1 Genomic imprinting and mammalian reproduction

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Abstract

Amongst animals, genomic imprinting is a uniquely mammalian phenomenon in which certain genes are monoallelically expressed according to their parent-of-origin. This silencing of certain alleles often involves differential methylation at regulatory regions associated with imprinted genes and must be recapitulated at every generation with the erasure and reapplication of these epigenetic marks in the germline. Imprinted genes encode regulatory proteins that play key roles in fetal growth and development, but they also exert wider effects on mammalian reproduction. Genetic knockout experiments have shown that certain paternally expressed imprinted genes regulate post-natal behavior in offspring as well as reproductive behaviors in males and females. These deficits involve changes in hypothalamic function affecting multiple areas and different neurochemical pathways. Paternally expressed genes are highly expressed in the hypothalamus which regulates growth, metabolism and reproduction and so are well placed to influence all aspects of reproduction from adults to the resultant offspring. Coadaptation between offspring and mother appears to have played an important role in the evolution of some paternally-expressed genes, but the influence of these genes on male reproductive behavior also suggests that they have evolved to regulate their own transmission to successive generations via the male germline.

Keywords: genomic imprinting; imprinted genes; reproduction; sexual behavior; maternal behavior; hypothalamus; paternally expressed genes; coadaptation; conflict

Introduction

In the early nineteen-eighties it became clear that the parental genomes in mammals were functionally non-equivalent (McGrath and Solter, 1984; Surani et al., 1984), a phenomenon termed 'genomic imprinting'. A subset of autosomal genes is expressed not in accordance with classical Mendelian laws of inheritance, but according to the sex of the parent from which they are inherited. Imprinted genes are thus not expressed biallelically but in non-stochastic, monoallelic fashion from either the maternally donated allele or the paternally donated allele. This parent-of-origin silencing of specific alleles is an important example of stable epigenetic regulation of gene expression and of crucial importance in mammalian development. Silencing at imprinted genes involves both DNA methylation and chromatin modification (Delaval and Feil, 2004) and imprinting research has revealed much about mechanisms of epigenetic regulation.

The original experiments by McGrath and Solter (1984) and Surani et al (1984) demonstrated that mouse embryos created with two male pronuclei (androgenetic – 'AG') or two female pronuclei (gynogenetic – 'GG') failed to reach term. The absence or doubling of expression of imprinted genes resulted in lethal phenotypes very early in development. Later research in which such AG or GG embryos were rescued by fusing them with wild-type blastocysts revealed the different developmental roles played by the two parental genomes. AG chimeras were much larger at birth than wild-type embryos while GG chimeras were much smaller (Allen et al., 1995; Barton et al., 1991), suggesting that the paternal and maternal genomes regulate offspring growth in opposite directions. Analysis of the fate of AG and GG cells in brains of these chimeras showed that GG cells segregated almost exclusively to the cortex and striatum, while AG cells were only found in the hypothalamus (Allen et al., 1995; Keverne et al., 1996). The differential fate of these AG and GG cells suggested that paternally and maternally expressed genes regulate the development and function of different brain areas. In recent years imprinted genes have been shown to have important roles in brain function and in behavior (Davies et al., 2008; Isles and Wilkinson, 2000; Wilkinson et al., 2007).

Imprinted genes present a particularly interesting genetic conundrum, as their haploid expression results in the loss of protection from mutation which diploidy confers (Orr, 1995) and so would seem to be a non-adaptive evolutionary step. The debate about how and why such a mechanism has evolved has been ongoing since genomic imprinting was first discovered (Hurst and McVean, 1997; Hurst and McVean, 1998; Moore and Haig, 1991; Moore and Mills, 2008) but the evolution of placentation and viviparity in mammals appears to coincide with the acquisition of genomic imprinting (Kaneko-Ishino et al., 2003). There are approximately 80 imprinted genes in eutherian mammals (Morison et al., 2005), of which only a subset are imprinted in marsupials (Renfree et al., 2008), and none in monotremes (Hore et al., 2007; Killian et al., 2001). The taxonomic distribution of imprinting coincides with the relative placental complexity and the development of viviparity in these different mammalian groups. Eutherians have elaborate placentas and prolonged gestation, marsupials have more rudimentary placentas and give birth to very altricial young, while monotremes are egg-laying mammals in which there is only a brief period of maternal nutrient supply after fertilization (Renfree et al., 2009). Moreover, almost all imprinted genes are expressed in the placenta (Bressan et al., 2009; Coan et al., 2005) and imprinted genes are also strongly expressed in the developing embryo. The importance of imprinted genes in development is indicated by the proteins they encode, which include growth factors, transcription regulators, apoptotic proteins and regulatory non-coding RNAs (Morison et al., 2005). The taxonomic patterns of genomic imprinting show that this regulatory mechanism has evolved in step with modes of reproduction in mammals, while the expression and functions of imprinted genes show that they play important developmental roles in reproduction. However experimental evidence has been also been accumulating that suggests that imprinted genes are involved in mammalian reproduction beyond just fetal development and placental functions. The influence of imprinted genes on brain and behavior extends to post-natal offspring behavior as well as adult reproductive behaviors. These findings raise new questions about the roles that imprinted genes play in behavior and consequently about the selective pressures which drove their evolution.

Genomic imprinting mechanisms and reproduction

The majority of imprinted genes are organized into clusters where gene expression is regulated from imprinting control regions (ICRs) associated with these clusters (Williamson et al., 2006). The clustering of imprinted genes and the shared ICRs suggest that imprinting has evolved as a regulatory mechanism to which genes are recruited, rather than evolving independently at each imprinted gene. ICRs contain differentially methylated regions (DMRs) rich in CpG repeats which are methylated on one of the parental alleles and not the other, and which determine the monoallelic expression of genes in the imprinted cluster. While other epigenetic mechanisms are also involved, such as histone modification and non-coding microRNAs, DNA methylation is the main mechanism by which imprinted gene expression is regulated (Delaval and Feil, 2004; Kacem and Feil, 2009). The imprints at each DMR must be recapitulated within the germline of each generation to reflect the gender of the individual and ensure the correct complement of imprinted genes in offspring. This is achieved in the germ line when imprints are erased and re-established in a sex specific manner during gametogenesis in the early embryo. In both males and females, primordial germ cells (PGCs) migrate to the genital ridge where they undergo widespread epigenetic modification including active demethylation of parental imprints (between embryonic days 10.5 and 12.5 in the mouse) (Sasaki and Matsui, 2008). After this demethylation of DMRs there is subsequent sexual dimorphism in imprint application. In females, DNA methylation at ICRs occurs after birth as the post-meiotic oocytes grow and mature (Hiura et al., 2006), while in males the paternal imprints are applied in the pre-meiotic prospermatogonia before birth. Moreover, the vast majority of imprinted ICRs are methylated during female gamete formation and only 3 DMRs have been identified which are methylated during spermatogenesis in males (Sasaki and Matsui, 2008). This asymmetry indicates that the mechanism of imprinting is primarily under maternal control and involves active silencing of maternal alleles in the female germ line.

As with other forms of epigenetic regulation, the methylation of imprinted DMRs during gametogenesis is mediated by DNA methyltransferases (DNMTs). DNMT3A mediates *de novo* methylation in both male and female gametes, although in males a second methyltransferase, DNMT3B, is also involved in methylation at the *Rasgrf1* imprinted locus (Kato et al., 2007). Furthermore, the methylation of all imprinted DMRs is dependent on DNMT3L, which is non-enzymatic but plays a crucial role in methylation at imprinted loci (Kaneda et al., 2004). DNMT3L appears to form a complex with DNMT3A/DNMT3B and interprets an existing mark (possibly histone-based) which is then converted into gender-specific DNA methylation at DMRs. The importance of appropriate levels of imprinting in mammalian gametogenesis is illustrated by the phenotype of DNMT3L mutant mice. Deleting DNMT3L and thus disrupting methylation at DMRs causes gross reproductive deficits in both sexes such that males are azoospermic and infertile, while females are unable to produce viable offspring (Schaefer et al., 2007). DNMT3L's important role in imprinting mechanisms is also evident from phylogenetic comparisons of the mammalian taxa: while DNMT3L is found in both eutherians and marsupials, there appears to be no ortholog in the monotremes which lack imprinted genes (Renfree et al., 2009; Yokomine et al., 2006).

Imprinting and reproduction in mammals

Almost all imprinted genes are expressed in either the embryo, the placenta or both and have been shown to have important regulatory roles in development. Imprinted genes are explicitly involved in mammalian reproduction through their influence on fetal development (Constancia et al., 2002) and placental function (Charalambous et al., 2010), however they also influence reproduction in other ways. Much of the research into the roles and functions of imprinted genes has involved the use of imprinted gene-knockout mice and while such studies have only been conducted in one experimental model, they indicate that imprinted genes are important regulators of reproductive behavior in the mouse and potentially also in other mammals. Due to monoallelic expression of imprinted genes, these knockouts also

provide unique opportunities to study imprinted genes independently in mother and offspring (fig. 1). Such knockout mouse studies have demonstrated that imprinted genes have important effects on brain function (Wilkinson et al., 2007) and behavior (Isles and Wilkinson, 2000) which include post-natal offspring behavior as well as reproductive behavior in both males and females. While direct effects on reproductive potential of global disruption of imprinting are evident in the infertility of DNMT3L-knockout mice in which appropriate imprinting is not established in the germline, behavioral effects suggest that imprinted genes also regulate different aspects of mammalian reproduction through effects on the brain (table 1). Such effects are particularly evident in knockouts of paternally expressed genes, perhaps due to their high levels of expression in the hypothalamus and related structures which mediate reproductive behaviors in mammals.

Imprinted gene effects in offspring

The first mouse knockouts of the imprinted genes *Igf2, Igf2r* and *H19* demonstrated that imprinted genes are involved in placentation and embryonic development (Barlow et al., 1991; Bartolomei et al., 1991; DeChiara et al., 1991), and subsequent studies also showed imprinted gene effects on post-natal behavior in offspring. One of the first such studies involved the paternally expressed gene *Peg1/Mest* on mouse chromosome 6. Although a paternally inherited mutation in this gene results in reduced weight at birth, this deficit is subsequently exacerbated by reduced post-natal growth rate relative to wild-type littermates (Lefebvre et al., 1998). This suggested that imprinted genes not only regulate resource extraction during gestation, but could also influence offspring physiology or behavior post-natally.

More detailed evidence for such post-natal effects came from studies of the paternally expressed gene *Peg3*, which encodes a large zinc-finger protein on mouse proximal chromosome 7 (Kuroiwa et al., 1996). Paternally-inherited deletions of *Peg3* cause deficits not only before birth but also post-natally, affecting growth, suckling and thermoregulation (Curley et al., 2004). *Peg3* mutant animals have small placentas and low birth weight which they fail to make up after birth. These pups remain smaller than their wild-type littermates

throughout post-natal nursing due to a suckling deficit which reduces their ability to take on milk. The *Peg3* mutation also affects thermoregulation as mutant pups are unable to maintain body temperature in response to maternal separation. Metabolism, appetite and thermoregulation are all mediated by the hypothalamus, an area where there is high *Peg3* expression during development (Li et al., 1999). The *Peg3* protein is involved in p53-mediated apoptosis (Deng and Wu, 2000) and these mutant animals show alterations in postnatal hypothalamic apoptosis (Broad et al., 2009), which suggests that aberrant neuronal pruning during development may disrupt normal functioning of the hypothalamus. Further disruption to hypothalamically-mediated behavior is seen in adolescence when *Peg3* females enter puberty later than their wild-type littermates (Curley et al., 2005).

The *GNAS* locus on mouse distal chromosome 2 encodes multiple transcripts with different parent-of-origin expression (Peters et al., 1999), including paternally expressed *Gnasxl* and *Nespas*, maternally expressed *Nesp,* and maternally expressed *Gnas*, which is only imprinted in adipose and endocrine tissues (Peters and Williamson, 2007). The relationship between these different transcripts is complex, however a lack of the protein XLαs, the *Gnasxl* product, causes deficits in post-natal behavior that bear some similarities to those seen in *Peg3* mutant pups (Plagge et al., 2004). Mice inheriting a paternal deletion of *Gnasxl* suffer post-natal growth retardation caused by metabolic deficits and an inability to suckle properly resulting in death no later than post-natal day 9. *Gnasxl* is expressed in the hypothalamus and pituitary that regulate energy homeostasis and in pontine and medullary nuclei which innervate the facial and jaw muscles involved in suckling. *Gnasxl* appears to be directly involved in the neural and endocrine control of post-natal suckling behavior and the similar phenotypes seen in the Peg1, Peg3 and Gnasxl mutants suggest that these paternally expressed genes are all involved in the ability of mouse pups to thrive post-natally.

Imprinted gene effects in females

As well as affecting offspring, deletion of paternally expressed *Peg1/Mest* also affects female maternal care (Lefebvre et al., 1998). Pups born to mutant mothers suffer elevated

196 post-natal mortality, even if they are wild-type offspring with a normal paternal *Peg1*/*Mest*
197 allele. The deletion affects multiple maternal behaviors and mutant females do not engage in
198 placentophagia after birth and exhibit reduced levels of pup investigation, pup retrieval to the
199 nest, nest building and post-natal nursing.
200 Similarly, the *Peg3* deletion also results in an adult phenotype which includes effects on
201 maternal care. Li et al (1999) noted very high levels of pre-weaning mortality in the offspring
202 of heterozygous *Peg3* mutant females despite these pups being wild-type, having been
203 fathered by wild-type males with normal *Peg3* expression. The elevated mortality thus could
204 not have been due to direct genetic effects in the wild-type offspring but rather to the
205 maternal responses of the females. Behavioral phenotyping of the *Peg3* mutant mothers
206 revealed a wide-ranging deficit affecting nest building, retrieval of scattered pups to the nest
207 and milk letdown. More detailed characterization of maternal behavior showed that overall
208 nursing levels are lower and that licking and grooming of pups is also reduced in these
209 females (Champagne et al., 2009). Licking and grooming is an important modulator of
210 offspring behavioral phenotypes (Francis et al., 1999) and it causes stable epigenetic
211 changes in gene expression (Weaver et al., 2004). The offspring and even grand-offspring of
212 these *Peg3* mutant mothers also display lower levels of maternal care, despite being wild-
213 type themselves (Curley et al., 2008). *Peg3* thus appears to exert an influence on offspring
214 behavioral phenotypes through its effects on maternal care. The *Peg3* mutation appears to
215 involve disruption of the oxytocin circuitry in the hypothalamus where *Peg3* is expressed.
216 Oxytocin is an important regulatory neuropeptide in maternal behavior and milk production
217 and the maternal effects of the knockout appear to be mediated by a reduction in the
218 number of oxytocinergic neurons in the paraventricular nucleus (Li et al., 1992) and a
219 reduction in oxytocin receptor density in the medial pre-optic area (Champagne et al., 2009).
220 *Peg3*'s apoptotic function suggests that developmental disruption in the hypothalamus leads
221 to these functional changes and then to the maternal behavior deficits. Indeed, *Peg3* mutant
222 mice have elevated levels of apoptosis in the medial preoptic area at postnatal days 4 and 6.
223 No changes are seen in paraventricular apoptosis at these time points, however this does

not discount the possibility of perturbations during embryonic development (Broad et al., 2009).

Recent studies of the paternally expressed gene *Magel2* have demonstrated that imprinted genes can influence female fertility and reproductive physiology as well as maternal care. The first knockout study of *Magel2* showed that these mutant mice have aberrant circadian rhythms due to abnormalities in the suprachiasmatic nucleus of the hypothalamus (Kozlov et al., 2007). Further phenotyping has shown they have a more wide-ranging deficit in hypothalamic function, with significant effects on female reproduction (Mercer and Wevrick, 2009). *Magel2* mutant females enter puberty late, despite similar post-weaning body weights in mutant and normal females. These females also have extended and irregular estrous cycles and enter reproductive decline at an early age, becoming infertile after 24 weeks when corpora lutea are no longer seen in the ovaries, even though mature follicles are present. *Magel2* mutant females were also slower to mate after pairing with males, produced smaller litters and had significantly higher litter mortality rates which were not connected to litter genotype.

Imprinted gene effects in males

The effects on female behavior described above all involve paternally expressed genes. All are strongly expressed in the hypothalamus in females and males, and thus it is not surprising that the phenotypes of several paternally expressed gene knockouts involve effects on male reproductive behavior too. As well as disrupting female maternal behavior, a paternal deletion of *Peg3* also causes reproductive deficits in males. *Peg3* mutant males are fertile but are unable to improve copulatory ability with sexual experience, unlike wild-type animals. Sexual experience typically results in shorter latencies and increased frequencies of sexual behaviors such as mounting and intromission in wild-type males but these behavioral changes are not seen in *Peg3* mutant males whose behavior once sexually experienced does not differ from that of virgin animals (Swaney et al., 2007). Wild-type male mice also develop preferences for the odors of receptive estrous females once sexually experienced, potentially enabling them to focus reproductive effort towards receptive

females. *Peg3* mutant males show no such changes in female-odor sensitivity (Swaney et al., 2008). While such a deficit may appear relatively minor, olfaction is the primary sensory modality in rodents as illustrated by the gross deficits in reproductive behavior which result from ablation of olfactory membranes (Keller et al., 2009). The *Peg3* mutation appears to disrupt plasticity in the main and accessory olfactory systems and in hypothalamic regions which regulate sexual behavior, where significant increases in female odor-elicited neural activity are seen in sexually experienced wild-type males but not in *Peg3* mutant males (Swaney et al., 2007; Swaney et al., 2008).

Although *Peg1* mutant mice have not been reported to suffer deficits in male reproductive behavior, studies in humans and mice suggest that this gene is involved in male fertility. The human homolog *PEG1* and its paternally expressed anti-sense transcript are strongly expressed in human testes and studies of male infertility have shown that hypermethylation of the *PEG1* DMR and consequent silencing are strongly associated with different classes of male infertility (Hammoud et al., 2009; Poplinski et al., 2009). In mice, pre-natal administration of the endocrine disruptor vinclozolin produces male offspring with significantly lower sperm counts with adults. Sperm from these males have greatly increased methylation at both the *Peg1* and *Peg3* DMRs suggesting that normal methylation patterns at these imprinted loci are necessary for male fertility (Stouder and Paoloni-Giacobino, 2009).

The *Magel2* mutants also have deficits in male reproductive behavior (Mercer and Wevrick, 2009), with some phenotypic similarities between mutant males and mutant females. Mutant males also become infertile by 24 weeks and take several days to mate after being paired with females. However the male phenotype also involves an olfactory deficit which includes a lack of preference for opposite-sex odors which is not seen in the female mutants. Basal forebrain neurochemistry animals is also affected by *Magel2* deletion which causes reductions in levels of both serotonin and dopamine in the hypothalamus, as well as reduced amygdala and nucleus accumbens volumes (Mercer et al., 2009). In line with the reproductive deficits, *Magel2*-knockout males also have reduced serum testosterone levels.

These hypothalamic and endocrine deficits are of interest given the involvement of the human homolog *MAGEL2* in Prader-Willi syndrome (PWS), a multigenic imprinting disorder involving paternal deletion or maternal duplication at chromosome 15q11-q13 region (Lee et al., 2000). PWS symptoms involve hypothalamic dysfunction affecting appetite and metabolism, as well as hypogonadism and infertility (Eiholzer et al., 2006). Aberrant expression of paternally expressed *NDN* is also involved in the etiology of PWS and mouse knockout studies have shown that paternal deletion of homologous *Necdin* disrupts reproductive hormone circuits. Muscatelli et al (2000) reported that *Necdin*-null mice (both male and female) have fewer oxytocin neurons in the paraventricular nucleus and fewer hypothalamic GnRH neurons. This latter deficit has recently been shown to be due to reduced migration of GnRH neurons to the hypothalamus during development (Miller et al., 2009), and lower numbers of both oxytocin and GnRH neurons have been reported in PWS patients. The *Magel2* and *Necdin* mutant phenotypes suggest that disruption of developmental expression contributes to PWS hypogonadism and provides further evidence that paternally expressed genes influence male reproductive behavior.

Reproduction and the evolution of genomic imprinting

There have been many theories proposed to explain the evolution of imprinting, each of which has different strengths and weaknesses regarding mechanisms of imprinting and imprinted gene-related phenotypes. The most widely cited of these is the conflict or kinship theory for the evolution of imprinting (Haig and Graham, 1991; Moore and Haig, 1991). This predicts that the conceptus (placenta and offspring), which inherits approximately half of its genes from its mother and half from its father, may be a site for potential conflict between the two parental genomes. The paternal genome would favor maximal maternal investment in offspring, to the possible detriment of the mother's long term health and any subsequent offspring conceived with other males. Conversely, the maternal genome would favor balanced investment of resources in all offspring across her entire reproductive career. The placenta is an important endocrine organ that interfaces and communicates with the

maternal brain to regulate maternal investment and so is functionally capable of influencing maternal behavior and physiology. The conceptus can thus be viewed as a parasite in which imprinted genes influence resource extraction from the mother in opposite directions. Paternally expressed genes are predicted to be growth-enhancing and maternally expressed genes are predicted to be growth-restricting. The first knockout studies showed that paternally expressed *Igf2* is indeed growth enhancing (DeChiara et al., 1991), while maternally expressed *Igf2r* and *H19* limit fetal growth (Lau et al., 1994; Leighton et al., 1995). While mutations at other imprinted loci also cause growth phenotypes which match the predictions of the conflict theory, there are some that do not (Hurst and McVean, 1997). Furthermore, some of the imprinted gene knockout mice also exhibit phenotypes which extend beyond birth and even into adulthood, where conflict between the parental genomes is less apparent. While it can be argued that post-natal, pre-weaning effects of imprinted genes are in line with the predictions of the conflict hypothesis (Isles and Holland, 2005), the adult phenotypes of some imprinted gene mutants are more difficult to reconcile with it.

The parent-of-origin transmission of the *Peg3* transgene allowed the effects of the mutation to be compared independently in mutant pups and mutant mothers. The phenotypes are remarkably complementary, with offspring deficits in pre-natal growth, suckling, and thermoregulation matched by reduced maternal food intake, milk letdown and nesting behavior (Curley et al., 2004). These result in similar outcomes for the offspring whether they or the mother carry the mutation. The different peri-natal behaviors are regulated by an endocrine interplay between the fetal hypothalamus, the maternal hypothalamus and the placenta (Keverne and Curley, 2008), all areas of high *Peg3* expression. *Peg3* appears not to have evolved in response to conflict between parental genomes but under selection pressures that favor coadaptation of behaviors between offspring and mother, leading to increased fitness for both. While no other imprinted gene mutants have such clearly dovetailing phenotypes, the effects of deletions of other paternally expressed genes on maternal behavior and post-natal behavior suggest that coadaptation may have played an important role in the evolution of imprinting at multiple loci. Detailed behavioral phenotyping

of other imprinted gene knockouts would help to substantiate the evolutionary significance of coadaptation and clarify whether other imprinted genes may also have evolved under such coadaptive selection pressures. Moreover, data from other species is required to confirm the importance of imprinted genes in mammalian reproduction and whether coadaptation has played a wider role in the evolution of imprinting.

Some imprinted genes seem to have evolved to regulate female reproductive behavior despite being paternally expressed. Such effects are not surprising given the high expression of paternally imprinted genes in the hypothalamus which regulates reproductive behavior and maternal care, however any adaptive effects will skip every other generation due to the silencing of paternally expressed genes in the female germline. Most of these genes have also been shown to influence male reproductive behavior to greater or lesser degrees. This is significant, as any effects of paternally expressed genes on male behavior would occur in every generation due to patrilineal transmission of actively expressed alleles at these loci. Any effects on male reproductive success would be magnified by a combination of paternal allele-only expression and reproductive skew, resulting in much faster spread of any paternally expressed alleles that regulate adaptive male reproductive behavior (Keverne, 2009). Paternal expression appears to have allowed the imprinted genes that govern the development and function of the hypothalamus to regulate both male and female reproduction, and so maximize reproductive success in both sexes.

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Figure 1. Non-Mendelian parent-of-origin expression allows imprinted genes to be studied independently in mother and offspring using knockout mice. Selective silencing of one parental allele means that heterozygous transgenic mice have either full mutant or full wild-type (WT) phenotypes depending on the imprinting status of the gene and the parent from which the mutation is inherited. By pairing different combinations of mutant-heterozygous, WT-heterozygous and WT individuals, mutant offspring can be born to wild-type mothers and wild-type offspring born to mutant mothers. a) Hypothetical mating combinations to study a paternally expressed gene knockout: i) crossing a WT father with a mutant-heterozygous mother produces WT and WT-heterozygous offspring; ii) crossing a mutant-heterozygous father with a WT mother produces WT and mutant-heterozygous offspring. b) Hypothetical mating combinations to study a maternally expressed gene knockout: i) crossing a WT father with a mutant-heterozygous mother produces WT and mutant-heterozygous offspring; ii) crossing a WT-father with a WT-heterozygous mother produces WT and mutant-heterozygous offspring.

Table 1. A summary table of the imprinted genes which have been linked to behavioral and neuroendocrine components of reproduction in mammals.

Gene	Expression	Offspring	Females	Males	References
Peg1	Paternal	Post-natal growth	Maternal behaviors	Male fertility and sperm viability	(Lefebvre et al., 1998; Hammoud et al., 2009; Poplinski et al., 2009)
Peg3	Paternal	Post-natal growth, suckling, thermo-regulation, puberty	Maternal behaviors, milk letdown, hypothalamic oxytocin	Sexual behavior, reproductive olfaction, hypothalamic plasticity	(Li et al., 1992; Curley et al., 2004; Champagne et al., 2009; Swaney et al., 2007; Swaney et al., 2008)
Gnasxl	Paternal	Post-natal growth, suckling	-	-	(Plagge et al., 2004)
Magel2	Paternal	-	Reproductive behavior, fertility, gonadal histology hypothalamic serotonin and dopamine, hypogonadism	Reproductive behavior, fertility, reproductive olfaction, hypothalamic serotonin and dopamine, testosterone levels, hypogonadism	(Mercer and Wevrick, 2009; Mercer et al., 2009)
Necdin	Paternal	-	Hypothalamic oxytocin and GnRH, hypogonadism	Hypothalamic oxytocin and GnRH, hypogonadism	(Muscatelli et al, 2000; Miller et al., 2009)