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Polledness intersex syndrome in goats – molecular and histological aspects

Iwona SZATKOWSKA[1], Daniel ZABORSKI[2], Witold Stanisław PROSKURA[1,*], Sara TABOR[1]

[1]Laboratory of Molecular Cytogenetics, Department of Ruminants Science, Faculty of Biotechnology and Animal Husbandry, West Pomeranian University of Technology, Szczecin, Poland

[2]Laboratory of Biostatistics, Department of Ruminant Science, Faculty of Biotechnology and Animal Husbandry, West Pomeranian University of Technology, Szczecin, Poland

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Abstract: Polledness intersex syndrome (PIS) is a phenomenon associated with 2 distinct conditions occurring in goats, namely polledness and intersexuality. The first is a trait exhibiting complete dominance, whereas the latter is recessive. The extreme case of intersexualism is complete sex reversal, which is observed only in females (60,XX). The trait PIS is inherited in a recessive manner and caused by deletion of 11.7 kbp DNA on chromosome 1q43. Female goats with PIS exhibit a broad range of developmental abnormalities of the sexual system, from only subtle changes to complete sex reversal. The phenotypic diversity in goats with PIS is most likely an effect of complexity of the sexual differentiation process, in which the gene-dosage effect may potentially play a crucial role, in addition to the numerous molecular factors regulating the developmental pathway. The occurrence may explain different degrees of masculinization. Nevertheless, the sex reversal phenomenon appearing in female goats (60,XX) with PIS is not considered a serious problem in breeding programs because properly planned selection of individuals for reproduction can allow for complete exclusion of risk of occurrence of this syndrome in offspring. On the other hand, female goats with PIS are still one of the most suitable animal models for studying complex processes of determination and differentiation of sex in mammals.

Key words: Goat, polledness intersex syndrome, sex reversal, SOX9

1. Introduction

Developmental abnormalities of the reproductive system in farm animals can have different backgrounds and frequencies of occurrence depending on species. In ruminants such as cattle and sheep, the most common sexual anomaly is freemartinism, which affects females born as a part of an opposite-sex twin pair. It occurs in 90% of opposite-sex twins in cattle and in 2%–20% of opposite-sex twins in sheep depending on breed (1,2). This phenomenon is caused by formation of arteriovenous anastomoses between placentas of opposite-sex twins during early fetal life that leads to direct interaction of their hormonal and immune systems and hematopoietic tissues. In consequence, the genital system of a female twin becomes masculinized. Sporadic cases of freemartinism have been also observed in goats, but with low frequency, not exceeding 1% (3,4).

Another developmental anomaly of the reproductive system, polledness intersex syndrome (PIS), occurs frequently in *Capra hircus*, but is observed very rarely in cattle and sheep. Potential association of polledness/hornedness with goat sexual activity has been investigated thoroughly. First, it was established that polledness (*P*, polled) is an autosomal dominant trait in both sexes (5). Next, Soller et al. (6) indicated that polledness may be associated with an intersexualism, which is a recessive trait observed only in polled females with a typical 60,XX karyotype. The selective breeding of polled goats was linked to more frequent occurrences of intersexualism. In turn, a disturbed male-biased sex ratio was reported, as a consequence of wrong classification of sex-reversed females as males (7). That phenomenon seemed to be more complicated, because polled homozygotic (*PP*) males were characterized by a 20% decrease of fertility, associated with underdevelopment of the epididymides (8). Surprisingly, those males were revealed later to be, in fact, females with complete sex reversal. Additionally, heterozygotic polled (*Pp*) goats of both sexes were indicated to have slightly better fertility potential compared with horned (*pp*) animals (8). Further studies revealed that polled intersexes, in fact, were cytogenetic females with developed testes, but lacked a sex-determining region Y (*SRY*) in their genomes, which is pivotal for the male developmental path (9). An identification of molecular background of PIS, which is a

* Correspondence: witold.proskura@zut.edu.pl

trait directly linked to polledness (5), allowed for better understanding of that phenomenon.

2. Molecular background of PIS

The polled intersex syndrome locus is located on goat chromosome 1q43 (10). Deep analysis revealed its significant homology with the 100 kbp human region associated with blepharophimosis-ptosis-epicanthus inversus syndrome, which is in fact caused by *FOXL2* gene dysfunction (11).

Further research indicated a deletion of 11.7 kbp to be the cause of occurrence of PIS (5). Although that region is considered noncoding because it mainly consists of 2 LINE sequences (2.2 and 2.6 kbp), its deletion affects chromatin structure and expression of *PISRT1*, *PFOXic*, and *FOXL2* genes (12,13). The deletion is located 25 kbp from *PISRT1* and 300 kbp from the *PFOXic* and *FOXL2* genes (12,13). Of these genes, only *FOXL2* is considered a typical gene, being transcribed and then translated into functional protein. It codes for a transcription factor containing a forkhead domain (14) and is thought, together with *RSPO1*, to be a major factor involved in ovarian differentiation (15). The first peak of *FOXL2* expression takes place in early stages of ovarian development, before the beginning of germ cells meiosis. The second peak is observed at the time of folliculogenesis, and the *FOXL2* gene remains highly expressed until adulthood (16). It is expressed mainly in ovarian pregranulosa cells, in which it is responsible for modulation of ovigerous cord fragmentation, primordial follicle formation, and follicular growth (17,18). A very interesting fact is that *FOXL2* is under the control of a conserved bidirectional promoter also driving *PFOXic* gene transcription (17).

PISRT1 is another gene considered to be regulated by PIS region. It is transcribed, translated, and then transported to the cytoplasm (19,20). Although its role in tissue differentiation and gonadal development has not been precisely established, it seems to be a very important factor. The promoter region of the *PISRT1* gene has been revealed to be highly conserved, based on comparison of *PISRT1* gene sequences of mice, goats, dogs, and humans. Moreover, high expression of the *PISRT1* gene is observed in properly developing fetal ovaries and testes, as well as in adults of both sexes (19,21). To investigate the hypothetical role of *PISRT1* in gonadal development and female-to-male sex reversal, transgenic PIS(-/-) goats were created. They had an additional copy of the *PISRT1* gene, under control of a phosphoglycerate kinase 1 promoter, so it was independent from PIS regulation (22). Gradual increase of *PISRT1* expression in males with proper testes, from final stages of the prenatal period to adulthood, implicated its potential role in correct gonadal differentiation. Although the additional copy of *PISRT1* was highly expressed in PIS(-/-) transgenic goats with complete female-to-male sex reversal, their testes were not properly developed. In turn, Boulanger et al. (22) concluded that the potential role of *PISRT1* in proper gonadal determination and differentiation remains unclear, and its contribution to the sex reversal phenomenon is doubtful. Nevertheless, *PISRT1* is known to enhance *FOXL2* expression in females, and its insufficiency in PIS(-/-) female goats is associated with significantly reduced *FOXL2* expression (22).

3. Molecular consequences of PIS deletion

A decrease of transcription of the *CYP19* gene coding for cytochrome p450 aromatase is the first molecular consequence of lack of the *FOXL2* gene expression in polled female (60,XX) goats with PIS. This can be observed as early as 36 days post coitum (DPC), in the early stages of fetal development (23). As a consequence, the ovarian cortex is dramatically reduced, whereas pre-Sertoli cells (the main component of fetal testes) are being properly differentiated. High expression of the *SOX9* gene (SRY-related HMG box), the main modulator of testis differentiation in tandem with SRY, is observed in these cells in females with PIS, which is similar to that observed in males of the same age. SOX9 is a protein belonging to SOX family of transcription factors, which fulfill many functions in organogenesis, but its key role is in induction and formation of testes through an activation of genes involved in these processes (24).

The main target of SOX9 is the promoter of the antimüllerian hormone (AMH) gene, which codes for the hormone responsible for regression of müllerian ducts in males (25). Additionally, *AMH* gene overexpression causes complete inhibition of *CYP19* gene transcriptional activity. That process takes place at 46 DPC in sex-reversed PIS goats (23). In completely masculinized gonads of females with PIS with completely differentiated Leydig cells located between the seminiferous cords, the genes involved in steroidogenesis are expressed the same as in gonads of typical males. One of these genes, *3β-HSD*, coding for 3-β-hydroxysteroid dehydrogenase, is characterized by an expression profile typical of males in gonads of females with PIS, and the same situation concerns testosterone synthesis (23). An initiation of typical male pathway of gonadal development in sex-reversed females is a result of male expression profile of genes involved in differentiation of Sertoli and Leydig cells. In consequence, the secreting and steroidogenic activities of those cells determine the development of masculine deferent canals and external sexual organs, while the observed degree of masculinization varies among particular cases (23,25).

During the neonatal and postnatal periods, expression of genes typical of the male in gonads of female-to-male sex reversals was similar to that observed in males of

analogous age. Considering that the testes of 1-month-old sex-reversed females contained gonadal cords with immature Sertoli cells and gonocytes, it is understandable that they exhibited an expression profile of *3β-HSD*, *AMH*, *CLDN11*, and *SOX9* genes similar to that observed in testes of typical males of the same age (22,26). However, the expressional activity of those genes drastically decreased during maturation, which was linked directly to the degeneration of the seminiferous epithelium, supporting cells, and interstitial cells. On the basis of study of the gene expression profile in gonads of female goats with PIS during their first year of life (26), a clear association exists between *SOX9* gene transcriptional activity and the functional state of Sertoli cells. Those cells were completely degraded and the *SOX9* expression level was strongly reduced in the 12-month-old PIS females, while the situation was totally opposite in the properly developed males of that age.

4. The degree of masculinization of PIS female gonads

First reports of the early process of masculinization in polled PIS goat females were based on investigations of anatomy, histology, and gene expression in intersex fetuses (23). At 36 DPC, no histological differences were observed between gonads of males (XY) and PIS(-/-) females (XX). Nevertheless, at as early as 40 DPC, significant changes of ovarian development were observed, including a reduction of the ovarian cortex and an initiation of formation of seminiferous cords with a presence of pre-Sertoli cells, similarly as in gonads of males of the same age. By 56 DPC, the changes were much greater: functional Leydig cells synthesizing androstenedione were present in the majority of PIS(-/-) females. Additionally, ovotestes with simultaneous presence of both seminiferous cords and reduced ovarian cortex were observed. That specific histological view of the gonads remained until birth. The same histological view at the same stage of development is observed in properly developing gonads of typical males. Nevertheless, at 70 DPC a reduction of seminiferous cords with disappearance of germ cells was clearly visible in PIS(-/-) females (23).

During both prenatal and postnatal periods, different degrees of masculinization were observed in particular PIS(-/-) female cases, but sex reversal was reported most often (26–28). Based on anatomical evaluation, those female-to-male sex reversals were classified as male pseudohermaphroditism, usually with developed testes rather than ovotestes.

In the first month of life, gonads of PIS(-/-) females mostly contained sex cords with basement membrane but lacking the lumen. Moreover, immature Sertoli cells, spermatogonia, and primordial germ cells were present. The latter were characterized by uncommon cytoplasmic content and dark dying nuclei (Figure 1), which can be interpreted as the sign of progressive elimination of primordial germ cells. Interestingly, the histological view of PIS(-/-) female gonads and the diameter of sex cords, which together indicated slight hypoplasia, were very similar to those described in males of the same age (26,29). Thus, the histological alterations observed during the neonatal period in gonads of PIS(-/-) females clearly indicated female-to-male sex reversal.

In the next months of life, the abnormalities of PIS(-/-) female gonads became easier to observe compared to the gonads of males of similar age (27,29). The first changes observed included progressive disappearance of genital cells, the lack of lumen in tightly packed seminiferous tubules, decreased number of interstitial cells in the gonadal stroma and its fibrosis, and partial loss of Sertoli cells. Somatically mature female-to-male sex-reversed goats with PIS(-/-) are very rare, because this condition is linked to abnormal functioning of the urinary and sexual

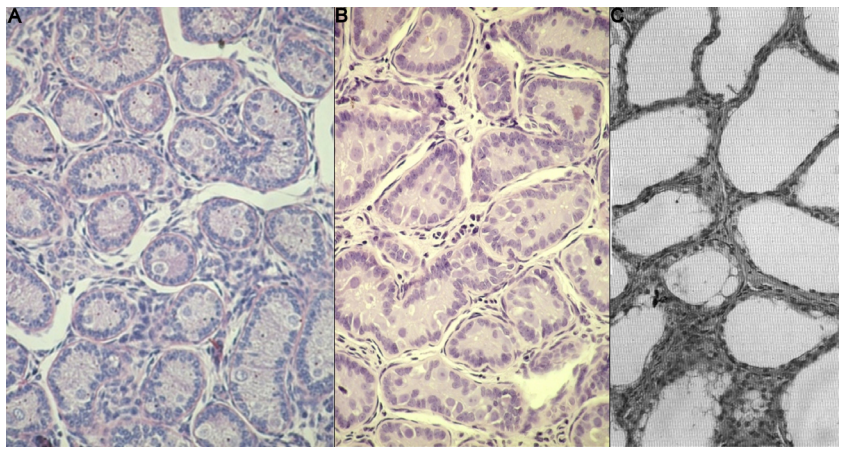

Figure 1. Histological view of gonads of 1- (A), 3- (B), and 12-month-old (C) PIS(-/-) goat females (30).

systems. Gonads of those individuals are usually very difficult for unambiguous identification (Figure 2), which is caused mainly by complete degeneration of Sertoli cells and total sterility of seminiferous tubules (26). In turn, in individual cases ovotestes were observed, which were simple to identify histologically because they consisted of 2 characteristic components: the testicular and the ovarian parts. The testicular area showed a presence of seminiferous tubules, exfoliation of Sertoli cells, and lack of germ cells, whereas in the ovarian area, structures similar to primary ovarian follicles, but lacking germ cells, were observed (23,26).

Apart from the presence of testes or ovotestes in particular sex-reversed PIS(-/-) females, the reproductive ducts may be masculinized to a different degree in particular cases. Three distinct conditions may be distinguished as: 1) the ducts being typical for males (the phenotypic sex being in agreement with the gonadal sex), 2) the ducts having a combination of masculine and feminine features, 3) the ducts being typical for females (the phenotypic sex being in contradiction with the gonadal sex) (30).

The development of external genital organs also may vary among particular PIS(-/-) sex-reversal cases. Nevertheless, the most common condition is a more or less developed male phenotype with a dysfunctional penis (lacking urethral process) and testes located in the abdominal cavity, inguinal region, or scrotum. Additionally, rare cases exhibit female phenotype with significantly enlarged clitoris and hypospadias (26).

The molecular background of ambiguous sexual development, including atypical gonads, reproductive ducts, and external genitalia, is well established in PIS(-/-) goat females. A similar situation applies to other syndromes (e.g., freemartinism) and other species (31,32). The phenotypic diversity in goats with PIS is most likely an effect of the complexity of the sexual differentiation process, in which the gene-dosage effect may potentially play a crucial role, in addition to the numerous molecular

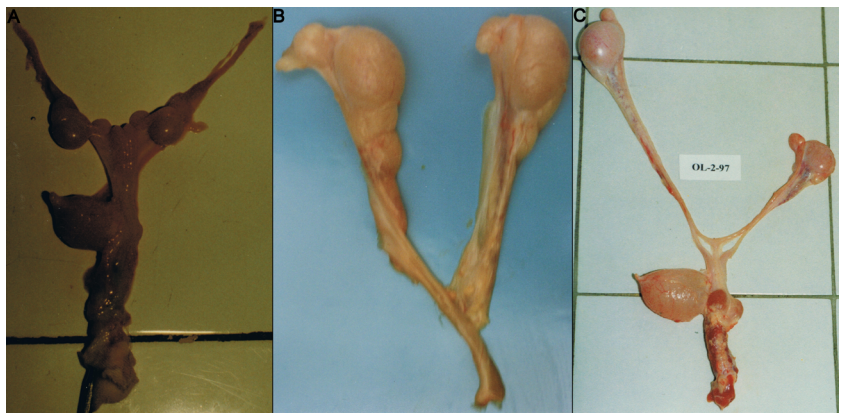

Figure 2. Female sexual system (A), female sexual system with male features (B), and male sexual system (C) observed in somatically mature female-to-male sex-reversed goats with PIS(-/-) (30).

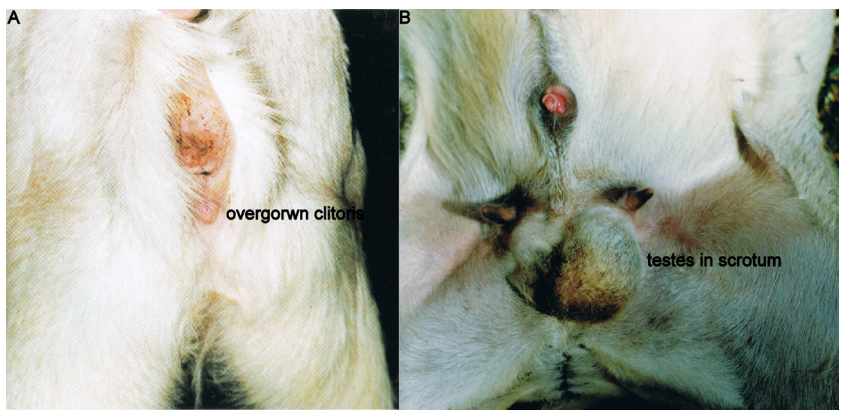

Figure 3. Female (A) and male (B) genitalia observed in somatically mature female-to-male sex-reversed goats with PIS(-/-) (30).

factors regulating the developmental pathway. The occurrence of that phenomenon may result in abnormal development of the sexual system, and also may explain different degrees of masculinization, including complete sex reversal, observed in PIS(-/-) goat females (23,33).

5. Conclusion

A sex-reversal phenomenon appearing in PIS(-/-) female goats (60,XX) is not considered to be a serious problem in breeding programs, because properly planned selection of individuals for reproduction can allow for complete exclusion of the risk of occurrence of this syndrome in offspring. That may be ensured by inclusion of at least 1 horned individual in every mated pair. If any intersexual offspring occur, it would not be PIS-related. On the other hand, female goats with PIS are still one of the most suitable animal models for studying the complex processes of determination and differentiation of sex in mammals.

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