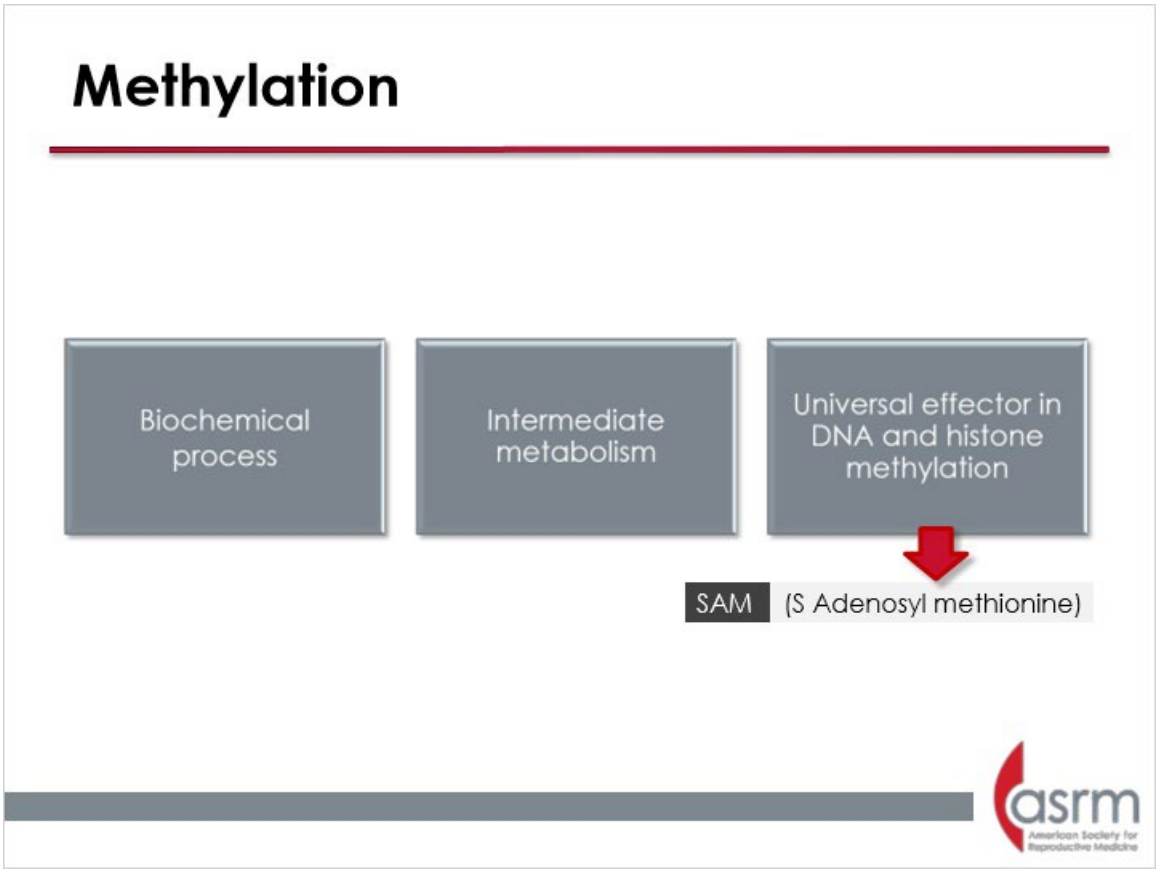
1.19 Major Imprinting Modifications



Notes:

There are 3 main methylation processes: imprinting erasure in the primordial germ cells, imprinting resetting during gametogenesis, and imprinting maintenance in the early stages of embryo development.

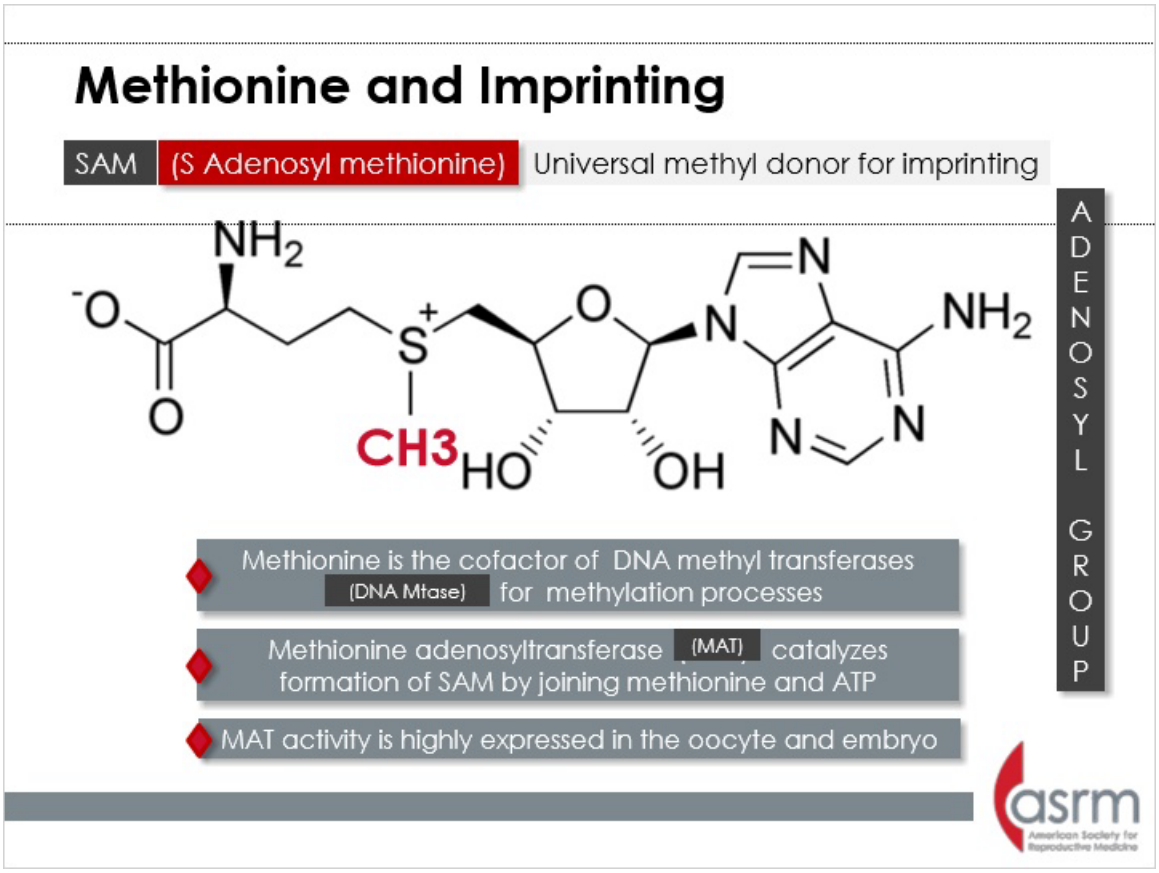
1.20 Methylation



Notes:

This explains the intermediate metabolism upstream of the methylation process. There is an activation of methionine (in the presence of ATP, adenosine triphosphate). This will lead to the formation of SAM: S Adenosyl methionine, the universal methylation agent. Then SAM will be used for methylation of DNA and histones and then SAH, S Adenosyl homocysteine will be released. Methionine is a methyl-donor-amino acid.

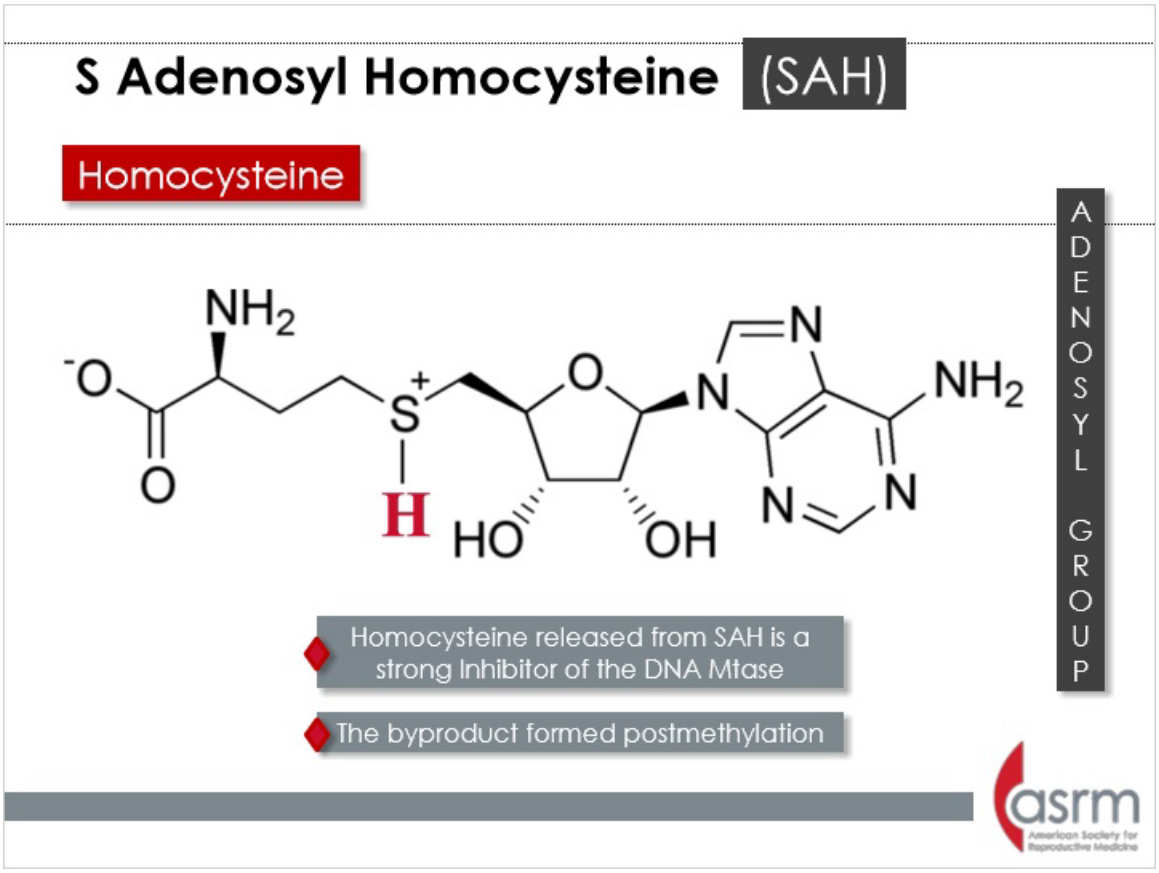
1.21 Methionine and Imprinting



Notes:

It's important to appreciate the biochemical aspects of imprinting. SAM is the universal methyl donor. The chemically active methyl group (CH_3) attached to the methionine sulfur atom can be transferred from one compound to another. Methionine is the cofactor of DNA methyl transferases (DNA Mtase) for methylation processes. Methionine adenosyl transferase (MAT) is an enzyme that catalyzes the formation of SAM by joining methionine and ATP. MAT activity is highly expressed in the oocyte and embryo.

1.22 S Adenosyl Homocysteine



Notes:

After methylation and release of the CH_3 group on DNA proteins (histones in the case of imprinting) S adenosyl homocysteine (SAH) is formed. SAH is then hydrolyzed and forms homocysteine (HCY), which is an inhibitor of methylation processes; in the adult it leads to numerous pathologies.

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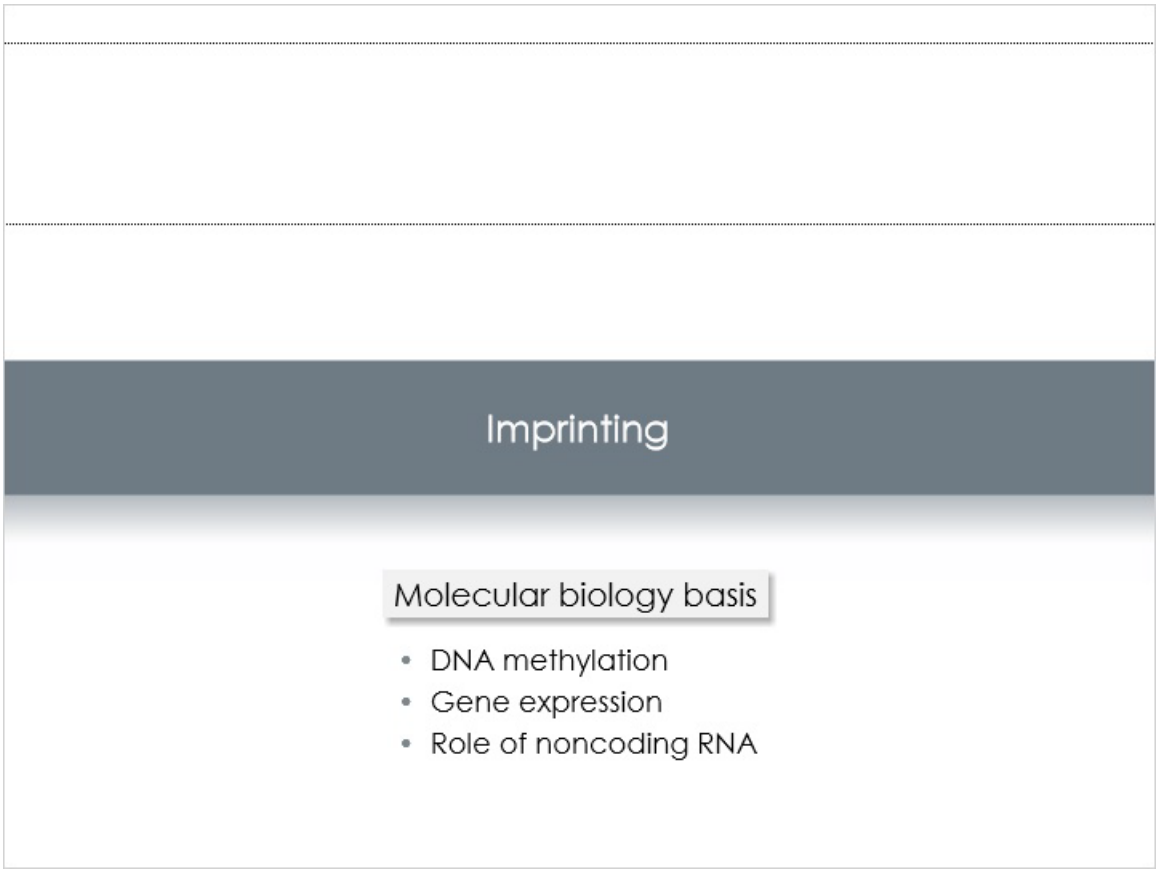
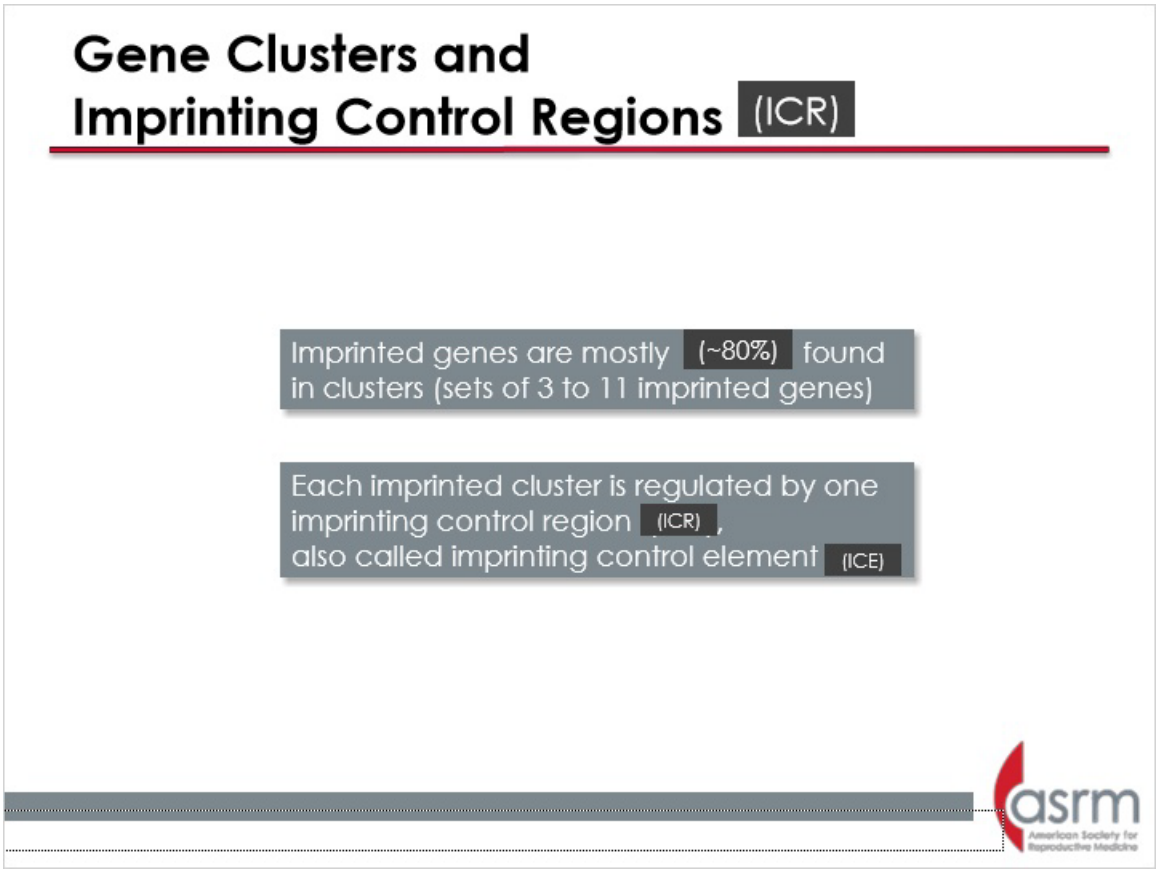


Notes:

This section will discuss the molecular biology aspects of imprinting: how gene expression is blocked at the DNA and histone level and downstream, the role of the cis noncoding RNAs.

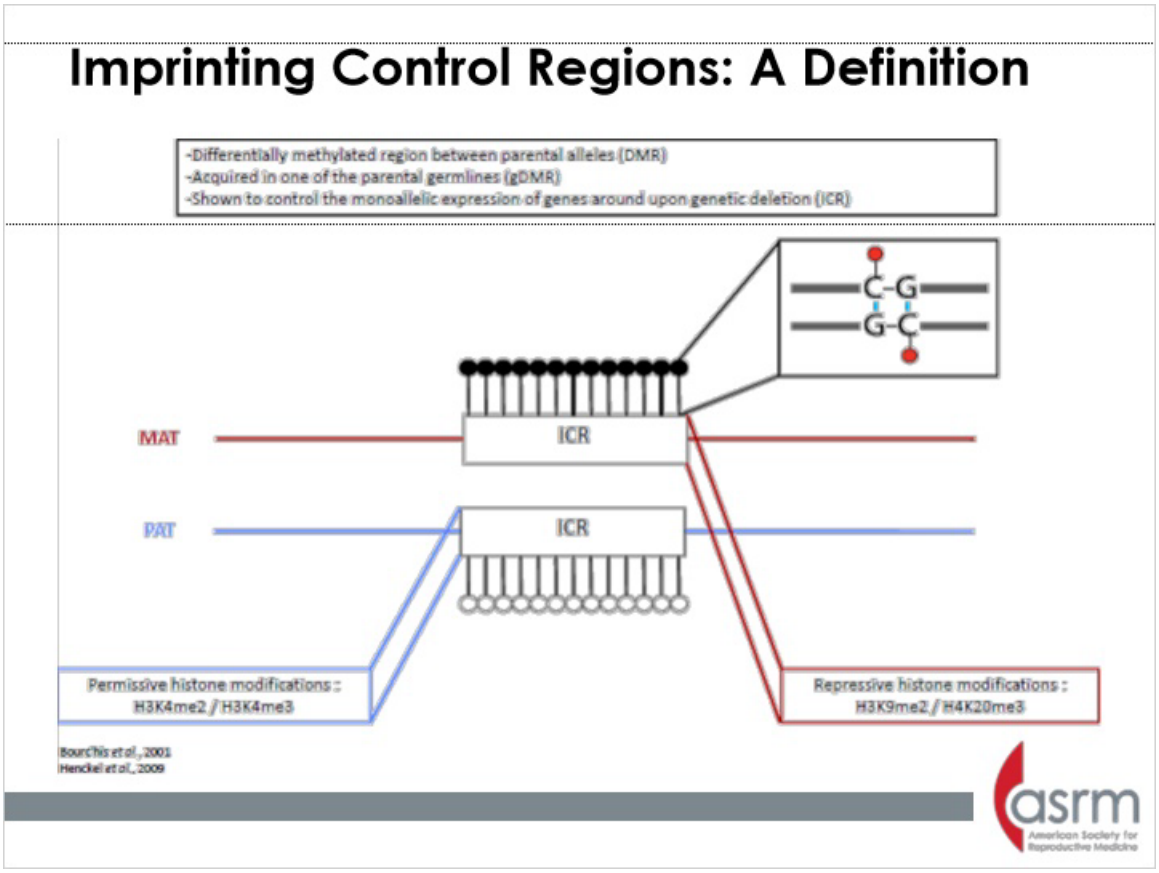
1.24 Gene Clusters and



Notes:

Imprinted genes are mostly (~80%) found in clusters, typically in sets of 3 to 11 imprinted genes. This grouping permits the genes to share common regulation. Each imprinted cluster is regulated by one imprinting control region (ICR) on the chromosome (also called imprinting control element [ICE]).

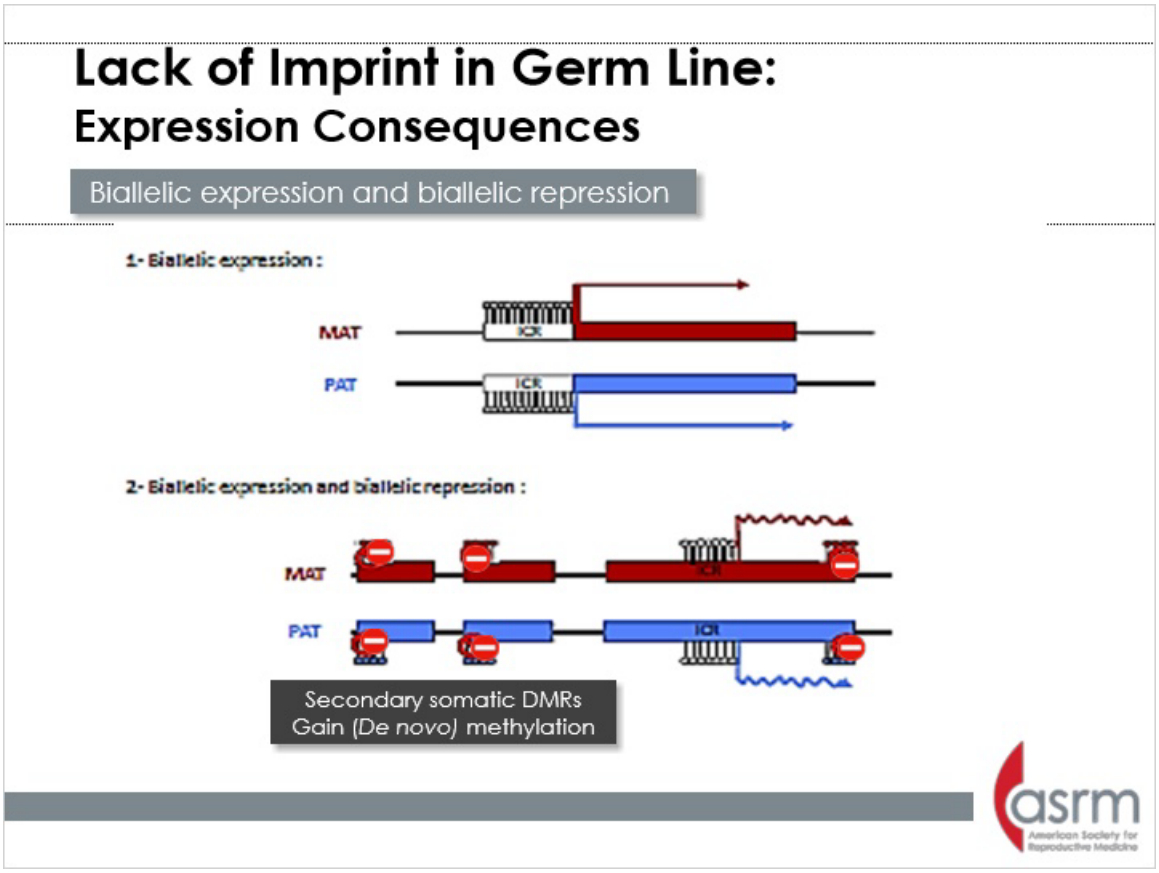
1.25 Imprinting Control Regions: A Definition



Notes:

Epigenetic marks (methylation) comprise both DNA methylation and histone methylation at the level of the ICR. DNA methylation is involved in the acquisition and/or maintenance of histone methylation at ICRs. DNA methylation also plays a central role in the control of repressive histone methylation at the ICR. DNA methylation might be a prerequisite for the acquisition of repressive histone marks.

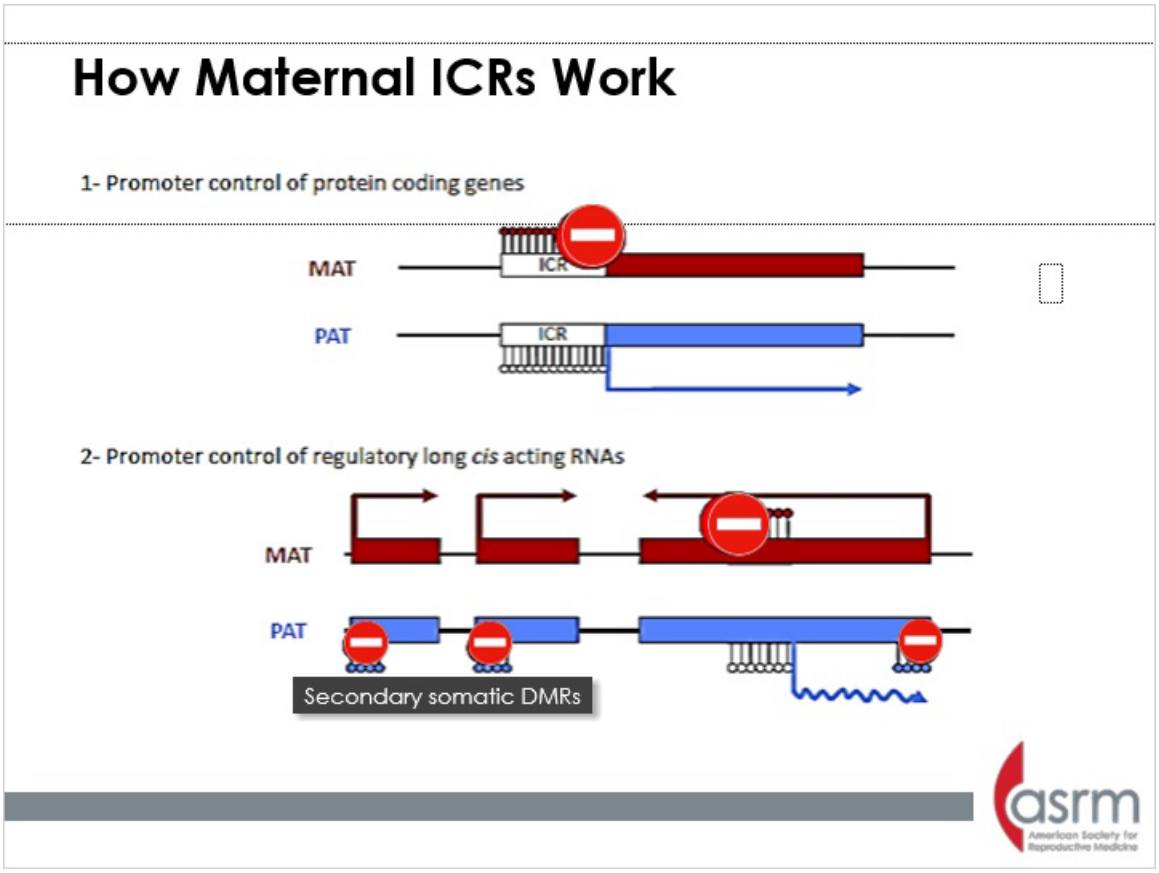
1.26 Lack of Imprint in Germ Line:



Notes:

For some imprinted genes it is possible to have a transitory double expression. During embryonic development this may depend on the organ and the age of the embryo.
In the first example, ICRs are permissive. This means that the pathway is open and all the genes downstream will be expressed. The exact opposite happens in the second example: both alleles will be repressed.

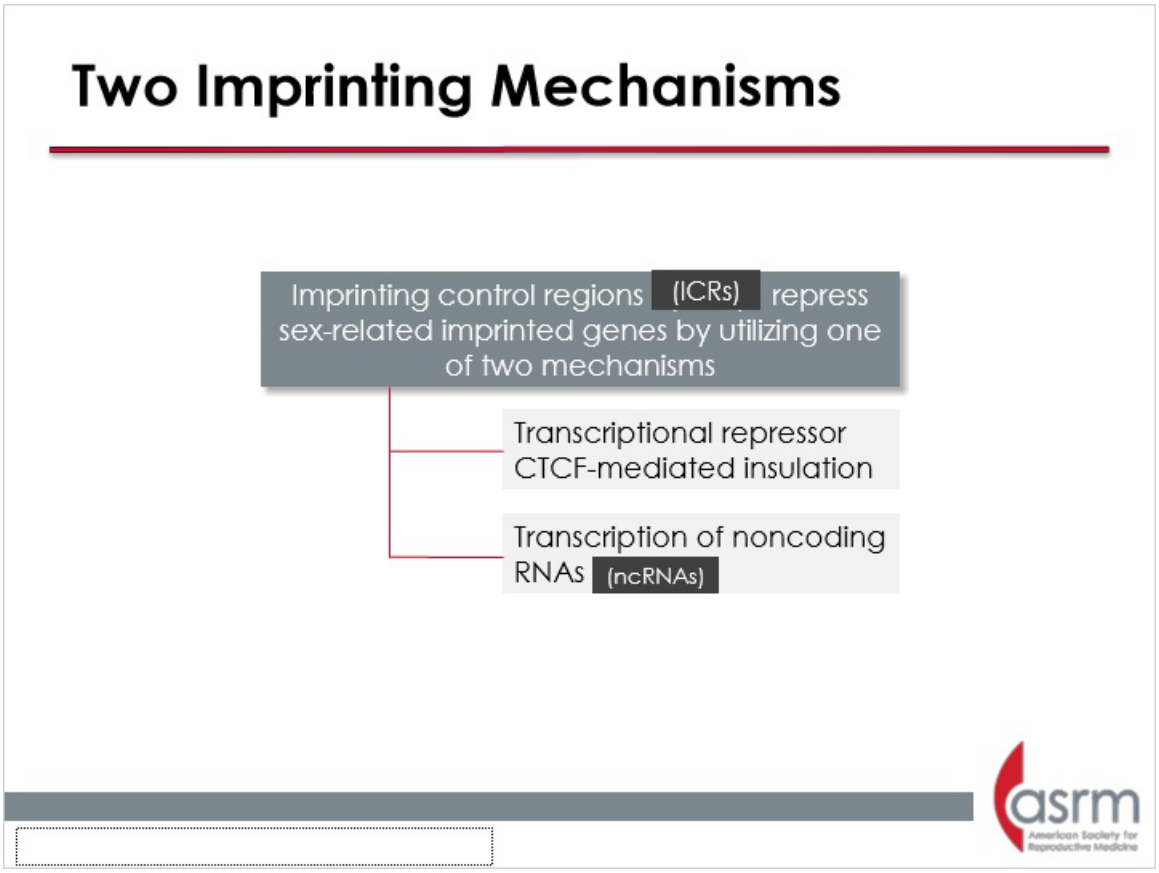
1.27 How Maternal ICRs Work



Notes:

Again, in the first example, the ICR will control directly the expression at the level of the promoter. And in the second, the control will be obtained in a second step through the interaction of a cis-acting noncoding RNA.

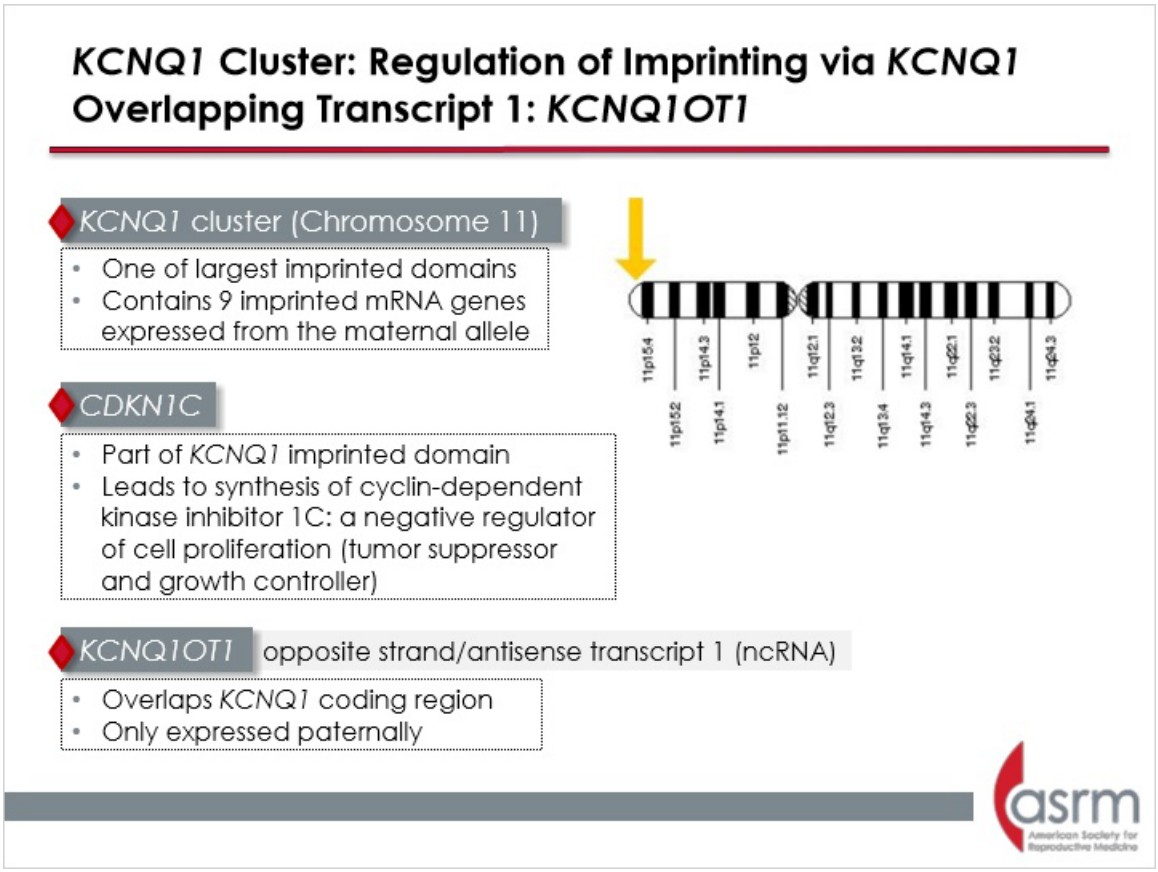
1.28 Two Imprinting Mechanisms



Notes:

Imprinting control regions (ICRs) are known to repress genes by utilizing one of two mechanisms, transcriptional repressor CTCF-mediated insulation or the transcription of noncoding RNAs (ncRNAs). Each of these mechanisms will be discussed.

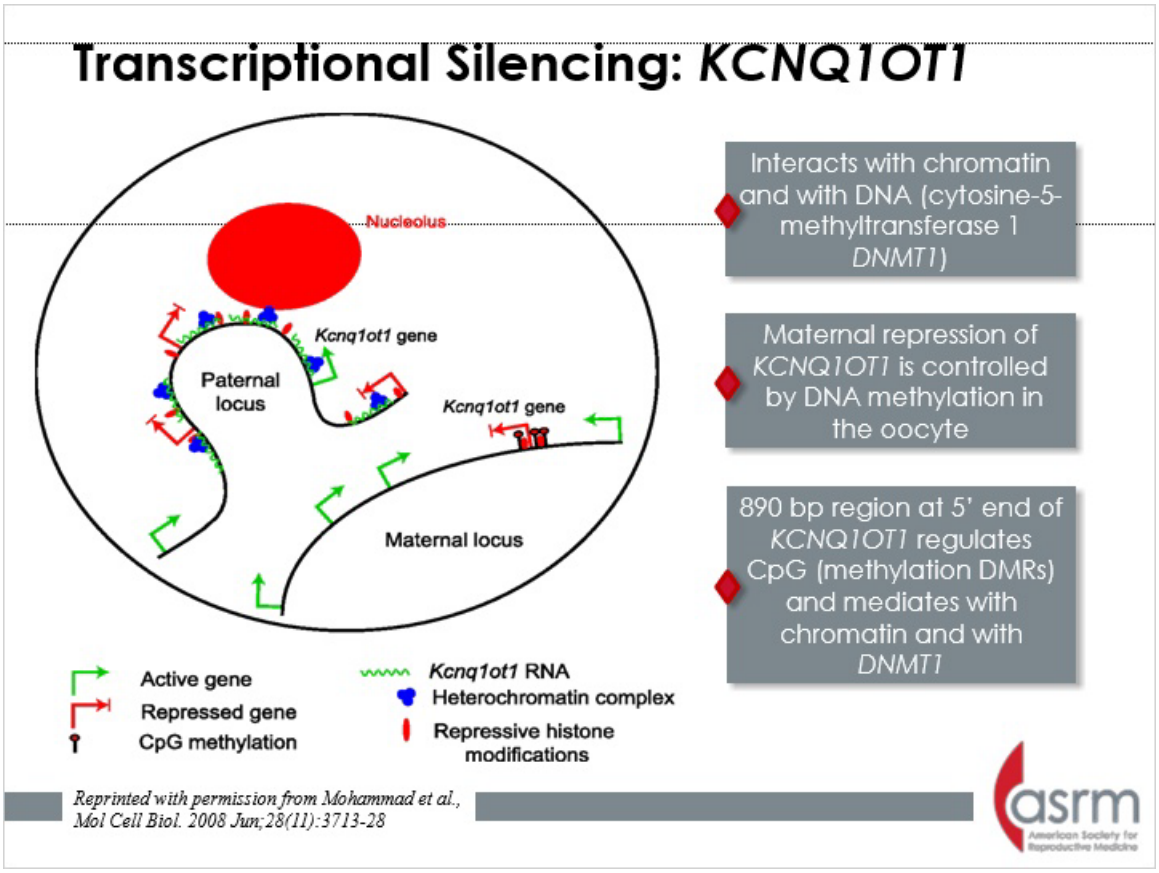
1.29 KCNQ1 Cluster: Regulation of Imprinting via KCNQ1 Overlapping Transcript 1: KCNQ1OT1



Notes:

The *KCNQ1* cluster is one of the largest imprinted domains. It contains 9 imprinted mRNA genes expressed from the maternal allele. The *KCNQ1* gene belongs to a large family of genes that provide instructions for making potassium channels. These channels, which transport positively charged atoms (ions) of potassium out of cells, play key roles in a cell's ability to generate and transmit electrical signals. The *CDKN1C* gene is part of the *KCNQ1* imprinted domain and provides instructions for synthesis of cyclin-dependent kinase inhibitor 1C, a negative regulator of cell proliferation. This protein acts as a tumor suppressor to keep cells from growing and dividing too fast or in an uncontrolled way. It also is involved in controlling growth before birth, preventing the developing fetus from becoming too large. The *CDKN1C* leads to *KCNQ1OT1* opposite strand/antisense transcript 1 ncRNA, which overlaps the *KCNQ1* coding region. This gene is only expressed paternally.

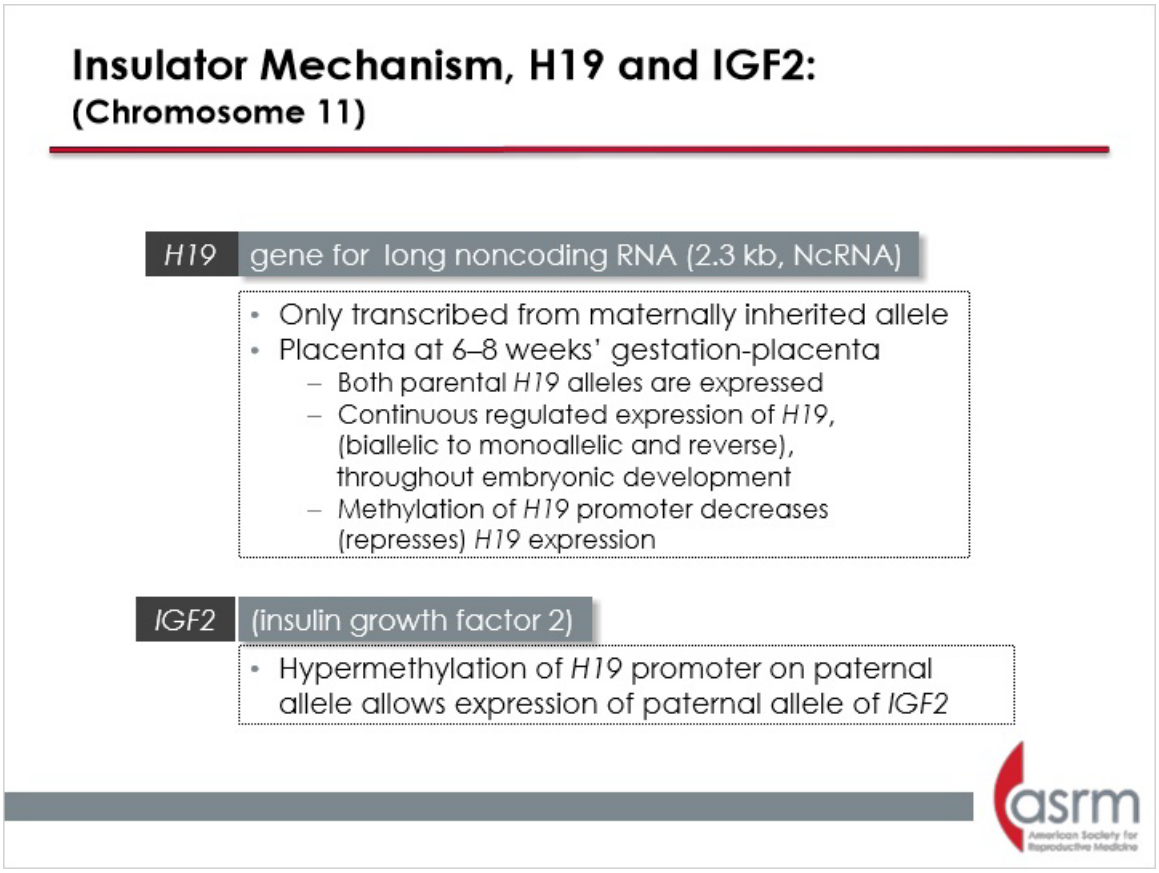
1.30 Transcriptional Silencing: KCNQ1OT1



Notes:

KCNQ1 overlapping transcript 1, *KCNQ1OT1,* interacts with chromatin and with the *DNMT1* (DNA [cytosine-5-]-methyltransferase 1) gene to induce transcriptional silencing of the KCNQ1 locus by regulating histone methylation. Maternal repression of *KCNQ1OT1* is controlled by DNA methylation in the oocyte. An 890 bp region at the 5' end of *KCNQ1OT1* gene regulates CpG methylation of differentially methylated regions (DMRs) and mediates the interaction of *KCNQ1OT1* with chromatin and with *DNMT1.*

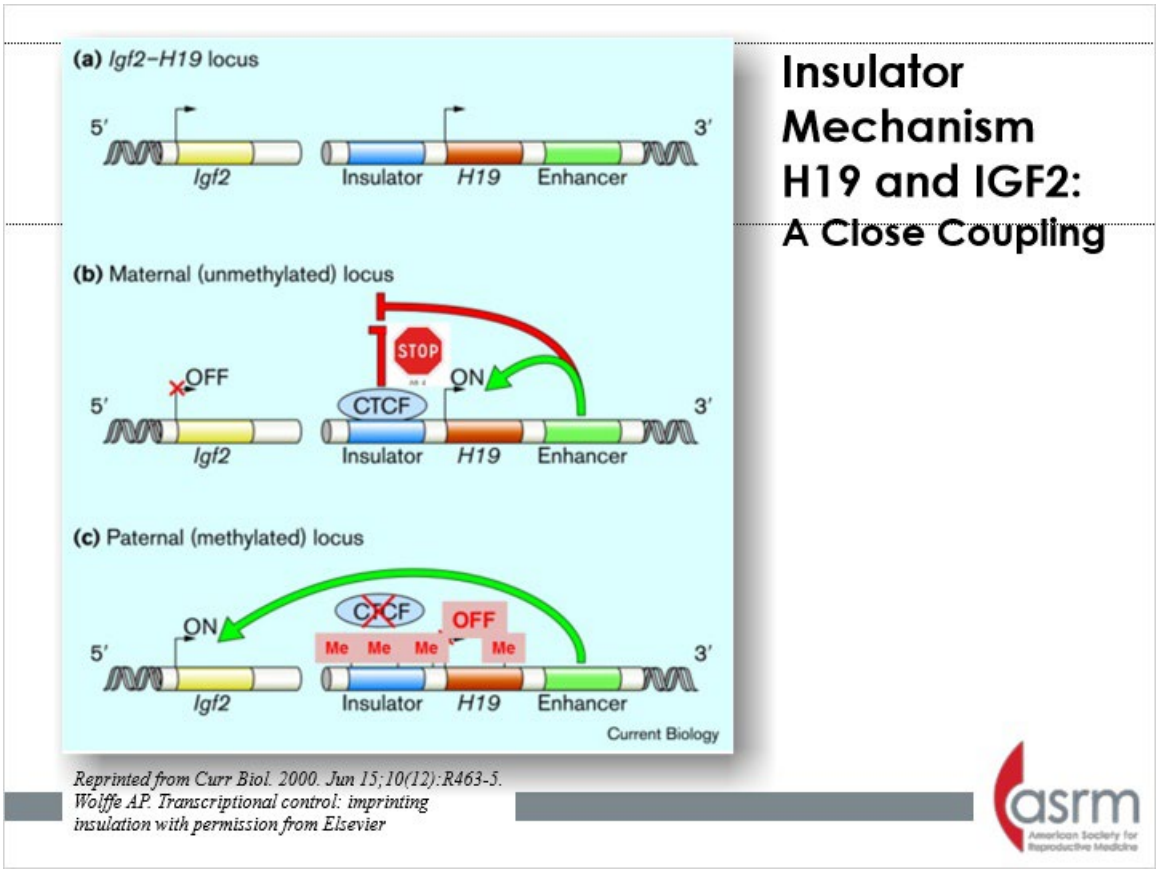
1.31 Insulator Mechanism, H19 and IGF2:



Notes:

The second process involves *H19* and Igf2. *H19* is a gene for a long noncoding RNA (2.3 kb, NcRNA). *H19* is only transcribed from the maternally inherited allele. In the 6–8 weeks' gestation placenta, both parental *H19* alleles are expressed. There is a continuous regulated expression of *H19*, (biallelic to monoallelic and reverse), throughout embryonic development. Methylation of the *H19* promoter decreases (represses) *H19* expression.
The insulin growth factor-2 (*IGF2)* gene plays an essential role in growth and development before birth. The hypermethylation of the *H19* promoter on the paternal allele allows the expression of the paternal allele of *IGF2*.

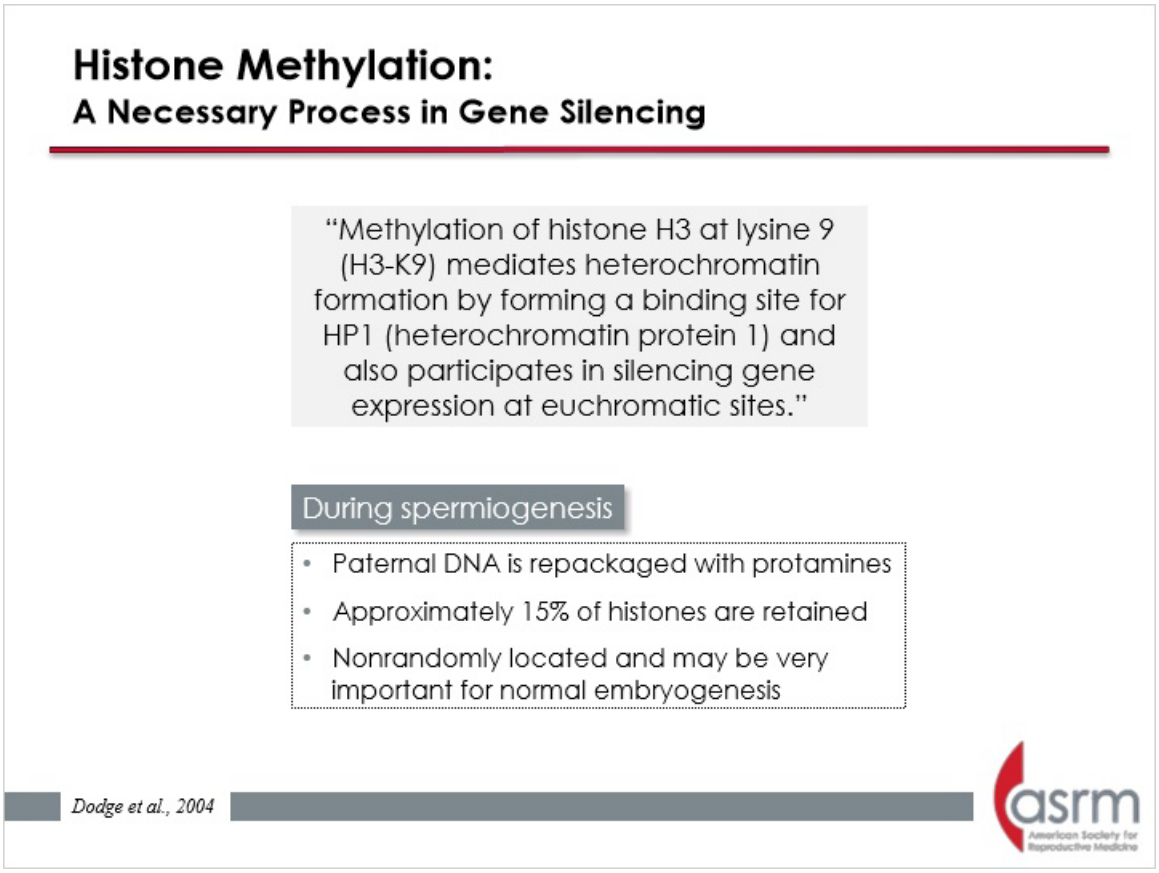
1.32 Insulator Mechanism H19 and IGF2: A Close Coupling



Notes:

This diagram illustrates the insulator mechanism in the *H19–IGF2* locus. Arrows indicate the start site and direction of transcription of each gene. In the maternal unmethylated locus, *CTCF* can bind to the insulator and prevent the 3' enhancer from activating the *IGF2* promoter. The enhancer can still efficiently activate the unmethylated *H19* promoter. In the paternal methylated locus, the insulator is heavily methylated. *CTCF* can no longer bind to the insulator because the DNA is methylated (indicated by 'Me') and the insulator becomes inactive. In addition, methylation of the *H19* gene sequence represses transcription directly. Now enhancer activity is focused on activation of the *IGF2* promoter.

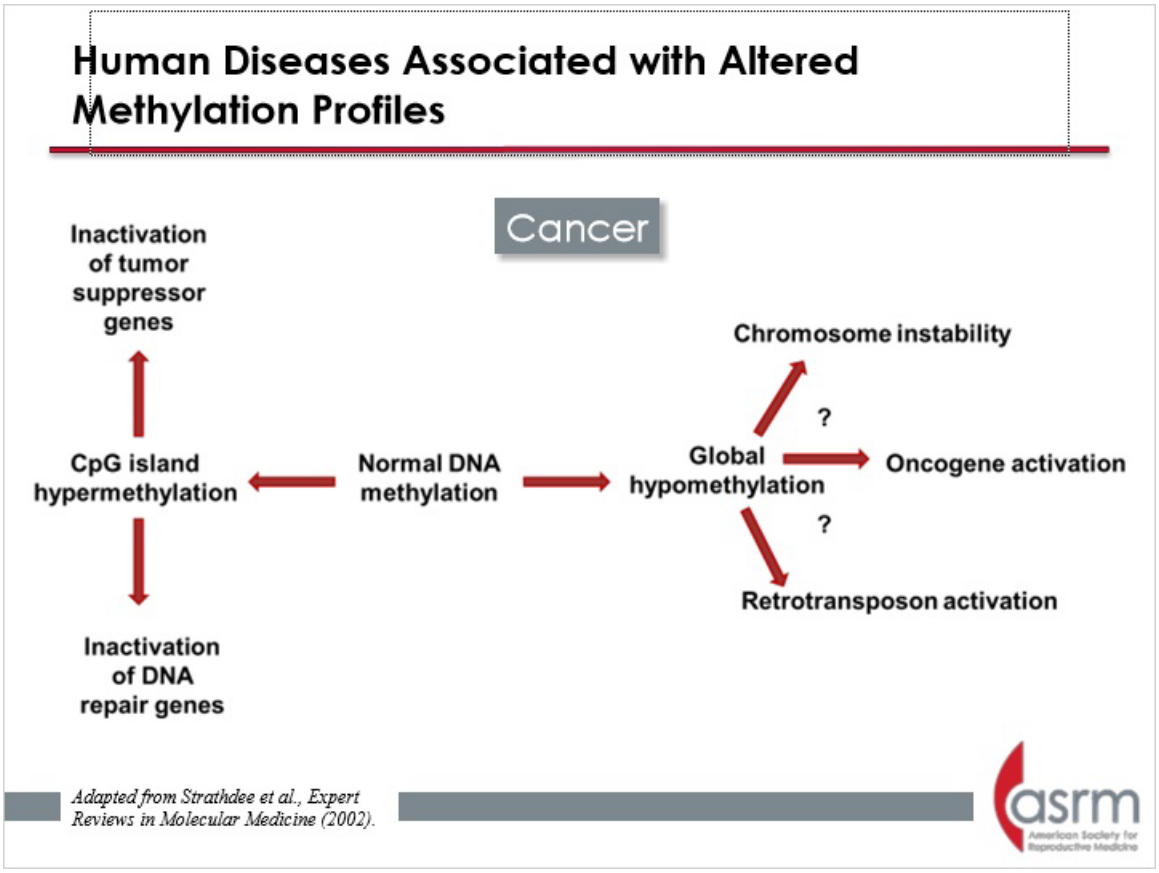
1.33 Histone Methylation:



Notes:

Histone H3 lysine 9 (H3-K9) methylation has been shown to correlate with transcriptional repression and serve as a specific binding site for heterochromatin protein 1 (HP1). Methylation of histone modulates interaction with DNA allowing or repressing transcription.
Importantly, during spermiogenesis, paternal DNA is repackaged with protamines but approximately 15% of histones are retained. They are nonrandomly located and may be very important for normal embryogenesis.

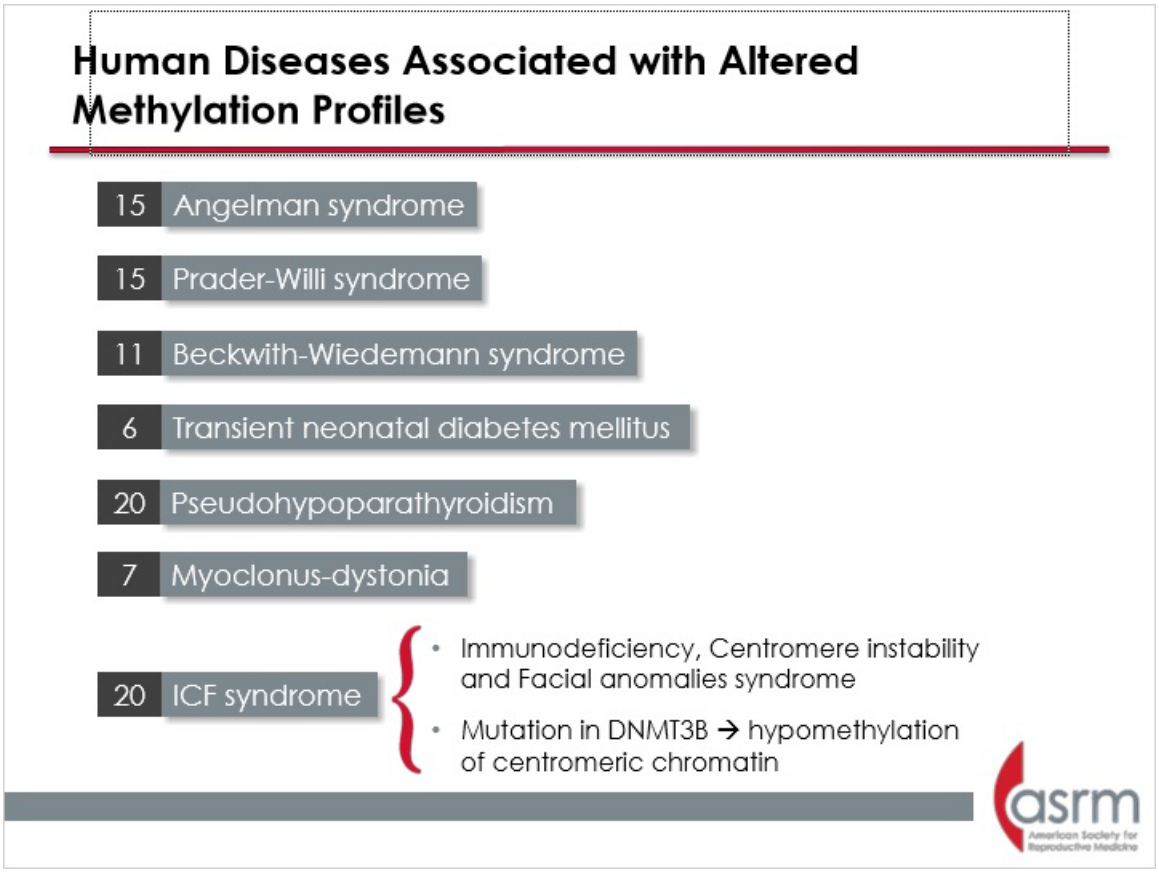
1.34 Human Diseases Associated with Altered Methylation Profiles



Notes:

There are several major diseases associated with issues in imprinting. All stem from altered methylation profiles.

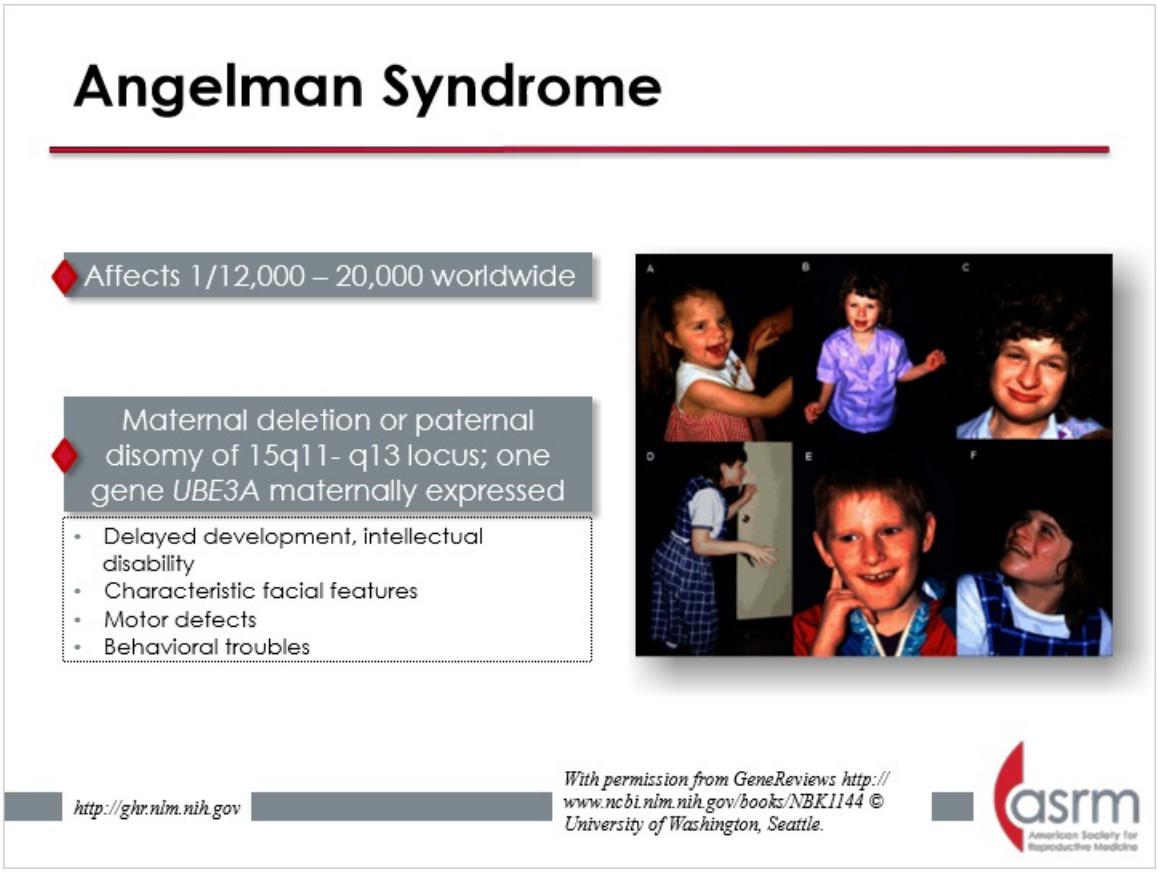
1.35 Human Diseases Associated with Altered Methylation Profiles



Notes:

Some of the more well-known syndromes are associated with altered methylation profiles in chromosomes 11 and 15, but other chromosomes are also affected.

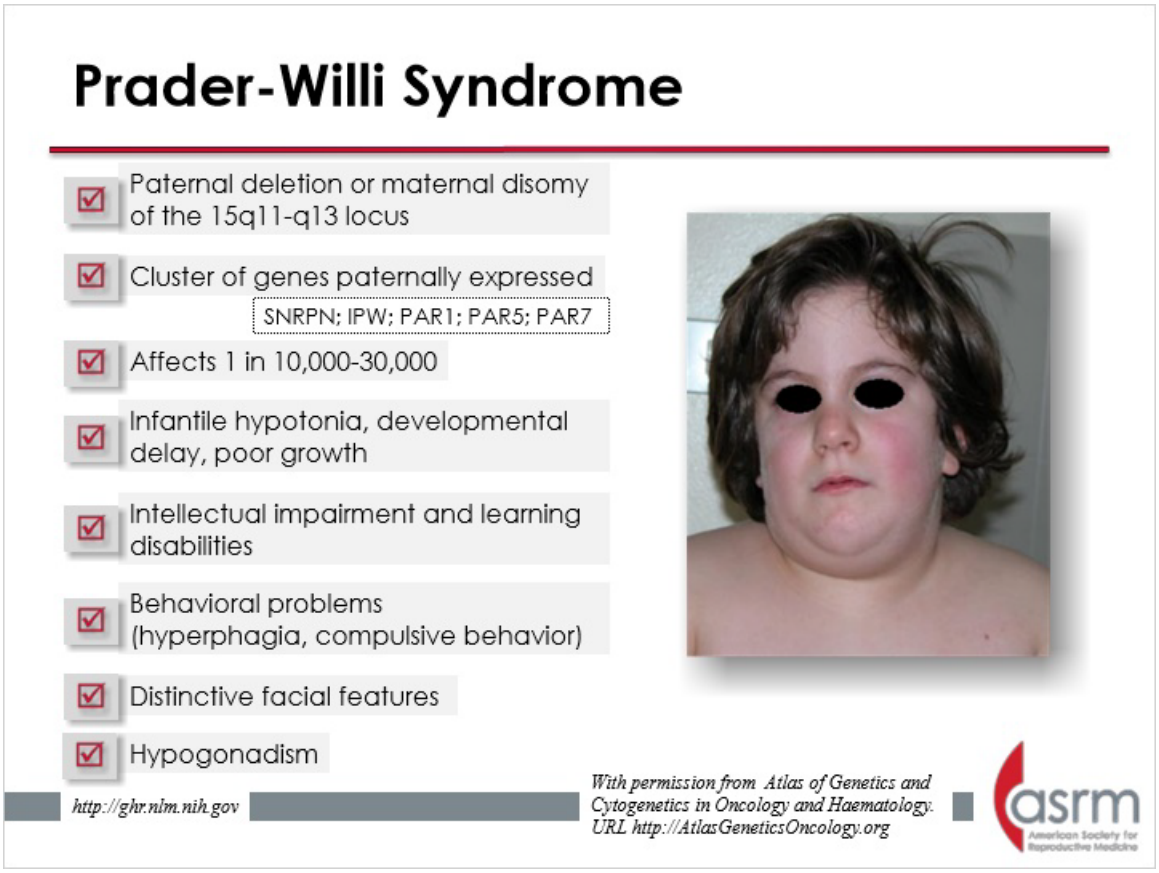
1.36 Angelman Syndrome



Notes:

Angelman syndrome affects 1/12,000–20,000 worldwide. Most cases of Angelman syndrome occur when a segment of the maternal chromosome 15 at the 15q11- q13 locus containing this gene is deleted. In other cases, Angelman syndrome is caused by a mutation in the maternal copy of the *UBE3A* gene. Angelman syndrome is a complex genetic disorder that primarily affects the nervous system. Characteristic features of this condition include delayed development, intellectual disability, severe speech impairment, and problems with movement and balance (ataxia). Most affected children also have recurrent seizures (epilepsy) and a small head size (microcephaly). Children with Angelman syndrome typically have a happy, excitable demeanor with frequent smiling, laughter, and hand-flapping movements. Hyperactivity, a short attention span, and a fascination with water are common.

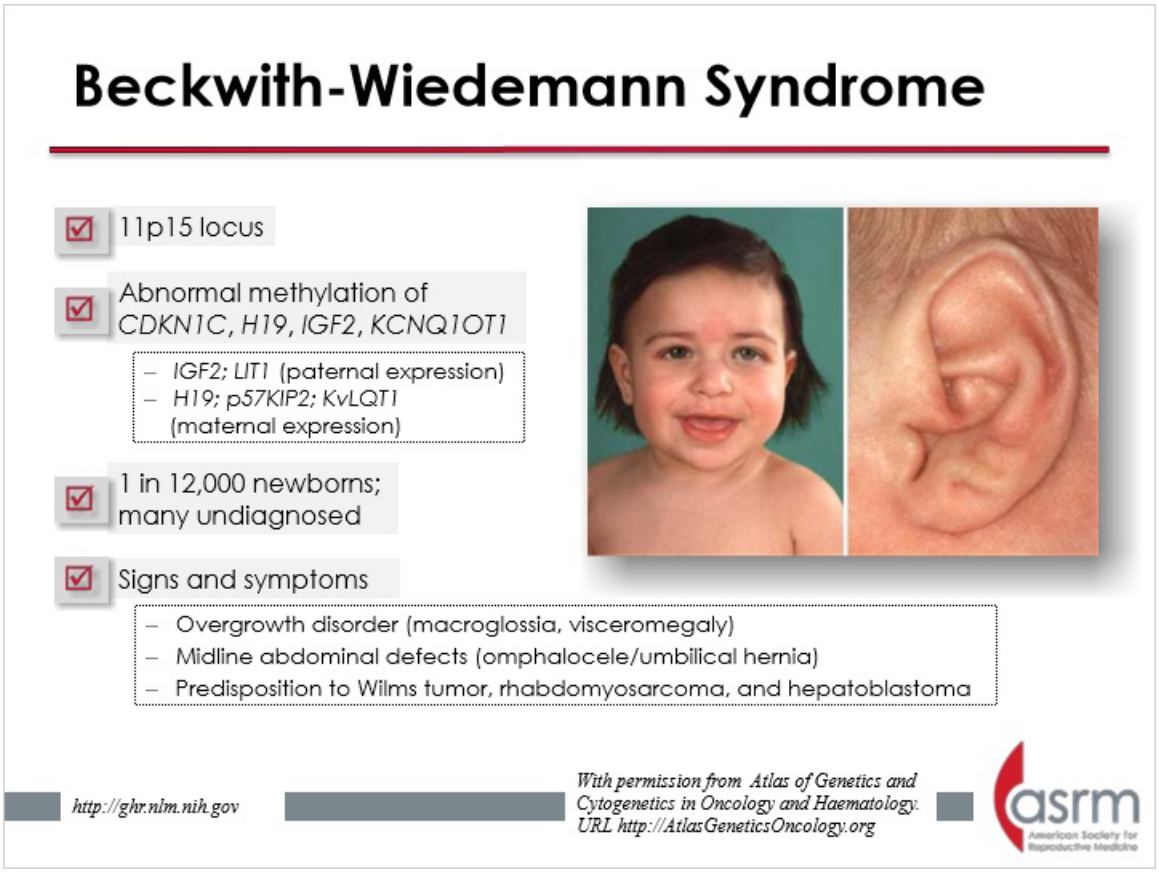
1.37 Prader-Willi Syndrome



Notes:

Prader-Willi syndrome involves paternal deletion or maternal disomy of the 15q11-q13 locus and affects 1 in 10,000 to 30,000. In infancy, this condition is characterized by hypotonia, feeding difficulties, poor growth, and delayed development. Beginning in childhood, affected individuals develop an insatiable appetite, which leads to chronic overeating (hyperphagia) and obesity. Individuals typically have mild to moderate intellectual impairment and learning disabilities. Behavioral problems are common, including temper outbursts, stubbornness, and compulsive behavior such as picking at the skin. Additional features of this condition include distinctive facial features (such as a narrow forehead, almond-shaped eyes, and a triangular mouth), short stature, and small hands and feet. Some people with Prader-Willi syndrome have unusually fair skin and light-colored hair. Both affected males and affected females have underdeveloped genitals, delayed or incomplete puberty, and infertility.

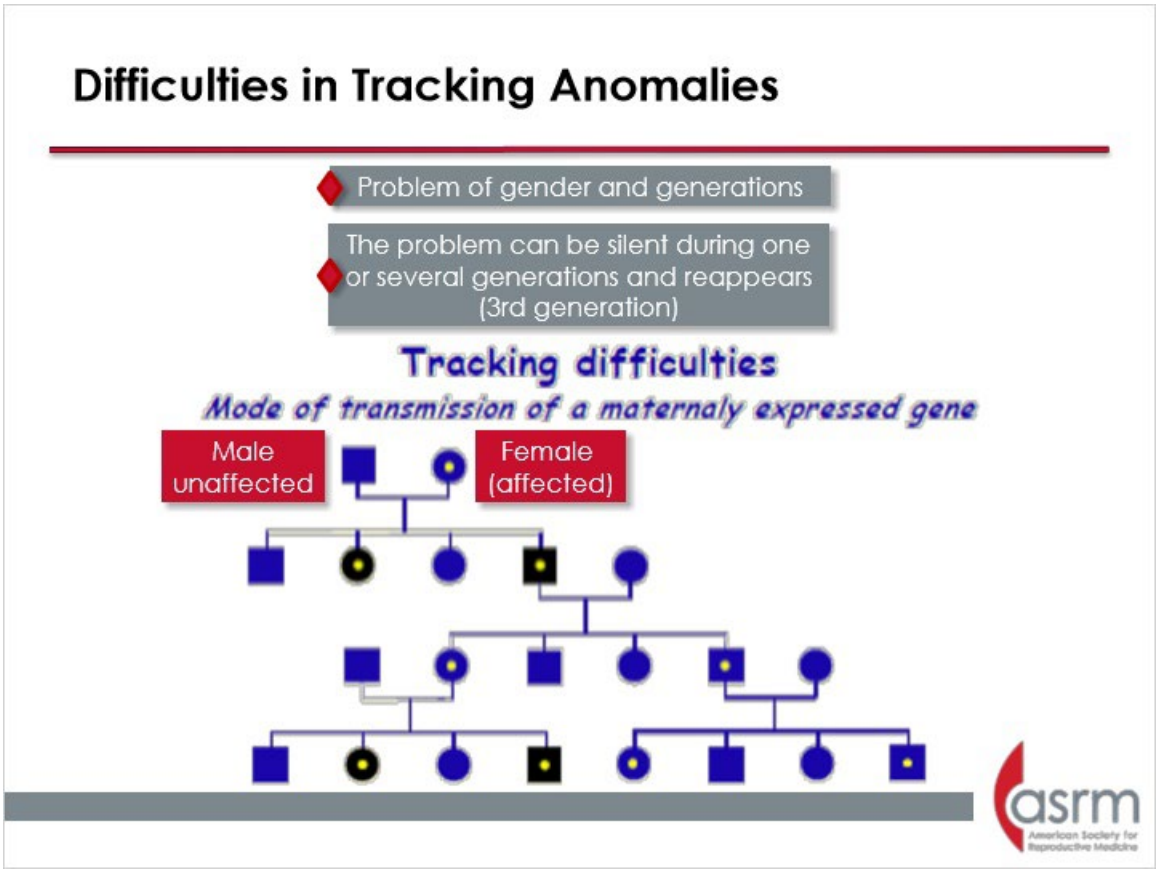
1.38 Beckwith-Wiedemann Syndrome



Notes:

As noted earlier, Beckwith-Wiedemann syndrome is often associated with changes in imprinting control regions on chromosome 11 at the 11p15 locus. Abnormal methylation disrupts the regulation of the cluster of genes involved in normal growth (*CDKN1C*, *H19*, *IGF2*, and *KCNQ1OT1)*, which leads to overgrowth and the other characteristic features of the syndrome. Beckwith-Wiedemann syndrome affects 1 in 12,000 newborns worldwide; however, some people with mild or unusual symptoms are never diagnosed. Common features include abdominal wall defects such as omphalocele and umbilical hernia, macroglossia, visceromegaly, creases or pits in the skin near the ears, hypoglycemia in infancy, and kidney abnormalities. Importantly, children with Beckwith-Wiedemann syndrome are at an increased risk of developing several types of cancerous and noncancerous tumors, particularly Wilms tumor, rhabdomyosarcoma, and hepatoblastoma.

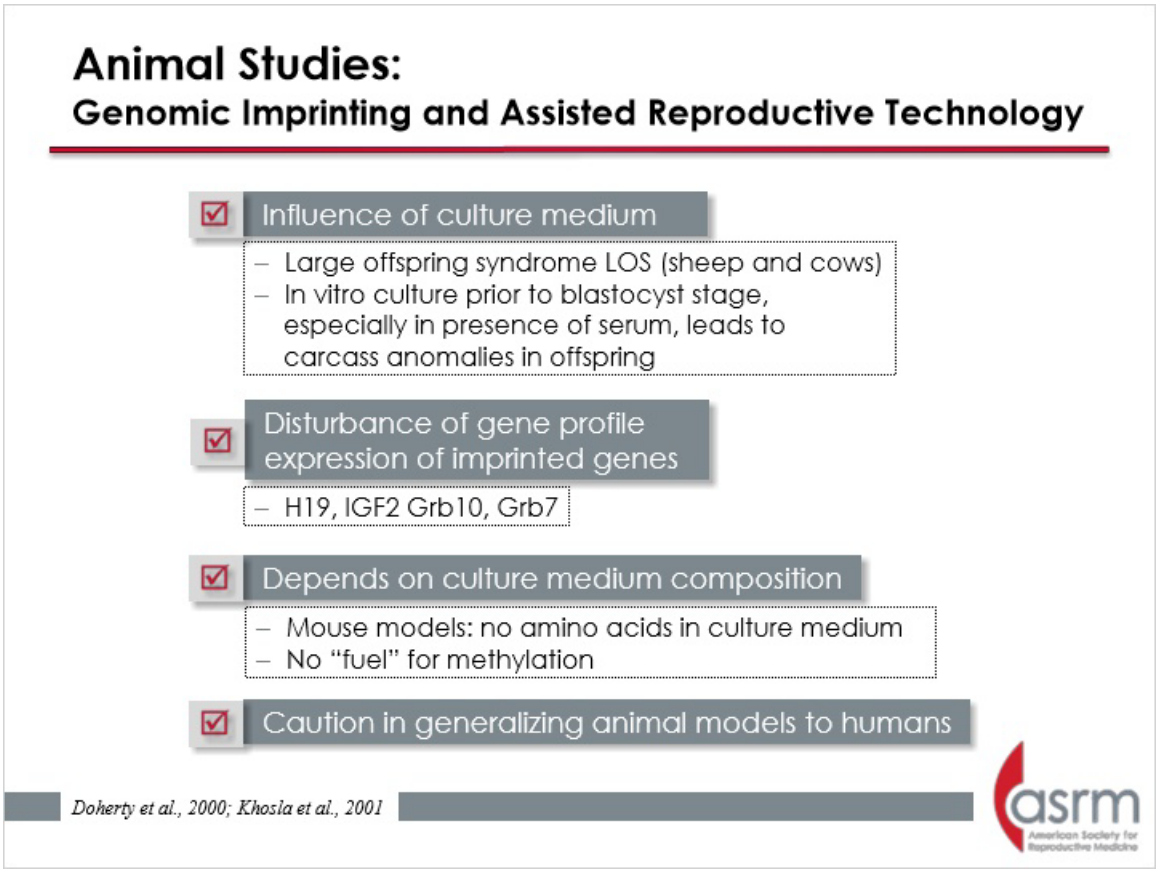
1.39 Difficulties in Tracking Anomalies



Notes:

This diagram shows how epigenetic problems can be silent during one or several generations and then reappear.

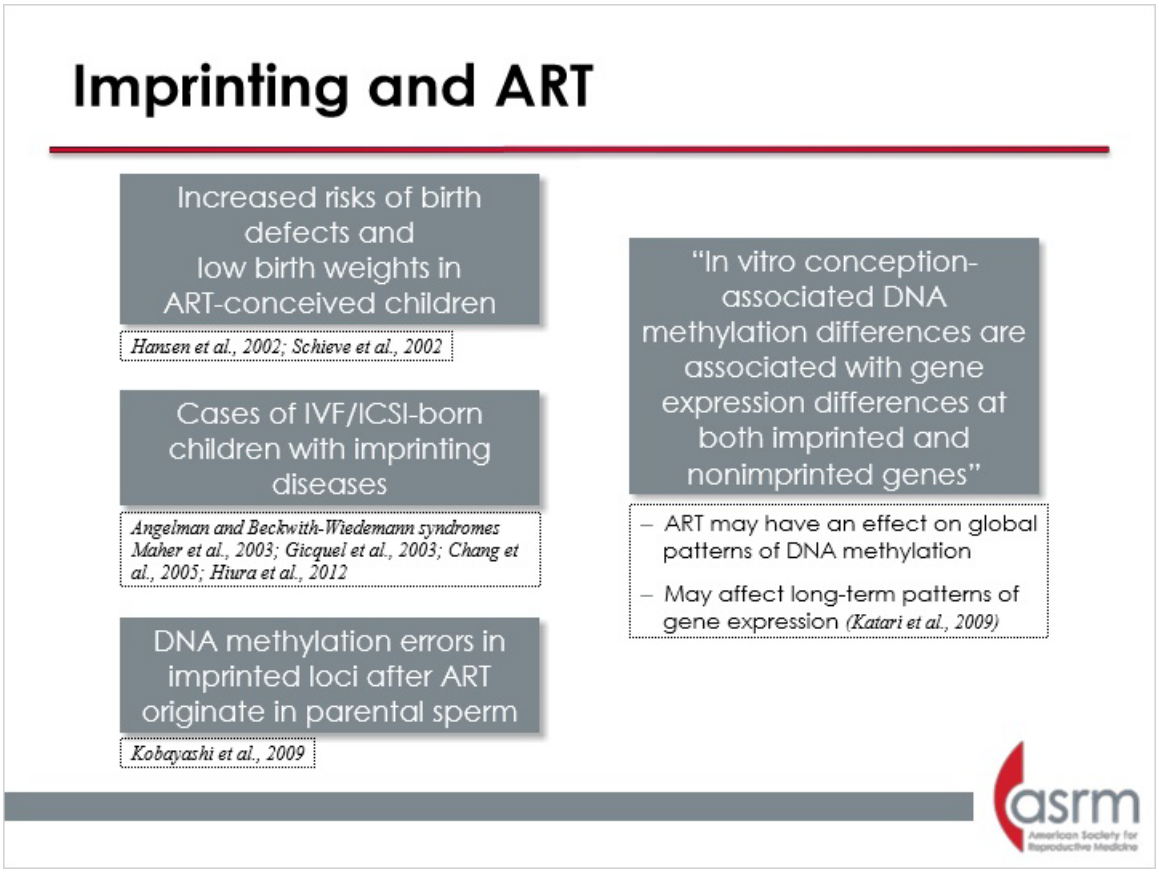
1.40 Animal Studies:



Notes:

In animal models of assisted reproductive technologies, there appears to be an influence of the embryo culture medium on imprinting regulation. Large Offspring syndrome is observed in both cows and sheep. In vitro culture prior to blastocyst stage, especially in the presence of serum, leads to carcass anomalies in the offspring. Culture of mouse embryos without amino acids, especially methionine, leads to alteration in the expression of imprinted genes. The mechanisms are related to anomalies in methylation. The mouse embryo has no ”fuel” for methylation, which affects imprinting. As noted earlier, imprinting anomalies can “escape” several generations and reappear. This has led some scientists to propose removing essential amino acids during the early stage of human in vitro culture. However, care must be taken in using animal models to generalize to humans.

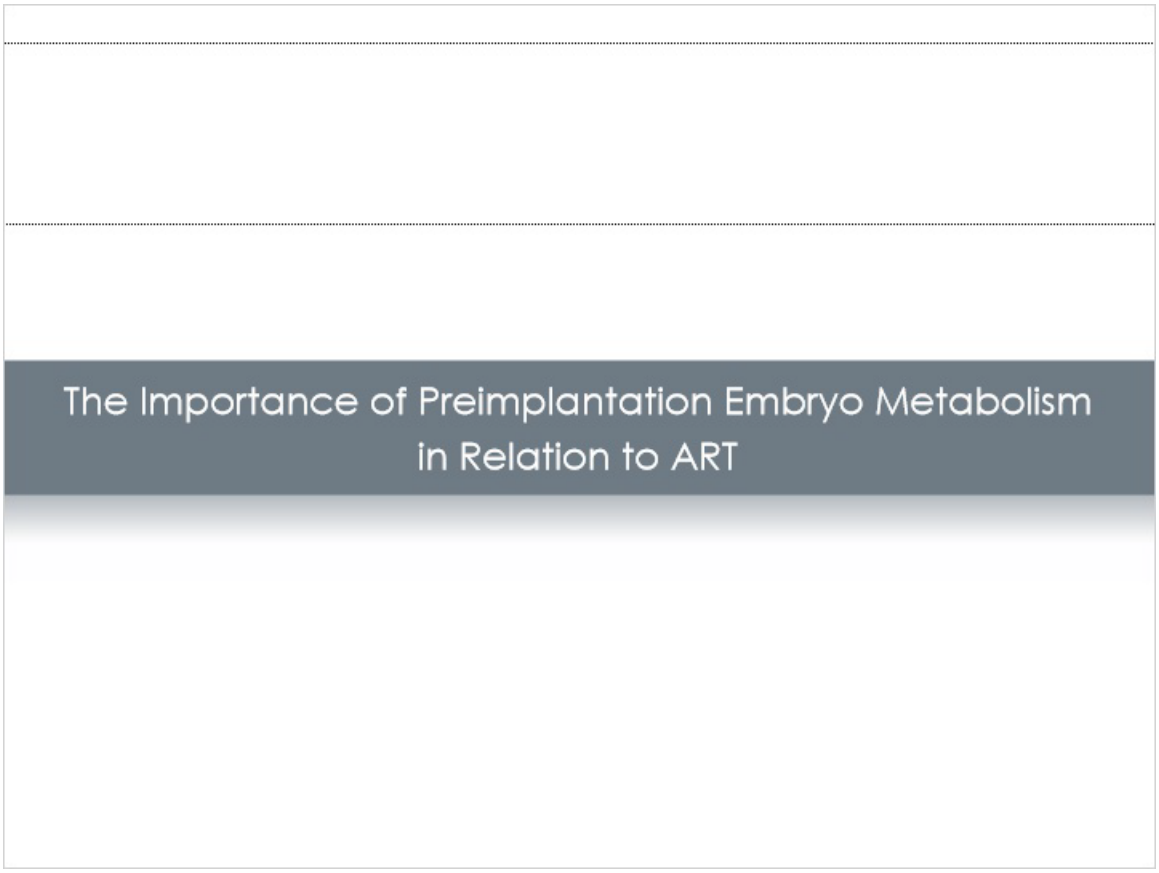
1.41 Imprinting and ART



Notes:

Several observations can be made regarding imprinting and ART in humans. Risks of birth defects and low birth weight are increased in ART-conceived children. The imprinting diseases of Angelman and Beckwith-Wiedemann syndromes are reported as more common in IVF/ICSI-born children. It has been found that DNA methylation errors in imprinted loci after ART originate in parental sperm. In vitro conception-associated DNA methylation differences are associated with gene expression differences at both imprinted and non-imprinted genes. ART may have an effect on global patterns of DNA methylation and may affect long-term patterns of gene expression.

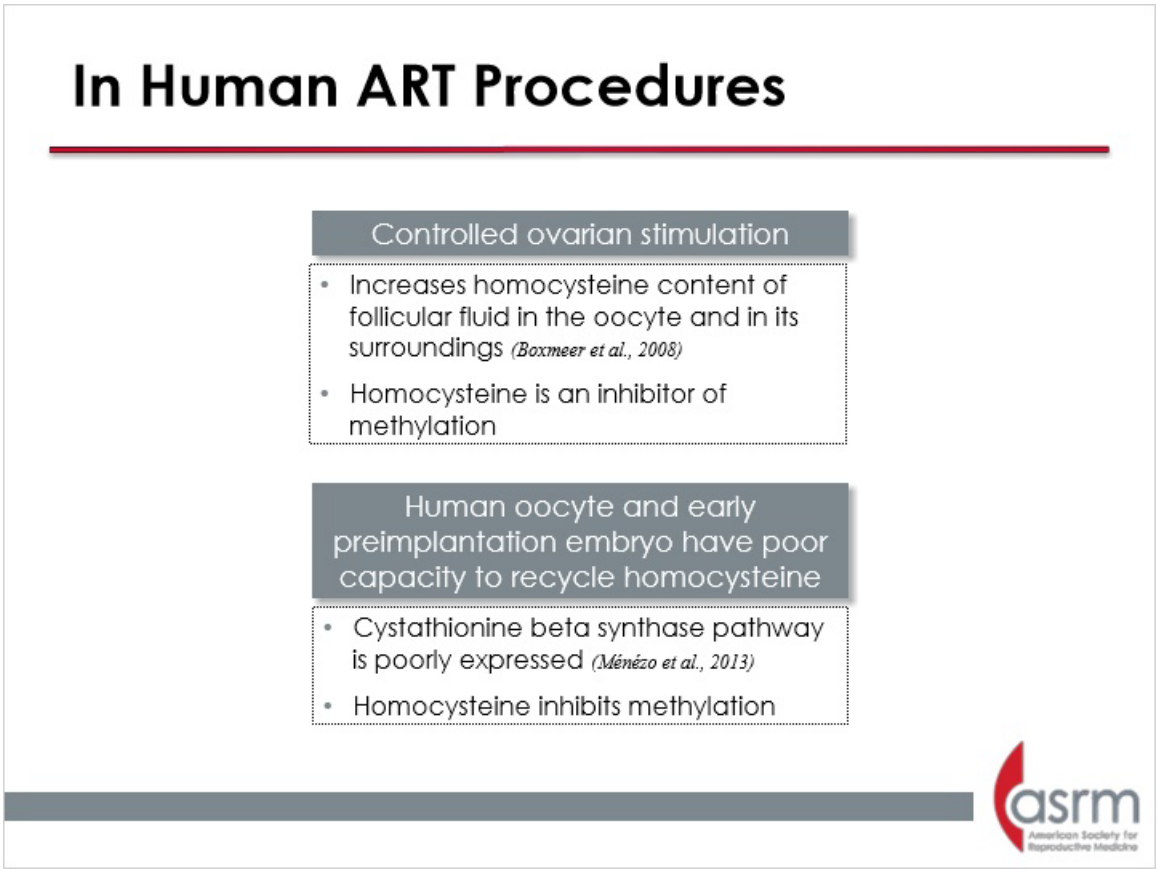
1.42 Untitled Slide



Notes:

Next is a discussion of the importance of preimplantation embryo metabolism in relation to ART.

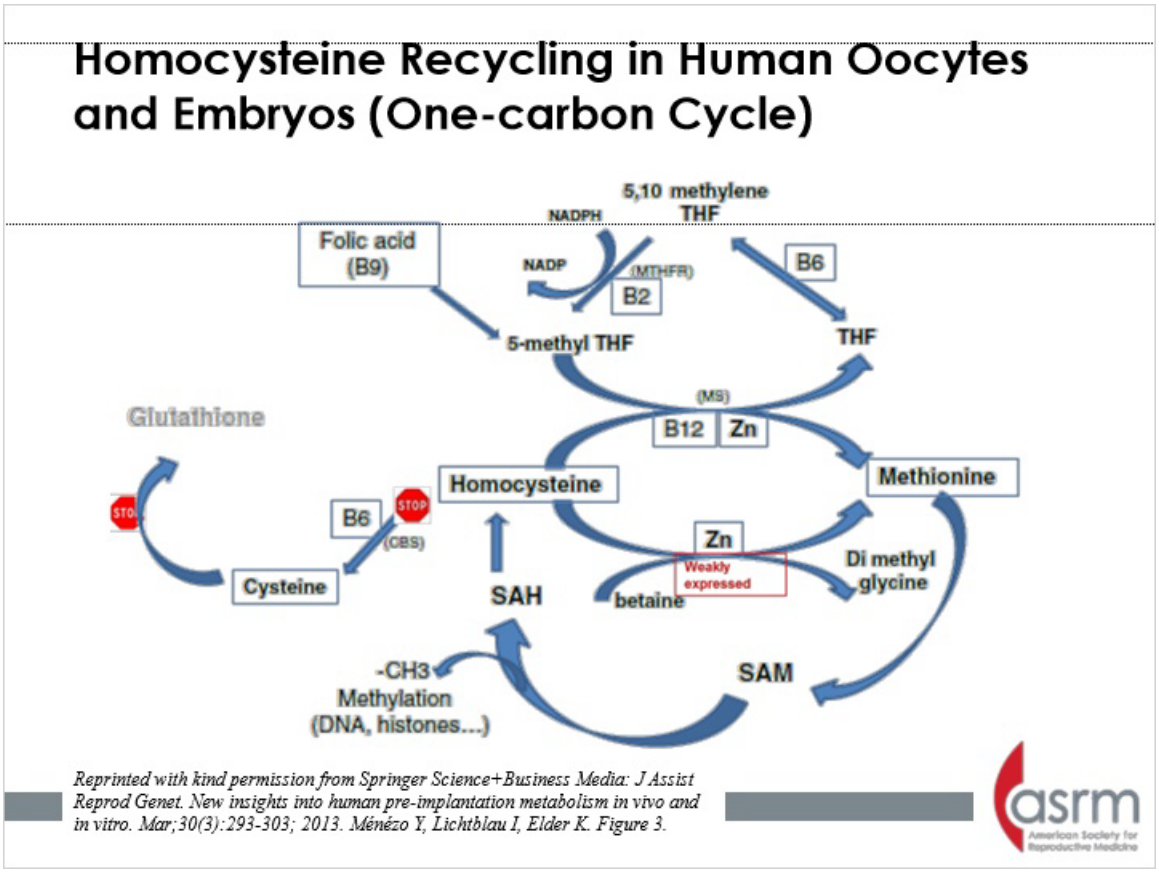
1.43 In Human ART Procedures



Notes:

The use of ART creates problems related to imprinting. First, controlled ovarian stimulation increases the homocysteine concentration in follicular fluid and thus in the oocyte. The second problem is that the human oocyte and early preimplantation embryo have a poor capacity for homocysteine recycling. This is important as homocysteine inhibits methylation.

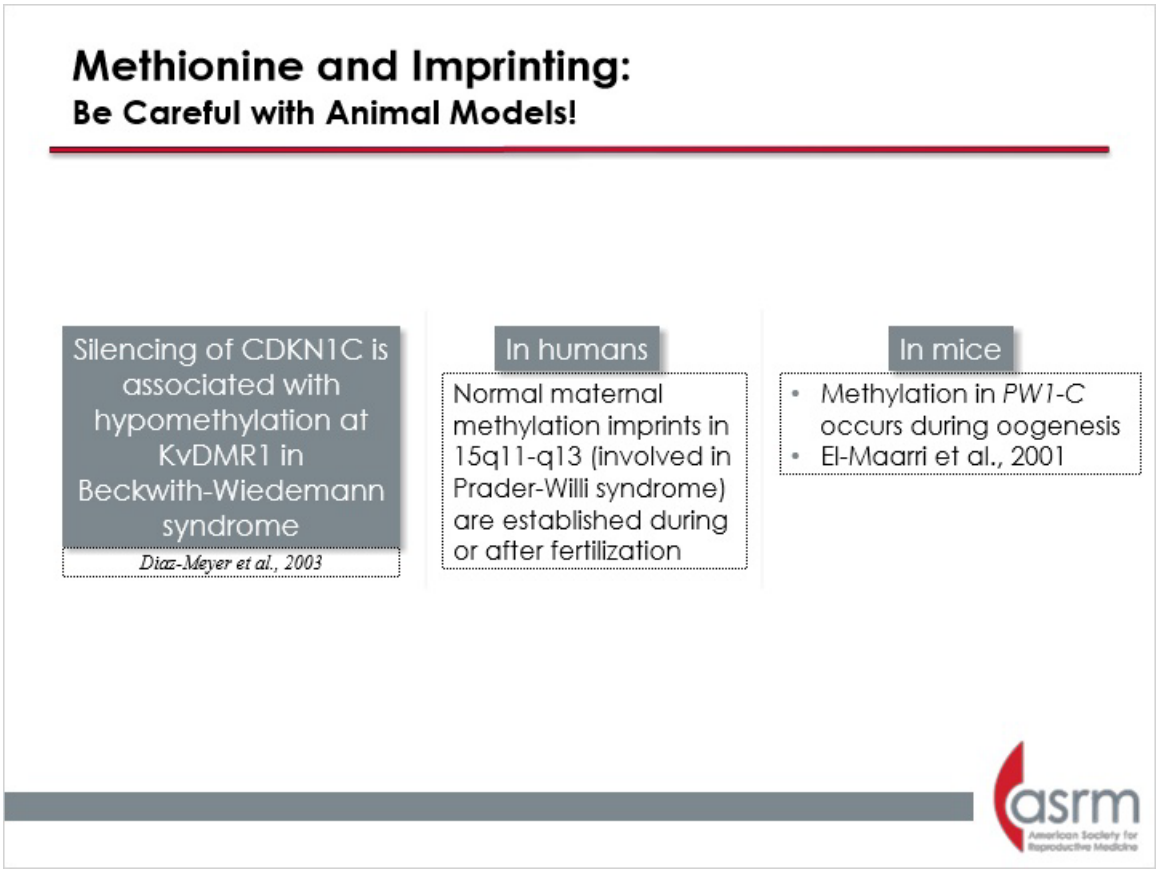
1.44 Homocysteine Recycling in Human Oocytes and Embryos (One-carbon Cycle)



Notes:

A critical biological function is the one-carbon cycle.
The cycle is composed of 4 molecules: methionine and homocysteine are the 2 key compounds.
Homocysteine is released from SAH after the methylation process has been performed. Homocysteine has to be evacuated from the oocyte/embryo as it prevents further methylation processes. It also inhibits the neosynthesis of SAM.
The one-carbon cycle allows a recycling of homocysteine into methionine.
Abnormalities in the one-carbon cycle might lead to hypomethylation in the embryo. In the adult it leads to hyperhomocysteinemia, leading to numerous diseases (cardiac) and hypofertility.
Notably, the cycle requires adequate cofactors: group B vitamins, including folic acid, and zinc. Further, human oocytes have a rather weak capacity to recycle homocysteine. Thus, it is important that women undergoing ART have adequate serum concentrations of vitamin B and zinc. Note that 15% of the Caucasian population has a zinc deficiency.

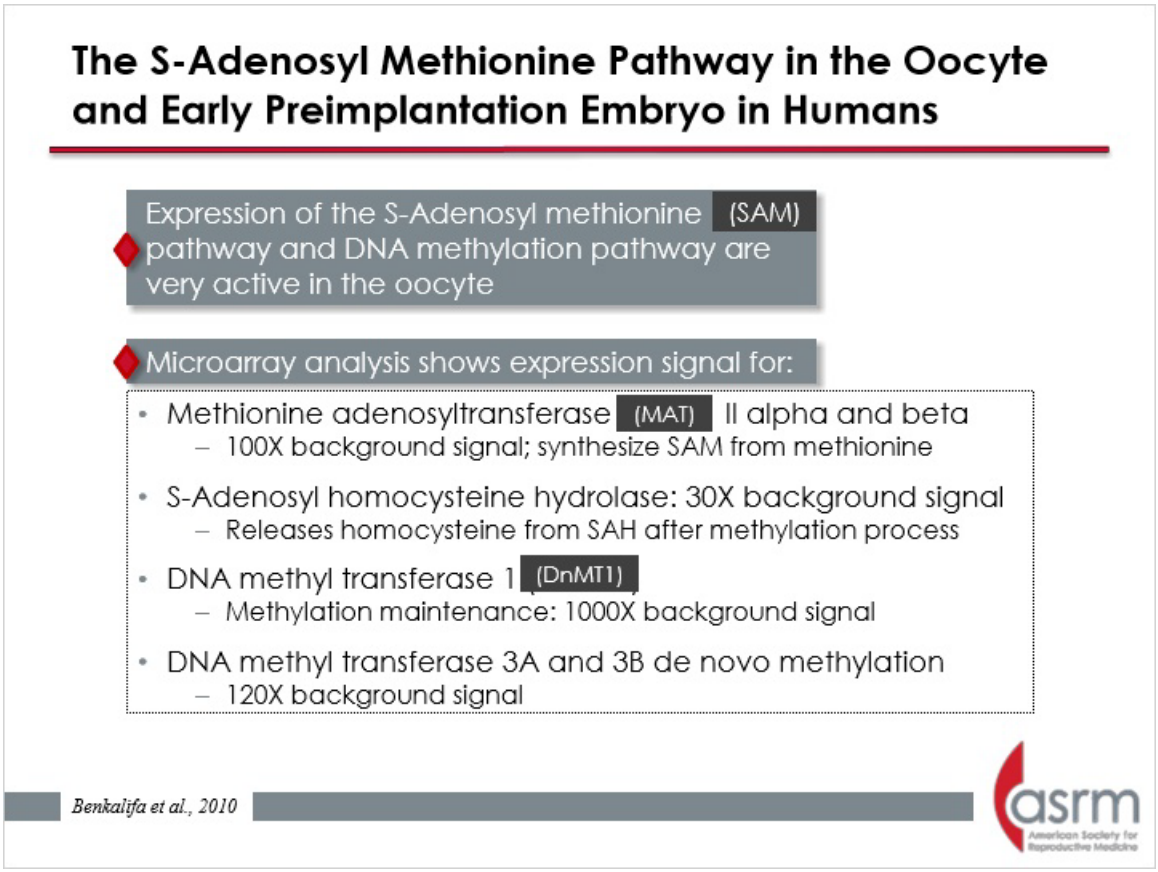
1.45 Methionine and Imprinting:



Notes:

Although animal models provide information about the imprinting process, care must be taken in recognizing limitations of in vitro conditions and the specific aspects of imprinting process in humans. Mice and humans do not always have the same mechanisms of action. For example: silencing of *CDKN1C* is associated with hypomethylation at *KvDMR1* in Beckwith-Wiedemann syndrome. In humans, normal maternal methylation imprints in 15q11-q13 (involved in Prader-Willi syndrome) are established during or after fertilization, whereas methylation in *PW1-C* occurs during oogenesis in the mouse. The methylation process (*de novo* and in majority maintenance) occurs during the very early stages of embryogenesis.

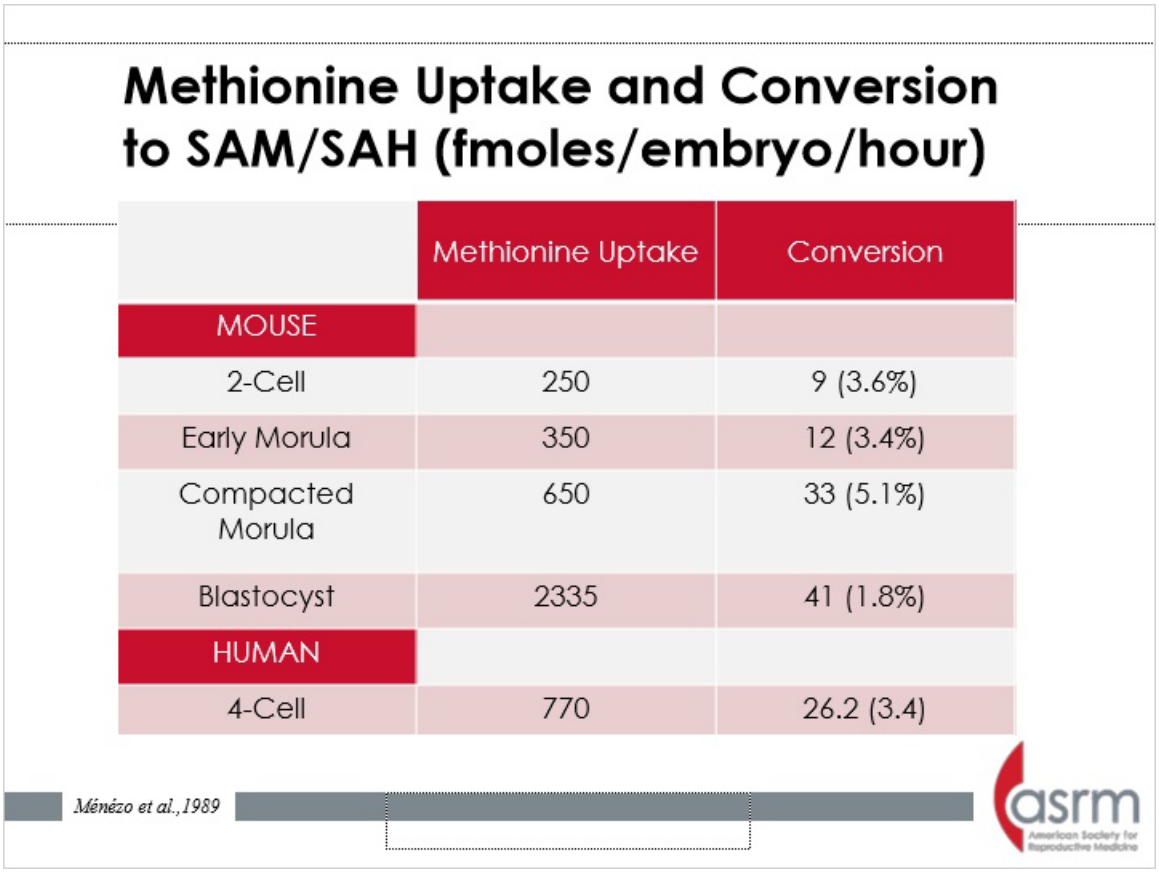
1.46 The S-Adenosyl Methionine Pathway in the Oocyte and Early Preimplantation Embryo in Humans



Notes:

In humans, the SAM synthesis pathway in the oocyte and early preimplantation embryo involves expression of some enzymes in the methylation process.
In the methionine synthesis of SAM, there is release of homocysteine from SAH. DNA methyltransferases are the enzymes involved in DNA methylation. Embryos develop using the reserves stored in the oocyte in the three days before genomic activation.

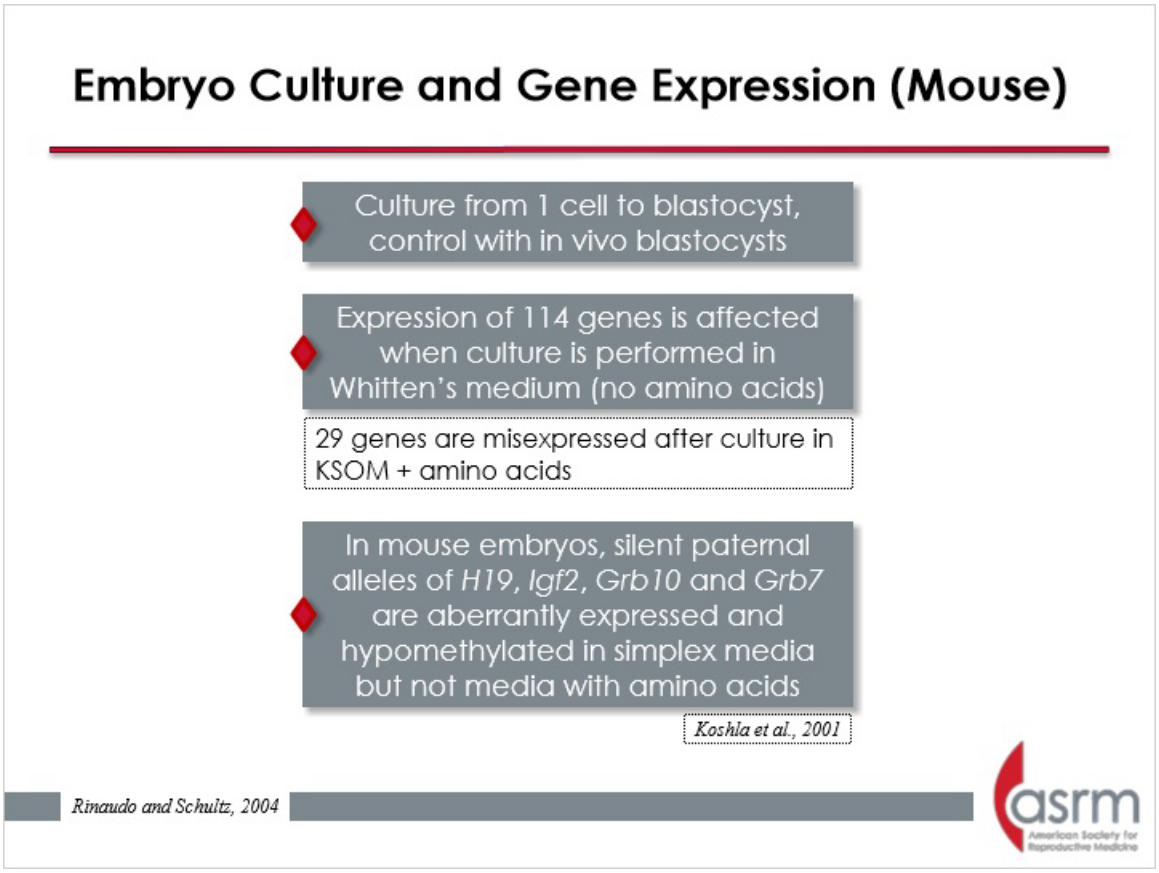
1.47 Methionine Uptake and Conversion to SAM/SAH (fmoles/embryo/hour)



Notes:

One study of SAM synthesis in mouse and human embryos (before genomic activation) is shown here. The process incorporated radiolabelled ^{35}S methionine and then chromatography analysis. The findings demonstrate that methionine is needed in culture media in order to provide fuel for methylation maintenance in the early embryo.

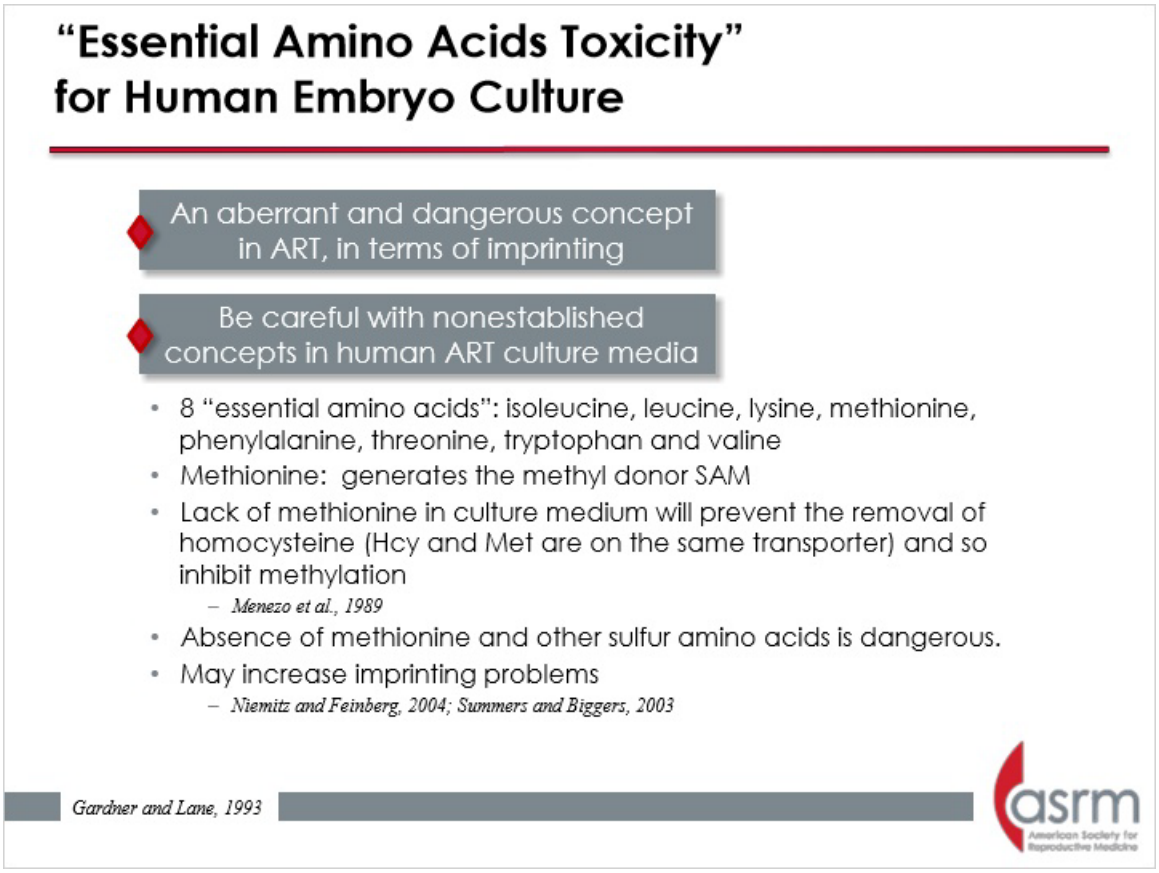
1.48 Embryo Culture and Gene Expression (Mouse)



Notes:

There are problems of gene expression in mouse embryo culture when no amino acids are provided in vitro.

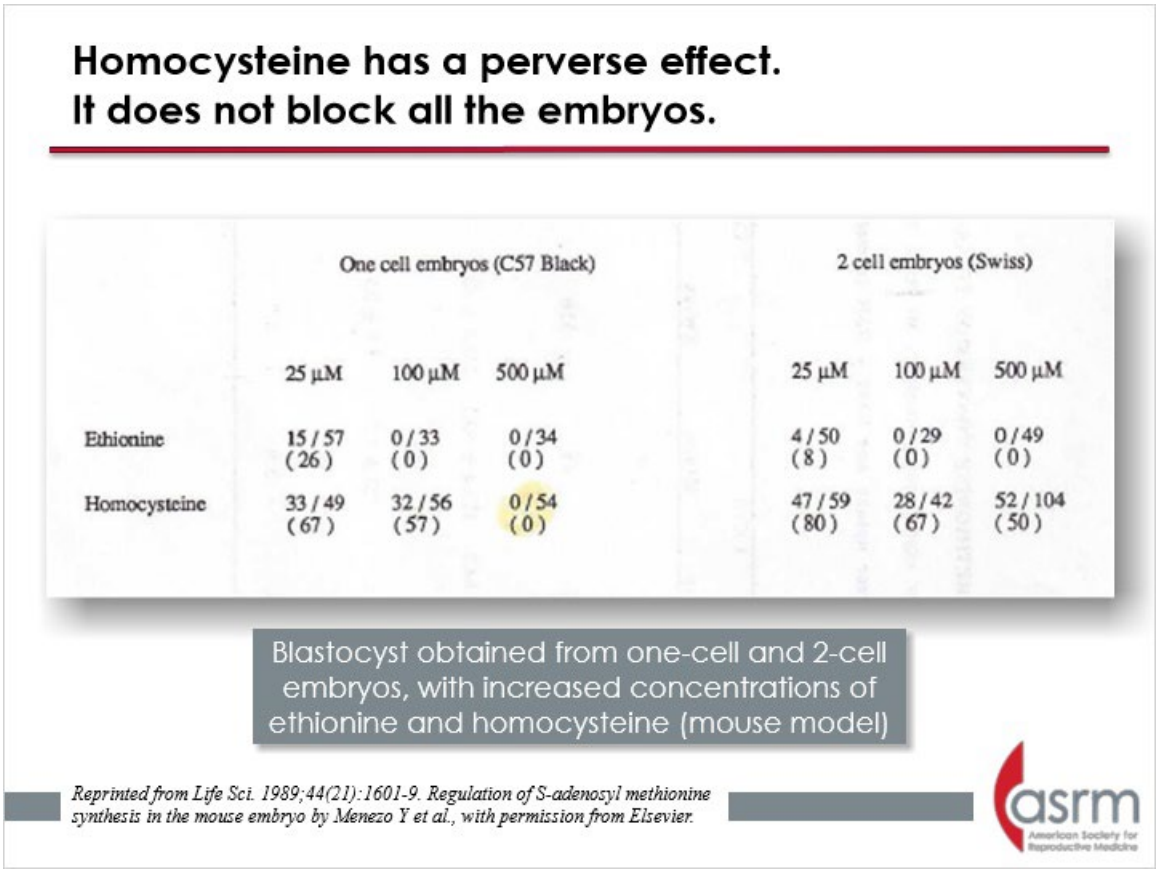
1.49 "Essential Amino Acids Toxicity"



Notes:

A dangerous concept in the ART literature regarding imprinting involves the concept of 8 "essential amino acids": isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine. Recall that methionine generates the methyl donor SAM. Lack of methionine in culture medium will prevent the removal of homocysteine (homocysteine and methionine are on the same transporter) and so inhibit methylation. The absence of methionine and other sulfur amino acids in culture media may increase imprinting problems.

1.50 Homocysteine has a perverse effect.



Notes:

An experimental demonstration showed that methylation is inhibited by homocysteine in the mouse model. Ethionine is a highly toxic inhibitor of methylation; it leads to ethylation instead of methylation. Homocysteine has a perverse effect in that not all the embryos appear to be affected (no developmental arrest) when the homocysteine concentration is moderately elevated. For the human model, consider the one-cell model shown on the left side of the table. This corresponds to culture before genomic activation. In human embryos, genomic activation starts at the 4-to 8-cell stage so it occurs after IVF and at least a total of 3 days in culture. It can be expected that elevated homocysteine will not block all the embryos. But, methylation might be affected.

1.51 Effect of Homocysteine (Hcy) on Methionine (Met) Incorporation and SAM Synthesis in the Mouse Embryo

Effect of Homocysteine (Hcy) on Methionine (Met) Incorporation and SAM Synthesis in the Mouse Embryo

Embryo Stage		Met uptake	% decrease	SAM	% conv.	Methylation index/ SAH/SAM %
	50μM Met/ 0 Hcy	281 (4)	--	9.5 (1.8)	3.4	15.7
1 Cell	50μM Met/ 50 Hcy	174.8 (15)	37.8	6.1 (.3)	3.5	13
	50μM Met/ 500 Hcy	78.2 (4)	72.2	4.9 (.5)	6.3	0

Reprinted from Life Sci. 1989;44(21):1601-9. Regulation of S-adenosyl methionine synthesis in the mouse embryo by Menezo Y et al., with permission from Elsevier.

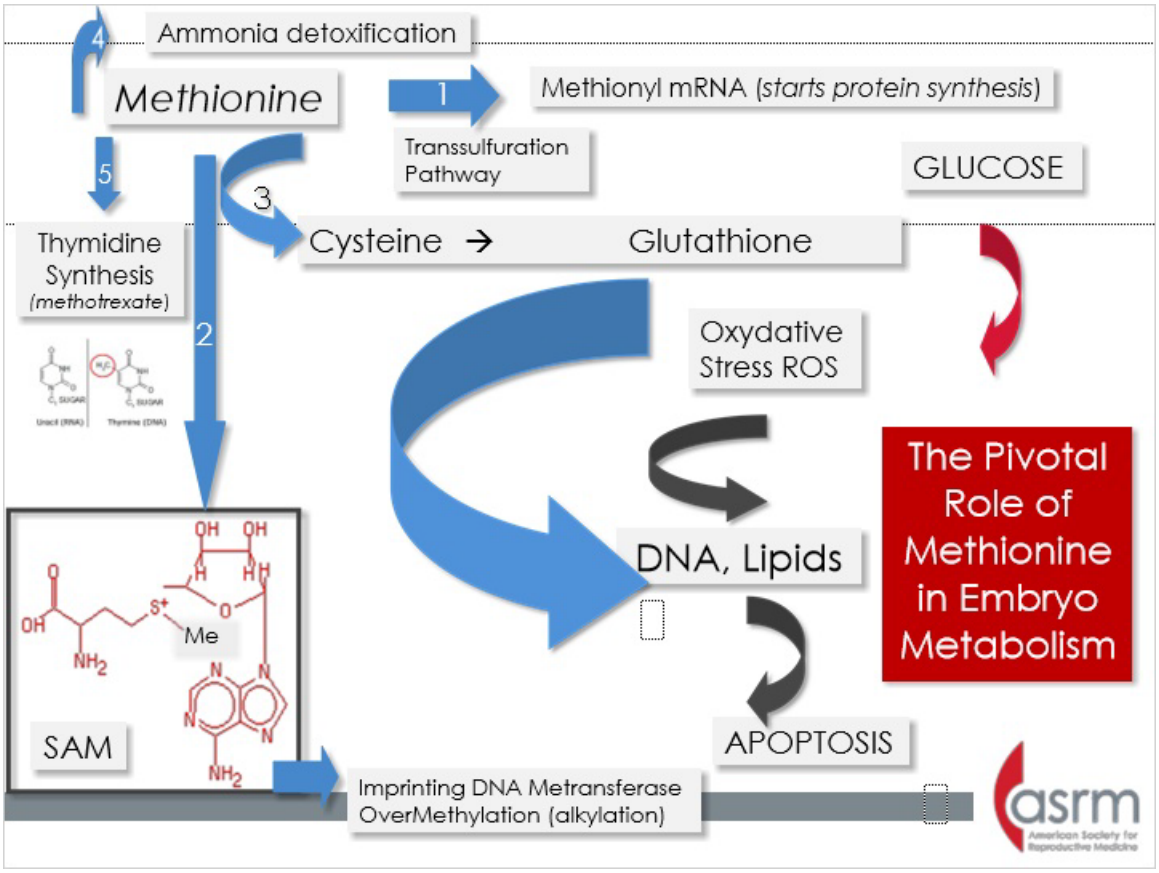
asrm American Society for Reproductive Medicine

Notes:

Methionine and homocysteine compete on the same amino acid transporter. If methionine is not added in the culture medium, homocysteine cannot be exchanged, remains in the embryo, and has to be actively recycled via the one-carbon cycle. Methylation is negatively affected by the presence of homocysteine. A high endogenous homocysteine concentration will lead to a drop in methylation (DNA, proteins and lipids), even if the conversion of Met into SAM has been increased. This corresponds to the human IVF situation and means that the “essential amino acids” toxicity concept is aberrant and dangerous in human IVF, if considering methionine as classified as an “essential amino acid.”

Considering that imprinting problems skip generations, a careful analysis of the health of the offspring originating from IVF in the 2nd and 3rd generations is mandatory.

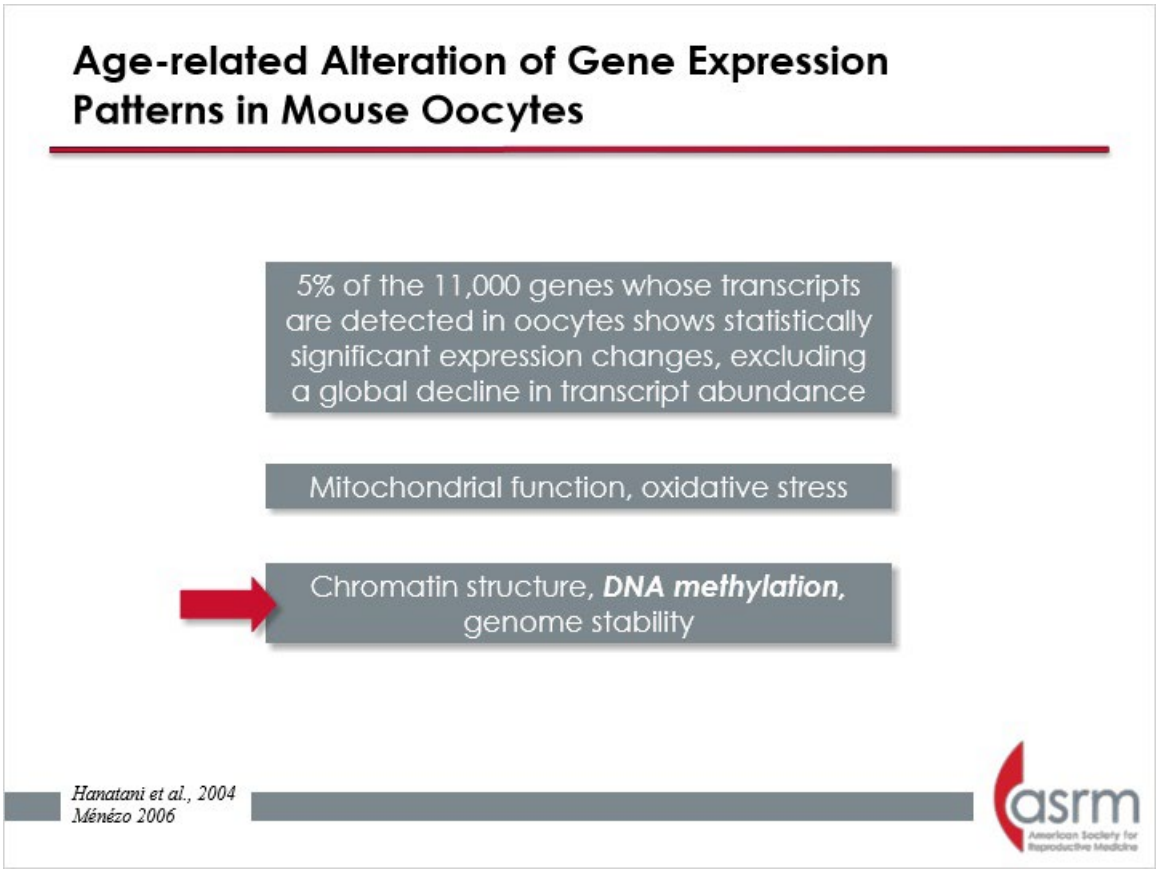
1.52 Methionyl mRNA



Notes:

Methionine has a pivotal role in the embryo; it is not only for imprinting but also for human embryo preimplantation metabolism. Imprinting is a precursor of SAM, the universal biological methylation agent. This includes the thymidine synthesis from uracil (DNA synthesis). Through the methionyl tRNA, methionine starts the protein synthesis. Methionine is also involved in prevention of ammonia accumulation in the embryo. Finally, as a carrier of SH group methionine has a role in the prevention of oxidative stress-related damages especially in the mitochondria.

1.53 Age-related Alteration of Gene Expression



Notes:

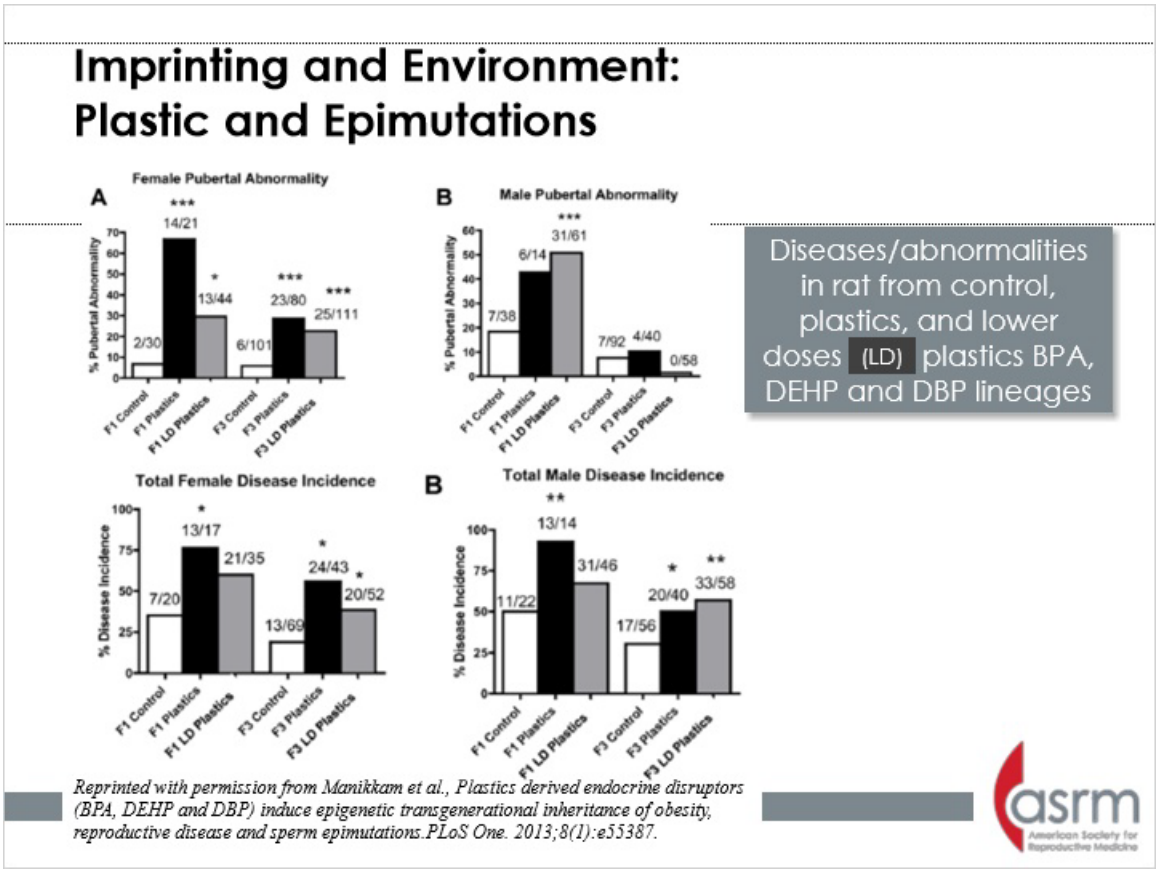
Another observation for ART is that there are age-related alterations in gene expression patterns in mouse oocytes. Five percent of the 11,000 genes whose transcripts are detected in oocytes show statistically significant expression changes, excluding a global decline in transcript abundance. Changes were noted in mitochondrial function, oxidative stress, chromatin structure, DNA methylation, and genome stability. As female ART patients are generally older than the overall population, this makes the culture conditions perhaps even more important.

1.54 Imprinting and Environment

Notes:

Current environment and lifestyle may play a role in increasing imprinting and other epigenetic problems.

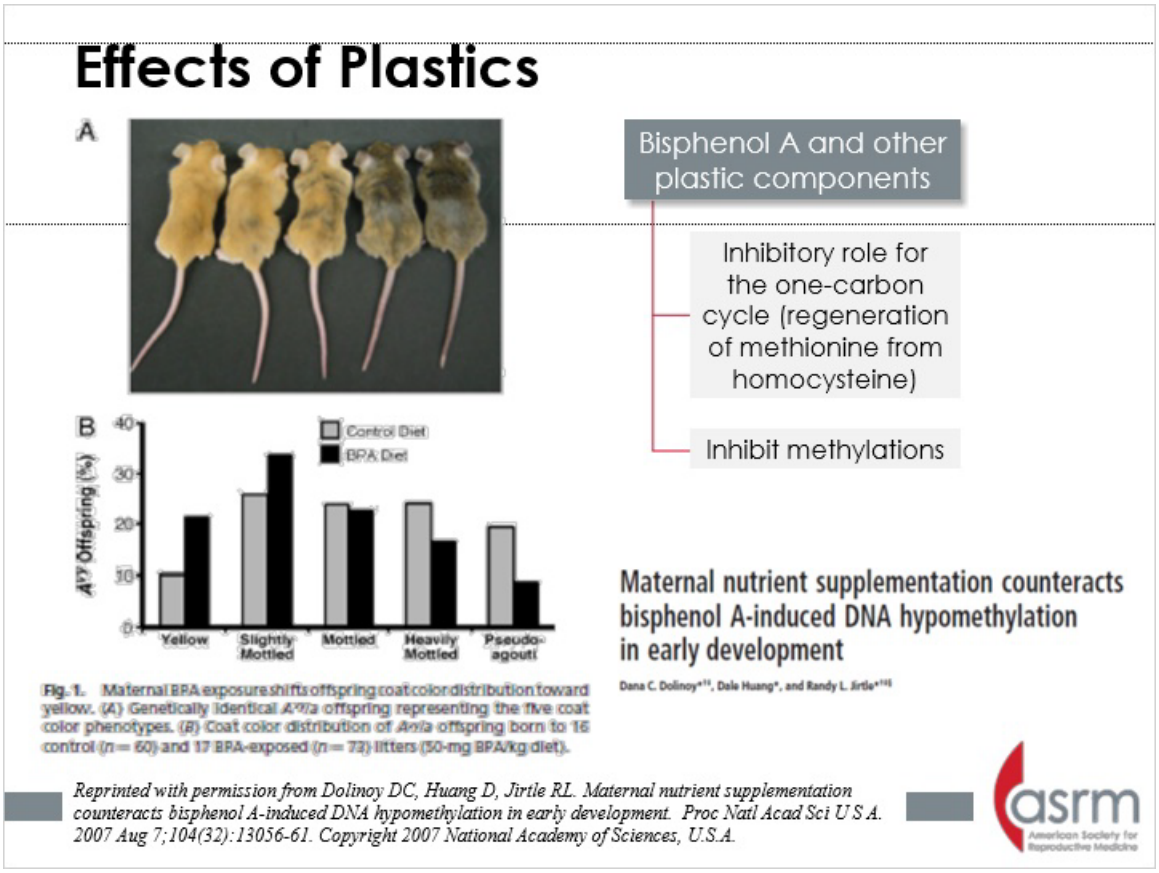
1.55 Imprinting and Environment:



Notes:

In rat models, plastics alter regulation of gene expression.

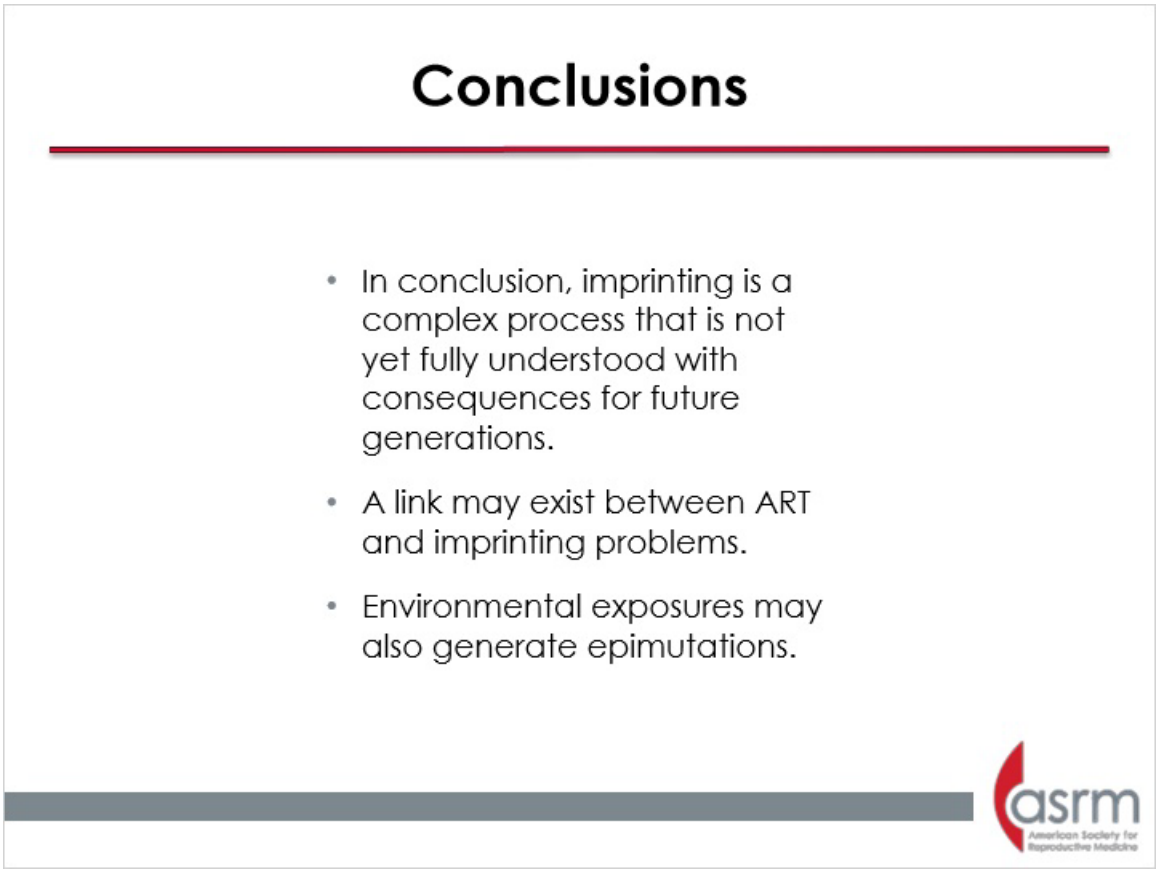
1.56 Effects of Plastics



Notes:

In this mouse model, maternal bisphenol A exposure shifted offspring coat color distribution toward yellow in genetically identical offspring. Coat color variations and pathologies are related to variations of epigenetic marks established early in development. Bisphenol A is clearly an inhibitor of methylation through decreased efficiency of the one-carbon cycle.

1.57 Conclusions



Notes:

In conclusion, imprinting is a complex process that is not yet fully understood with consequences for future generations. A link may exist between ART and imprinting problems, but this has not been established. Environmental exposures may also generate epimutations.

1.58 Thank You

Notes:

Thank you for participating in this educational activity.