Rabbit as a reproductive model for human health

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Abstract

The renaissance of the laboratory rabbit as a reproductive model for human health is closely related to the growing evidence of periconceptional metabolic programming and its determining effects on offspring and adult health. Advantages of rabbit reproduction are the exact timing of fertilization and pregnancy stages, high cell numbers and yield in blastocysts, relatively late implantation at a time when gastrulation is already proceeding, detailed morphologic and molecular knowledge on gastrulation stages, and a hemochorial placenta structured similarly to the human placenta. To understand, for example, the mechanisms of periconceptional programming and its effects on metabolic health in adulthood, these advantages help to elucidate even subtle changes in metabolism and development during the pre- and peri-implantation period and during gastrulation in individual embryos. Gastrulation represents a central turning point in ontogenesis in which a limited number of cells program the development of the three germ layers and, hence, the embryo proper. Newly developed transgenic and molecular tools offer promising chances for further scientific progress to be attained with this reproductive model species.

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Introduction

After mice (59.3%) and rats (17.7%), the laboratory rabbit is the third most often used experimental mammal (2.78%) within the EU (EU report 2010: <http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=COM:2010:0511:REV1:EN:PDF> (<http://www.ncbi.nlm.nih.gov/projects/genome/guide/rabbit>). The vast majority of experimental rabbits are used for 'other human diseases', i.e. not for categories like cardiovascular diseases, nervous and mental disorders, cancer or animal diseases. In toxicological testing, the rabbit is often the compulsory second laboratory species besides mice or rats, and the preferred one in the testing of chemicals regarding acute dermal irritation (OECD Guideline Test No. 404). The European rabbit (*Oryctolagus cuniculus*) belongs taxonomically to the mammalian order lagomorpha and – together with hares – to the family Leporidae. The rabbit–primate phylogenetic distance is the same as the rodent–primate one. However, because rodent sequences evolved more rapidly, rabbit gene sequences are more similar to human sequences than rodent ones (Graur *et al.* 1996).

Historically, the rabbit was a 'classical' species in the first decades of embryology and reproductive biology,

starting from the late 19th century. Hensen's (or the 'primitive') node, the 'organizer' of gastrulation and, as a result, of the main body axes, was first described in the rabbit by Hensen in 1876 (Viebahn 2001); and Brachet (1913) described the *in vitro* development of rabbit 'blastodermic vesicles'. The teratogenic effects of thalidomide were found in the laboratory rabbit but not in rodents (Somers 1962, Hay 1964, Schumacher *et al.* 1968, Gottschewski & Zimmermann 1973, Lenz 1988). Paradigmatic research in reproductive biology using the rabbit as a model animal is associated with a number of well-known pioneers of reproductive biology, such as C E Adams (sperm and oocyte transport, prenatal mortality), C R Austin (capacitation of spermatozoa, oocyte maturation and fertilization), R A Beatty (genetics of gametes), J M Bedford (sperm morphology), G Pincus (fertilization in the rabbit; 1932), E S E Hafez (fertilizability of ova, embryo survival), D W Bishop (oviduct physiology), H M Beier (uteroglobin, endocrine regulation of uterine secretion), M C Chang (experimental embryology and endocrinology), C Lutwak-Mann (biochemical analysis of blastocyst and uterine fluid), C Thibault (IVF), R R Maurer and M Kane (embryo *in vitro* culture), J C Daniel Jr (preimplantation embryo

development *in vivo* and *in vitro*, uterine secretions), H-W Denker (trophoblast–endometrial interactions at implantation), A C Enders (ultrastructure of implantation and placentation), F Seidel (developmental competence of isolated blastomeres), G H M Gottschewski (normo- and teratogenesis in the rabbit), A Jost (sexual differentiation), and M F Hay and H Tuchmann-Duplessis (teratogenesis) (non-exhaustive list of names and disciplines). In the last 30 years, however, isolation of embryonic stem (ES) cells, allowing the easy generation of knockout phenotypes, and an abundance of molecular data and markers have promoted the mouse as the most often used laboratory animal in reproductive and, particularly, developmental biology. Nevertheless, there are numerous advantages that make the rabbit a highly suitable animal model for studies in reproduction.

Ovulation and preimplantation embryo development

Female rabbits have a uterus duplex, i.e. two separated functional uteri and cervixes, with a vagina simplex. The uterus duplex allows the transfer of two embryo groups to the same recipient female. The native fertility of rabbits is high (>8–9 embryos per female, depending on breed and strain) and can be increased by routinely employed hormonal treatments in order to reduce the number of donor rabbits (Fischer & Meuser-Odenkirchen 1988, Ramin *et al.* 2010). Even in females suffering from metabolic diseases like diabetes mellitus, high blastocyst yields can be achieved by hormonal follicle stimulation (at day 6 post coitum (p.c.) 13.3 blastocysts in diabetic and 21.9 in control rabbits; Ramin *et al.* (2010)).

The size of the genital tract facilitated seminal landmark investigations on the endocrine and paracrine regulation of embryo–maternal interactions and uterine receptivity in early pregnancy and on hormone responsiveness of endometrial secretion, as highlighted by progesterone-regulated uteroglobin (Beier 1968, 2000). Recently, it was shown that the rabbit is a good model for Asherman's syndrome, or uterine synechia, defined as a partial or complete obliteration of the uterine cavity and/or the cervical canal, and which can cause infertility and recurrent pregnancy losses (Fernandez H, Krouf M, Morel O, Ali MH & Chavatte-Palmer P, unpublished observations). So far, synechia, which are frequently observed after uterine curettage in women, had been reported in the mare only.

The rabbit belongs to the few species in which ovulation is induced by mating, resulting in an exactly defined pregnancy and embryonic age (hours or days p.c.). The rabbit has a short reproductive cycle. Pregnancy lasts for 31 days, with female sexual maturity occurring, depending on strains, at about 4–5 months of age. Without mating/insemination, pseudopregnancy can be triggered by hormonal treatment (LH, hCG), resulting in functional corpora lutea for 12 days. Fertilization and implantation can be achieved from

day 15 after unsuccessful mating or pseudopregnancy (Fischer *et al.* 1986).

Fertilization, cleavage and prenatal mortality have been investigated extensively by C E Adams (Adams 1960a, 1960b, 1962, 1982). Fertilization occurs at ~10 h p.c. (Harper 1961), with the second polar body being visible at about 14 h p.c. Until the 16-cell stage, blastomeres divide every 7 to 8 h. Morulae (~60 h p.c.) have cell numbers >32. Compaction, coinciding with the establishment of the first cellular junctions, occurs at ~68 h p.c. and blastocyst formation (appearance of blastocyst cavity and differentiation of embryoblast (ICM) and trophoblast) at 72 h p.c. The size of day 4 blastocysts is ~1 mm and expands to a diameter of 2 to 6 mm until implantation at day 6 and 18 h (Denker 1977). Measurements of cell numbers at day 5 differ between 1291 and 9536, and on day 6 between 80 000 and 100 000 (Fischer *et al.* 1986). Embryoblast cell numbers in the embryonic disc at day 6 p.c. are ~2000 in stage 1 blastocysts (Ramin *et al.* 2010) and 7000 in stage 2 (Thieme *et al.* 2012a). Shortly prior to implantation, the polar trophoblast covering the embryonic disc (Raubert trophoblast) is shed, exposing the embryoblast cells to the uterine luminal epithelium (Williams & Biggers 1990, Tscheudschilsuren *et al.* 1999). The rabbit embryo enzymatically dissolves the zona pellucida during day 4 (Denker & Gerdes 1979) and replaces it by the neozona during day 5 p.c. The peri-implantation blastocyst is covered by three extracellular coverings: the neozona, remnants of the mucoprotein layer deposited during tubal passage and uterine secretions (Denker 1977, Fischer *et al.* 1991).

The size of the expanded rabbit blastocyst (the largest spherical blastocyst in mammals) allows the collection of sufficient material for morphological and molecular analyses of individual blastocysts from single females. Diameter and developmental stage can be reliably analyzed and allow a firm evaluation of physiological parameters such as protein synthesis (Jung & Fischer 1988), cellular transport (Biggers *et al.* 1988) or glucose uptake (Navarrete Santos *et al.* 2004b) in intact blastocysts and in separated embryoblast and trophoblast cells (Navarrete Santos *et al.* 2008, Thieme *et al.* 2012a).

Molecular events during preimplantation embryo development

Embryonic genome activation (EGA) in the rabbit spans several cell cycles, as in most mammals including humans but opposite to the mouse (Telford *et al.* 1990). Such interspecies differences are significant for EGA regulation, since each DNA replication offers specific opportunities for nucleo-cytoplasmic interactions involved in genome reprogramming. The rabbit genome is fully active from the 8- to 16-cell stage

onward (Manes 1973, Pacheco-Trigon *et al.* 2002). Gene reprogramming during the preimplantation period of development has been partly described using a dedicated array (Leandri *et al.* 2009), providing a first representative description of gene reprogramming in this species. It is characterized by overlap between maternal and embryonic information. The degradation of maternal transcripts progresses through the entire cleavage period, with different kinetics for different genes. Embryonic transcripts also accumulate with different kinetics, with a significant proportion displaying a sharp and transient increase at EGA. Epigenetic events during this period are highly dynamic. The use of the rabbit embryo has brought new insights into the general mechanisms that affect gene expression and/or further development in mammals, i.e. pericentromeric heterochromatin organization, DNA methylation and X chromosome inactivation (XCI).

The rabbit somatic structure of pericentromeric heterochromatin with pericentromeres that do not cluster into chromocenters is close to the human organization and different from the mouse (Andrey *et al.* 2010). In the embryo, the somatic-like organization of pericentromeric heterochromatin appears concomitantly to EGA (Yang, personal communication).

The quantitative study of DNA methylation dynamics has shown that, in the zygote, the methylation of the maternal pronucleus DNA remains constant due to the maintenance of methylase activity during the S phase. Conversely, paternal DNA is first actively but partially demethylated before DNA replication, and subsequently remains constant during the S phase when the maintenance methylase activity is active (Reis Silva *et al.* 2011). A passive DNA demethylation then occurs between the four- and 16-cell stage. In the blastocyst, as in most mammalian species, DNA methylation is higher in embryoblast cells than in trophoblast cells. These data demonstrate that both heterochromatin structure and DNA methylation levels are highly modified concomitantly with the onset of EGA. Because the kinetics of EGA in the rabbit is close to most mammalian embryos but different from the mouse, the rabbit embryo is now used as a pertinent model to analyze the environmental effects on these epigenetic changes. For example, *in vitro* development was shown to modify DNA demethylation dynamics in the paternal pronucleus and during cleavages (Reis Silva *et al.* 2012).

XCI in female mammals ensures dosage compensation for X-linked genes. Developmental regulation of this major epigenetic process had been analyzed in detail in the mouse where the maternal imprint of *Xist*, the non-coding transcript responsible for X inactivation, results in the early inactivation (four- to eight-cell stage) of the paternal X chromosome. This imprinted inactivation persists in the extraembryonic tissues while in the pluripotent embryoblast cells, paternal X chromosome reactivation precedes a random inactivation in each cell.

This situation was considered to be representative for the eutherian situation. The use of the rabbit embryo as an alternative model for *in vivo* developed embryos, however, evidenced a high diversity in the early regulation of X inactivation among eutherians. In particular, both the early imprint of *Xist* and the reactivation of the previously inactivated X chromosome in the pluripotent cells of the inner cell mass were shown to be mouse specific. It may be that the maternal imprint of *Xist* in mice evolved to avoid premature maternal X inactivation in a species with very early EGA and *Xist* expression (Okamoto *et al.* 2011). In the rabbit embryo, *Xist* is expressed from both X chromosomes and accumulates in the blastocyst on both X chromosomes in a high proportion of cells. *Xist* expression becomes monoallelic only at the late blastocyst stage, first in the trophoblast, and then in the embryoblast. The functional consequence of *Xist* expression, i.e. repression of X-linked genes, seems to occur only at the blastocyst stage. Both the nonimprinted early expression of *Xist* and the delay in X-linked genes inactivation are common to rabbit and human embryos when compared with the mouse (Okamoto *et al.* 2011).

Gastrulation

A central turning point in blastocyst differentiation and vertebrate ontogenesis is gastrulation. The body plan with regard to axis formation and the starting point for germ layer formation (ecto-, meso- and endoderm) is set. The anterior–posterior (cranio–caudal) body axis (with dorsal–ventral polarity) and, consequently, left–right axis in mammals is first and definitively fixed and the mesoderm emerges as the earliest germ layer to be identified.

The embryo proper of most gastrulation-stage mammals, including the human and rabbit, has the shape of a flat disc with two cell layers (epiblast and hypoblast; for review, see Viebahn (1999)). Gastrulation in the rabbit starts at day 6 p.c., i.e. at a stage that is easily accessible, as implantation has not yet started. Seven gastrulation stages have been identified so far (Fig. 1), using morphological and molecular methods (Viebahn *et al.* 1995, 2002, Schafer-Haas & Viebahn 2000, Viebahn 2004) and the staging system developed by Hamburger & Hamilton (1992) for the chick as a template to facilitate intra-amniote interspecific comparison. Implantation takes place at stage 4. Stage 0 is a blastocyst with no apparent axial differentiation in the embryonic disc (e.g. at day 5.5 p.c.). The onset of gastrulation (stage 1), found at day 6.0 p.c., is characterized by a crescent-like dense area in the anterior part of the embryonic disc (anterior marginal crescent; Viebahn *et al.* (1995)), followed about 6 h later by a sickle-shaped elongation of reduced density at the posterior pole (posterior gastrula extension, PGE; stage 2; Viebahn *et al.* (2002)). Appearance of the primitive streak in the

Figure 1 Schematic drawing of the gastrulation stages 0 to 7 in the rabbit. For details, see text.

midline of the PGE, generating the first mesoderm cells, defines stage 3. The mesoderm is formed by epithelio-mesenchymal transition of epiblast cells under the 'fine-tuning' influence of the hypoblast at stages 1 and 2 (Idkowiak *et al.* 2004). Movement, migration, and epithelio-mesenchymal transformation of epiblast cells, resulting in primitive streak formation, characterize stage 3 (Viebahn *et al.* 2002) until the primitive streak encompasses up to half of the longitudinal axis of the embryonic disc. The appearance of Hensen's node at the tip of the primitive streak characterizes stage 4 and coincides with the time when implantation starts. This classification finishes with stage 7 when the first somites become visible.

In principle, this series of stages is found in all mammals including rodents in which, however, the embryonic disc is forced into the complex shape of the so-called egg cylinder in which the anterior and posterior poles of the embryo come to lie in close proximity to each other and an additional (proximo-distal) body axis has to be taken into account (Beddington & Robertson 1999). Results obtained on the epithelio-mesenchymal transition or on extraembryonic control of gastrulation in the mouse blastocyst are, therefore, difficult to extrapolate to other mammalian embryos including the human. Early cell-lineage decision during embryonic development is another example where the embryos of different mammals clearly vary between species, as recently emphasized by Rossant (2011).

Rabbit blastocysts can be exactly staged in living embryos *in vivo* and *in vitro* and are amenable to experimentation under standard culture conditions (Halacheva *et al.* 2011). Gene expression profiling of stage 0 to 3 blastocysts (Viebahn *et al.* 2002, Idkowiak *et al.* 2004, Ramin *et al.* 2010) and cell-lineage-specific molecular phenotyping (Navarrete Santos *et al.* 2008, Thieme *et al.* 2012a) have begun to emerge and will gradually generate a broad spectrum of molecular tools for this species.

Feto-placental interactions and placental function and imaging

Compared with other mammals, the rabbit blastocyst attaches to the uterine epithelium late in embryogenesis.

Implantation starts at day 6 plus 18 h; definitive mesometrial chorioallantoic placentation occurs at day 8 p.c. (Denker 1977). Peri-implantation embryo-maternal dialog depends on timely controlled mutual temporal and local signals. When interspecies comparisons are made, the expression of proprotein convertase 5/6 (PC 5/6), for example, required for uterine remodeling both in decidualizing cells and uterine epithelium in women and the rabbit, is of importance, since in mice PC 5/6 is only involved in decidualization, suggesting that the rabbit may be a very appropriate model to decipher molecular events during implantation (Nicholls *et al.* 2011).

The rabbit placenta develops as a discoid, hemochorial placenta such as that of the human and rodents (Duval 1889). Its hemodichorial structure (two cellular layers of chorion between the maternal and the fetal blood) is closer to that of the human in the last trimester (hemomonochorial) than the placenta of rodents (hemotrichorial). It shares with the rodents a labyrinth

Figure 2 Schematic representation of the maternal and fetal blood flow in the rabbit placenta. Fetal blood reaches the placenta through two umbilical arteries that join the chorionic plaque. These arteries subsequently branch off and form a capillary network within the labyrinth that flow from the chorionic plaque toward the decidua. A venous network brings back the oxygenated blood to the chorionic plaque and umbilical cord. In contrast to fetal blood that remains within the blood vessels, the maternal blood flows within maternal vascular spaces, along trophoblastic columns from the chorial to the basal plaque. This counter-flow between maternal and fetal blood flow optimizes feto-maternal exchanges.

(exchange region) as opposed to the microvillous chamber in humans (Enders & Blankenship 1999, Foote & Carney 2000; Fig. 2). As in primates, placental growth is most rapid in the first half of pregnancy, whereas fetal weight gain occurs in the second half due to a large increase in feto-maternal exchanges, mostly due to increased convolution of the exchange surface (McArdle *et al.* 2009).

Hemodynamic changes occurring during pregnancy are comparable with the human, with an important increase in maternal blood pressure throughout gestation. The placental giant cells, which originate from the trophoblast and which are present in the rabbit placenta from day 10 p.c., but not in the human placenta, are involved in the remodeling of the spiral arteries (Larsen 1962) replacing the endothelial cells, as in the invasive trophoblast in the human and as demonstrated in rodents. Moreover, a structural correlation between the expression of genes involved in the placental renin-angiotensin system and hemodynamic changes, observed by stereological approaches, underline the importance of the rabbit as a pertinent model for the study of pathologies that affect maternal blood pressure or placental perfusion during pregnancy (McArdle *et al.* 2009, 2010).

The relative large size of the rabbit compared with other laboratory animals makes it possible to monitor fetal and placental growth using conventional ultrasound equipment (Chavatte-Palmer *et al.* 2008, Polisca *et al.* 2010). Uterine artery ligations have been used to induce intra-uterine growth retardation (IUGR) and to evaluate modifications in blood flow using standard

Doppler ultrasound technology (Eixarch *et al.* 2009, 2011). 3D Power Doppler, which enables the quantification of blood flow within the human and sheep placenta (Morel *et al.* 2010), is also able to detect variation in vascularization within the rabbit placenta (Lecarpentier E, Morel O, Tarrade A, Dahirel M, Bonneau M, Gayat E, Evai-Brion D, Chavatte-Palmer P & Tsatsaris V, unpublished observations; Fig. 3).

The rabbit as a tool for investigating hormonal regulation of the embryo-maternal dialog and the effects of maternal metabolic disorders on offspring development and health

Within the Developmental Origins of Health and Disease concept (Barker 1998), a focus is increasingly laid on the periconceptional and feto-placental periods. The periconceptional period is particularly sensitive to metabolic disruption with long-term impacts on pre- and postnatal development and adult health (Kwong *et al.* 2000, Watkins *et al.* 2008a, 2008b, 2010). Endocrine and metabolic circuits characterize embryo-maternal interactions in these pregnancy stages, and confounders have been described, with most attention recently being paid to endocrine disruptors (cf. Fowler *et al.* 2012) and prevalent metabolic diseases such as diabetes mellitus and adiposity. Adipokines, a subset of cytokines derived from adipose tissue, affect metabolism and obesity particularly, as also fertility in women (Mitchell *et al.* 2005). Plasma levels are known to be decreased in patients suffering from type II diabetes and obesity.

Figure 3 Photograph of 3D ultrasound of the placenta. Successive steps of quantitative 3D Doppler acquisition of the rabbit placenta, using a Voluson E8 (GE Medical Systems) ultrasound machine with a transabdominal multifrequency probe (RNA 5–9 MHz) enabling automatic 3D volume and power Doppler acquisitions. The orange color on the ultrasound images indicates blood flow. (1) Localization of the placenta and fetal heart on a 2D, power Doppler image, for 3D automatic scanning. (2) Reconstruction by automatic rotation of the image (15°) and manual drawing of the volume, to get the total placental volume. In this image, a, b and c represent the placenta visualized from the 3-dimensional planes, whereas d represents the complete volume. (3) 3D reconstruction showing internal blood flow (this can be turned around on the screen). (4) Computer-calculated vascular indices: vascularization index (VI), flow index (FI) and vascularization flow index (VFI). These indices are correlated to the blood flow.

Adiponectin, the receptors *adipoR1* and *adipoR2*, and the adiponectin paralogues *CTRP2* and *CTRP7* are expressed in rabbit endometrium and in blastocysts with a specific expression pattern in the embryonic disc (Schmidt *et al.* 2008). Adiponectin impairs insulin sensitivity in humans and rodents (Cnop *et al.* 2003). In addition, activation of IRS1-associated PI3 kinase ameliorates insulin sensitivity by enhanced glucose uptake in myocytes (Kurth-Kraczek *et al.* 1999, Maeda *et al.* 2002). In rabbit blastocysts, the translocation of glucose transporter 4 (GLUT4) and glucose uptake are activated by adiponectin *in vitro* (Fischer *et al.* 2010).

A possible explanation for periconceptional programming is the plasticity of the limited number of embryonic cells at this early stage in ontogenesis, whose modified metabolism induced by the environment may result in a larger shift in postnatal phenotype than at later stages when cellular mass is greater (Fleming *et al.* 2012). Later on, feto-placental interactions have been shown to be essential for modulating early effects of the environment (Vaughan *et al.* 2012). Due to its advantages in terms of early embryo development and placental structure and function, the rabbit is a pertinent model to study the impact of maternal metabolic disorders on offspring development as shown below using three examples.

Diabetes mellitus

Embryo development has recently been investigated in rabbits in which a diabetes mellitus type 1 has been induced experimentally by treatment with alloxan (Ramin *et al.* 2010, Thieme *et al.* 2012a). Expression and signaling of insulin, IGFs and GLUTs had earlier been studied (Navarrete Santos *et al.* 2004a, 2004b, 2008). The rabbit blastocyst expresses most GLUT isoforms (GLUT1, 3, 4, 8), insulin receptor (IR), IGF1, IGF2, IGF1R, IGF2R, but not insulin itself. Insulin is exclusively delivered by maternal secretions to the preimplantation embryo. Classical IR signaling via MAP kinases and PI3 kinase was found. Noteworthy, insulin/IGF signaling has cell-lineage-specific adjustments: insulin stimulates Erk1/2 in embryoblast and trophoblast and Akt in trophoblast but not embryoblast, whereas IGF1 activates both kinases exclusively in the embryoblast. While IGF1 acts primarily as a mitogen, insulin induces mitogenic and metabolic effects.

In peripheral blood of experimentally induced diabetic rabbits, hypoinsulinemia and hyperglycemia are measurable 48 h after the destruction of pancreatic β -cells. Within the uterine lumen, insulin is no longer measurable and the glucose concentration is increased by a factor of 3 (Ramin *et al.* 2010). An increase of uterine and blastocyst IGF1 and IGF2 (Thieme *et al.* 2012b) in these females may partly compensate for the reduced insulin/IGF sensitivity. Maternal diabetes downregulates IR and IGF1R signaling, delays blastocyst development and gastrulation and increases apoptotic

cell death in the embryonic disc. The 'double face' of IGFs – growth factor of the embryoblast (Kaye 1997, Markham & Kaye 2003, Navarrete Santos *et al.* 2004a) and pro-apoptotic effects in bovine blastocysts (Velazquez *et al.* 2011) – needs further clarification to improve understanding of potential mechanism(s) of periconceptional programming and diabetogenous embryopathies.

Ovulation and blastocyst numbers were significantly reduced in experimentally induced diabetic rabbits, mirroring subfertility of diabetic women. Insulin induced Wnt3a, Wnt4 and the mesoderm-specific transcription factor *Brachyury* in a temporal- and stage-specific expression pattern in gastrulating blastocysts. If cultured *in vitro*, only blastocysts cultured with insulin reached the Wnt3a, Wnt4 and *Brachyury* expression levels of stage 2 *in vivo* blastocysts (Thieme *et al.* 2012a), indicating that insulin is required for early gastrulation and mesoderm formation in rabbit blastocysts. Further work is currently ongoing to analyze subsequent feto-placental and post-natal development.

Maternal dyslipidemia

Because of its lipid metabolism which is close to that of humans, with high LDL-cholesterol, and its sensitivity to developing atherosclerosis, the rabbit has often been used to study lipid metabolism-related diseases. In terms of reproduction, it is therefore a particularly suitable model to study the fetal and placental effects of maternal high-fat and/or high-cholesterol diets. Work by Napoli *et al.* (2000) and Palinski *et al.* (2001) has confirmed, using the rabbit model, the importance of maternal hypercholesterolemia in predisposing offspring to atherosclerosis and the role of preventive maternal treatment. Maternal hypercholesterolemia is also associated with IUGR in rabbits (Montoudis *et al.* 1999) and affects placental transfer of fatty acids and GLUT expression in the placenta (Kevorkova *et al.* 2007, Marseille-Tremblay *et al.* 2007, Ethier-Chiasson *et al.* 2008). When the high-cholesterol diet is enriched with poly-unsaturated fatty acids and is administered to females prior to puberty, gene expression is altered in the female's embryos, which subsequently develop IUGR leading to overweight in adult offspring (Picone *et al.* 2011). The cellular and molecular mechanisms involved are currently being studied.

Obesity

Although the use of high-fat diets in rabbits has been reported to induce lipid metabolism disorders and obesity (Antic *et al.* 1999, Zhao *et al.* 2008, Zheng *et al.* 2009), this is not the case in all studies and diets supplemented with fats due to spontaneous reduction of food intake (Armitage *et al.* 2005, Picone *et al.* 2011).

As a consequence, high-fat and high-carbohydrate diets have been designed to induce obesity. In a recent study, it was shown that maternal obesity induced fetal IUGR (as already observed in other species), but also that mammary gland differentiation in the dam was advanced, with abnormal accumulation of milk and secretory products as early as mid-gestation (Hue-Beauvais *et al.* 2011). These observations contrast with those made in mice where obesity impaired maternal mammary development (Flint *et al.* 2005). Mammary gland development and milk composition have been described extensively in the rabbit (Denamur 1963, Houdebine *et al.* 1985, Baranyi *et al.* 1995). An important aspect of rabbit lactation for biomedical research is that rabbit dams only suckle once a day, which greatly simplifies the study of neonatal milk intake and/or oral supplementation with drugs or components at the time of suckling.

Conclusions

There are clear advantages in the use of the rabbit. Embryo and feto-placental development are similar to the human, making the rabbit a particularly suitable model for the impact of embryo and fetal development on offspring and adult health. Significant insights into various aspects of reproduction have been gained already in this species due to 1) exact staging of early embryonic developmental and maternal pregnancy stages, 2) large-sized blastocysts amenable to micro-manipulation, 3) cell-lineage-specific analyses, 4) gastrulation stages representative of mammalian development, and 5) placental morphology and function similar to the human.

More recently, functional genomic tools have also become available in this species (Duranthon *et al.* 2012). A deep coverage draft (7×) sequence of the rabbit genome has been released (<http://www.broadinstitute.org/models/europeanrabbit/>), whose annotation is in progress (http://www.ensembl.org/Oryctolagus_cuniculus/). Concomitantly, transcriptomic data have been published (<http://www.ncbi.nlm.nih.gov/dbEST/> and <http://www.ebi.ac.uk/ena/data/view>). A microarray dedicated to the embryo, although limited in size, has successfully been used to analyze gene expression in the early embryo (Leandri *et al.* 2009) and effects of the embryo's environment (Picone *et al.* 2011). More recently, a larger array has been made commercially available (Agilent Technologies, Palo Alto, CA, USA). This array is in the course of improvement (more than 12 000 genes) and is available for use in the context of developmental biology and periconceptional/fetal programming (Duranthon, personal communication). Moreover, ES cell isolation has been reported (Honda *et al.* 2008, Intawicha *et al.* 2009), while the production of chimera using these cells remains rare (Zakhartchenko *et al.* 2011) and no germinal chimera have been reported yet. However,

isolation of iPS cells should help the development of functional genomics analyses in this species (Honda *et al.* 2010). Finally, the concomitant development of genome data together with new transgenesis strategies (Duranthon *et al.* 2012), notably the possibility to target specific genes by injection of zinc finger nucleases into embryo cytoplasm (Flisikowska *et al.* 2011), should promote the rabbit to its legitimate place among the standard models for developmental biology and reproductive pathologies.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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