Livestock Epigenetics

Epigenetics is an accelerating area of research with broad application to better understanding basic biological functions. In recent years, the study of epigenetics in livestock has increased our knowledge of animal health, reproduction, production, and development, which has far-ranging implications for the development of healthier foods to cutting-edge biomedical advances. Livestock Epigenetics brings together the wide array of research being done in livestock species and provides a valuable synthesis of the field.

Livestock Epigenetics is designed to cover a comprehensive and essential variety of topics on the epigenetics of key domestic species, including cattle, pigs, sheep, chickens, and horses. Chapters range from topics related to animal development and reproduction, molecular and statistical aspects of imprinted genes, and the role of epigenetics on animal health and nutrition.

The material found in these chapters sheds new light on our understanding of basic animal genetic and biological processes.

Livestock Epigenetics is written by a global team of leading researchers in the field of epigenetics. The collected work will be a valuable guide to this growing and evolving field for years to come.

Editor

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Contents

Contributors vii
Preface xi

1. Epigenetics of Mammalian Gamete and Embryo Development 3
   Nelida Rodriguez-Osorio, Sule Dogan, and Erdogan Memili

2. Epigenetics of Cloned Preimplantation Embryos of Domestic Animals 27
   X. Cindy Tian and Sadie L. Marjani

3. Roles of Imprinted Genes in Fertility and Promises of the Genome-Wide Technologies 43
   Ashley Driver, Wen Huang, and Hasan Khatib

   Pasqualino Loi, Antonella D’Agostino, Marta Czernik, Federica Zacchini, Paola Toschi, Antonella Fidanza, and Grazyna Ptak

5. The DLK1-DIO3 Imprinted Gene Cluster and the Callipyge Phenotype in Sheep 73
   Christopher A. Bidwell, Ross L. Tellam, Jolena N. Waddell, Tony Vuocolo, Tracy S. Hadfield, and Noelle E. Cockett

6. Genomic Imprinting and Imprinted Gene Clusters in the Bovine Genome 89
   Ikhide G. Imumorin, Sunday O. Peters, and Marcos De Donato

7. Imprinting in Genome Analysis: Modeling Parent-of-Origin Effects in QTL Studies 113
   Suzanne Rowe, Stephen Bishop, and D. J. de Koning

8. Epigenetics and Animal Health 131
   Juan Luo, Ying Yu, and Jiuzhou Song
vi Contents

9. Epigenetics and microRNAs in Animal Health 147
   Fei Tian and Jiuzhou Song

10. Nutrients and Epigenetics in Bovine Cells 161
    Cong-jun Li, Robert W. Li, and Ted H. Elsasser

Index 179
Color plates appear between pages 148 and 149.
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Preface

In recent years, we have witnessed remarkable positive changes in the perception of the value of livestock genetic and epigenetic research among federal and nonfederal agencies and in the scientific community more generally. First, it is becoming widely accepted that human health benefits from the promotion of animal health. Human consumption of healthy meat, eggs, dairy, and other animal products necessitates healthy animals. Second, the wide range of phenotypic and genotypic diversity in livestock populations facilitates the use of these species as models for certain human diseases and health traits. There are plentiful historic and contemporary examples of domestic animals being used in biomedical research, including vaccinations (cowpox virus), xenotransplantations (heart valves of pigs), reproductive biology and cloning (Dolly, the first cloned mammal), developmental biology (limb patterning in chicken), metabolic diseases (malignant hyperthermia in the pig), and neurodegenerative diseases (prions were first isolated from sheep and goat) (http://www.adsbm.msu.edu/).

At present, epigenetic studies are focused on understanding the mechanisms of actions of chromatin modifications that regulate the machinery of gene expression. Although DNA mutations are crucial in the development of diseases and other phenotypes, it is becoming increasingly evident that these mutations are not sufficient to fully explain disease risks and that “additional” factors are equally important. A major element of the “additional” factors is epigenetics, which plays a key role in phenotypic variations, some of which appear to be transgenerationally inherited in almost all organisms. The acceleration of epigenetic research over the last few years is impressive. Indeed, it is not easy to find a biological question that does not engage an epigenetic component.

There is ample and accumulating evidence of the influence of environment, including diet, on gene actions through the manipulation of the epigenome. Currently, the best known example of an epigenetically sensitive gene affected by an environmental factor is Agouti viable yellow and the maternal diet in the mouse. Interestingly, it was found that methyl supplements in the diet of pregnant mice increased the methylation level of the Agouti gene, consequently causing coat color changes in the offspring (Cooney et al., 2002; Waterland et al., 2008). Further studies in rats showed that high-fat diet consumption by sires induced increased body and body fat weights and higher glucose tolerance and insulin resistance in their female offspring (Ng et al., 2010). Another fascinating example of nutritional effects on DNA methylation and
gene expression is the social honey bee *Apis mellifera*. Although the queen bee and her workers are genetically identical, whole genome DNA methylation analysis of the brain revealed that hundreds of genes were differentially expressed between the queen and the workers (Lyko et al., 2010). The methylomes of the adult queen bees are most likely determined by the phenyl butyrate component of the royal jelly which regulates the epigenetic networks controlling gene expression in the brain (Lyko et al., 2010).

The majority of epigenetic studies have been performed in mouse and humans and to a lesser extent in livestock species, despite the contributions of livestock research to the body of knowledge in genetics and gene regulation. Therefore, the objective of this book is to introduce the most up-to-date research on epigenetics in livestock species.

The first four chapters cover the influence of epigenetic mechanisms on the developmental competency of spermatozoa, oocytes, and embryos in mammals; the epigenetic aberrations in cloned embryos from domestic species, including cattle, pigs, rabbits, sheep, goats, and horses; roles of imprinted genes in early embryonic development; and the use of sheep as an animal model to monitor the phenotypic and epigenetic effects of in vitro embryo production and culture. Chapters 5–7 present a very comprehensive review of the molecular and statistical aspects of imprinted genes and parent-of-origin effects on production traits. The callipyge trait in sheep, which was the first demonstration of a non-Mendelian mode of inheritance in mammals, is extensively reviewed in Chapter 5. A thorough review of imprinted genes in the bovine genome, including sequence characteristics, current imprinting status, regulations of imprinted gene clusters, and the effects of imprinted genes on quantitative traits in cattle, is presented in Chapter 6. A detailed description of the quantitative genetic properties of imprinted genes and the detection of these effects in experimental crosses, general pedigrees, and association studies is presented in Chapter 7. Chapters 8 and 9 summarize and discuss the roles of epigenetics in health and disease, the prospects of epigenetics in disease prevention and disease resistance, and recent findings on the relationships between microRNAs and epigenetics. Also, some evidence of microRNAs targeting the epigenetic machinery and the effects of epigenetic regulation on microRNA biogenesis in the context of disease diagnosis and therapy are discussed. Chapter 10 presents information on the role of dietary components in changing epigenetic patterns and the impacts on functional genomic research in bovines and on farm animal industries.

This book is designed to cover a comprehensive and essential variety of topics on the epigenetics of domestic species, including cattle, sheep, chicken, and horses. With the rapid growth of epigenetics research and with the possible use of epigenetics therapy in the near future, it is hoped that this book will serve students at both undergraduate and graduate levels, researchers in genetics and epigenetics, and others interested in this emerging science.

I wish to thank my colleagues Erdogan Memili, Cindy Tian, Ashley Driver, Wen Huang, Pasqualino Loi, Christopher Bidwell, Ross Tellam, Ikhide Imumorin, DJ de Koning, Jiuzhou Song, and Congjun Li from Europe, Australia, and the USA who have put enormous efforts into this project.

Hasan Khatib
Livestock Epigenetics
Chapter 1
Epigenetics of Mammalian Gamete and Embryo Development

Nelida Rodriguez-Osorio, Sule Dogan, and Erdogan Memili

SUMMARY

Roots of mammalian development stem from successful gametogenesis and embryogenesis. Many aspects of developmentally regulated events in gamete and embryo biology involve epigenetic changes that impact gene expression and thus function. From the moment an oocyte and a sperm cell come together to form a zygote, up to the formation of the blastocyst, there are dramatic epigenetic changes that determine the success of the developmental program. This chapter will first provide an overview of oogenesis, spermatogenesis, and the process of fertilization,
which, when successfully accomplished, leads to embryogenesis. We will then review the epigenetic mechanisms regulating life at the onset of development, particularly DNA methylation, posttranslational modifications of core histones, chromatin remodeling, and a related concept, noncoding RNAs. We will address the influence of epigenetic mechanisms on the developmental competency of spermatozoa, oocytes, and embryos in mammals. A better understanding of the epigenome of gametes and embryos will lead to identification of biological networks that play important roles in disease and development and help improve fertility and health.

“...at fertilization, the diploid genome contains all the information necessary to regulate (or cause) individual ontogenesis, requiring only an appropriately permissive and supportive environment for full genomic expression to occur.” (Moss, 1981)

GAMETOGENESIS

Gametes, spermatozoa, and oocytes are formed in the gonads (testes and ovaries), through a process that starts during the embryonic and fetal development of the animal, comes to a halt during the animal’s infancy, and is resumed once the individual reaches puberty. The formation of gametes is called gametogenesis and is a complex process that involves a series of common events for both males and females followed by a very distinctive pathway in the formation of sperm cells or eggs.

For male and female mammalian embryos, gametes are formed as a cell line that differentiates from the somatic cells early in development (Surani et al., 2004). Surprisingly, these “primitive gametes,” known as primordial germ cells (PGCs), are not originated in the primitive gonads or urogenital ridges; they are actually a group of cells from the epiblast that are located in the extraembryonic mesoderm, at the base of the allantois in the posterior part of the embryo (Gardner and Rossant, 1979). This group of cells later migrates into the left and right urogenital ridges (Hahnel and Eddy, 1986; McLaren and Lawson, 2005). The migration of PGCs has been extensively characterized due to their peculiar and high alkaline phosphatase activity, which allows us to identify them and follow their ameboid migratory movements (Ginsburg et al., 1990). The migration process occurs during days 7–14 of gestation in the mouse embryo (De Felici, 2009; De Miguel et al., 2009) and between days 30 and 64 of gestation in the bovine embryo (Aerts and Bols, 2008). During their migration, PGCs actively proliferate through mitosis; in the mouse, the population of PGCs reaching the gonads is estimated at several thousand (Tam and Snow, 1981). The migration of PGCs is completed toward the end of embryonic gastrulation (Matsui, 2010). Therefore, the complex series of developmental events in PGCs should proceed precisely in a spatially and temporally dependent manner (Matoba and Ogura, 2011).

Several genes are thought to be involved in PGC differentiation and in their migration. Recent evidence suggests that members of the bone morphogenetic protein (BMP) family play important roles in early development of PGC precursors. BMP4 and BMP8B, secreted from the extraembryonic ectoderm, and BMP2, from the visceral endoderm, seem to be crucial for early specification of PGC precursors from other somatic cells (De Felici, 2009; Hayashi et al., 2007; Kurimoto et al., 2008; Ohnata et al., 2009; Saitou, 2009). The protein Prdm14 (PRDI-BF1-RIZ domain containing 14) also plays a key role in germ cell specification and differentiation of PGC precursors. In Prdm14 null embryos, PGC-like cells are initially formed; however, they do not undergo differentiation and cannot undergo proper epigenetic reprogramming into PGCs. Therefore, Prdm14 null female and male mice are infertile (Edson et al., 2009). E-cadherin is also important in primordial germ cell formation, and migration treatment of PGC precursors with a blocking monoclonal antibody for E-cadherin, ECCD-1, prevented the formation of PGCs, indicating that E-cadherin–mediated cell–cell interaction among the precursors is essential for PGC formation (Okamura et al., 2003). RNA binding proteins, cell adhesion proteins, tyrosine kinase receptors, and G protein–coupled receptors facilitate PGC migration and early
colonization of the gonads; PGCs express NANOG and the cell surface markers SSEA1, EMA1, and TG1 (Nicholas et al., 2009).

The beginning of gametogenesis is identical for both male and female embryos. Before their arrival to the urogenital ridge, XX-female and XY-male PGCs appear to behave identically in all aspects. They both originate from epiblastic cells at the base of the allantois, and they both migrate toward the urogenital ridges. This suggests that formation, migration, and entry of the PGCs into the genital ridge is not a sexually dimorphic process (Edson et al., 2009). However, the next steps of meiosis and gamete formation are initiated at very different time points and in a different way in males and females (Kocer et al., 2009).

**SPERMATOGENESIS**

In the male embryo, primitive germ cells soon stop their divisions after colonizing the primitive testicle and enter a period of mitotic quiescence. Quiescent male germ cells are called *prospermatogonia* or gonocytes (De Felici, 2009), and they remain in their mitotic “slumber” until the male reaches puberty, when the spermatogenic cycle is initiated within the seminiferous tubule, the functional unit of the testis. Serial cross-sections of a seminiferous tubule show that sperm cells differentiate in distinctive associations. Each association is a stage of the seminiferous epithelial cycle. In other words, a spermatogenic cycle is the time it takes for the recurrence of the same cellular stage within the same segment of the tubule. Each stage of the cycle follows in an orderly sequence along the length of the tubule. The number of stages in the spermatogenic cycle is species-specific with 12 stages in the mouse and bull and 6 stages in man (Phillips et al., 2010). During each spermatogenic cycle, spermatogonia proliferate by mitosis, and, after several stages, primary spermatocytes are formed. Each primary spermatocyte will enter meiosis and through the first meiotic division will produce two secondary spermatocytes, each of which will finish meiosis becoming round haploid spermatides. The last part of the process is spermiation, characterized by the loss of most of the cytoplasm and organelles, the formation of a tail, and the delivery of these tailed cells into the seminiferous tubule lumen (Lie et al., 2009). Spermatozoa will then be transported into the epididymis, where they will be stored and acquire forward motility. However, final maturation of sperm cells is only completed in the female reproductive tract.

**OOGENESIS**

Contrary to the mitotic arrest of the male germ cells, PGCs in the female embryo continue to divide mitotically for a while until they enter meiosis and pass through leptotene, zygotene, and pachytene stages before arresting in diplotene stage (McLaren, 2003). The peak number of female PGCs is reached at the transition from mitosis to meiosis (Gondos, 1981), but this number is drastically reduced before birth as a result of apoptosis (Hartshorne et al., 2009; Morita and Tilly, 1999). In the cow, the maximum number of PGCs was estimated at 2,100,000 during the mitosis-to-meiosis transition, but it is reduced to around 130,000 at birth (Erickson, 1966). In humans, the maximum number of PGCs is considered to be established during the fifth month of fetal development at 7,000,000, but only around 2,000,000 are thought to remain at birth (Tilly, 1996). The concept that female mammals are born with a fixed supply of oocytes that are depleted during each estrous (or menstrual in the human) cycle declining with age has been an accepted dogma of reproductive biology for many years. However, in 2004, a controversial study published by Johnson and collaborators suggesting that neo-oogenesis takes place during adult life in the mouse ovary from germline stem cells in the surface epithelium of the ovary challenged this dogma (Johnson et al., 2004). To this day, several studies have supported this theory (Abban and Johnson, 2009; De Felici, 2010; Fu et al., 2008; Lee et al., 2007; Tilly et al., 2009; Virant-Klun and Skutella, 2010), whereas others failed to find evidence that any cells contribute to the formation of new oocytes in the adult (Begum et al., 2008; Bristol-Gould et al., 2006; Eggan et al., 2006; Notarianni, 2011).
Table 1.1. Timeline of oogenesis in some mammals.\(^a\)

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Species</th>
<th>Mouse</th>
<th>Cattle</th>
<th>Human</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGCs</td>
<td></td>
<td>13.5 days</td>
<td>90 days</td>
<td>30 days</td>
</tr>
<tr>
<td>Oogonium</td>
<td></td>
<td>18–20 days</td>
<td>80–90 days</td>
<td>112–130 days</td>
</tr>
<tr>
<td>Primary oocyte</td>
<td></td>
<td>21 days</td>
<td>210 days</td>
<td>140 days</td>
</tr>
<tr>
<td>Secondary oocyte</td>
<td></td>
<td>Puberty</td>
<td>Puberty</td>
<td>Puberty</td>
</tr>
<tr>
<td>Ootid</td>
<td></td>
<td>Fertilization</td>
<td>Fertilization</td>
<td>Fertilization</td>
</tr>
<tr>
<td>Ovum</td>
<td></td>
<td>After fertilization</td>
<td>After fertilization</td>
<td>After fertilization</td>
</tr>
</tbody>
</table>

\(^a\)The stages from PGC migration until primary oocyte formation are expressed in days during the female embryo development. Although secondary oocytes are observed before puberty, the majority of oocyte maturation occurs after puberty.

Mammalian oogenesis is accomplished through three developmental stages: the initiation of meiosis, the formation of a follicle around each oocyte during the perinatal period, and the cyclic growth of the follicles and the maturation of the oocytes within. The events that coordinate the initiation of meiosis are not completely understood; however, several studies have proposed that retinoic acid is the molecular switch that determines meiotic entry in the developing ovary (Bowles et al., 2006; Koubova et al., 2006; Wang and Tilly, 2010). Once each oocyte has arrested meiosis in the diplotene stage, a single layer of pregranulosa cells surrounds the oocyte, forming a primordial follicle (Hirshfield, 1991). The formation of primordial follicles is known as ovarian follicular assembly and occurs at around day 112–130 of gestation in humans (Hartshorne et al., 2009) and 80–90 days of gestation in the bovine fetus (Braw-Tal and Yossefi, 1997; Nilsson and Skinner, 2009), but it occurs in the days immediately following birth in rodents (Pepling, 2006; Pepling and Spradling, 2001). Oocytes remain in their meiotic arrest until the female reaches puberty. During each estrous or menstrual cycle, a cohort of follicles is recruited; these follicles will grow and develop an antrum or cavity, therefore being known as antral follicles. From this cohort, only a subset of follicles (in polytocos species) or only one follicle (in monotypocous species) is selected for dominance and ovulation, becoming preovulatory follicles (McGee and Hsueh, 2000). Prior to ovulation, oocytes resume meiosis; this can be recognized by dissolution of the nuclear envelope, known as germinal vesicle breakdown. However, meiosis is stopped again and oocytes are ovulated at the metaphase of the second meiotic division; therefore, they are known as MII oocytes. The final stage of meiosis will only be completed if the oocyte is fertilized (see Oocyte activation below). Thus, after being formed in the embryo and remaining in “meiotic stand by” for months or even years, the oocyte can only complete its journey with fertilization. The main processes during oogenesis in mammals and the differences between some of the model species are summarized in Table 1.1.

FERTILIZATION AND EGG ACTIVATION

Once delivered into the female reproductive tract, sperm cells have to travel a long distance and swim against a series of obstacles (the low vaginal pH, the cervix, and the presence of macrophages in the uterus) that serve as the selection machinery preventing abnormal spermatozoa from reaching the egg. During their transit from the uterus to the oviduct, their last destination, spermatozoa go through a process called capacitation, or the acquisition of fertilization capability, described independently by both Chang (1951) and Austin (1951, 1952). When spermatozoa reach the oviduct, there are still two more barriers they need to overcome in
order to reach the egg. First, oocytes are surrounded by layers of cells, which together form the **cumulus cell oocyte complex**. Sperm cells need to pass the cumulus cell layers in order to reach the last barrier separating them from the oocyte, the **zona pellucida**, a transparent glycoprotein coat that surrounds and protects the oocyte.

Fertilization consists of a series of events that begins when the sperm makes contact with the cumulus cells and ends with the fusion of paternal and maternal chromosomes at metaphase of the first mitotic division of the zygote. The events of fertilization require just over 24 hours and include a series of steps, the first of which is the passage through the cumulus cells. The second step is the penetration of the zona pellucida, a receptor–ligand interaction with a high degree of species specificity, in which the zona pellucida glycoproteins ZP1, ZP2, and ZP3 (that were formed during oocyte maturation) play the leading role (Wassarman et al., 2004). The last step is the binding and fusion of the sperm and oocyte.

The zona pellucida is made out of three glycoproteins in rodents (Wassarman, 1988) and four in humans and bovines (Conner et al., 2005; Goudet et al., 2008; Lefievre et al., 2004). One of the zona pellucida glycoproteins, ZP3, is a well-known, species-specific receptor. Although ZP3 is highly conserved in mammals, its differential glycosylation pattern in each species only allows the entry of spermatozoa from the same species (Goudet et al., 2008; Litscher et al., 2009). Interaction of spermatozoa with ZP3 causes the **acrosome reaction**, which is characterized by the loss of the acrosome from the sperm head and the liberation of several enzymes that allow the sperm the final entry through the zona pellucida. The ability of ZP3 to induce the acrosome reaction resides in its C-terminal fragment; whereas in rodents O-linked glycans are critical for ZP3-induced acrosome reaction, in humans N-linked glycans are the ones involved in ZP3-mediated acrosome reaction (Gupta and Bhandari, 2011).

Only acrosome-reacted sperm can fuse with the oocyte. However, after all the obstacles that they encounter, only a few (probably <10) sperm cells do actually reach the egg. The complete molecular control of the sperm–oocyte binding and fusion is not known; however, several molecules have been implicated in this process, including the tetraspanin protein family members CD9 and CD81, GPI-anchored proteins (Evans, 2002; Primakoff and Myles, 2007), and the ADAM family of proteins, now known as fertilin (Primakoff and Myles, 2000) and the protein Izumo1 (Ikawa et al., 2008; Inoue et al., 2005, 2011).

When the mammalian oocyte is fertilized, it is still arrested at metaphase II and would remain so, unless the sperm’s entrance triggers a release of calcium from storage sites into the ooplasm in a wave-like pattern (Kline and Kline, 1992; Swann and Yu, 2008). The repeated oscillations of cytosolic Ca\(^{2+}\) give rise to a set of events collectively known as **oocyte activation**. Oocyte activation includes mainly two very important events for zygote formation. First, the release of cortical granules (enzyme-filled vesicles that lie just below the oocyte’s plasma membrane) modifies the zona pellucida glycoproteins rendering the zona impenetrable by any other spermatozoa (Abbott and Ducibella, 2001); this is known as the **block to polyspermy** and has been widely studied in several species (Gardner and Evans, 2006; Gardner et al., 2007; White et al., 2010). Second, the calcium oscillations trigger the **cell cycle resumption**, which is the culmination of meiosis and the extrusion of the second polar body. Several studies point to the importance of sperm phospholipase C zeta in oocyte activation (Kashir et al., 2010). Within around 24 hours of fertilization, the paternal and maternal genomes are assembled into pronuclei, which replicate their DNA, and their chromosomes come together at **syngamy**, the last step of fertilization that culminates with the formation of the one-cell embryo, the **zygote**.

**EMBRYOGENESIS**

From a cytological perspective, early embryo development is a process of series of repeated cell divisions known as **cleavage**. Each cell in an early mammalian embryo is called a **blastomere**, and there are no morphologic differences among individual blastomeres (Chen et al., 2010).
However, from a molecular point of view, there are many complex processes taking place in each individual blastomere in order to attain cell division and begin cell differentiation.

During the first cell cycles, the preimplantation embryo is controlled by maternal genomic information that is accumulated during oogenesis (Telford et al., 1990). Around the 2-cell stage in the mouse embryo (Schultz, 1993, 2002), the 4- to 8-cell stage in the human embryo (Schultz, 2002; Telford et al., 1990), and the 8-cell stage in the bovine embryo (Memili and First, 2000), the embryo starts transcribing its own RNA. This process has been called embryonic genome activation (EGA), maternal-to-zygotic transition, or zygotic gene activation. The developmental program initially directed by oocyte-stored proteins and transcripts is replaced by a new program controlled by the expression of the new embryonic genes. The goal of the EGA is to transform the highly differentiated oocyte into a set of totipotent blastomeres. The EGA is a crucial condition for following successful embryonic development (Kanka, 2003). The length of the first cell cycles is particularly variable. Around 18–36 hours are needed to complete the first cell cleavage. The second- and third-cell stages are shorter, taking from 8 to 12 hours to be accomplished. The fourth cell cycle may span from 9 to 40 hours (Lequarre et al., 2003).

At about the 8-cell stage in mouse embryos (Hamatani et al., 2004) and 16-cell stage in human and bovine embryos, an amorphous mass of cells undergoes “compaction,” in which loosely associated cells adhere tightly to generate an organized mulberry-shaped cell mass called the morula. Then, the cells on the outside of the sphere begin to express membrane transport molecules, including sodium pumps causing fluid to accumulate inside the embryo, which signals the formation of a blastocyst. The blastocyst is composed of two distinctive groups

**Figure 1.1.** Timeline of preimplantation development in the mouse, bovine, and human embryos. Pronuclear formation is assumed as day zero. (a) Fertilization; (b) pronuclear formation; (c) zygote; (d) two-cell embryo; (e) four-cell embryo; (f) eight-cell embryo; (g) M orula; (h) early blastocyst; (g) late blastocyst. (For color detail, please see the color plate section.)