Sex reversal in non-human placental mammals

Pietro PARMA[a#], Frédéric VEYRUNES[b#] & Eric PAILHOUX[c#]

[a]Department of Agricultural and Environmental Sciences, Milan University, Milan, Italy.

[b]Institut des Sciences de l'Evolution de Montpellier, CNRS / Université Montpellier / IRD (UMR 5554), France.

[c]UMR BDR, INRA, ENVA, Université Paris Saclay, 78350, Jouy en Josas, France.

[#]All three authors contributed equally to this review article.

Corresponding author:

Eric PAILHOUX

Tel.: 33 (1) 34 65 25 39

E-mail : Eric.Pailhoux@jouy.inra.fr

Abstract

Gonads are very peculiar organs given their bi-potential competence. Indeed, early differentiating genital ridges evolve into either of two very distinct organs that are testis and ovary. Accumulating evidence now demonstrate that both genetic pathways must repress the other in order to differentiate properly, meaning that if this repression is disrupted or attenuated, the other pathway may completely or partially be expressed leading to disorders of sex development. Among these disorders are the cases of XY male-to-female and XX female-to-male sex reversals as well as true hermaphrodites, in which there is a discrepancy between the chromosomal and gonadal sexes. Here, we review known cases of XY and XX sex reversals described in mammals, focusing mostly on domestic animals where sex reversal pathologies occur and wild species in which deviations from the usual XX/XY system have been documented.

Keywords

Sex determination, Sex reversal, Gonad differentiation, placental mammals.

1 - Introduction

In mammals, sexual development begins at fertilization when an X- or a Y-bearing sperm fertilizes an ovum carrying an X chromosome to produce respectively a genetically XX female or XY male zygote. This crucial step will influence the fate of the future gonad into an ovary or a testis for XX or XY embryos respectively. In therian mammals (marsupials and placentals), testis differentiation is activated by a genetic factor, called the Testis Determining Factor (TDF), and identified more than 20 years ago as the *SRY* gene (Sex-determining Region of Y), a gene carried by the Y chromosome [Sinclair et al., 1990; Foster et al., 1992]. SRY triggers a cascade of molecular events, the *SOX9* pathway, inducing the undifferentiated uro-genital ridge, a gonadal primordium characterizing all embryos, to differentiate into testis [Sekido and Lovell-Badge, 2008; Lavery et al., 2011; Vidal et al., 2001]. Subsequently, the hormonal products of specific testicular cells, i.e. AMH (Anti-Müllerian Hormone) produced by Sertoli cells and androgens by Leydig cells, lead the male phenotype development of the embryo. In XX individuals where *SRY* is not present, a specific female molecular cascade, the β-catenin pathway, directs ovarian development thus ensuring a female phenotype differentiation (for review [Wilhelm et al., 2007]).

To summarize, mammalian sexual development is therefore dependent on a three-step process: (i) the sex chromosome complement at fertilization; (ii) gonad differentiation into testis or ovary in accordance with the chromosomal sex and especially with SRY; and (iii) differentiation of sex-specific phenotypic features including external and internal genitalia in accordance with the gonadal sex. This schematic view is highly challenged today by accumulating evidence showing that some sex-specific phenotypic features could be directly under the control of the sex chromosome content (for review and an example see [Arnold et al., 2013; Renfree et al., 2014]). Nevertheless, at any of the three steps of sexual development, the process can go awry, leading to disorders of sex development (DSD), a generic definition encompassing any problem noted at birth when the genitalia are atypical in relation to the chromosomes or gonads [Hughes, 2008]. Sex reversal pathologies are included in DSDs, and here, we will restrict this review to the true "sex reversals", i.e., involving a discrepancy between the chromosomal sex (XY or XX) and the gonadal sex (testis or ovary), and thus the phenotypic sex (male or female). Hence, sex reversals hereafter include XY females and XX males, as well as intersex/hermaphroditic cases that may be observed in both categories.

Since the discovery of the *Sry* gene in the early 90's, considerable progress has been made in our understanding of gonad development in mammals (reviewed in [Brennan and Capel, 2004; Wainwright & Wilhelm, 2010; Quinn and Koopman, 2012]). Most of the major advances in the field (e.g. identification of *SRY*, *SOX9*, *WT1*, *MAP3K1*, *RSPO1*) have stemmed from the analysis of sex reversed human patients or genetically manipulated laboratory mice. Most often, genes were identified in human patients and their involvement in the sex determining gene cascade were afterwards validated in the mouse model where knock-out/knock-in strategies can be performed (with ease) [Warr & Greenfield, 2012]. However, even if many genes involved in gonad differentiation have been discovered, many sex reversal cases in humans and also in other mammals, remain unexplained. This suggests that (i) other genes involved in sex differentiation pathways are yet waiting to be found; (ii) some cases are due to complex regulatory mutations difficult to identify; or (iii) single SNPs changing only one amino-acid, that while now easier to detect by exome sequencing remain difficult to validate by functional studies.

We will review most of known cases of XY and XX sex reversal in a large diversity of placental mammals excluding the human species, as this themed issue includes an article from McElreavey's lab dealing with sex reversal in humans. We will show that other mammalian models (mostly domestic animals where sex reversal occurs), or even non-model mammals (wild species that present rare cases of deviation from the usual XX/XY system) provide invaluable opportunities to unravel new gene interactions involved in the initiation of gonadal development.

2 - XY sex reversal in domestic species

As presented below, XY sex reversal condition has been described in numerous mammalian species leading, as in humans, to a great phenotypic variability. The genetic characterization of this condition had considerably evolved with the discovery of the testis-determining factor *SRY* in 1990 [Sinclair et al., 1990], thus permitting to distinguish two categories (i) XY *SRY*-defective females and (ii) XY females harboring a normal *SRY* allele.

2.1 - XY sex reversal in cattle

Probably the first case of XY sex reversal was reported in cattle (*Bos taurus*) in the mid-1960s [Henricson and Akesson, 1967]. The authors described two heifers (Swedish Red and White breed) with an XY sex chromosome constitution in blood cells, skin, and lung-derived fibroblasts. In cattle, the analysis of chromosomes in tissues other than blood is a priority in order to exclude XX/XY chimeric condition that represents the most common sex chromosome aberration, and leads to abnormal sexual development [Padula, 2005; Peretti et al., 2008; Ducos et al., 2008; Villagomez et al., 2009; Favetta et al., 2012] (XX/XY chimerism in cattle is mainly a consequence of chorionic fusion and placental vascular anastomoses between two twins of different sex; a phenomenon known as freemartinism). The heifers had hypoplastic-dysplastic and dysgenetic left and right ovaries with abnormal follicle development, and were classified as gonadal dysgenesis. Another case was reported about 10 years later: a female with no estrus and an XY karyotype in blood cells. However, the search for sex chromatin in cells from other tissues was ineffective. The left gonad was quite normal with primary and secondary follicles, and undifferentiated germ cells, but no oocytes; the right gonad was absent. Therefore, the case was classified as partially hypoplastic [Chapman et al., 1978]. Partially hypoplastic gonads are not uncommon in cattle. When only one gonad is present, it is the left one in 97% of cases [Roberts, 1971; Settergreen, 1964]. Subsequently, several cases were reported: a female with no estrus, pure XY sex chromosome constitution, and streak gonads [Sharma et al., 1980], an XY female with hypoplastic ovaries [MacMillan et al., 1984], plus two other reports from congress abstracts with unfortunately no further descriptions [Gustavsson et al., 1981; Eldridge et al., 1984]. In 1989, Murakami and colleagues reported the analysis of 18 sterile females, and one of these was described with a pure XY chromosome constitution, while from the anatomical point of view, the ovaries, uterus, and vagina were present [Murakami et al., 1989]. Nevertheless, this female showed a feeble estrus. This situation is fairly common in XY cattle and represents an exception with respect to other animals where two X chromosomes are usually required to promote ovarian development. Subsequently, following the discovery of *SRY* as being TDF [Sinclair et al., 1990], five XY females were reported. The first one possessed bilateral streak gonads associated with a normal female genital development. The molecular genetic analysis revealed an XY constitution and the presence of the *Amgy* and *Zfy* genes [Kondoh et al., 1992]. These two genes are closely linked to *SRY* in humans. The authors failed

to identify the *SRY* gene probably because they used PCR primers designed for the human *SRY* gene; since the cattle *SRY* gene sequence was still unknown at that time. The second one was a phenotypic XY female with both hypoplastic gonads: the right one resembled an ovary and the left one an undeveloped testis [Hare et al., 1994]. In this subject, testosterone levels remained low even after stimulation. The last three cases were first reported in 1996 and concerned females in appearance, but sterile with no estrus [Kawakura et al., 1996]. The availability of the cattle *SRY* locus allowed the characterization of these females as *SRY*-negative despite the presence of an XY chromosome constitution. A more accurate characterization of these three subjects was reported a year later [Kawakura et al., 1997]. By using two FISH probes, BC1.2 and btDZY-3 that localized respectively to the telomeric region [Cotinot et al., 1991] and to the centromeric region of the short arm of the Y cattle chromosome [Perret et al., 1990], the authors showed that this Y chromosome was represented by an Yp isochromosome. This condition excludes the presence of *SRY*, which is localized on Yq12.3 in cattle [Xiao et al., 1995; Di Meo et al., 2005]. Noteworthy, other studies reported the characterization of “XY females” [Nes, 1966; Sysa et al., 1974; Balakrishnan et al. 1979; Long and David, 1981; Peter et al., 1993], but they are excluded from this review as they could not be properly defined as XY females with gonadal dysgenesis, and may instead refer to other syndromes, such as PMDS (Persistent Müllerian Ducts Syndrome), AR mutation (Androgen Receptor) and XX/XY freemartinism, the most common chromosomal abnormality in cattle.

2.2 - XY sex reversal in horses

XY sex reversal in horses appears in a higher frequency than in any other domestic mammals, with an occurrence of 12–30% of the specimens harboring cytogenetic abnormalities [Bowling et al., 1987; Power and Leadon, 1990]. The first report was published 40 years ago with an XY mare associated with rudimental gonads [Chandley et al., 1975]. Two years later, the analysis of 12 phenotypic normal mares with reproductive failure allowed the identification of one animal with a 64,XY karyotype and small ovaries [Hughes and Trommershausen-Smith, 1977]. In 1980, the first molecular analysis of an XY mare with gonadal dysgenesis, showed that it retained the H-Y+ phenotype [Sharp et al., 1980]. Interestingly, this sex reversed female gave birth to a normal XX daughter. This is not surprising in horses as sex

reversed females may present very different phenotypes: fertile with ovaries, sterile with ovaries, or hermaphrodite with ovotestes. The great majority of XY mares involves sporadic cases, but at least one familial case was described [Kent et al., 1986]. Before the identification of the *SRY* gene, there were four other reports of XY sex reversal in horses [Power, 1986; Long, 1988; Kent et al., 1988a; Kent et al., 1988b]; notably, on basis of the observation of 38 XY mares, Kent and collaborators proposed a classification of gonadal dysgenetic mares in four groups according to the gonad anatomy, the H-Y antigen reaction, and the level of testosterone, [Kent et al., 1988a, 1988b]. Then, the discovery of *SRY* allowed to investigate these cases in more detail. A pioneering work described the deletion of this gene in an XY mare with non-functional ovaries [Pailhoux et al., 1995]; this was followed by many other studies that considered several Y-linked and X-linked loci (*SRY*, *AMEL-X/Y*, *ZFX/Y*) [Abe et al., 1999; Makinen et al., 1999; Makinen et al., 2001; Bugno et al., 2003; Raudsepp et al., 2010; Villagomez et al., 2011; Anaya et al., 2014; Pieńkowska-Schelling et al., 2014]. All these studies detected *SRY* deletions, most of them consisting in a ~21 kb deletion on the Y chromosome [Raudsepp et al., 2010]. The high frequency of this deletion, the fact that *SRY* is not located in the vicinity of the pseudo-autosomal boundary in horses (contrary to human *SRY*) and that an XX sex reversed *SRY*-positive stallion has never been described, argued against an XY chromosomal interchange during paternal meiosis as proposed [Fergusson-Smith, 1966] and then demonstrated [Sinclair et al., 1990] in humans. Accordingly, Raudsepp and colleagues hypothesized that the prevalence of deletions involving *SRY* was related to the intrinsic organization of the horse Y chromosome, i.e., with palindromic structures [Rozen et al., 2003] surrounding *SRY* that facilitate deletions [Raudsepp et al., 2010]. However, among the XY sex reversed horses, a few are *SRY*-positive, and when sequenced, the *SRY* open-reading frame was intact [Raudsepp et al., 2010]. The associated phenotypes seem to be more variable in this condition than in the XY *SRY*-negative ones, suggesting yet unknown causative mutations on different genes involved in primary sex-determination (as in humans).

2.3 - XY sex reversal in River buffalo

More recently, two cases of XY females in the river buffalo *Bubalus bubalis* were reported [Iannuzzi et al., 2001]. The first subject was a five-year-old female with severe reproductive problems that were diagnosed as slight hypoplasia of derivative

Müllerian ducts and very small gonads with ovarian structures by subsequent anatomical observations. Blood cells showed an XY sex-chromosome complement [Parma et al., 2004], and further investigations revealed the presence of an intact *SRY* gene (our unpublished data). A few years later, the same research group reported a second case, an XY three-year-old female, with this time no internal reproductive organs [Iannuzzi et al., 2004]. The presence of the *SRY* locus was confirmed by FISH and the authors hypothesized that a *SRY* mutation was responsible for the clinical features. However, this hypothesis was subsequently rejected, when they found that the open-reading frame of *SRY* was identical to the wild-type allele (i.e. without any mutation) [Di Meo et al., 2008].

2.4 - XY sex reversal in dogs

While XX sex reversal occurs regularly in dogs (see detailed section in this review, below), at least two cases of XY sex reversal have also been reported: a dog characterized by the presence of an ovary with atretic follicles devoid of oocytes and a hyperplastic testis with Sertoli cell tumor [Chaffaux and Cribiu, 1991]; and more recently, a gray wolf with bilateral ovotestes [Kang et al., 2012]. Interestingly, the latter originated from a cloning strategy [Oh et al., 2008].

2.5 - XY sex reversal in other species

Single cases of XY sex reversal have been anecdotally reported in other species: a sterile ewe with streak gonads, and a cat with two ovotestes as well as derivatives of Müllerian and Wolffian ducts. In both cases, the *Sry* gene was present and the sequences were normal [Ferrer et al., 2009; Schlafer et al., 2011].

3 - XY sex reversal in mammals following evolution of unusual sex determination systems

In mammals, the XX/XY system is the norm, but despite the apparent stability of this system, a handful number of species (all rodents) harbour unusual sex determination systems that have evolved through millions years of natural selection (Table 1; [reviewed in Fredga, 1994; Jimenez et al., 2013]). For instance, at the opposite of the sporadic pathological cases associated to gonadal malformation or dysfunction described above, XY sex reversal naturally occurs independently in at least four

mammalian species without affecting fertility. These concern the wood lemming *Myopus schisticolor* (the first species to be described with such a system) [Fredga et al., 1976, Fredga, 1988, 1994], the arctic lemming *Dicrostonyx torquatus* [Gileva, 1987, Fredga, 1988, 1994], several species of South American field mice *Akodon* [Bianchi, 2002; Ortiz et al., 2009], and the African pygmy mouse *Mus minutoides* [Veyrunes et al., 2010]. A fifth species has been described, the Cabrera's vole *Microtus cabrerae*, with four fertile XY females found in a population [Burgos et al. 1988]. However, despite extensive search no new sex reversed females have been identified since then, suggesting that the mutation converting XY individuals into females has been lost. In addition, *Microtus cabrerae* is the only known mammalian species to have presumably non-functional copies of *Sry* on the X chromosome [Bullejos et al. 1997]. The prevalence of fully fertile XY females is very high, from 10 up to 75%, depending on the species and populations [e.g. Bianchi, 2002; Veyrunes et al., 2010, 2013]. Histological and molecular analyses revealed that XY ovaries display a typical ovarian structure, with no sign of any testicular organization, confirming a complete sex reversed phenotype in these species [Rahmoun et al., 2014]. However, it is noteworthy that in *Myopus schisticolor* XY females have smaller ovaries with less oocytes and follicles than XX females [Fredga et al., 2000], whereas in *Mus minutoides*, it is the contrary, the ovarian volume of XY females is larger, with more follicles compared to XX [Rahmoun et al., 2014].

In fact, the sex determination systems of the four taxa are relatively similar since the sex reversal is caused independently by the presence of a third sex chromosome: a variant of X, named X*, that blocks the male program initiated by the Y chromosome. This occurs even in *Akodon* where the sex reversal mutation was previously misinterpreted to be on the Y chromosome, but see [Ortiz et al., 2009]. The Y chromosome of all these females is normal and contains the *Sry* gene. So while males are XY, there are three types of females with different sex chromosome complements: XX, XX* and X*Y. Chromosome banding and FISH techniques revealed that the X and the X* chromosomes can be cytogenetically distinguished in all four species except *Dicrostonyx torquatus* [Herbst et al., 1978; Liu et al., 1998; Ortiz et al., 2009; Veyrunes et al., 2010]. These sex determination systems can be characterized as polygenic [Moore and Roberts, 2013]: two sex determining genes segregate in the populations: the regular mammalian male determiner *Sry* on the Y chromosome, and a still unknown dominant female determiner on the X*. The genetic

bases of the sex reversal may therefore involve mutations on the same X-linked gene(s) in the four lineages independently.

In *Myopus schisticolor*, the X* is characterized by an inversion in the short arm and a deletion of about 1Mb in the Xp^{21-23} region that was demonstrated to contain the *Cct7* gene, a member of the chaperonin gene family assisting protein folding in the cytosol of eukaryotes and mainly expressed in testis, making it a potential candidate for the sex reversal mutation [Liu et al., 2001]. However, recent studies weakened this finding, since they uncovered an intense duplication activity of this gene in vertebrates; notably the mouse *Cct7* homologue is located on chromosome 6 with pseudogene sequences on the X [Mukherjee et al., 2010]. In *Mus minutoides*, the expression of key sex-specific genes (*Foxl2*, *Sox9*, *Wnt4* and *Dmrt1*) was investigated in adult gonads and did not reveal any unusual pattern; in addition, the X-linked gene *Dax1*, that has been described as inducing XY sex reversal when duplicated in humans [Bardoni et al., 1994; Swain et al., 1996], showed no sign of change in either copy number or expression [Rahmoun et al., 2014]. These first results stress the serious need for further molecular analyses to identify the mutation(s) in these non-model mammals, and notably at the embryonic stages when gonads start to differentiate.

In mammals, most XY females are sterile or have very poor fertility. They generally suffer from the lack of an X chromosome meiotic partner and the presence of a Y chromosome. This leads to meiotic disruption or arrest, decreased oocyte survival, poor preimplantation rates, increased embryonic loss [Villemure et al., 2007; Alton et al., 2008; Xu et al., 2012], and for those that manage to go beyond meiosis, fertility is still greatly reduced as 25% of embryos produced (YY) are non-viable. Clearly, the X*Y females of the four taxa manage to bypass the meiotic constraints somehow, but they are still expected to face the high reproductive cost of the YY embryo loss. In this sense, the long-term evolution of such systems may appear as a paradox, unless mechanisms compensating for the cost have evolved in these lineages. They actually did, and unexpectedly, in a very diverse way according to the different species. In *Myopus schisticolor*, a unique mechanism of meiotic double disjunction occurs in X*Y females, in which the Y chromosome is eliminated from the germ line and only X*-carrying ovocytes are formed, producing only XX* and X*Y daughters, thus preventing the formation of YY embryos [Fredga, 1983]. In *Dicrostonyx torquatus*, X*Y females have a greater ovulation rate compared to XX [Fredga, 1994]. In

Akodon azarae, X*Y females have both a longer reproductive lifespan and a higher rate of preimplantation embryonic development [Espinosa and Vitullo, 2001]. Finally, in *Mus minutoides*, X*Y females show enhanced reproductive performance compared to XX and XX* females, resulting from a higher probability of breeding, an earlier first litter, a larger litter size, and a greater ovulation rate [Saunders et al., 2014]. In addition, in the latter species, it has been shown that the sex chromosome complement may directly influence some behavioral traits, independently of the gonads and their hormones. Hence, X*Y females are much more aggressive than the other females, and show masculinized behaviors related to anxiety and exploratory activity [Saunders et al., 2016].

4 - XX sex reversal in domestic species

Following a general view, XX sex reversal results from the development of testicular tissue in a genetically programmed XX female individual. From a genetics point of view, this condition could result either from gain-of-function (GoF) mutations leading to the up-regulation of a crucial male gonadal-differentiating gene, or from loss-of-function (LoF) mutations of key female genes. Whatever the genetics cause of XX sex reversal in mammals (GoF or LoF), the resulting adult is an infertile male because XX testes in domestic mammals are always devoided of germ cells. In all studied species, XX males appear more or less masculinized, depending on the proportion of testicular *versus* ovarian tissue development. The gonadal phenotypes thus vary from slightly masculinized with an ovo-testis on one side and an ovary on the other (classified as XX true hermaphrodites) to fully masculinized animal with two descended testes and a male appearance (XX males without ambiguities of the genitalia) with many intermediate phenotypes such as animals with only testes-like gonads most often abdominal and hypoplasic (XX males with ambiguous external and internal genitalia). As presented below, XX sex reversal is regularly encountered in different mammalian species in addition to humans. The phenotypes are highly variable and the genetics causes heterogeneous.

4.1 - XX sex reversal in dogs

The XX *SRY*-negative sex reversal condition is frequently observed in dogs which represent a very useful animal model to identify the causes that induce the

undifferentiated gonad to develop into testicular tissue without the crucial action of *SRY*. The first review about intersexuality in dogs, in which 45 different cases were reported, appeared in 1976 [Hare, 1976]. Out of the 45, six harbored an XX chromosomal content (established with different techniques) with left and right abdominal testes. Previously, another XX pug with abdominal ovotestes had been reported [Stewart et al., 1972]. All these subjects belonged to different breeds and represented sporadic cases. The first familial case was reported three years later [Williamson, 1979]. It was a Kerry Blue Terrier family where some males presented an XX chromosomal constitution and testes-like gonads with apparently normal seminiferous tubules. It is only 20 years later that another familial case was identified, belonging to a pedigree where interestingly two other sporadic independent cases were noticed [Melniczek et al.,1999]. In this pedigree, an autosomal recessive transmission was supposed. Noteworthy, most of these cases have been described before the discovery of the *SRY* gene, and thus, we cannot exclude that some of them were *SRY*-positive. By 2012, 29 new cases of *SRY*-negative XX male dogs were described in an exhaustive review [Meyers-Wallen et al., 2012], followed by seven other reports of XX dogs with testicular or ovo-testicular DSD [Marcinkowska-Swojak et al., 2015; Salamon et al., 2015; Pérez-Gutiérrez et al., 2015; Del Carro et al., 2014; Rossi et al., 2014; Groppetti et al., 2012; Max et al., 2011]. Notably, many investigations have been conducted on a large American Cocker Spaniel pedigree [Meyers-Wellen, 1999]. The analyses (i) confirmed the absence of the *SRY* gene, (ii) ruled out several sex reversal candidate genes [Kothapalli et al., 2003, 2004, 2005, 2006; Pujar et al., 2005] and iii) identified by a linkage analysis a candidate region on the canine chromosome Cfa29 [Pujar et al., 2007]. Unfortunately, further investigations were unable to identify the causative mutation in this genomic region. Searching for mutation on *RSPO1* gene in a single case was not conclusive [De Lorenzi et al., 2008]. The occurrence of a Robertsonian translocation involving Cfa5 and Cfa23 in an XX sex reversed dog [Switonski et al., 2011] suggested that another genetic factor responsible for this phenotype was located on Cfa23, as this chromosome carries three important genes involved in sex determination (*FOXL2*, *PISRT1* and *CTNNB1*). More recently it has been shown that a duplication covering the whole *SOX9* open reading frame as well as a large part of the 5' region was responsible for XX sex reversal in dogs [Rossi et al., 2014, 2015]. Duplication of *Sox9* is thus the only causative mutation for XX sex reversal in dogs identified so far,

but it is certainly not the only one as many other cases do not carry this duplication [Marcinkowska-Swojak et al., 2015; Szczerbal et al., 2016].

From an anatomical point of view, XX sex reversed dogs show a wide variability. About 90% of cases occur with bilateral ovotestes and the remaining 10% with bilateral testes (Fig. 1). Furthermore, some rare reports concern ovary/ovotestis and testis/ovotestis. Finally, regarding masculinization of internal and external genitalia, their degree of masculinization is fully related to the portions of testicular tissue, as also demonstrated in XX sex reversed specimens from other mammalian species.

4.2 - XX sex reversal in pigs

Sex reversed pigs, also called "intersexes" in the past, have generally been characterized as male pseudo-hermaphrodites having testis-like gonads, Müllerian and Wolffian duct derivatives and male accessory sexual glands exhibiting various degrees of development [Backstrom and Henricson, 1971; Hunter et al., 1982]. True hermaphrodites with both male and female gonadal tissue have also been described [Hunter et al., 1985] and also rare fertile female cases harboring one normal ovary on one side and an ovotestis on the other side [Hulland, 1964]. In most cases, sex reversed pigs have a female karyotype, showing a 38,XX chromosomal constitution [McFeely et al., 1967]. At slaughter, the frequency of intersexuality ranges from 0.1 to 0.6% in pigs of different breeds [Koch, 1963]. In a large study in Holland, Breeuwsma found 0.4% intersexes [Breeuwsma, 1971]. The clinical frequency (0.2%) observed in breeding herds is probably an underestimation of the real frequency since the diagnosis of true hermaphroditism is difficult to ascertain because many cases present a normal female external genitalia. Economic losses due to intersexuality are significant and result from sterility, genital infections reducing growth and viability and downgrading of carcasses because of sexual odor.

Since 1992, we have carried out specific studies on pig sex reversal that we will shortly resume thereafter in order to propose a personal analysis. These studies have used an experimental pig unit of INRA where 4 lines of pigs have been crossed altogether since 1988 for genetic selection of 4 different traits (the founder population of this herd was of 42 related sows and 19 none-related boars). Intersex animals were detected as of the fourth generation at a frequency of ~1% and our study was carried out on a 6 years period (1992-1997). We firstly autopsied ~30 sex reversed adults and we kept their parents alive in order to isolate and breed them separately

(creation of an "Intersex" line). All piglets from this "Intersex line" were autopsied at 5-weeks after birth allowing to observe another 30 sex reversed familial cases [Pailhoux et al., 1997]. *SRY* gene detection was carried out on all sex reversed cases and only 3 were found positive among the 30 sporadic cases, showing that a small proportion (~10%) of sporadic sex reversed pigs is due to a 38XX, 38XY chimerism or mosaicism (2 cases on 30) or to the abnormal presence of *SRY* into a 38,XX genome (1 case on 30) [Pailhoux et al., 1994b; Pailhoux et al., 1997]. As expected all familial cases harbored a pure female 38,XX genotype, without *SRY*. Phenotypically, all the 57 cases studied ranged from true hermaphrodite (48%) to males with (50%) and without (2%) ambiguities. True hermaphrodites with both testicular and ovarian tissue in the same animal or in the same gonad have been observed and rare cases (n=3) of pregnancy in these hermaphrodite sows have been obtained; especially in animals having one normal ovary and an ovotestis, female fertility has been restored after surgically removing the sex reversed gonad [Pailhoux et al., 1997].

By the past, several authors have presented evidences that pig sex reversal may be inherited [Koch et al., 1957; Breeuwsma, 1971] since certain boars produce a relatively high incidence of intersexes in their offspring. Sittman and collaborators favored the hypothesis of recessive sex reversal genes at very few loci [Sittman et al., 1980] whilst Johnston and collaborators argued that a single recessive gene is the genetic basis of the defect [Johnston et al., 1958]. Moreover, the existence of crossbred intersexes suggests that the mutation giving rise to intersexuality is the same in several European breeds of pigs [Sittman et al., 1980]. At the end of our INRA's study, we observed frequencies of 17% of XX sex reversed cases on all 38,XX animals (23/135) in crosses with unaffected sows and 29% (9/31) in crosses with affected (fertile XX true hermaphrodites) mothers ([Pailhoux et al., 1997] and unpublished observations). The fact that the frequency shows a twofold increase when the mother is affected argues toward a monogenic recessive mode of inheritance, but the observed frequencies (17% and 29%) were found statistically different from the expected ones (25% and 50%). Thus, if XX sex reversal in pigs has a monogenic recessive mode of inheritance, the penetrance of the trait is incomplete (i.e.: some cases remaining as normal females even with a homozygous mutated genotype). Interestingly, more than 10 years later and thanks to the development of a commercial porcine high throughput SNP60 BeadChip, XX sex reversal in pigs was mapped on porcine chromosome 12 onto the *SOX9* locus [Rousseau et al., 2013]. In

this recent study, familial cases from the INRA's study have been genotyped with other 26 half/full-sib families collected between 2006 and 2010 from the Large-White population of some French breeding companies. Despite the large familial system comprising 89 sex reversal cases from two different origins (INRA's study and 26 unrelated half/full-sib families), no genome-association could be found when both populations were studied independently. But a unique strong association with the *SOX9* locus was revealed when both populations were analyzed as a pooled population. In other word, the association between sex reversal in pigs and the *SOX9* gene was only highlighted with more than 80 affected cases, that is very surprising when compared with some "real monogenic" recessive traits mapped by using very few affected animals (from 3 to 12, depending on the disease) [Charlier et al., 2008]. Agreeing with this supposed complexity of the mode of inheritance, no single homozygous chromosome segment could be identified among the affected piglets of the design, and numerous haplotypes seem to be linked with the XX sex reversal trait even if three of them were found associated at a frequency of ~64% [Rousseau et al., 2013]. Two hypotheses could explain this situation, (i) the mutation is very ancient and has invaded different haplotypes; (ii) or there are different mutations of the same *SOX9* locus leading to a sex reversal phenotype. The fact that, to our knowledge, XX sex reversal has never been described in wild boar argued against the first hypothesis. Moreover, an 18-bp indel has been recently described in the 5'-untranslated region of the *SOX9* gene in pigs [Brenig et al., 2015]. This 18-bp fragment contains a well-conserved cAMP-responsive element (CRE) and *in vitro* experiments demonstrate that the deletion of these 18 base pairs decreased *SOX9* transcriptional and translational activities [Brenig et al., 2015]. Consequently, the presence or not of this 18-bp element into an haplotype which harbors another regulatory mutation enhancing *SOX9* up-regulation in XX gonads, could explained why XX pigs could be sex reversed or not even if they carried an apparently equivalent haplotype.

In conclusion, it seems that XX sex reversal in pigs is linked to an abnormal up-regulation of *SOX9* during the ovarian differentiation process. The developmental stage where *SOX9* up-regulation occurs seems to be variable and could account for the huge variability of observed phenotypes. Indeed, the fact that some XX males with no internal nor external genital ambiguities were observed suggests that, in some cases, *SOX9* up-regulation occurred as early as when gonads start to

differentiate. By contrast, when we produced XX sex reversed fetuses at 50 and 70 d*pc* from the genitors of the "Intersex" line at INRA [Pailhoux et al., 2001a], we noticed a frequency of only 4% of sex reversed fetuses among 38,XX ones, while the same genitors produced at least 17% of affected 5-weeks old piglets, suggesting that most of cases initiate the sex reversal process, and thus *SOX9* up-regulation, after mid-gestation; so more than one month after the crucial stage of gonadal differentiation, that is 28 d*pc* in the pig species [Parma et al., 1999]. In addition to the developmental stage, several evidences suggest that different *SOX9* regulatory mutations may be involved in the occurrence of this pathology. If true, the combination of these different mutations could also explain the huge phenotypic variability of this trait. *SOX9* gain-of-function mutations have already been shown to be responsible for XX sex reversal in dogs and humans [Rossi et al., 2014]. In humans, the duplication of a 42-kb region named *RevSex* and located ~600kb upstream *SOX9* has been involved in XX sex reversal [Hyon et al., 2015]. It will be thus very interesting to study the pig *RevSex* equivalent region in order to determine if some genetic variations of this region could be linked with sex reversal haplotypes in pigs.

4.3 - XX sex reversal in goats

XX sex reversal in goats has been studied for a long time. The first reports are from the end of the nineteenth century but before 1944, XX sex reversal was studied independently from the polled phenotype (e.g. [Petit, 1894]). A crucial step was reached with the discovery that the same dominant mutation named "P" for "Polled" is a pleiotropic one, responsible for (i) an absence of horns in both sexes since one mutated allele is present (dominant trait; "Pp"), and (ii) a female-to-male sex reversal limited to XX animals homozygous for the mutated allele (recessive trait, XX "PP")[Asdell, 1944]. Following Asdell's hypothesis, intersexes and sterile males have been counted as XX animals and the *sex-ratio* (from crosses using two Pp parents), that was biased in favor of supposed XY before, then remained biased in favor of supposed XX [Brandsch, 1959]. This was due to the fact that ~80% of XY "PP" males are also infertile due to epididymal obstructions [Soller et al., 1963]. Finally, cytogenetic studies later revealed that the *sex-ratio* remains at equilibrium. Karyotype analyses thus confirmed that intersexes and sterile males with testicular hypoplasia were XX "PP", and sterile males with a normal testicular volume were XY "PP" [Soller

et al., 1969]. During the 1960s, it has also been shown that polled "Pp" females have a higher prolificacy (6–7%) compared to horned "pp" females [Soller and Kempenich, 1964; Ricordeau, 1969], while "PP" XY males (when fertile) are more prolific (8%) than "Pp" or "pp" males [Ricordeau, 1969]. This reproductive advantage for some animals bearing the P allele, while homozygous “PP” XX individuals are sex reversed and completely infertile, may explain why this mutation has been maintained in goats, even if it couldn't be fixed (fixation that has been attempted by artificial selection in some European countries at the beginning of the 20th for fixing the polled trait in goat herds).

The demonstration that *SRY* is the TDF in the 1990s [Sinclair et al., 1990] and the advent of micro-satellite genetic markers permitting construction of genetic maps, encouraged us to re-analyze this goat pathology to characterize the causal mutation. In a first step, we and others demonstrated that XX sex reversed goats do not harbor *SRY* in their genome [Pailhoux et al., 1994a; Just et al., 1994]. Then, by developing genetic markers in goats we mapped the "P" mutation on goat chromosome 1q41-q45 in a region corresponding to human chromosome 3q23 that encompass the Blepharophimosis Ptosis Epicanthus-inversus Syndrome (BPES, MIM #110100); a human disease affecting ovarian function also associated with cranio-facial malformations [Vaiman et al., 1996; Vaiman et al., 1999; Schibler et al., 2000; Crisponi et al., 2001]. The "P" mutation has since been renamed PIS for Polled Intersex Syndrome [Vaiman et al., 1996]. PIS was then identified as an 11.7 kb deletion located in a gene-desert region 300 kb upstream of the *FOXL2* gene [Pailhoux et al., 2001b], previously shown (on the same 2001 year) to be responsible for BPES [Crisponi et al., 2001]. The PIS mutation is a regulatory one, affecting the transcriptional expression of *FOXL2* and three long non-coding RNAs (*PISRT1*, *PISRT2* and *PFOXic*) [Pailhoux et al., 2001b; Pannetier et al., 2012] (Fig. 2A). Moreover, the transcriptional effects of the PIS mutation depend on the considered tissue, i.e., transcriptional silencing in XX gonads; transcriptional enhancing in developing horn buds and more generally in the cephalic skin [Pailhoux et al., 2001b]. Whatever the considered tissue, the four genes sensible to PIS (*FOXL2*, *PFOXic*, *PISRT1*, *PISRT2*) were found to be co-regulated, all four silenced in the gonads and all four up-regulated in the cephalic skin. Accumulating evidence seem to demonstrate that the 3 lncRNAs are all involved in a complex regulation of the *FOXL2* gene with (i) *PISRT1* and *PISRT2* acting at ~300 kb upstream of *FOXL2* and

probably involved in the primary expression of *FOXL2* by a close cooperation with the proximal *FOXL2* promoter [Boulanger et al., 2008; D'Haene et al., 2009; Pannetier et al., 2012]; and (ii) *PFOXic*, that should be viewed as a "transcriptional relic" arising from the bi-directional *FOXL2* promoter, itself involved in the *FOXL2* proximal regulation [Pannetier et al., 2005] (Fig. 2B). In the future, the complexity of *FOXL2* gene regulation should be deciphered in order to unravel unexplained cases of sex reversal especially in humans. Indeed, one could imagine mutational events in ovarian *FOXL2*-regulators abolishing *FOXL2* primary up-regulation specifically in the XX gonad, leading only to testis differentiation and thus to non-syndromic XX sex reversal.

Following the discovery that the PIS mutation was responsible for XX sex reversal in goats [Pailhoux et al., 2001b], two *Foxl2* KO mouse lines have been engineered independently [Schmidt et al., 2004; Uda et al., 2004] showing that, similarly with *FOXL2*$^{+/-}$ BPES type I patients, *Foxl2*$^{-/-}$ mice have no eyelids (instead of eyelids malformations) and a blockage of ovarian primary follicle formation (instead of primary ovarian insufficiency in humans). During many years, we tried to understand why *FOXL2/Foxl2* ovarian loss-of-function leads to folliculogenesis arrest in mice and to XX sex reversal in goats. In other words, why *Foxl2* is not involved in early ovarian differentiation in mice even if FOXL2 primary expression occurs as early as 12.0 d*pc*? After having incriminated the putative involvement of the three lncRNAs in addition to *FOXL2*, we finally demonstrate that *FOXL2* loss-of-function alone (i.e., independently of the lncRNAs) leads to XX sex reversal in goats (by contrast with mice) associated with an absence of eyelids development as described for mice [Boulanger et al., 2014]. Finally, following the demonstration that XX sex reversal in PIS$^{-/-}$ animals is indeed a consequence of *FOXL2* disruption, we look for early ovarian *FOXL2* target genes by sequencing the transcriptome of early-differentiating ovaries with (XX PIS$^{+/+}$) or without (XX PIS$^{-/-}$) *FOXL2* [Elzaiat et al., 2014]. According to these transcriptomic analyses, we demonstrated that FOXL2 normally represses the *DMRT1* gene in early developing ovaries, thus avoiding *SOX9* up-regulation and testis differentiation. This feature of the female pathway also has impact on the male counterpart where DMRT1 seems required for *SOX9* up-regulation in goats [Elzaiat et al; 2014], which contrasts with what is found in mice [Sekido and Lovell-Badge, 2008]. Moreover, in the goat species, SRY is predicted to be involved in testicular maintenance by repressing *FOXL2* during all the life in the Sertoli cells in order to

protect them from trans-differentiation [Montazer-Torbati et al., 2010; Elzaiat et al., 2014]. In summary, all results obtained in goats lead us to propose a sex-determining model that differs from the model currently admitted for mice (Fig. 3). Future studies will endeavor to demonstrate this model and to test its veracity on different non-rodent mammalian species.

4.4 - XX sex reversal in other species

More than 20 years ago, an XX sex reversed llama (*Lama glama*) with gonads entirely composed of testicular tissue was reported, but without information on the presence or absence of the *SRY* gene; thus we cannot exclude an XX *SRY*-positive case [Wilker et al., 1994]. Five years later, another llama was characterized with an XX karyotype, absence of *SRY* gene (tested by PCR) and partially masculinized external genitalia. However, the histological analysis of the gonad revealed only ovarian tissue, without any evidence of testicular tissue [Drew et al., 1999]. Consequently, this subject cannot be termed as XX sex reversed.

Horses are also regularly described with XX sex reversal syndrome, but phenotypes can be variable: a *Sry*-negative with bilateral ovotestes [Meyers-Wallen et al., 1997], a *Sry*-negative with hypoplastic ovotestes [Bannasch et al., 2007], and a *Sry*-negative with a normal-sized left ovary and a testis-like right gonad [Bodvarsdottir et al., 2009]. More recently, six new cases of XX sex reversed *SRY*-negative horses were reported [Villagomez et al., 2011]. For three of them, there is no information about the gonads' structure, two possessed bilateral ovotestes and the last one showed hypoplastic retained testicles. The last report concerns a *Sry*-negative with two bilateral ovotestes. In this case, the *RSPO1* coding region was also sequenced but no causative mutation was identified [Ciotola et al., 2012].

In 2009, a roe deer (*Capreolus capreolus*) was identified with an abnormal sexual development [Pajares et al., 2009]. Even if no cytogenetic analysis was performed, a PCR test excluded the presence of the *SRY* gene, and the histological examination of the gonad revealed the clear presence of testicular tissue in an ovotestis context. More recently, another interesting case of XX sex reversed roe deer was reported [Kropatsch et al., 2013]. This animal possessed two abdominal testes but no evidence of *SRY* and another Y-linked gene, *AMEL-Y*, was detectable by PCR. Using a whole genome sequencing approach, authors could identify 42 genetic variations in several genes previously shown to be involved in sex determination (*AR*, *DMRT1*,

FGF9, *FOXL2*, *RSPO1*, *SOX3*, *SOX9*, *SOX10* and *WT1*), but none was clearly established to be responsible for the pathological condition. More interestingly, a copy number variation analysis revealed a duplication of the *SOX9* locus that included not only the coding region but also about 1 Mb of the 5'-upstream region and 1,5 Mb of the 3'-downstream region. In humans, *SOX9* duplications have already been identified as a causative mutation for XX sex reversal, but most of these duplications are relegated in the 5'-upstream region of *SOX9* [Vetro et al., 2015], only one case concerns a duplication of the *SOX9* coding region [Huang et al., 1999].

5 - XX sex reversal in mammals following evolution of unusual sex determination systems

In mammals, atypical sex determination systems with XX sex reversal are even rarer than XY sex reversal ones. They are only known from three species of the same genus: the mole voles *Ellobius* (Arvicolinae, Rodentia). This genus probably exhibits the weirdest sex determination systems, described for the first time by Matthey in 1953. It comprises five species: *E. lutescens* which has an odd karyotype (2n = 17) identical in males and females with only one X chromosome (X0/X0), *E. tancrei*, *E. talpinus* and *E. alaicus* with also an indistinguishable karyotype between males and females, but this time with two Xs in both sexes (males being sex reversed XX), and finally *E. fuscocapillus* that retains a standard XX/XY sex chromosome system (e.g. [Kolomiets et al., 1991; Vogel et al., 1998; Just et al., 2002, 2007]). Hence the four former species lack the Y chromosome, and even the *Sry* gene [Just et al., 1995]. No other Y-born genes have been found in the genome, meaning that the Y chromosome has completely disappeared [Vogel et al., 1998; Just et al., 2002].

Tokudaia osimensis, a spiny rat from a remote Japanese island, has also a sex determination system similar to that of *Ellobius lutescens* (X0/X0), but in this case, at least four original Y-linked genes, *Zfy*, *Tspy*, *Eif2s3Y* and *Kdm5d* (but not *Sry*) were translocated to the X chromosome [Arakawa et al., 2002; Kuroiwa et al., 2010]. In *Ellobius*, in the absence of *Sry*, other genes having a role in the mammalian sex determining pathway, and known to be involved in XX sex reversal in humans or mammalian models, have been investigated to identify the gene that has replaced *Sry*, and became the new TDF. So far, all candidate genes analyzed by segregation analysis (i.e., *Sf1, Dax1, Dmrt1, Sox9, Sox3, Foxl2, Pisrt1*) have been excluded,

notwithstanding their role in gonad differentiation [Baumstark et al., 2001, 2005; Just et al., 2002, 2007]. Another candidate to test is *Cbx2*, a gene member of the mammalian Polycomb group which acts upstream of *Sry* and represses the ovarian development. Even if the loss-of-function mutations in *Cbx2* cause the opposite sex reversal pattern, i.e., XY females [Katoh-Fukui et al., 1998; Biason-Lauber et al., 2009], in the other species with a *Sry*-independent sex determining program, *Tokudaia osimensis*, additional copies have been detected in X0 males compared to X0 females, suggesting that differences in gene dosage might determine the development fate of the undifferentiated gonads in this species [Kuroiwa et al., 2011]. Interestingly, some authors have sequenced the *cis*-regulatory element of *Sox9*, named TESCO (for testis-specific enhancer of *Sox9* core), which is synergistically activated by *Sry* and *Sf1* in different species of *Ellobius*. They found that all species of the genus display a 14-bp deletion that increases activity of TESCO in HEK293T cells (via generation of new transcription factor binding sites). They proposed a model whereby the deletion may have triggered upregulation of *Sox9* in XX gonads leading to destabilization of the XX/XY sex determination system that has facilitated the evolution of *Sry*-independent sex determination mechanisms in some *Ellobius* species [Bagheri-Fam et al., 2012]. The novel TDF in *Ellobius* still remains to be identified.

Finally, the last case is unique among mammals despite a typical XX/XY sex determination system. It does not concern XX sex reversal, but a constitutive hermaphroditism. In the European mole species of the genus *Talpa*, and in some other talpid species from Europe, North America and Asia, all XX female gonads develop as ovotestes in the absence of a Y chromosome [Jimenez et al., 1993; Sanchez et al., 1996; Rubenstein et al., 2003; Carmona et al., 2008]. The ovotestes are composed of a small portion of histologically normal ovarian tissue, and of dysgenic testicular tissue (variable in size, but generally large). The ovarian portion contains follicles and represents the fertile component of the gonad, but its development is unusual since meiosis is initiated postnatally [Zurita et al., 2007]. The testicular portion contains no germ cells, numerous testis cord-like structures with resembling immature Sertoli cells but that do not produce AMH or SOX9, and abundant Leydig cells producing high levels of testosterone [Barrionuevo et al., 2004; Carmona et al., 2009]. The sexual tract has some male features such as rudimentary epididymides and peniform clitoris, but the rest of the tract is typically female

[Jimenez et al., 1993; Beolchini et al., 2000]. A time-course description of male and female gonad organogenesis in *Talpa* was reported by [Barrionuevo et al., 2004]. Additionally, two sex reversed XX males of the Iberian mole *Talpa occidentalis* were identified along normal XY males and “normal” XX females with ovotestes [Jimenez et al., 1988]. But no other XX males have been found since then, suggesting that these two males must refer to an exceptional familial case of complete sex reversal according to the authors.

6 - Conclusion

As in humans, different sex reversal conditions have been described in a broad range of mammalian species. Even if numerous cases have been described by the past, the number of cases will certainly increase in the future as a consequence of genomic selection [Georges, 2001]. Indeed, a higher number of farm mammals will be genotyped in the future, allowing a growing detection of individuals showing discrepancies between their genotyped sex chromosome complement and their morphological sex. Sex reversal mostly occurs following mutational events and studying sex reversed animals should help to decipher the genetic cascades underlying gonadal differentiation in both sexes. However in rare cases, in wild mammals, the sex reversal condition seems to be stable, "fixed" by natural selection. How to explain that sex reversal, leading mainly to infertility in domestic mammals as in humans, could become the norm in some species?

As previously stated, a deletion or mutation of *SRY* leads to male-to-female sex reversal with extremely divergent gonadal phenotypes between species, and to a lesser extent, among conspecifics as well. Indeed, the range of phenotypes extends from an absence of gonads (gonad dysgenesis/agenesis as illustrated in humans) to normal ovarian development associated with sub-fertile condition as described in the laboratory mouse, to even super-fertile females like in the African pygmy mouse. The XY del/mut-*SRY* condition remains highly similar to the X0 condition (X monosomy condition, Turner's syndrome). Consequently, one could predict that haplo-insufficiency of one or several X-linked genes is incompatible with ovarian development in some mammalian species. In other words, at least one or more X-linked genes escaping X-inactivation is/are crucial, at a double dose, for proper ovarian differentiation. It is thus not surprising to observe a huge variability of gonadal

phenotypes in XY del/mut-*SRY* condition, according to the fact that (i) the genes escaping X-inactivation are not the same in the different mammalian species; (ii) the number of these genes are different depending of the species [Berletch et al., 2011]; (iii) some X-linked genes show an heterogeneous pattern of inactivation and are thus expressed to different extents between organs and females [Carrel and Willard, 2005]; and (iv) some X-linked genes have orthologous autosomal copies in some species and consequently could compensate for X-linked gene haplo-insufficiency. Interestingly, in human, where no less than 15% of the X-linked genes escape inactivation (i.e., the two copies are required) and an additional 10% shows a variable inactivation pattern [Carrel and Willard, 2005], all XY del/mut-*SRY* or X0 genotypes induce gonad dysgenesis, associated with other physical anomalies. While, in the mouse *Mus musculus*, only 3% escape inactivation [Yang et al., 2010; Berletch et al., 2010], and most of the X0 females are sub-fertile and present very few abnormal phenotypes [Deckers et al., 1981; Burgoyne et al., 1983]. Hence, one could hypothesize that these striking differences may explain why unusual sex determination systems have been found only in rodents (in addition of being the most speciose group of mammals). The almost complete inactivation process of the rodent X chromosome allows tolerating monosomies without complications, as shown by the fertile XY females found in the pygmy mouse, two species of lemmings and in *Akodon*, and by the X0 condition in *Ellobius lutescens* or *Microtus oregoni* (Table 1).

At the opposite, XX males in mammals (including human) are always sterile, regardless the underlying genetic cause of the sex reversal. This is due to the fact that the very few genes that remain on the mammalian Y chromosome are highly specialized in reproduction and spermatogenesis-related functions [Graves, 2006; Hughes et al., 2010; Yamauchi et al., 2014]. Hence the absence of the Y chromosome precludes the development of mature sperm and normal fertilization. However, one group of species, the mole voles *Ellobius*, proves us wrong as it managed to loss the entire Y chromosome without having affected reproduction and maleness. This surely must have happened in stages, in which sex and spermatogenesis genes were lost and each function replaced by other genes in the genome. Interestingly, a recent study demonstrated that it is possible to bypass the requirement for the mouse Y chromosome in male assisted reproduction, by replacing *Sry* by transgenic activation of its downstream autosomal target *Sox9*, and *Eif2s3y* by transgenic overexpression of its X-linked homolog *Eif2s3x* [Yamauchi et

al., 2016]. Identifying the new sex determining gene in these Y-less mole voles would undoubtedly provide a better understanding of the mammalian sex determination program. Likewise, all cases of sporadic sex reversal in domestic animals and of sex reversal evolution in rodent species offer as many opportunities to unravel new gene interactions underlying gonad development.

Acknowledgments

The authors would like to thank Pr. Silvana Arrighi (University of Milano) for kindly providing original unpublished histological images of a sex reversed dog, and Janice Britton-Davidian and Paul Saunders for reviewing the English and their valuable comments on the manuscript.

References

Abe S, Miyake YI, Kageyama SI, Watanabe G, Taya K, Kawakura K: Deletion of the *Sry* region on the Y chromosome detected in a case of equine gonadal hypoplasia (XY female) with abnormal hormonal profiles. Equine Vet J 31:336-338 (1999).

Alton M, Lau MP, Villemure M, Taketo T: The behavior of the X- and Y-chromosomes in the oocyte during meiotic prophase in the b6.Y(tir)sex reversed mouse ovary. Reproduction 135:241-252 (2008).

Anaya G, Moreno-Millán M, Bugno-Poniewierska M, Pawlina K, Membrillo A, Molina A, Demyda-Peyrás S: Sex reversal syndrome in the horse: Four new cases of feminization in individuals carrying a 64,XY *Sry* negative chromosomal complement. Anim Reprod Sci 151:22-27 (2014).

Arakawa Y, Nishida-Umehara C, Matsuda Y, Sutou S, Suzuki H: X-chromosomal localization of mammalian Y-linked genes in two X0 species of the Ryukyu spiny rat. Cytogenet Genome Res 99:303-309 (2002).

Arnold AP, Chen X, Link JC, Itoh Y, Reue K: Cell-autonomous sex determination outside of the gonad. Dev Dyn 242:371-379 (2013).

Asdell S: The genetic sex of intersexual goats and a probable linkage with the gene for hornlessness. Science 99:124 (1944).

Bäckström L, Henricson B: Intersexuality in the pig. Acta Vet Scand 12:257-273 (1971).

Bagheri-Fam S, Sreenivasan R, Bernard P, Knower KC, Sekido R, Lovell-Badge R, Just W, Harley VR: *Sox9* gene regulation and the loss of the XY/XX sex-determining mechanism in the mole vole *Ellobius lutescens*. Chromosome Res 20:191-199 (2012).

Balakrishnan CR, Yadav BR, Bhatii AA, Nair KGS: Unusual chromosome constitution of a bovine freemartin. Indian J Dairy Sci 32: 191-219 (1979).

Bannasch D, Rinaldo C, Millon L, Latson K, Spangler T, Hubberty S, Galuppo L, Lowenstine L: *Sry* negative 64,XX intersex phenotype in an american saddlebred horse. Vet J 173:437-439 (2007).

Bardoni B, Zanaria E, Guioli S, Floridia G, Worley KC, Tonini G, Ferrante E, Chiumello G, McCabe ER, Fraccaro M: A dosage sensitive locus at chromosome Xp21 is involved in male to female sex reversal. Nat Genet 7:497-501 (1994).

Barrionuevo FJ, Zurita F, Burgos M, Jiménez R: Testis-like development of gonads in female moles. New insights on mammalian gonad organogenesis. Dev Biol 268:39-52 (2004).

Baumstark A, Akhverdyan M, Schulze A, Reisert I, Vogel W, Just W: Exclusion of *Sox9* as the testis determining factor in *Ellobius lutescens*: Evidence for another testis determining gene besides *Sry* and *Sox9*. Mol Genet Metab 72:61-66 (2001).

Baumstark A, Hameister H, Hakhverdyan M, Bakloushinskaya I, Just W: Characterization of *Pisrt1/Foxl2* in *Ellobius lutescens* and exclusion as sex-determining genes. Mamm Genome 16:281-289 (2005).

Beolchini F, Rebecchi L, Capanna E, Bertolani R: Female gonad of moles, genus *Talpa* (Insectivora, Mammalia): Ovary or ovotestis? J Exp Zool 286:745-754 (2000).

Berletch JB, Yang F, Disteche CM: Escape from X inactivation in mice and humans. Genome Biol 11:213 (2010).

Berletch JB, Yang F, Xu J, Carrel L, Disteche CM: Genes that escape from X inactivation. Hum Genet 130:237-245 (2011).

Bianchi NO: *Akodon* sex reversed females: The never ending story. Cytogenet Genome Res 96:60-65 (2002).

Biason-Lauber A, Konrad D, Meyer M, DeBeaufort C, Schoenle EJ: Ovaries and female phenotype in a girl with 46,XY karyotype and mutations in the *CBX2* gene. Am J Hum Genet 84:658-663 (2009).

Bodvarsdottir SK, Imsland F, Thorisson B, Steinarsdottir M, Eyfjord JE: 64,XX, sr gamma-, and Zf gamma-negative icelandic horse likely to be true hermaphrodite. Journal of Equine Veterinary Science 29:734-738 (2009).

Boulanger L, Kocer A, Daniel N, Pannetier M, Chesné P, Heyman Y, Renault L, Mandon-Pépin B, Chavatte-Palmer P, Vignon X, Vilotte JL, Cotinot C, Renard JP, Pailhoux E: Attempt to rescue sex reversal by transgenic expression of the *Pisrt1* gene in XX PIS-/- goats. Sex Dev 2:142-151 (2008).

Boulanger L, Pannetier M, Gall L, Allais-Bonnet A, Elzaiat M, Le Bourhis D, Daniel N, Richard C, Cotinot C, Ghyselinck NB, Pailhoux E: *Foxl2* is a female sex-determining gene in the goat. Curr Biol 24:404-408 (2014).

Bowling AT, Millon L, Hughes JP: An update of chromosomal abnormalities in mares. J Reprod Fertil Suppl 35:149-155 (1987).

Brandsch H: Die Vererbung geschlechtlicher Missbildung und des Hornes bei der Hausziege in ihrer gegenseitigen Beziehung. Versuch einer genetischen Analyse. Arch Geflügelz Kleintierk 8: 310–362 (1959).

Breeuwsma AJ: Studies on intersexuality in pigs. Tijdschr Diergeneeskd 96:514-519 (1971).

Brenig B, Duan Y, Xing Y, Ding N, Huang L, Schütz E: Porcine *Sox9* gene expression is influenced by an 18 bp indel in the 5'-untranslated region. PLoS One 10:e0139583 (2015).

Brennan J, Capel B: One tissue, two fates: Molecular genetic events that underlie testis versus ovary development. Nat Rev Genet 5:509-521 (2004).

Bugno M, Klukowska J, Słota E, Tischner M, Switoński M: A sporadic case of the sex reversed mare (64,XY; *Sry*-negative): Molecular and cytogenetic studies of the Y chromosome. Theriogenology 59:1597-1603 (2003).

Bullejos M, Sánchez A, Burgos M, Hera C, Jiménez R, Díaz de la Guardia R: Multiple, polymorphic copies of *Sry* in both males and females of the vole *Microtus cabrerae*. Cytogenet Cell Genet 79:167-171 (1997).

Burgos M, Jiménez R, Díaz de la Guardia R: XY females in *Microtus cabrerae* (Rodentia, Microtidae): A case of possibly Y-linked sex reversal. Cytogenet Cell Genet 49:275-277 (1988).

Burgoyne PS, Evans EP, Holland K: X0 monosomy is associated with reduced birthweight and lowered weight gain in the mouse. J Reprod Fertil 68:381-385 (1983).

Carmona FD, Lupiáñez DG, Real FM, Burgos M, Zurita F, Jiménez R: *Sox9* is not required for the cellular events of testicular organogenesis in XX mole ovotestes. J Exp Zool B Mol Dev Evol 312:734-748 (2009).

Carmona FD, Motokawa M, Tokita M, Tsuchiya K, Jiménez R, Sánchez-Villagra MR: The evolution of female mole ovotestes evidences high plasticity of mammalian gonad development. J Exp Zool B Mol Dev Evol 310:259-266 (2008).

Carrel L, Willard HF: X-inactivation profile reveals extensive variability in X-linked gene expression in females. Nature 434:400-404 (2005).

Castiglia R, Makundi R, Corti M: The origin of an unusual sex chromosome constitution in *Acomys* sp. (Rodentia, Muridae) from Tanzania. Genetica 131:201-207 (2007).

Chaffaux S, Cribiu EP: Clinical, histological and cytogenetic observations on 9 intersex dogs. Genetics Selection Evolution 23:S81-S84 (1991).

Chandley AC, Fletcher J, Rossdale PD, Peace CK, Ricketts SW, McEnery RJ, Thorne JP, Short RV, Allen WR: Chromosome abnormalities as a cause of infertility in mares. J Reprod Fertil Suppl:377-383 (1975).

Chapman HM, Bruere AN, Jaine PM: XY gonadal dysgenesis in a charolais heifer. Animal Reproduction Science 1:9-18 (1978).

Charlier C, Coppieters W, Rollin F, Desmecht D, Agerholm JS, Cambisano N, Carta E, Dardano S, Dive M, Fasquelle C, Frennet JC, Hanset R, Hubin X, Jorgensen C, Karim L, Kent M, Harvey K, Pearce BR, Simon P, Tama N, Nie H, Vandeputte S, Lien S, Longeri M, Fredholm M, Harvey RJ, Georges M: Highly effective snp-based association mapping and management of recessive defects in livestock. Nat Genet 40:449-454 (2008).

Chen Y, Dong Y, Xiang X, Zhang X, Zhu B: Sex determination of *Microtus mandarinus mandarinus* is independent of *Sry* gene. Mamm Genome 19:61-68 (2008).

Ciotola F, Albarella S, Pasolini MP, Auletta L, Esposito L, Iannuzzi L, Peretti V: Molecular and cytogenetic studies in a case of XX *Sry*-negative sex reversal in an arabian horse. Sex Dev 6:104-107 (2012).

Cotinot C, Kirszenbaum M, Leonard M, Gianquinto L, Vaiman M: Isolation of bovine Y-derived sequence: Potential use in embryo sexing. Genomics 10:646-653 (1991).

Crisponi L, Deiana M, Loi A, Chiappe F, Uda M, Amati P, Bisceglia L, Zelante L, Nagaraja R, Porcu S, Ristaldi M, Marzella R, Rocchi M, Nicolino M, Lienhardt-Roussie A, Nivelon A, Verloes A, Schlessinger D, Gasparini P, Bonneau D, Cao A, Pilia G: The putative forkhead transcription factor *Foxl2* is mutated in blepharophimosis/ptosis/epicanthus inversus syndrome. Nat Genet 27:159-166 (2001).

D'haene B, Attanasio C, Beysen D, Dostie J, Lemire E, Bouchard P, Field M, Jones K, Lorenz B, Menten B, Buysse K, Pattyn F, Friedli M, Ucla C, Rossier C, Wyss C, Speleman F, De Paepe A, Dekker J, Antonarakis SE, De Baere E: Disease-causing 7.4 kb cis-regulatory deletion disrupting conserved non-coding sequences and their interaction with the *Foxl2* promotor: Implications for mutation screening. PLoS Genet 5:e1000522 (2009).

De Lorenzi L, Groppetti D, Arrighi S, Pujar S, Nicoloso L, Molteni L, Pecile A, Cremonesi F, Parma P, Meyers-Wallen V: Mutations in the *Rspo1* coding region are not the main cause of canine *Sry*-negative XX sex reversal in several breeds. Sexual Development 2:84-95 (2008).

Deckers JFM, Vanderkroon PHW, Douglas LT: Some characteristics of the X0 mouse (*Mus musculus* l) .2. Reproduction - fertility and gametic segregation. Genetica 57:3-11 (1981).

Del Carro AP, Rosset E, Josson-Schramme A, Lambert V, Buff S: First description of scrotal testicles in a dog affected by 78, XX testicular disorder of sex development. Reproduction in Domestic Animals 49:E48-E52 (2014).

Di Meo GP, Perucatti A, Di Palo R, Iannuzzi A, Ciotola F, Peretti V, Neglia G, Campanile G, Zicarelli L, Iannuzzi L: Sex chromosome abnormalities and sterility in river buffalo. Cytogenetic and Genome Research 120:127-131 (2008).

Di Meo GP, Perucatti A, Floriot S, Incarnato D, Rullo R, Caputi Jambrenghi A, Ferretti L, Vonghia G, Cribiu E, Eggen A, Iannuzzi L: Chromosome evolution and improved cytogenetic maps of the y chromosome in cattle, zebu, river buffalo, sheep and goat. Chromosome Res 13:349-355 (2005).

Drew ML, Meyers-Wallen VN, Acland GM, Guyer CL, Steinheimer DN: Presumptive *Sry*-negative XX sex reversal in a llama with multiple congenital anomalies. Journal of the American Veterinary Medical Association 215:1134-1139 (1999).

Ducos A, Revay T, Kovacs A, Hidas A, Pinton A, Bonnet-Garnier A, Molteni L, Slota E, Switonski M, Arruga MV, van Haeringen WA, Nicolae I, Chaves R, Guedes-Pinto H, Andersson M, Iannuzzi L: Cytogenetic screening of livestock populations in europe: An overview. Cytogenet Genome Res 120:26-41 (2008).

Eldridge FE, Koenig JLF, Harris NB: H-Y negative, 60,XY. Heifer. 6th European Colloquium on cytogenetics of domestic animals. July 16-20 Zurich; pp 217 (1984).

Elzaiat M, Jouneau L, Thépot D, Klopp C, Allais-Bonnet A, Cabau C, André M, Chaffaux S, Cribiu EP, Pailhoux E, Pannetier M: High-throughput sequencing analyses of XX genital ridges lacking *Foxl2* reveal *Dmrt1* up-regulation before *Sox9* expression during the sex reversal process in goats. Biol Reprod 91:153 (2014).

Espinosa MB, Vitullo AD: Fast-developing preimplantation embryo progeny from heterogametic females in mammals. Zygote 9:289-292 (2001).

Favetta LA, Villagómez DA, Iannuzzi L, Di Meo G, Webb A, Crain S, King WA: Disorders of sexual development and abnormal early development in domestic food-producing mammals: The role of chromosome abnormalities, environment and stress factors. Sex Dev 6:18-32 (2012).

Ferguson-Smith MA: X-Y chromosomal interchange in aetiology to true hermaphroditism and of XX klinefelters syndrome. Lancet 2:475-476 (1966).

Ferrer LM, Monteagudo LV, García de Jalón JA, Tejedor MT, Ramos JJ, Lacasta D: A case of ovine female XY sex reversal syndrome not related to anomalies in the sex-determining region y (*Sry*). Cytogenet Genome Res 126:329-332 (2009).

Foster JW, Brennan FE, Hampikian GK, Goodfellow PN, Sinclair AH, Lovell-Badge R, Selwood L, Renfree MB, Cooper DW, Graves JA: Evolution of sex determination and the Y chromosome: *Sry*-related sequences in marsupials. Nature 359:531-533 (1992).

Fredga K: Aberrant sex chromosome mechanisms in mammals. Evolutionary aspects. Differentiation 23 Suppl:S23-30 (1983).

Fredga K: Aberrant chromosomal sex-determining mechanisms in mammals, with special reference to species with xy females. Philos Trans R Soc Lond B Biol Sci 322:83-95 (1988).

Fredga K: Bizarre mammalian sex-determining mechanisms. In *The differences between the sexes* (eds R. V. Short & E. Balaban), pp. 419–431. Cambridge, UK: Cambridge University Press (1994).

Fredga K, Gropp A, Winking H, Frank F: Fertile XX- and XY-type females in the wood lemming *Myopus schisticolor*. Nature 261:225-227 (1976).

Fredga K, Setterfield L, Mittwoch U: Gonadal development and birth weight in X*X and X*Y females of the wood lemming, *Myopus schisticolor*. Cytogenet Cell Genet 91:97-101 (2000).

Georges M: Recent progress in livestock genomics and potential impact on breeding programs. Theriogenology 55:15-21 (2001).

Gileva EA: Meiotic drive in the sex-chromosome system of the varying lemming, *Dicrostonyx torquatus Pall*. (Rodentia, Microtinae). Heredity 59:383-389 (1987).

Graves J: Sex chromosome specialization and degeneration in mammals. Cell 124:901-914 (2006).

Groppetti D, Genualdo V, Bosi G, Pecile A, Iannuzzi A, Perucatti A, De Lorenzi L, Parma P, Arrighi S: XX *Sry*-negative true hermaphrodism in two dogs: Clinical, morphological, genetic and cytogenetic studies. Sex Dev 6:135-142 (2012).

Gustavsson I, Settergren I, Gustafsson H, Larsson K: Testicular feminization and XY gonadal dysgenesis in Swedish Red and White cattle. J of Anim Sci 53(Suppl 1): 160 (1981).

Hare JE, Baird JD, Duignan P, Saunders J, Floetenmeyer R, Basrur PK: XY gonadal dysgenesis and tetralogy of fallot in an angus calf. Can Vet J 35:510-512 (1994).

Hare WCD: Intersexuality in dog. Canadian Veterinary Journal-Revue Veterinaire Canadienne 17:7-15 (1976).

Henricson B, Akesson A: Two heifers with gonadal dysgenesis and the sex chromosomal constitution XY. Acta Vet Scand 8:262-272 (1967).

Herbst EW, Fredga K, Frank F, Winking H, Gropp A: Cytological identification of two X-chromosome types in the wood lemming (*Myopus schisticolor*). Chromosoma 69:185-191 (1978).

Huang B, Wang S, Ning Y, Lamb AN, Bartley J: Autosomal XX sex reversal caused by duplication of *Sox9*. Am J Med Genet 87:349-353 (1999).

Hughes IA: Disorders of sex development: A new definition and classification. Best Pract Res Clin Endocrinol Metab 22:119-134 (2008).

Hughes JF, Skaletsky H, Pyntikova T, Graves TA, van Daalen SK, Minx PJ, Fulton RS, McGrath SD, Locke DP, Friedman C, Trask BJ, Mardis ER, Warren WC, Repping S, Rozen S, Wilson RK, Page DC: Chimpanzee and human Y chromosomes are remarkably divergent in structure and gene content. Nature 463:536-539 (2010).

Hughes JP, Trommershausen-Smith A: Infertility in the horse associated with chromosomal abnormalities. Aust Vet J 53:253-257 (1977).

Hulland TJ: Pregnancy in a hermaphrodite sow. The Canadian veterinary journal La revue veterinaire canadienne 5:39-41 (1964).

Hunter RH, Baker TG, Cook B: Morphology, histology and steroid hormones of the gonads in intersex pigs. J Reprod Fertil 64:217-222 (1982).

Hunter RH, Cook B, Baker TG: Intersexuality in five pigs, with particular reference to oestrous cycles, the ovotestis, steroid hormone secretion and potential fertility. J Endocrinol 106:233-242 (1985).

Hyon C, Chantot-Bastaraud S, Harbuz R, Bhouri R, Perrot N, Peycelon M, Sibony M, Rojo S, Piguel X, Bilan F, Gilbert-Dussardier B, Kitzis A, McElreavey K, Siffroi JP, Bashamboo A: Refining the regulatory region upstream of *Sox9* associated with 46,XX testicular disorders of sex development (dsd). Am J Med Genet A 167A:1851-1858 (2015).

Iannuzzi L, Di Meo GP, Perucatti A, Di Palo R, Zicarelli L: 50,XY gonadal dysgenesis (swyer's syndrome) in a female river buffalo (*Bubalus bubalis*). Vet Rec 148:634-635 (2001).

Iannuzzi L, Di Meo GP, Perucatti A, Incarnato D, Di Palo R, Zicarelli L: Reproductive disturbances and sex chromosome abnormalities in two female river buffaloes. Vet Rec 154:823-824 (2004).

Jimenéz R, Burgos M, Caballero L, Diaz de la Guardia R: Sex reversal in a wild population of *Talpa occidentalis* (Insectivora, Mammalia). Genet Res 52:135-140 (1988).

Jiménez R, Barrionuevo FJ, Burgos M: Natural exceptions to normal gonad development in mammals. Sex Dev 7:147-162 (2013).

Jiménez R, Burgos M, Sánchez A, Sinclair AH, Alarcón FJ, Marín JJ, Ortega E, Díaz de la Guardia R: Fertile females of the mole *Talpa occidentalis* are phenotypic intersexes with ovotestes. Development 118:1303-1311 (1993).

Johnston EF, Zeller JH, Cantwell G: Sex anomalies in swine. J Heredity 49:254-261 (1958).

Just W, Baumstark A, Hameister H, Schreiner B, Reisert I, Hakhverdyan M, Vogel W: The sex determination in *Ellobius lutescens* remains bizarre. Cytogenet Genome Res 96:146-153 (2002).

Just W, Baumstark A, Süss A, Graphodatsky A, Rens W, Schäfer N, Bakloushinskaya I, Hameister H, Vogel W: *Ellobius lutescens*: Sex determination and sex chromosome. Sex Dev 1:211-221 (2007).

Just W, De Almeida CC, Goldshmidt B, Vogel W: The male pseudohermaphrodite XX polled goat is *Zfy* and *Sry* negative. Hereditas 120:71-75 (1994).

Just W, Rau W, Vogel W, Akhverdian M, Fredga K, Graves J, Lyapunova E: Absence of *Sry* in species of the vole *Ellobius*. Nat Genet 11:117-118 (1995).

Kang JT, Kim HJ, Oh HJ, Hong SG, Park JE, Kim MJ, Kim MK, Jang G, Kim DY, Lee BC: *Sry*-positive 78, XY ovotesticular disorder of sex development in a wolf cloned by nuclear transfer. J Vet Sci 13:211-213 (2012).

Katoh-Fukui Y, Tsuchiya R, Shiroishi T, Nakahara Y, Hashimoto N, Noguchi K, Higashinakagawa T: Male-to-female sex reversal in m33 mutant mice. Nature 393:688-692 (1998).

Kawakura K, Miyake Y, Murakami RK, Kondoh S, Hirata TI, Kaneda Y: Abnormal structure of the Y chromosome detected in bovine gonadal hypoplasia (XY female) by FISH. Cytogenet Cell Genet 76:36-38 (1997).

Kawakura K, Miyake YI, Murakami RK, Kondoh S, Hirata TI, Kaneda Y: Deletion of the *Sry* region on the Y chromosome detected in bovine gonadal hypoplasia (XY female) by PCR. Cytogenet Cell Genet 72:183-184 (1996).

Kent MG, Schneller HE, Hegsted RL, Johnston SD, Wachtel SS: Concentration of serum testosterone in XY sex reversed horses. J Endocrinol Invest 11:609-613 (1988a).

Kent MG, Shoffner RN, Buoen L, Weber AF: XY sex reversal syndrome in the domestic horse. Cytogenet Cell Genet 42:8-18 (1986).

Kent MG, Shoffner RN, Hunter A, Elliston KO, Schroder W, Tolley E, Wachtel SS: XY sex reversal syndrome in the mare: Clinical and behavioral studies, h-y phenotype. Hum Genet 79:321-328 (1988b).

Koch W: Intersexuality in mammals. In: Intersexuality (ed. by Overzier CJ), p.563. Academic Press, New York (1963).

Koch P, Fischer H, Schumann H: In "Genetical pathology of farm animals". Paul Parey, Hamburg, p.436 (1957).

Kolomiets OL, Vorontsov NN, Lyapunova EA, Mazurova TF: Ultrastructure, meiotic behavior, and evolution of sex-chromosomes of the genus Ellobius. Genetica 84:179-189 (1991).

Kondoh S, Miyake Y, Nakahori Y, Nakagome Y, Kaneda Y: Cytogenetical and molecular biological studies on a bovine XY female. J Vet Med Sci 54:1077-1080 (1992).

Kothapalli K, Kirkness E, Pujar S, Van Wormer R, Meyers-Wallen VN: Exclusion of candidate genes for canine *Sry*-negative XX sex reversal. J Hered 96:759-763 (2005).

Kothapalli KS, Kirkness E, Natale LJ, Meyers-Wallen VN: Exclusion of *Pisrt1* as a candidate locus for canine *Sry*-negative XX sex reversal. Anim Genet 34:467-469 (2003).

Kothapalli KS, Kirkness EF, Pujar S, Meyers-Wallen VN: Exclusion of *Wt1* as a candidate gene for canine *Sry*-negative XX sex reversal. Anim Genet 35:466-467 (2004).

Kothapalli KS, Kirkness EF, Vanwormer R, Meyers-Wallen VN: Exclusion of *Dmrt1* as a candidate gene for canine *Sry*-negative XX sex reversal. Vet J 171:559-561 (2006).

Kropatsch R, Dekomien G, Akkad DA, Gerding WM, Petrasch-Parwez E, Young ND, Altmüller J, Nürnberg P, Gasser RB, Epplen JT: *Sox9* duplication linked to intersex in deer. PLoS One 8:e73734 (2013).

Kuroiwa A, Handa S, Nishiyama C, Chiba E, Yamada F, Abe S, Matsuda Y: Additional copies of *Cbx2* in the genomes of males of mammals lacking *Sry*, the amami spiny rat (*Tokudaia osimensis*) and the Tokunoshima spiny rat (*Tokudaia tokunoshimensis*). Chromosome Res 19:635-644 (2011).

Kuroiwa A, Ishiguchi Y, Yamada F, Shintaro A, Matsuda Y: The process of a Y-loss event in an X0/X0 mammal, the Ryukyu spiny rat. Chromosoma 119:519-526 (2010).

Lavery R, Lardenois A, Ranc-Jianmotamedi F, Pauper E, Gregoire EP, Vigier C, Moreilhon C, Primig M, Chaboissier MC: XY *Sox9* embryonic loss-of-function mouse mutants show complete sex reversal and produce partially fertile XY oocytes. Dev Biol 354:111-122 (2011).

Liu WS, Eriksson L, Fredga K: XY sex reversal in the wood lemming is associated with deletion of Xp21-23 as revealed by chromosome microdissection and fluorescence *in situ* hybridization. Chromosome Res 6:379-383 (1998).

Liu WS, Nordqvist K, Lau YF, Fredga K: Characterization of the Xp21-23 region in the wood lemming, a region involved in XY sex reversal. J Exp Zool 290:551-557 (2001).

Long SE: Chromosome anomalies and infertility in the mare. Equine Vet J 20:89-93 (1988).

Long SE, David JS: Testicular feminisation in an ayrshire cow. Vet Rec 109:116-118 (1981).

Mäkinen A, Hasegawa T, Mäkilä M, Katila T: Infertility in two mares with XY and XXX sex chromosomes. Equine Vet J 31:346-349 (1999).

Mäkinen A, Suojala L, Niini T, Katila T, Tozaki T, Miyake Y, Hasegawa T: X chromosome detection in an X0 mare using a human X paint probe, and PCR detection of *Sry* and amelogenin genes in 3 XY mares. Equine Vet J 33:527-530 (2001).

Marcinkowska-Swojak M, Szczerbal I, Pausch H, Nowacka-Woszuk J, Flisikowski K, Dzimira S, Nizanski W, Payan-Carreira R, Fries R, Kozlowski P, Switonski M: Copy number variation in the region harboring *Sox9* gene in dogs with testicular/ovotesticular disorder of sex development (78,XX; *Sry*-negative). Sci Rep 5:14696 (2015).

Matthey R: La formule chromosomique et le problème de la determination sexuelle chez *Ellobius lutescens* (Thomas) (Rodentia,-Muridae-Microtinae). Arch Julius Klaus-Stift Vererb Forsch 28 :65-73 (1953)

Max A, Grabiec A, Sacharczuk M, Sysa P, Jurka P, Krzyżewska A: 78,XX testicular DSD syndrome in a mongrel dog. Reprod Domest Anim 47:e7-e11 (2012).

McFeely RA, Hare WC, Biggers JD: Chromosome studies in 14 cases of intersex in domestic mammals. Cytogenetics 6:242-253 (1967).

Melniczek JR, Dambach D, Prociuk U, Jezyk PF, Henthorn PS, Patterson DF, Giger U: *Sry*-negative XX sex reversal in a family of Norwegian elkhounds. J Vet Intern Med 13:564-569 (1999).

Meyers-Wallen V, Schlafer D, Barr I, Lovell-Badge R, Keyzner A: *Sry*-negative XX sex reversal in purebred dogs. Mol Reprod Dev 53:266-273 (1999).

Meyers-Wallen VN: Gonadal and sex differentiation abnormalities of dogs and cats. Sex Dev 6:46-60 (2012).

Meyers-Wallen VN, Hurtgen J, Schlafer D, Tulleners E, Cleland WR, Ruth GR, Acland GM: *Sry*-negative XX true hermaphroditism in a pasa fino horse. Equine Vet J 29:404-408 (1997).

Montazer-Torbati F, Kocer A, Auguste A, Renault L, Charpigny G, Pailhoux E, Pannetier M: A study of goat *Sry* protein expression suggests putative new roles for this gene in the developing testis of a species with long-lasting *Sry* expression. Dev Dyn 239:3324-3335 (2010).

Moore EC, Roberts RB: Polygenic sex determination. Curr Biol 23:R510-512 (2013).

Mukherjee K, Conway de Macario E, Macario AJ, Brocchieri L: Chaperonin genes on the rise: New divergent classes and intense duplication in human and other vertebrate genomes. BMC Evol Biol 10:64 (2010).

Murakami R, Miyake Y, Kaneda Y: Cases of xy female, single-birth freemartin and trisomy (61, XX, +20) observed in cytogenetical studies on 18 sterile heifers. Nihon Juigaku Zasshi 51:941-945 (1989).

Nes S: Testicular Feminisation in cattle. Nord Vet Med 18: 19-29 (1966).

Oh HJ, Kim MK, Jang G, Kim HJ, Hong SG, Park JE, Park K, Park C, Sohn SH, Kim DY, Shin NS, Lee BC: Cloning endangered gray wolves (*Canis lupus*) from somatic cells collected postmortem. Theriogenology 70:638-647 (2008).

Ortiz MI, Pinna-Senn E, Dalmasso G, Lisanti JA: Chromosomal aspects and inheritance of the XY female condition in *Akodon azarae* (Rodentia, Sigmodontinae). Mammalian Biology 74:125-129 (2009).

Padula AM: The freemartin syndrome: An update. Anim Reprod Sci 87:93-109 (2005).

Pailhoux E, Cribiu EP, Chaffaux S, Darre R, Fellous M, Cotinot C: Molecular analysis of 60,XX pseudohermaphrodite polled goats for the presence of *Sry* and *Zfy* genes. J Reprod Fertil 100:491-496 (1994a).

Pailhoux E, Cribiu EP, Parma P, Cotinot C: Molecular analysis of an XY mare with gonadal dysgenesis. Hereditas 122:109-112 (1995).

Pailhoux E, Parma P, Sundström J, Vigier B, Servel N, Kuopio T, Locatelli A, Pelliniemi LJ, Cotinot C: Time course of female-to-male sex reversal in 38,XX fetal and postnatal pigs. Dev Dyn 222:328-340 (2001a).

Pailhoux E, Pelliniemi L, Barbosa A, Parma P, Kuopio T, Cotinot C: Relevance of intersexuality to breeding and reproductive biotechnology programs; XX sex reversal in pigs. Theriogenology 47:93-102 (1997).

Pailhoux E, Popescu P, Parma P, Boscher J, Legault C, Molteni L, Fellous M, Cotinot C: Genetic analysis of 38XX males with genital ambiguities and true hermaphrodites in pigs. Anim Genet 25:299-305 (1994b).

Pailhoux E, Vigier B, Chaffaux S, Servel N, Taourit S, Furet J, Fellous M, Grosclaude F, Cribiu E, Cotinot C, Vaiman D: A 11.7-kb deletion triggers intersexuality and polledness in goats. Nat Genet 29:453-458 (2001b).

Pajares G, Balseiro A, Pérez-Pardal L, Gamarra JA, Monteagudo LV, Goyache F, Royo LJ: *Sry*-negative XX true hermaphroditism in a roe deer. Anim Reprod Sci 112:190-197 (2009).

Pannetier M, Elzaiat M, Thépot D, Pailhoux E: Telling the story of XX sex reversal in the goat: Highlighting the sex-crossroad in domestic mammals. Sex Dev 6:33-45 (2012).

Pannetier M, Renault L, Jolivet G, Cotinot C, Pailhoux E: Ovarian-specific expression of a new gene regulated by the goat pis region and transcribed by a *Foxl2* bidirectional promoter. Genomics 85:715-726 (2005).

Parma P, Feligini M, Greppi G, Enne G: The complete coding region sequence of river buffalo (*Bubalus bubalis*) *Sry* gene. DNA Seq 15:77-80 (2004).

Parma P, Pailhoux E, Cotinot C: Reverse Transcription-Polymerase Chain Reaction analysis of genes involved in gonadal differentiation in pigs. Biol Reprod 61:741-748 (1999).

Peretti V, Ciotola F, Albarella S, Paciello O, Dario C, Barbieri V, Iannuzzi L: XX/XY chimerism in cattle: Clinical and cytogenetic studies. Sex Dev 2:24-30 (2008).

Pérez-Gutiérrez JF, Monteagudo LV, Rodríguez-Bertos A, García-Pérez E, Sánchez-Calabuig MJ, García-Botey C, Whyte A, de la Muela MS: Bilateral ovotestes in a 78, XX *Sry*-negative beagle dog. J Am Anim Hosp Assoc 51:267-271 (2015).

Perret J, Shia YC, Fries R, Vassart G, Georges M: A polymorphic satellite sequence maps to the pericentric region of the bovine Y chromosome. Genomics 6:482-490 (1990).

Peter AT, Scheidt AB, Campbell JW, Hahn KA: Male pseudohermaphroditism of the testicular feminization type in a heifer. Can Vet J 34:304-305 (1993).

Petit G: Nouvelle observation d'hermaphrodisme complexe des voies génitales chez un bouc. Recl Méd Vét Ec Alfort 71: 247–249 (1894).

Pieńkowska-Schelling A, Becker D, Bracher V, Pineroli B, Schelling C: Cytogenetical and molecular analyses in a horse with *Sry*-negative sex reversal. Schweiz Arch Tierheilkd 156:341-344 (2014).

Power MM: XY sex reversal in a mare. Equine Vet J 18:233-236 (1986).

Power MM, Leadon DP: Diploid-triploid chimaerism (64, XX/96,XXY) in an intersex foal. Equine Vet J 22:211-214 (1990).

Pujar S, Kothapalli KS, Göring HH, Meyers-Wallen VN: Linkage to Cfa29 detected in a genome-wide linkage screen of a canine pedigree segregating *Sry*-negative XX sex reversal. J Hered 98:438-444 (2007).

Pujar S, Kothapalli KS, Kirkness E, Van Wormer RH, Meyers-Wallen VN: Exclusion of *Lhx9* as a candidate gene for *Sry*-negative XX sex reversal in the American cocker spaniel model. J Hered 96:452-454 (2005).

Quinn A, Koopman P: The molecular genetics of sex determination and sex reversal in mammals. Semin Reprod Med 30:351-363 (2012).

Rahmoun M, Perez J, Saunders PA, Boizet-Bonhoure B, Wilhelm D, Poulat F, Veyrunes F: Anatomical and molecular analyses of XY ovaries from the African pygmy mouse *Mus minutoides*. Sex Dev 8:356-363 (2014).

Raudsepp T, Durkin K, Lear TL, Das PJ, Avila F, Kachroo P, Chowdhary BP: Molecular heterogeneity of XY sex reversal in horses. Anim Genet 41 Suppl 2:41-52 (2010).

Renfree MB, Chew KY, Shaw G: Hormone-independent pathways of sexual differentiation. Sex Dev 8:327-336 (2014).

Ricordeau G, Bouillon J, Carpentier M, Lajous A, Guillimin P: Surprolificité des génotypes sans cornes dans les races caprines alpine Saanen, alpine chamoisée et poitevine. Ann Genet Sel Anim 1:391-395 (1969).

Roberts SJ: Veterinary Obstetrics and Genital Diseases. Ithaca, New York, pp 459-476 (1971).

Rossi E, Radi O, De Lorenzi L, Iannuzzi A, Camerino G, Zuffardi O, Parma P: A revised genome assembly of the region 5' to canine *Sox9* includes the revsex orthologous region. Sex Dev 9:155-161 (2015).

Rossi E, Radi O, De Lorenzi L, Vetro A, Groppetti D, Bigliardi E, Luvoni GC, Rota A, Camerino G, Zuffardi O, Parma P: *Sox9* duplications are a relevant cause of *Sry*-negative XX sex reversal dogs. PLoS One 9:e101244 (2014).

Rousseau S, Iannuccelli N, Mercat MJ, Naylies C, Thouly JC, Servin B, Milan D, Pailhoux E, Riquet J: A genome-wide association study points out the causal implication of *Sox9* in the sex reversal phenotype in XX pigs. PLoS One 8:e79882 (2013).

Rozen S, Skaletsky H, Marszalek JD, Minx PJ, Cordum HS, Waterston RH, Wilson RK, Page DC: Abundant gene conversion between arms of palindromes in human and ape Y chromosomes. Nature 423:873-876 (2003).

Rubenstein NM, Cunha GR, Wang YZ, Campbell KL, Conley AJ, Catania KC, Glickman SE, Place NJ: Variation in ovarian morphology in four species of new world moles with a peniform clitoris. Reproduction 126:713-719 (2003).

Salamon S, Nowacka-Woszuk J, Switonski M: Polymorphism of the *Ctnnb1* and *Foxl2* genes is not associated with canine XX testicular/ovotesticular disorder of sex development. Folia Biol (Krakow) 63:57-62 (2015).

Sánchez A, Bullejos M, Burgos M, Hera C, Stamatopoulos C, Diaz De la Guardia R, Jiménez R: Females of four mole species of genus *Talpa* (Insectivora, Mammalia) are true hermaphrodites with ovotestes. Mol Reprod Dev 44:289-294 (1996).

Saunders PA, Franco T, Sottas C, Maurice T, Ganem G, Veyrunes F: Masculinised behaviour of XY females in a mammal with naturally occurring sex reversal. Sci Rep 6:22881 (2016).

Saunders PA, Perez J, Rahmoun M, Ronce O, Crochet PA, Veyrunes F: XY females do better than the XX in the African pygmy mouse, *Mus minutoides*. Evolution 68:2119-2127 (2014).

Schibler L, Cribiu E, Oustry-Vaiman A, Furet J, Vaiman D: Fine mapping suggests that the goat polled intersex syndrome and the human blepharophimosis ptosis epicanthus syndrome map to a 100-kb homologous region. Genome Res 10:311-318 (2000).

Schlafer DH, Valentine B, Fahnestock G, Froenicke L, Grahn RA, Lyons LA, Meyers-Wallen VN: A case of *Sry*-positive 38,XY true hermaphroditism (XY sex reversal) in a cat. Vet Pathol 48:817-822 (2011).

Schmidt D, Ovitt C, Anlag K, Fehsenfeld S, Gredsted L, Treier A, Treier M: The murine winged-helix transcription factor *Foxl2* is required for granulosa cell differentiation and ovary maintenance. Development 131:933-942 (2004).

Sekido R, Lovell-Badge R: Sex determination involves synergistic action of *Sry* and *Sf1* on a specific *Sox9* enhancer. Nature 453:930-934 (2008).

Settergreen I: The ovarian morphology in clinical bovine hypoplasia with some aspects of its endocrine relations. Acta Vet Scand 1(Suppl 5):1-107 (1964).

Sharma AK, Vijaykumar NK, Khar SK, Verma SK, Nigam JM: Xy gonadal dysgenesis in a heifer. Vet Rec 107:328-330 (1980).

Sharp AJ, Wachtel SS, Benirschke K: H-Y antigen in a fertile XY female horse. J Reprod Fertil 58:157-160 (1980).

Sinclair A, Berta P, Palmer M, Hawkins J, Griffiths B, Smith M, Foster J, Frischauf A, Lovell-Badge R, Goodfellow P: A gene from the human sex-determining region encodes a protein with homology to a conserved DNA-binding motif. Nature 346:240-244 (1990).

Sittmann K, Breeuwsma AJ, te Brake JH: On the inheritance of intersexuality in swine. Can J Genet Cytol 22:507-527 (1980).

Soller M, Kempenich O: Polledness and litter size in saanen goats. J Hered 55:301-304 (1964).

Soller M, Laor M, Barnea R, Weiss Y, Ayalon N: Polledness and infertility in male saanen goats. J Hered 54:237-240 (1963).

Soller M, Padeh B, Wysoki M, Ayalon N: Cytogenetics of saanen goats showing abnormal development of the reproductive tract associated with the dominant gene for polledness. Cytogenetics 8:51-67 (1969).

Stewart RW, Menges RW, Selby LA, Rhoades JD, Crenshaw DB: Canine intersexuality in a pug breeding kennel. Cornell Vet 62:464-473 (1972).

Swain A, Zanaria E, Hacker A, Lovell-Badge R, Camerino G: Mouse *Dax1* expression is consistent with a role in sex determination as well as in adrenal and hypothalamus function. Nat Genet 12:404-409 (1996).

Switonski M, Szczerbal I, Nizanski W, Kociucka B, Bartz M, Dzimira S, Mikolajewska N: Robertsonian translocation in a sex reversal dog (XX, *Sry* negative) may indicate that the causative mutation for this intersexuality syndrome resides on canine chromosome 23 (Cfa23). Sex Dev 5:141-146 (2011).

Sysa P, Bernacki Z, Kunska A: Intersexuality in cattle-a case of male pseudohermaphroditismus with a 60,XY karotype. Vet Rec 94:30-31 (1974).

Szczerbal I, Nowacka-Woszuk J, Dzimira S, Atamaniuk W, Nizanski W, Switonski M: A rare case of testicular disorder of sex development in a dog (78,xx; sry-negative) with male external genitalia and detection of copy number variation in the region upstream of the sox9 gene. Sex Dev 10:74-78 (2016).

Uda M, Ottolenghi C, Crisponi L, Garcia J, Deiana M, Kimber W, Forabosco A, Cao A, Schlessinger D, Pilia G: *Foxl2* disruption causes mouse ovarian failure by pervasive blockage of follicle development. Hum Mol Genet 13:1171-1181 (2004).

Vaiman D, Koutita O, Oustry A, Elsen J, Manfredi E, Fellous M, Cribiu E: Genetic mapping of the autosomal region involved in XX sex reversal and horn development in goats. Mamm Genome 7:133-137 (1996).

Vaiman D, Schibler L, Oustry-Vaiman A, Pailhoux E, Goldammer T, Stevanovic M, Furet JP, Schwerin M, Cotinot C, Fellous M, Cribiu EP: High-resolution human/goat comparative map of the goat polled/intersex syndrome (PIS): The human homologue is contained in a human YAC from Hsa3q23. Genomics 56:31-39 (1999).

Vetro A, Dehghani MR, Kraoua L, Giorda R, Beri S, Cardarelli L, Merico M, Manolakos E, Parada-Bustamante A, Castro A, Radi O, Camerino G, Brusco A, Sabaghian M, Sofocleous C, Forzano F, Palumbo P, Palumbo O, Calvano S, Zelante L, Grammatico P, Giglio S, Basly M, Chaabouni M, Carella M, Russo G, Bonaglia MC, Zuffardi O: Testis development in the absence of *Sry*: Chromosomal rearrangements at *Sox9* and *Sox3*. Eur J Hum Genet 23:1025-1032 (2015).

Veyrunes F, Chevret P, Catalan J, Castiglia R, Watson J, Dobigny G, Robinson T, Britton-Davidian J: A novel sex determination system in a close relative of the house mouse. Proc Biol Sci 277:1049-1056 (2010).

Veyrunes F, Perez J, Paintsil SN, Fichet-Calvet E, Britton-Davidian J: Insights into the evolutionary history of the X-linked sex reversal mutation in *Mus minutoides*: Clues from sequence analyses of the Y-linked *Sry* gene. Sex Dev 7:244-252 (2013).

Vidal V, Chaboissier M, de Rooij D, Schedl A: *Sox9* induces testis development in XX transgenic mice. Nat Genet 28:216-217 (2001).

Villagómez DA, Lear TL, Chenier T, Lee S, McGee RB, Cahill J, Foster RA, Reyes E, St John E, King WA: Equine disorders of sexual development in 17 mares including XX, *Sry*-negative, XY, *Sry*-negative and XY, *Sry*-positive genotypes. Sex Dev 5:16-25 (2011).

Villagómez DA, Parma P, Radi O, Di Meo G, Pinton A, Iannuzzi L, King WA: Classical and molecular cytogenetics of disorders of sex development in domestic animals. Cytogenet Genome Res 126:110-131 (2009).

Villemure M, Chen HY, Kurokawa M, Fissore RA, Taketo T: The presence of X- and Y-chromosomes in oocytes leads to impairment in the progression of the second meiotic division. Dev Biol 301:1-13 (2007).

Vogel W, Jainta S, Rau W, Geerkens C, Baumstark A, Correa-Cerro LS, Ebenhoch C, Just W: Sex determination in *Ellobius lutescens*: The story of an enigma. Cytogenet Cell Genet 80:214-221 (1998).

Wainwright EN, Wilhelm D: The game plan: Cellular and molecular mechanisms of mammalian testis development. Curr Top Dev Biol 90:231-262 (2010).

Warr N, Greenfield A: The molecular and cellular basis of gonadal sex reversal in mice and humans. Wiley Interdiscip Rev Dev Biol 1:559-577 (2012).

Wilhelm D, Palmer S, Koopman P: Sex determination and gonadal development in mammals. Physiol Rev 87:1-28 (2007).

Wilker CE, Meyers-Wallen VN, Schlafer DH, Dykes NL, Kovacs A, Ball BA: XX sex reversal in a llama. J Am Vet Med Assoc 204:112-115 (1994).

Williamson JH: Intersexuality in a family of Kerry blue terriers. Journal of Heredity 70:138-139 (1979).

Xiao C, Kato Y, Sato S, Sutou S: Mapping of bovine *Sry* gene on the distal tip of the long arm and murine *Sry* on the short arm of the Y chromosome by the method of fluorescence *in situ* hybridization (FISH). Anim Sci Technol 66: 441-444 (1995).

Xu B, Obata Y, Cao F, Taketo T: The presence of the Y-chromosome, not the absence of the second X-chromosome, alters the mRNA levels stored in the fully grown XY mouse oocyte. PLoS One 7:e40481 (2012).

Yamauchi Y, Riel JM, Ruthig VA, Ortega EA, Mitchell MJ, Ward MA: Two genes substitute for the mouse Y chromosome for spermatogenesis and reproduction. Science 351:514-516 (2016).

Yamauchi Y, Riel JM, Stoytcheva Z, Ward MA: Two Y genes can replace the entire Y chromosome for assisted reproduction in the mouse. Science 343:69-72 (2014).

Yang F, Babak T, Shendure J, Disteche CM: Global survey of escape from X inactivation by RNA-sequencing in mouse. Genome Res 20:614-622 (2010).

Zurita F, Carmona FD, Lupiáñez DG, Barrionuevo FJ, Guioli S, Burgos M, Jiménez R: Meiosis onset is postponed to postnatal stages during ovotestis development in female moles. Sex Dev 1:66-76 (2007).

Figure Legends

Fig. 1. Gonad and internal genitalia of an hermaphrodite XX sex reversed dog.
A Gonadal histology showing peripherally located ovarian follicles (OF) whereas the major internal part of the gonad is occupied by testicular tissue (TT) without germ cells (Sertoli cell only). **B** Internal genital tract histology showing either female derivative as uterine horn (UH) and male derivative as vas deferens (VD). Both ducts are included within a unique connective envelope. Scale bars: 1000µm.

Fig. 2. Schematic representation of the PIS (Polled Intersex Syndrome) locus in goats. **A** The PIS locus is delimited by two genes, *MRPS22* on the left and *PIK3CB* on the right. It encompasses the PIS 11.7 kb deletion (red bar) and four PIS-sensible genes *PISRT1*, *PISRT2*, *PFOXic* and *FOXL2*. *PISRT2* are uncharacterized transcripts corresponding to an active transcriptional activity on a ~100 kb region upstream of *PISRT1*. The sense of gene transcription is indicated by arrows. Green and red rectangles represent exonic parts of the genes (green: non coding exons; red: coding exon). **B** Working model of the PIS locus assuming that all three non-coding genes are linked to *FOXL2* distal (*PISRT1*, *PISRT2*) or proximal (*PFOXic*) transcriptional regulation. Transcriptional activation of the distal region (*PISRT1*, *PISRT2*) via conserved elements (CE) is supposed to be the primarily event (**1**) leading to *FOXL2* transcription (**2**) by DNA-looping and cooperative interaction between distal and proximal transcription factors. Once *FOXL2* remains activated, its level of expression could be finely tuned thanks to the proximal bidirectional promoter (**3**) that transcribed *PFOXic* in the opposite orientation of *FOXL2* when *FOXL2* transcription should be limited.

Fig. 3. A genetic model for gonadal differentiation in goats. In an XX context without *SRY* (right column), FOXL2 inhibits *DMRT1* thus avoiding *SOX9* up-regulation. Both *FOXL2* and *RSPO1/WNT4/CTNNB1* pathways trigger ovarian differentiation. In XY (left column), SRY inhibits *FOXL2* thus permitting *DMRT1* expression that represents a pre-requisite for *SOX9* up-regulation in synergy with SF1 and SRY itself. *SOX9* up-regulation then leads to downregulation of the *CTNNB1* pathway, and to testis differentiation.

Table 1: List of mammalian species with unusual sex determination systems.

	Female sex chromosomes	Male sex chromosomes	References
XY sex reversal			
Wood Lemming *Myopus schisticolor* (Arvicolinae)	XX, XX*, X*Y[a]	XY	[Fredga et al., 1976; reviewed in Fredga 1988, 1994]
Collared Lemming *Dicrostonyx torquatus* (Arvicolinae)	XX, XX*, X*Y[a]	XY	[reviewed in Fredga 1983, 1988, 1994]
South American field mice *Akodon* sp., 9 species (Sigmodontinae)	XX, XX*, X*Y[a]	XY	[reviewed in Bianchi, 2002]
African pygmy mouse *Mus minutoides* (Murinae)	XX, XX*, X*Y[a]	XY	[Veyrunes et al., 2010]
XX sex reversal			
Mole voles *Ellobius tancrei, E. talpinus, E. alaicus* (Arvicolinae)	XX	XX	[reviewed in Just et al., 2007]
other systems			
Mole vole *Ellobius lutescens* (Arvicolinae)	X0	X0	[Matthey, 1953; Just et al., 1995, 2002, 2007]
Ryukyu spiny rats *Tokudaia osimensis, T. tokunoshimensis* (Murinae)	X0	X0	[Arakawa et al., 2002; Kuroiwa et al., 2010, 2011]
Creeping vole *Microtus oregoni* (Arvicolinae)	X0	XY	[reviewed in Fredga 1983,1994]
Mandarin vole *Microtus mandarinus* (Arvicolinae)	XX, X0[b]	XY[c]	[Chen et al., 2008]
Ngurui spiny mouse *Acomys ngurui* (Deomyinae)	XX/X0 mosaic[d]	XY/X0 mosaic[d]	[Castiglia et al., 2007]

[a] large proportion of the females are X*Y (the asterisks designates a still unknown sex reversal mutation on the X chromosome), the other females are XX or XX*

[b] females are XX or X0

[c] males are XY, but the *Sry* gene is apparently absent

[d] females have an excess of X0 somatic cells (97%), while males are mosaic X0 or XY in somatic cells, and only XY in the germinal lineage

Fig. 1. « Sex-reversal in placental mammals »; Parma, Veyrunes, Pailhoux.

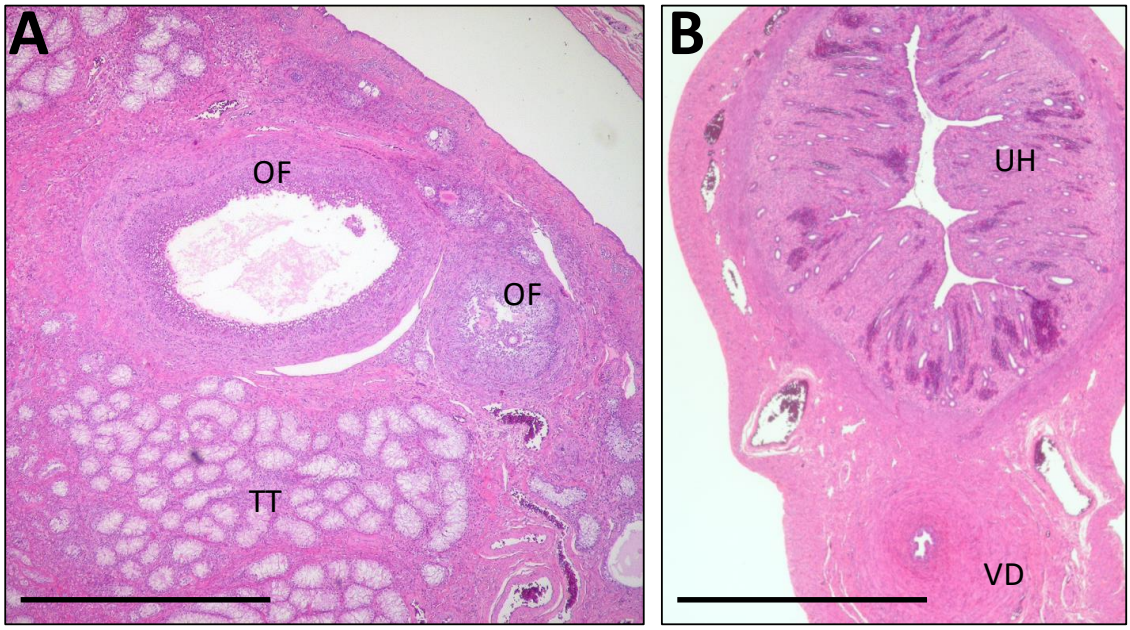

Fig. 2. « Sex-reversal in placental mammals »; Parma, Veyrunes, Pailhoux.

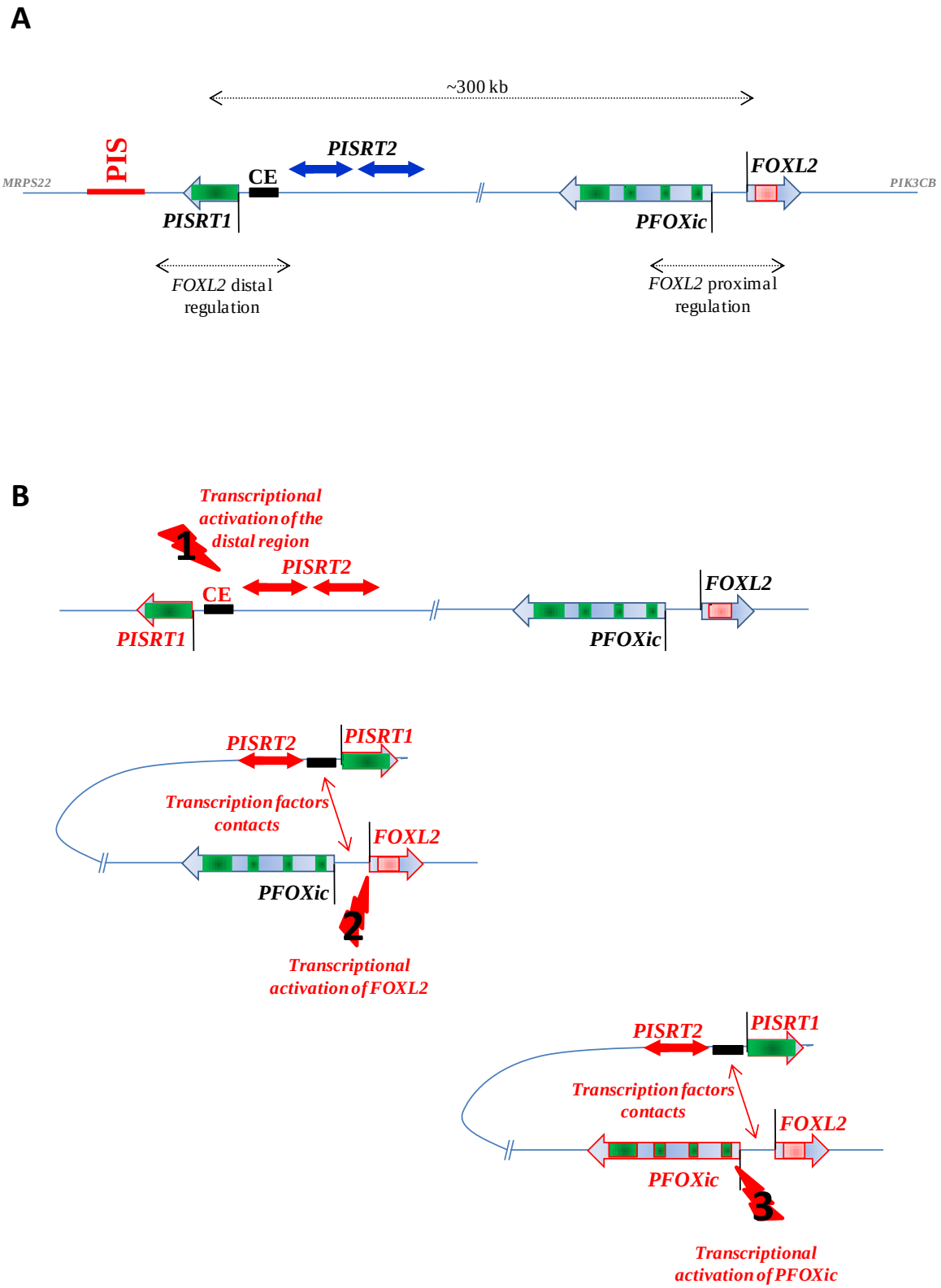

Fig. 3. « Sex-reversal in placental mammals »; Parma, Veyrunes, Pailhoux.