Chapter 2

Genetics in Domestic Animal Reproduction

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Abstract

Reproduction and early development in domestic animals are biological processes in which complex genetic events like formation of the germ cells, meiosis, syngamy, zygote formation, cleavage, midblastula transition, dose compensation of sex chromosomes, genetic imprinting, and multiple cell differentiation take place. Many of these processes have great impact in veterinary reproductive medicine and are influenced by the assisted reproductive techniques applied. Altered environmental and metabolic conditions caused by intensive livestock farming influence reproductive success by altered gene expression via epigenetic changes.

Keywords: reproductive genetics, developmental genetics, domestic animals

1. Genetics during germ cell production

Genetics during germ cell production: Mammalian oocytes and sperms originate from the primordial germ cells (PGCs) located in the embryonic mesoderm. Their development is initiated by signals from the extra embryonic ectoderm and the visceral endoderm. In mammals the primordial germ cells invade the genital ridge where they proliferate by mitosis and give rise to either oogonia or spermatogonia. Migration, proliferation, and colonization of PGCs to the developing gonads are controlled by many factors and depend as well on the interaction of PGCs and their surrounding somatic cells. Around the period of PGC migration into the genital ridges sex determination starts. Absence of the expression of the Y-linked gene "sex determining region of Y" (SRY, a transcription factor of the high mobility group box family) leads to female differentiation, therefore the gonads develop into ovaries [1]. Presence of SRY gene expression leads to differentiation of the gonads into testes. As soon as PGCs are formed, the initially bi-potential gonad will continue its differentiation mostly under the influence of somatic cell-derived transcription factors. In female animals after colonization

of the gonad, PGCs will undergo a phase of mitotic proliferation leading to the formation of germ cell nests. Following this event, around birth, the germ cell nests break down and mitotic divisions stop. Germ cells initiate meiosis, become primary oocytes which are surrounded by somatic cells leading to the formation of primordial follicles which continue the female program of development.

Meiosis can be divided into two divisions: meiotic division I and II. The prophase of meiotic division I is extended and can be divided into the following parts: leptotene, zygotene, pachytene, diplotene, and diakinesis. During leptotene, progressive condensation of the chromosomes takes place and the telomeres become associated with the inner nuclear membrane. In zygotene, the homologous chromosomes pair due to the formation of the synaptonemal complex in a zipper-like way. The pachytene is characterized by further condensation of the chromosomes and by occurrence of cross-over events leading to intrachromosomal recombination. In the course of the diplotene, the chromosomes separate again except small anchoring regions the so-named chiasmata. Oocytes progressed to the diplotene stage enter into a prolonged resting phase called dictyotene. Oocytes remain at the dictyate stage of meiosis I throughout oogenesis, until luteinizing hormone (LH) induces final oocyte maturation to metaphase II [2]. Cell division during oocyte meiosis is asymmetrically leading to one oocyte containing most of the cytoplasm and three polar bodies. Formation of the second polar body is a requisite of oocyte maturity.

Spermatogenesis, the process that gives rise to fertile sperm, occurs in a similar manner in many animal species, including domestic mammals and birds. Male germ cell development starts with emergence of primordial germ cells (PGCs), which migrate and associate with somatic cells that form the testes. Within the gonad, PGCs localize to regions that are competent to serve as stem cell (SC) niches, where they develop into spermatogonial SCs (SSCs). SCs divide to form new SCs and spermatogonial daughter cells. The daughter cells proliferate, undergoing sequential rounds of mitosis and incomplete cytokinesis to form syncytial groups of spermatogonia. Following a series of synchronous mitotic amplification divisions, spermatogonia differentiate into spermatocytes, which then undergo meiosis. As in the earlier mitotic divisions, meiotic cytokinesis is incomplete, resulting in syncytia of interconnected haploid spermatids. Postmeiotic spermatids undergo an extensive period of differentiation, or spermiogenesis, in which they form sperm-specific organelles, including the sperm head, acrosome, basal body, specialized mitochondria, and flagellum. At the end of spermiogenesis, mature sperm are separated from each other by removal of the remnants of incomplete cytokinesis, released from the gonad, and stored prior to delivery by ejaculation.

In both, oogenesis and spermatogenesis meiosis leads to intrachromosomal recombination (cross-over occurring in prophasis of meiosis I) and in interchromosomal recombination (random mixture of maternal and paternal chromosomes during both meiotic divisions), reducing the diploid chromosome complement to a haploid level. As a result of oogenesis, one haploid oocyte and three haploid polar bodies develop. In spermatogenesis four haploid sperm come into being. In female eutherian mammals meiosis is continued by luteal hormone stimulation and finished during fertilization. In male eutherian mammals meiosis takes place all the time.

2. Genetics during fertilization

During their active and passive migration in the femal genital tract, the sperms obtain their fertilization capacity by capacitation and hyper activation two processes which seem to proceed largely independent. The glycoprotein composition changes during capacitation of the cell membrane. In domestic mammalian species fertilization takes place in the ampulla of the oviduct where the metaphase II oocyte surrounded by the acellular zona pellucida and the cellular cumulus (corona radiata) is present. The sperm leading to fertilization has to fulfill its acrosomal reaction. This is mediated by partial fusion of the cell membrane with the outer acrosomal membrane leading to the release of hyaluronidase. This enzyme enables the sperm to cross the corona radiata. At the zona pellucida binding to species specific zona proteins is necessary (species specificity). After detachment of the cell and outer acrosomal membrane the inner acrosomal membrane rises to the top delivering the protease Acrosin which enables the penetration of the zona pellucida. Now the sperm enters to the perivitelline space and the inner acrosomal membrane and the cell membrane of the oocyte fuse. In natural fertilization, only the head of the sperm enters the cytoplasm of the oocyte meaning that no mitochondria from the sperm can participate (maternal inheritance). After entrance of the first sperm, depolarization of the cell membrane, degranulation of the corticalis granula, shrinkage of the ooplasm, and zona hardening takes place in order to avoid multiple fertilizations. Fertilization by more than one sperm would lead to aneuploid (n = 3, 4, 5…) chromosome complement. These embryos stop development before reaching the blastocyst stage. After entrance of the sperm the oocyte finishes the second meiotic division. Female and male pronuclei form and fuse to form a diploid nucleus in a process named syngamy leading to the zygote entering now into cleavage.

3. Genetics of eutherian sex determination and dose compensation

Sex determination in eutherian mammals is mediated by the chromosomes X and Y. Absence of the Y chromosome (SRY gene) leads to female development. Male genital development is mediated by production of the anti-Müllerian hormone (AMH) produced by the Sertoli cells of the embryonal testes. This peptide hormone, belonging to the transforming growth factors (TGF) family, leads to the regression of the Müllerian duct from which in the female the oviduct, the uterus, and the vagina are formed.

Aberrant sex chromosome numbers can arrive by false segregation during meiosis leading to multiple X or Y chromosome numbers. If one or more Y chromosomes are present the sex is male, if no Y chromosome is present the sex is female. Additional X chromosomes will be inactivated to a large extend (only a few genes escape from this inactivation) according to the Lyon hypothesis. This inactivation is mediated by the noncoding X inactive-specific transcript (XIST) RNA. The corresponding gene is located at the X Inactivation Center (XIC) at the X chromosome. Coating of the X chromosome to be inactivated by the XIST RNA leads to the deactivation of most of the genes: Only the XIST gene itself and about one quarter of the genes present mainly in the pseudo autosomal regions of the X chromosome escape

from this inactivation. Before inactivation starts small quantities of XIST RNA are produced by both X chromosomes. After inactivation XIST is expressed from the inactivated X chromosome, whereas XIST expression from the active X chromosome ceases. Cytologically the inactivated X chromosome can be visualized as Barr body. The XO syndrome named Turner syndrome is sometimes observed in mares which are not fertile. In cats, the coat color gene is located at the X chromosome leading to the absence of tortoiseshell and calico coats in the X0 (Turner syndrome) female cat. In male cats, this coloring is only possible in the rare case of the Klinefelter syndrome [3]. These animals typically have an extra X chromosome (XXY) and their cells undergo an X-inactivation process like that in females. Additional Y chromosomes are described in humans as a result of a nondisjunction in meiosis II. This chromosomal aberration seems to have less influence since only a few genes are transcribed from the Y chromosome. Nevertheless, in humans, a higher testosterone concentration was observed in chromosome pair in their pseudoautosomal region(s) (PAR). In the horse this region was very helpful in mapping of the euchromatic region of the equine Y- chromosome. These investigations were very helpful for the prediction of male fertility in horses and are therefore considered in horse breeding [4, 5].

Rarely as a consequence of a translocation, the SRY region containing the testes determining factor (TDF) to the X chromosome during meiosis a male cytological having two X chromosomes can be created. These XX men are infertile since they lack other genes necessary for the production of mobile spermatozoa [6].

Before fertilization the XIST gene in the ripe oocyte is inactive, meaning that the X chromosome is active. Fertilization by a male (Y) sperm does not change this status. If a femal (X) sperm enters the oocyte one active XIST gene is now present producing XIST RNA which inactivates also the second X chromosome. This status is not stable since for maintenance of inactivation a protein named EED (polycomb protein EED, responsible for maintaining the transcriptional repressive state of genes over successive cell generations) also coded at the X chromosome is necessary [7, 8]. Therefore, both X chromosomes and also the XIST genes become active again at the morula stage leading to random inactivation of either the matenal or paternal X chromosome. That means that female eutherian mammals are mosaics in relation to maternal or paternal X inactivation, a phenomenon which becomes evident in X coded genes like coat color of the domestic cat.

4. Genomic imprinting

Genomic imprinting became evident by nuclear transplantation experiments at the pronuclear stage in mice [9]. Enucleated oocytes reconstructed with either only two femal or two male pronuclei exhibited different developmental potential concerning the embryo proper and the extraembryonic tissue. Further investigations revealed the nature of this phenomenon: In imprinted genes the female and male promotor regions have different methylation status leading to different gene expression in the early embryo. In humans and mice about 80 imprinted genes are known many of which are involved in embryonic and placental growth and development. Sometimes hybrid offspring of two different species may exhibit unusual

growth due to the novel combination of imprinted genes. This becomes especially evident in reciprocal Lion-Tiger crosses [10]. The majority of imprinted genes are found in clusters, the so-called imprinted domains, suggesting a high degree of coordinated regulation. Imprinting is a dynamic process: imprints can be generated and removed at different time points of development from one generation to the other. In the germ line the imprints are erased and then reestablished according to the sex of the individual. In the developing sperms (during spermatogenesis), paternal imprints are established, whereas in developing oocytes (during oogenesis) a maternal imprints are established. In mammals genomic imprinting is present in therian mammals (marsupials and placental mammals) [11, 12].

5. Genetics during preimplantative eutherian embryo development

The eutherian oocyte is oligolecithal. After fertilization, the femal and male pronuclei fuse to establish the diploid zygote nucleus. The eutherian zygote undergoes now rotational holoblastic cleavage. These fast cell divisions consist only in synthesis phase and mitosis and subdivide the former ooplasm onto the blastomeres. Zygotic transcription starts dependent on the species at the two-, four-, or eight-cell stage. This process is named midblastula transition (MBT). So far the early embryo translates only maternally inherited mRNAs just present in the oocyte. So far the zygotic chromatin is hypo-acetylated and methylated, which means that most of the genes are repressed in a heterochromatic state. Now embryo starts to transcribe its own DNA and its cells become motile and the cell divisions loose synchrony. At the eight-cell stage most of the blastomeres become polarized and develop tight junctions with the other blastomeres. At the 16-cell state, the embryo is named morula. The outer cells become bound tightly together by the formation of cell junctions (compact morula). This process initiates the differentiation of two distinct cell populations: outside cells characterized by polarity and undifferentiated inside cells. The outer cells develop into trophoblast cells, which are epithelial cells connected by tight junctions in order to seal the extracellular passage. They express Na+/K+ ATPases and aquaporins at the apical and basal membranes in order to exchange sodium in from the outside by cellular potassium. This leads to electrochemical gradient which is equalized by water influx from the outside leading to blastocoel formation. The embryo is now called a blastocyst. At least in the horse expansion of the blastocyst seems to be under hormonal control [13, 14]. After implantation the trophoblast cells will develop into the fetal part of the placenta. The undifferentiated inner cells are displaced by the water influx to one side of the cavity to form the inner cell mass (ICM). It will develop into the embryo proper and some extraembryonic membranes.

So far the blastocyst like all previous embryonic stages are still surrounded by the acellular zona pellucida since the size of the embryos increased only marginally. Now a rapid expansion mainly due to fluid accumulation takes place and therefore the embryo hatches (human, mouse, pig, cow, sheep, goat, camels) or the zona pellucida is replaced by an embryonic capsule participating in enlargement. Hatched embryos show early placenta formation whereas encapsulated embryos exhibit prolonged preimplantative phase. At that time in most species the maternal recognition of pregnancy (MRP) takes place ensuring the maternal changes necessary for further development.

6. Genetics during mammalian placentation and fetal development

Mammalian placentation can be rated according to the implantation depth and surface expansion. Concerning implantation depth the invasiveness of the chorion is the crucial factor: epitheliochorial (pig, horse, ruminants), endotheliochorial (dog, cat), or hemochorial (human) describe implantational depth. Surface expansion is also influenced by placental capacities and number of fetuses: diffuse, cotelydonaria, zonaria, and discoidal. Implantational depth influences the quality of exchange of substances: The human hemochorial placenta enables the transfer of maternal antibodies into the fetal blood which is very important during the first weeks after birth. From the fetal face also a transfer of fetal cells into the maternal blood stream is probable. This microchimerism can persist even for some years and might be responsible for occurrence of autoimmune diseases in women [15]. In carnivoral placentation, such a microchimerism seems to be probable but was not investigated so far. The circumstances concerning the antibodies seem to be similar like in humans: "In carnivora and rodentia the blood of the newborn has roughly the same globin content as the maternal blood, whereas in those animals in which the placental barrier is relatively thick, such as the cow and pig, the fetus receives few if any antibodies before it is born" [16]. In horses despite their epitheliochorial placentation a microchimerism was proven recently by detection of SRY by means of digital PCR [17]. One of the most probable explanations for this microchimerism seems to consist in the migration of the chorionic girdle cells forming the endometrial cups.

Another form of microchimerism often arrives in cattle during twin pregnancies with different fetal sex named Freemartin syndrome: In most bovine twin pregnancies anastomoses form between the fetal circulations leading to exchange of male hormones (anti-Müllerian hormone, testosterone). Depending on the time of anastomose formation during pregnancy the femal fetus becomes masculinized resulting in intersexuality and infertility. In other domestic ruminantia the occurrence of freemartin syndrome is very rare [18].

7. Genetic alterations caused by assisted reproduction techniques

During *in vitro* maturation (IVM) and *in vitro* fertilization (IVF) the gamets respectively the early embryo are exposed to artificial environments which can differ in both directions from the natural conditions. Absence of essential substances often leads to the termination of development, whereas supernutrition can result in epigenetic changes which manifest later in pregnancy and during birth. One of the most frequent abnormities connected with IVF is the large offspring syndrome in cattle and sheep resulting in oversized fetuses [19].

Somatic nuclear transplantation of domestic animals can cause incomplete epigenetic reprogramming of the somatic nucleus leading to incapacity of blastocyst formation. Therefore, blastocyst rates are relatively low around 2–3%. High rates of embryonic loss, abortions due to placental aberrations, and high postnatal mortality were observed in cloned animals. Directed epigenetic changes during somatic cell culture can ameliorate the blastocyst rate obtained (up to 10%) and reduce the problems during pregnancy and birth [20].

During intracytoplasmic sperm injection (ICSI) a mature oocyte is injected with one sperm or a haploid progenitor of the sperm. This leads to omission of the natural selection of sperms in the femal genital tract and sometimes to fertilization with a sperm that under natural conditions never would be able to fertilize an oocyte. Using ICSI no acrosomal reaction and hyperactivation is necessary. Even dead sperms can be used if the nucleus is intact. Different from natural fertilization the whole sperm is injected into the oocyte leading to a mixture of mitochondria. There seems to be a mechanism to favor the mitochondria originally contributed by the oocyte (also in cloned animals). Nevertheless also coexistence and the reverse are possible [21]. In humans oocytes from aged women can be ameliorated by cytoplasma donation from an oocyte of a young women leading to a mixture of different mitochondria similar to the situation after nuclear transplantation.

In all these techniques applied in animal-assisted reproduction, the ability of blastocyst formation is the critical point to rate the success of the method. In human-assisted reproduction, earlier stages are transferred in multiple numbers often leading to twin and multiple pregnancies.

Different other techniques like oocyte transfer (OT) gamete intrafollopian transfer (GIFT), and zygote intrafollopian transfer (ZIFT) have been developed. Depending on the time of incubation of the gametes and the abandonment of embryo culture these techniques have reduced potential for epigenetic alterations.

PCR techniques using genetic material from blastomeres after the puncture of the embryo before transfer can be used for sex determination, preimplantative diagnostic, and marker-assisted selection in animal breeding.

8. Genetics in poultry reproduction

In birds and monotremes the sex is determined genetically but the exact mechanism is still unknown. All species show a ZZ/ZW sex chromosome system. Contrary to therian mammals it is characterized by female (ZW) heterogamety and male homogamety (ZZ). So far different hypothesis exist: Avian sex might be determined by dosage of a Z-coded gene or by a W-coded gene or may be both. A SRY gene or homolog is absent in birds and monotremes [22–24].

High performance chickens are bred by heterosis breed: In grandparent linages different traits (like high laying performance or good eggshell quality) are fixed by linage breed. In the parent generation the most suitable crossing between those linages will be determined. In case of the recurrent selection one father or mother line will be mated to a standard line. In the reciprocal recurrent selection each parent combination will be tested as mother and father line and rated according to the performance of the progeny.

Due to the chicken genome sequencing finished in 2005 many genes affecting vitality and disease resistance are going to be identified and become focused by the breeding organizations. This might lead to a reduction of therapeutic agents in poultry production.

9. Genetics in fish reproduction

Most of the fishes fostered in aquaculture belong to the teleosts. Many of them show hermaphroditism meaning that both sexes are present in one individuum but active at a different time during life. Most of the farmed fishes in aquaculture just release their eggs and sperms at the same time nearby in the water. In farms the eggs and sperms will be delivered artificially by abdominal massage and admixed together. This is the most suitable state for artificial manipulation like chromosome manipulation and gene transfer. Egg size is very important in fish breeding since large fry have to be fed later. Fertilization takes place by entering of the sperm through the micropyle of the fish egg, a narrow channel through the outer egg membrane. It is accomplished by the fish sperm swimming to the egg surface, locating the entry to the micopyle, and swimming down it to make contact with and penetrating the inner egg membranes. The membrane lifts after penetration of the first sperm which seems to be the mechanism for avoidance of polyspermy. Generation of triploid teleosts has become a widely used tool in order to protect wild fishes from hybridization since these animals are sterile. Production of monosex individuals has a similar effect [25]. Genetic engineering will have a great potential for the generation of disease resistance and low oxygen tolerating fish in the future. Interestingly somatic nuclear transplantation in teleosts leads to nucleocytoplasmic hybrids, meaning that the cytoplasm of the oocyte is able to inherit certain features. The molecular basis of this phenomenon is not elucidated so far [25].

10. Further perspectives of genetics in domestic animal reproduction

In many of the domestic animal species genome projects are ongoing or just finalized meaning that genes important for reproduction can be detected easier in order to investigate their specific role and the influences by the environment and metabolic state of the animal. In dairy cows classical selection has shown a negative correlation between daily milk yield and fertility parameters. In this regard, the metabolic state of the animal might be the limiting factor; therefore, the expression of reproductive genes under these conditions should be of high interest [26]. Modulation of gene expression is mediated not only by epigenetics (DNA methylation, histone modulation) but to a large extent also by micro-RNAs affecting mRNA stability. In this context the reproductive performance is influenced by the quality of the allele inherited, their epigenetic status, as well as the metabolic status of the animal.

In assisted reproduction sexing of sperms and embryos will become more important. Improvement of sexing technologies will allow a wider use also in domestic species which so far were excluded due to reproductive necessities (insemination doses, sensitivity of the sperm cells, survival time of the sperm cells). In embryos noninvasive techniques for sexing and preimplantative diagnostic may be possible in the near future. Selection of the sperm used for fertilization in intracytoplasmic sperm injection (ICSI) might change from motility alone to other, more complex criteria. *In vitro* culture conditions will be improved by adaption to natural conditions avoiding aberrant gene expression leading to phenomenon like the

large offspring syndrome. In animal cloning the reprogramming of the donor cells will be improved in order to reach blastocyst rates around 20%.

In the social context the techniques applied in assisted reproduction should be rated in the correct light by understanding their way of action and their advantages in comparison to the objective risks.

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