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Comparison of Sex Determination in Vertebrates (Nonmammals)

Aleksandr F. Smirnov and Antonina V. Trukhina

Abstract

The chapter is devoted to the consideration of sex determination in vertebrate groups of nonmammals: fish, amphibians, reptiles, and birds. Attention is drawn to the fact that all these groups of animals, unlike mammals, are implemented hormonal control options for primary sex determination, and there is a possibility of sex reversion. Determination of gonadal development in vertebrates like testis or ovary was initially controlled mainly by sex hormones (fish and amphibians). Later, various sex determining genes were involved in this process. The system was quite plastic and was able to respond to changes in external conditions (reptiles). The appearance of heteromorphic sex chromosomes (birds) has led to the emergence of some specific W chromosomal signal, which provides estrogen control of the development of a heterogametic sex. In mammals, the control of the primary determination of sex (the appearance of the gonad) becomes purely genetic, and the role of sex hormones is reduced to the differentiation of testis or ovaries.

Keywords: sex determination, sex hormones, sex chromosomes, sex determining genes

1. Introduction

Gender is a set of morphological and physiological characteristics of the organism, providing reproduction, the essence of which is to fertilization, i.e. the fusion of male and female germ cells (gametes) in zygote, which develops into a new organism. Differentiation of sex (its phenotypic manifestation) includes two successive stages: the primary determination of sex and the appearance of secondary (external) sexual characteristics (actual differentiation). It is believed that the concept of this process is conservative. Sex determination is both a genetic and ecological process, with the sex of the individual being determined by an alternative physiological solution. It is assumed that there are two main mechanisms for determining sex: genetic (GSD—genetic sex determination) and environmental (ESD—environmental sex determination). Genetic sex is determined at the time of conception and depends on genetic differences between males and females, and ecological sex depends on external conditions in the absence of significant genetic differences and is determined after fertilization in response to environmental conditions. For birds and mammals, only the GSD is characteristic, and for crocodiles—TSD (one of the forms of ESD). In addition, there are two varieties of the genetic sex determination system: with heterogametic males (XY, mammals) and heterogametic females (ZW, birds). It should be noted that amphibians have both

genetic systems, and for lizards, snakes, turtles, and bony fish, all possible variants of sex determination are described [1–3].

Sex steroid hormones including androgens, estrogens, and progesterone are present in all vertebrates which play essential roles in modulating a variety of behavior and processes, such as embryonic development, sexual differentiation, growth, aggression, reproduction, learning, memory, social communication, and so on. Many signaling actions of these sex steroid hormones are mediated by their receptors that belong to the superfamily of steroid nuclear receptors. Once a sex steroid hormone ligand binds to its receptor, the receptor becomes phosphorylated and is translocated into the nucleus, where it binds to specific DNA sequences and activates gene transcription. Androgens have a critical physiological role in reproductive biology and sexual differentiation, particularly in the development of male secondary sex characteristics [4, 5].

It is assumed that sex determination is a combination of hormonal and genetic factors and is divided conditionally into appropriate stages. This phenomenon is reflected in the possibility of sex inversion—the possibility of its complete or partial hormonal alteration. For fishes and amphibians, there is the sensitivity of normal development of the gonads to androgens and estrogens. In reptiles, birds and marsupials, only estrogens are effective. The appearance of the gonads of placental mammals does not depend on sex hormones. This trend is associated with the stability of growing offspring or incubation of eggs [6].

The proposed chapter will consider the system of sex determination in fish, amphibians, reptiles, and birds in comparing the role of hormonal and genetic mechanisms, possibilities, and mechanisms of sex inversion.

2. Features of sex determination in fishes

Fishes are perhaps the most complex group of animals in the mechanism of sex determination. Only bony fish include over 30,000 species. It is the largest group of vertebrates. They are divided into three groups in accordance with the laws of sex determination: (1) gonochoristic species whose sex is determined genetically or through environmental factors; (2) sequential hermaphrodites (about 2% of all existing species), changing the sex of males to females (protandrous), the sex of females to males (protogynous), or in both directions (serial) in the process of ontogenesis; (3) unisexual type of sex determination (characteristic only for Amazon mollies (*Poecilia formosa*)). Gonochoristic genetics of sex in fish is largely unclear. Functional hermaphroditism occurs in many different species of animals such as echinoderms, crustaceans, molluscs, and fish; however, it is lost in vertebrates during the transition from amphibians to mammals. From here, fishes provide a unique model for studying the mechanism of hermaphroditism in vertebrates. Unfortunately, only one species of fish (Japanese medaka—*Oryzias latipes*) was identified by a primary system of sex determination [7, 8].

The Japanese medaka (*Oryzias latipes*) and Maebashi medaka (*Oryzias curvinitus*)—species with heterogametic male sex with homomorphic sex chromosomes that are a very early stage of evolution, the recently described Y-chromosome plot, containing hypothetical gene *dmy*. This gene is specifically expressed in the gonads and is essential for embryo development in male type. Gene *dmrt1bY* (*dmy*) homologous (about 80%) of the *dmrt1* gene in other species of vertebrates represents the equivalent of *sry* gene in mammals. It is important that medaka *dmy/dmrt1bY* is a unique system. This species is described as ontology mammalian *sox9* gene, but in contrast to amniotes and amphibians, this does not play a role in determining the testes. Sex determination system of medaka is unstable. Medaka has interesting

significant genetic divergence: *dmy* gene is absent in some lines of the Japanese medaka (over 10%) and other types of fish of the genus *Oryzias*. In some laboratory lines, the proportion of homogametic males (XX) exceeds 20%. It is believed that gene *dmy* has occurred as a result of the *dmrt1* gene duplication and transposition of part of its copy size to 280 kbp about 10 million years ago. The products of these genes differ only in one amino acid replacement (Ser26/Thr), which may have led to such differences by gender. It has been shown that the rate of synonymous substitutions in the *dmy* is 1.78 times greater than that of *dmrt1* and this is consistent with the hypothesis of evolution through males (male-driven evolution hypothesis). In birds and salmon, it has the same orientation. The speed ratio of nonsynonymous substitutions (dN) to synonymous (dS) is also higher in comparison with *dmy/dmrt1*.

Only two sex determining genes in vertebrates were described: *sry* and *dmy*. It is believed that the protein DMY performs two different functions in germ and somatic cells. In somatic cells surrounding germ ones, it affects the proliferation of the latter (for example, influencing a cascade of genes involved in the transmission of the estrogen signal). Another feature is the induction of development of pre-Sertoli cells (cells surrounding the primary germ cells (PGCs)) in the gonad heterogametic XY sex. In this case, there is an analogy with *sry*, which is involved in the activation of other genes that support the development of Sertoli cells. In medaka, there are other female-specific genes and male-specific genes (**Figure 1**). Moreover, the latter gene is located in autosomes. Some ideas of the diversity of sex determining genes among medaka given.

In this species, the sex determined region of the Y chromosome is only 260 kb (1% of the total length of the Y chromosome (59 Mbps)). In this area, there is suppression of recombination. In medaka, all XY individuals carry mutations in the gene *dmy* form ovaries. In individuals with altered *gsdf*-gene, sex inversion is also observed. It is believed that for medaka, the normal gene *dmrt1* (*dmy*) initiates the formation of the testes and controls their maintenance with *gsdf*. The study of sex chromosomes in six species of medaka from the group *celebensis* with XX/XY-sex determination showed that *O. marmoratus* and *O. profundicola* sex chromosomes homologous sex chromosomes of *O. latipes* from the LG10 linkage group. Four species *O. celebensis*, *O. matanensis*, *O. wolasi* and *O. woworae* marked homology

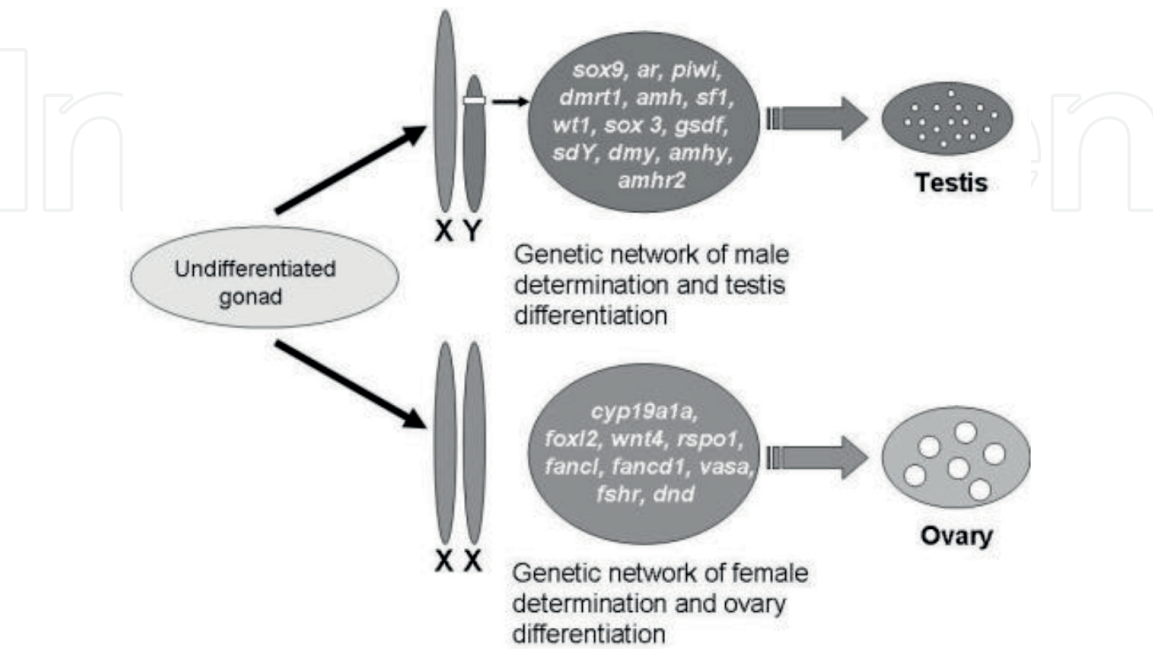


Figure 1.
A schematic diagram of sex determination and gonad (testis or ovary) differentiation in fish with XX/XY sex determination system (adopted from Mei and Gui [10]).

with the chromosomes of LG 24, which involves the transformation of chromosomes from *O. latipes* LG to 24 LG10 within this group. All six studied species share a common sex determined gene (SD). It is shown that genomic predecessor is the Y-chromosomal gene *sox3* and this process involves specific insertion (430 bp).

The zebrafish testes derived from *dmrt1* mutant fish fail to express the anti-Müllerian hormone (*amh*) gene, a key testis-expressed gene, and over-express the ovary-associated gene *foxl2*. Therefore, zebrafish *dmrt1* shares similar roles in male sexual development as other organisms in regulating sex determination and testis differentiation.

In other fishes, e.g., salmonids, there appear to be an early stage of differentiation of sex chromosomes. In rainbow trout (*Oncorhynchus mykiss*) with monofactorial XX/XY system of sex determination, a new gene *sdY* responsible for the development of testes is described. This gene is partially similar to the gene regulator of interferon 9. It has been found that highly conserved in *sdY* salmon is male Y-chromosomal gene for the majority of these species. It is assumed that it is the main testis determining gene for this group of fishes. For the two species of whitefish (subfamily *Coregoninae*), the *sdY* gene is found in both males and females. This implies that there is an alternative system of sex determination in this family. Among other candidate genes for sex determination, gene antimüllerian hormone (*amh*) tilapia is discussed. Fishes with hermaphrodite sex determination (*Labridae*, fish-clowns—amphiprion (*Amphiprion*), and gobies—*Trimma okinawae*) have got bisexual gonads capable of restructuring with the participation of aromatase and gonadotropin receptors. For some species, such as blue tilapia (*Oreochromis aureus*), sex determined putative gene is located on the genetic map of a sex determining region consisting of more than 550 minisatellite markers [7, 9].

In vertebrates, until recently, only four sex determining genes were discovered: *sry* (in mammals), *dmrt1* (in domestic chicken), *dmy* (the Japanese medaka), and *dm-w* (the frog). Recently, four candidate genes were found for this role (and all fish): Patagonian atherin have *amhy*, Luzon ricefish (*Oryzias luzonensis*) have *gsdf*, and puffer (*Takifugu* or *Fugu*)—*amhr2* and rainbow trout—*sdY*. In the Nile tilapia (*Oreochromis niloticus*) gene *gdf*, (gonadal soma derived factor (*gsdf*)) also induces the development of the testes. Assume that the Atlantic salmon *sdY* gene product activates genes *gsdf* and *amh/mi*, thereby reducing the activity of aromatase (*cyp19a* gene), leading to the appearance of males. Where *sdY* is missing, aromatase is synthesized in quantities sufficient for the emergence of the females [8, 10].

Sex determining genes in fish are not conservative. It is believed that the reason for this is the more frequent variation of sex chromosomes in fish than other cold-blooded animals and mammals (**Figure 1**).

These objects sex determination has a high plasticity and is, therefore, possible sex reversal, even in species with established regulatory genes. Striped Danio (*Danio rerio*) experimental data are in good agreement with polygenic sex determination (PSD) when the sex is determined by allelic combinations of several loci. Typically, these loci are dispersed throughout the genome, but some species of bony fish are placed in special sex chromosomes. In hermaphroditic fish, ovotestis develops first, and then secondary sex determination occurs. So, the black bass individuals (genus *Micropterus*) in the first 2 years of life are males, but in the third year, 50% of them are transformed into females. Sex determining male genes such as *dmrt1*, *amh*, and *amhr2* are activated during differentiation of the testis, and their expression is maintained at high level during the period of functioning as males. High dose estrogen E2 induces the development of ovarian and testicular tissue degradation [11, 12].

In fish, there are two systems of sex determination: XX/XY and ZW/ZZ. The most common one is the last. Exploring the flatfish *Cynoglossus semilaevis* as a model

species with genetic sex determination system of ZW-type and the simultaneous presence of ESD, it was found that about 14% of females at a temperature of 22°C become males (pseudomales). It is believed that there is *dmrt1* gene (double sex and mab-3 related transcription factor 1) which is the sex determining gene in this species. It was also shown that pseudomales change the level of methylation of a certain portion of the Z chromosome, resulting in the intensity of transcription in this area as in normal males. In females, on the contrary, the activity of the corresponding plot of W chromosome by methylation is suppressed. Unusual WXZ-system is described for the swordtail (*Xiphophorus helleri*). Not so many fish species had morphologically different sex chromosomes (about 10%) and in most species they are in the early stages of their differentiation. For many members of this class, sex is determined by the environment, and even changes under the influence of behavioral factors. There are species with heterogametic male and female [13].

Fish is characterized by plasticity of germ and somatic cells. This plasticity is maintained throughout the life cycle. Furthermore, they have described the influence of factors on this process such as temperature, pH, density of population, etc. It should be noted that the temperature sensitivity of fish is different from that of reptiles, especially because these types of monosexual populations are rare, even under extreme conditions. TSD in fish is less common than previously thought. The effect of estrogens, acting via estrogen receptors (ER) and directly or indirectly regulating P450arom and AMH, is particularly noticeable. It is noted that the analysis of the differences between gonochoristic and hermaphroditic fish species will help to understand the mechanism of plasticity of sex determination in vertebrates. In addition, there is the idea that gender in fish depending

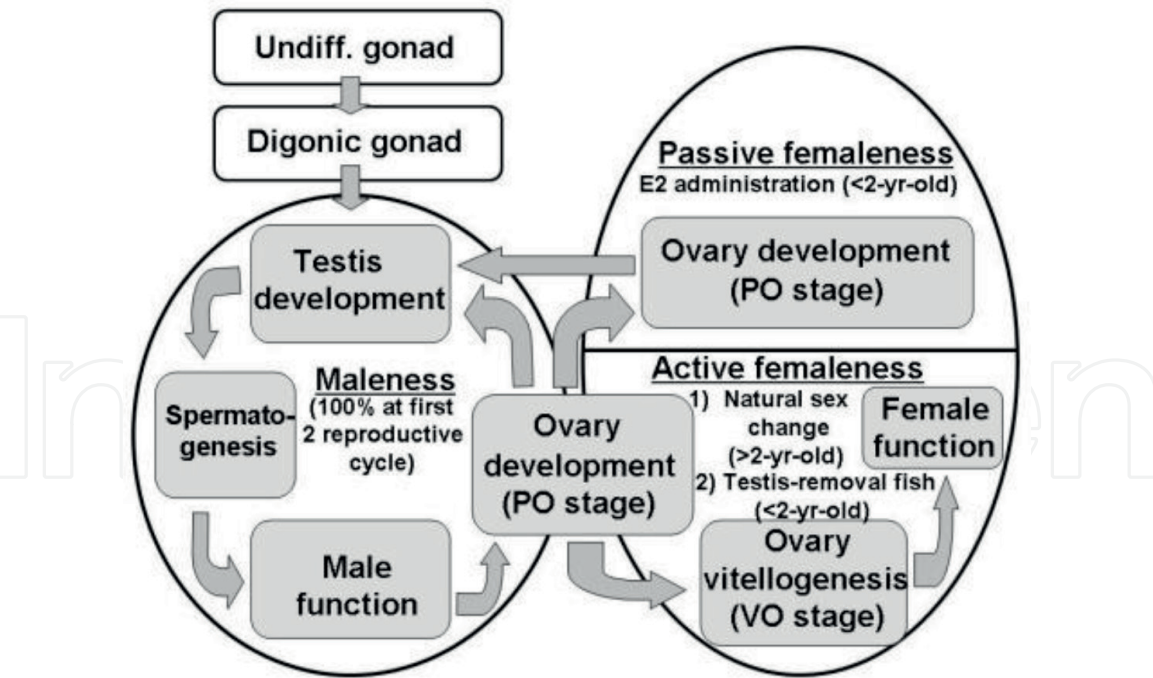


Figure 2.
The profiles of gonadal development in three different sexual phases in hermaphroditic Japanese black porgy, *Acanthopagrus schlegelii*. **Maleness:** the fishes are functional males in the first two spawning seasons. The testis exists at all stages of the reproductive cycles in maleness. **Active femaleness:** the fishes are functional females following the natural sex change that occurs in fish older than 2 years or when induced by the removal of the testis of the digonic gonad. The ovary could reach to the stage of vitellogenesis, vitellogenic, and mature oocytes. **Passive femaleness:** Long-term E2 (4–6 mg/kg feed) administration for 2–3 months results in the appearance of a dominant ovary (with the primary oocytes) with a regressed testis in fish younger than 2 years old, and no vitellogenic oocytes are observed in E2-induced sex-changing fish. A reversible sex change (from passive femaleness to maleness) exists after E2 administration has been withdrawn. Undiff. gonad, undifferentiated gonad; E2, estradiol-17β; PO, primary oocyte stage; and VO, vitellogenic oocyte stage (adopted from Wu and Chang [16]).

on species is a complex trait under the control of one or many genetic factors in addition to environmental effects [9, 14]. In the Chinese tongue sole (*Cynoglossus semilaevis*), genetic ZZ females may change into pseudomales, thereby increasing aquaculture costs because of the lower growth rate of the males than that of the females. A new locus was identified to regulate sex reversal interactively with the SNPCyn_Z_6676874; the linkage between these two loci and the absence of W sperm for pseudomales clearly elucidate the genetic architecture of sex reversal in the tongue sole [15]. Sexual determination in zebrafish is unique in that laboratory strains lack a sex chromosome, and no sex determining gene has been identified. GPER (estrogen receptor) is not required for normal sex differentiation, gonad development, or gonad function in zebrafish [16]. Genetic studies suggest that gonadal sexual fate is not only established by competition for primacy between two sexes via antagonistic signaling pathways during embryonic development but also requires active maintenance to suppress the opposite sex during adulthood. Documented in about 2% of teleost species spanning over 20 families, functional sex change generally occurs in three ways: protogynous (female-to-male), protandrous (male-to-female), and sequentially bi-directional. Most sequentially hermaphroditic fish are protogynous. Sex change in all hermaphroditic species involves radical gonadal transformation, and follows diverse ontogenetic pathways in different lineages particularly where sequential hermaphroditism has independently evolved. Gonadal transition in sex-changing fish is accompanied by changes in plasma concentrations of gonadal steroids. These steroids control gonad differentiation and maintain sexual phenotypes in teleost fish, wherein 17 β -estradiol (E2) and 11-ketotestosterone (11-KT) function as the major estrogen and androgen, respectively. The balance between estrogen and androgen production is expected to control sexual fate of the gonads during sex change. For example,

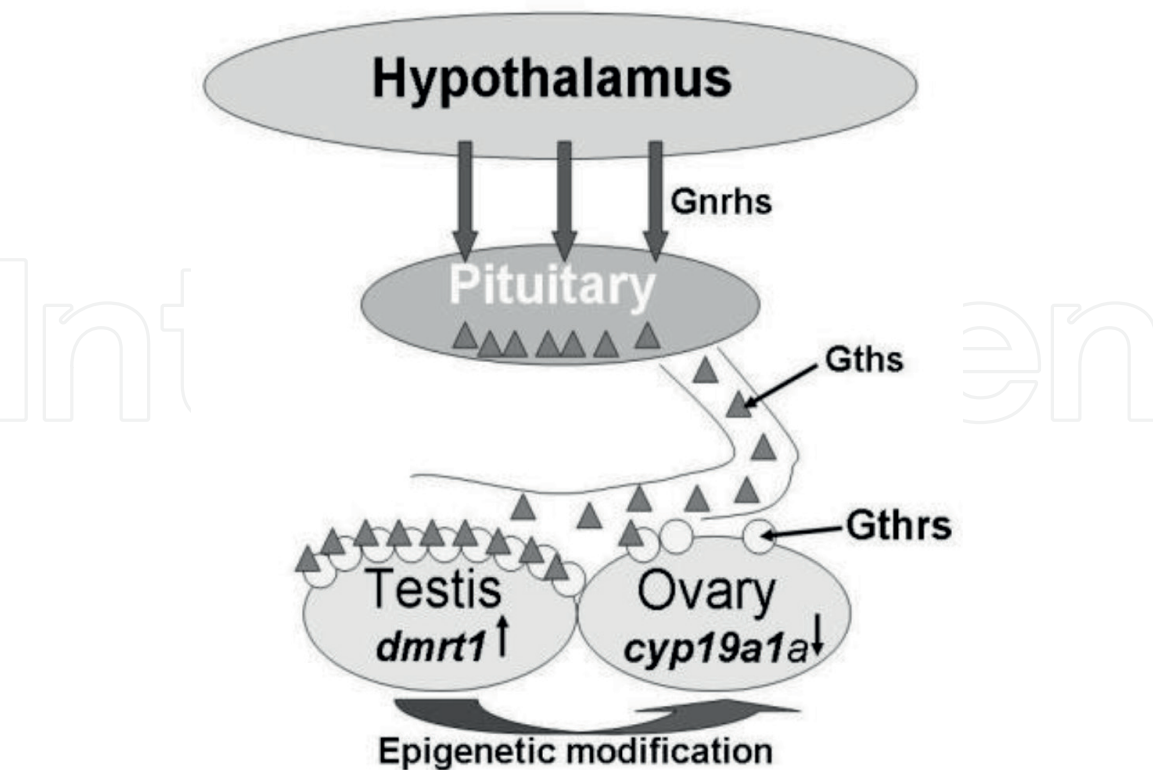


Figure 3. The potential mechanism for sexual fate decision through the GnRHs—GTHs—Dmrt1 axis (brain-pituitary-testis axis). The model shows that the male fate decision is controlled by gonadotropins through the GnRHs—GTHs—Dmrt1 axis. The testis may stimulate the epigenetic modification of the ovary by DNA methylation of the *cyp19a1a* promoter to suppress the *cyp19a1a* expression. GnRHs, gonadotropin-releasing hormones; GTHs, gonadotropins; and Gthrs, gonadotropin receptors (adopted from Wu and Chang [16]).

factors regulating *cyp19a1a* expression are strong candidates for the trigger that initiates gonadal sex change; *cyp19a1a* promoter regions contain binding motifs for numerous factors that potentially regulate its expression [15].

However, hermaphroditic fishes have a plastic sex, and a stable sex is difficult to maintain with sex steroids. The black porgy regulated the dynamic development of both sexes; only one sex can grow while the other sex exists in a rudimentary stage (Figure 2). The sexual fate of the digonic gonad is determined by the male fate maintenance and through the GnRh—Gth—Dmrt1 signaling. Altogether, testicular *dmrt1* and ovarian *cyp19a1a* expression are critical to the sexual fate of a male phase and female phase, respectively (Figure 3).

3. Sex in amphibians

Amphibians have two sex determined systems: XX/XY and ZZ/ZW. Most tailed amphibians (order *Caudata*) have XX/XY-system. For 63 species of 1500, sex was determined and only 20 species have differing sex chromosomes. Males of some New Zealand frogs (*Leiopelma hamiltoni* and *L. hochstetteri*) have heterogametic sex. In most amphibians, sex chromosomes are homomorphic (undifferentiated) in both sexes and are characterized by frequent turnover. This is in sharp contrast to sex chromosomes in two major vertebrate groups, the mammals and birds, where they are heteromorphic in one sex and are highly conserved. Thus, amphibians are excellent research materials on the turnover of sex sensitive to a resistant state, indicating the relationship between sex chromosome turnover and sex ratio control.

Models of sex differentiation in amphibians can be divided into three types: (1) a direct development of the undifferentiated gonads into testes or ovaries, (2) the development of the undifferentiated gonad into the ovary and subsequent development of the testis through the ovary, and (3) the development of the testes through the intersex phase (prodifferentiating type) [17]. For a long time, genes that determine sex could not be found in amphibians. Recently, for smooth clawed frog (*Xenopus laevis*), the candidate gene has been found suitable for such a role. It is believed that it is involved in the development of the ovary. African clawed frog has a ZZ/ZW system of sex determination. Its *dm-w* gene was described. It is localized in the X chromosome and possessed a DM-domain. The nucleotide sequence of gene encoding a DNA-binding domain has 89% identity with *dmrt1*, but there is not similarity in trans-activational region *dm-w* and *dmrt1*: genes are expressed exclusively in the primordial gonads, and *dm-w* is expressed more actively than in the gonads of ZW-larvae. The gene *dmrt1* (*dmrt1α* and *dmrt1β*) is located in autosome and there are no differences in its expression in males and females. The product of this gene enhances the expression of *cyp19* and *foxl2* ones. A similar gene was not detected in other species of amphibians. It is assumed that in these frogs, homo- and heterodimer products of *dmrt1* and *dm-w* participate in the sex determination [18, 19] (Figure 4).

In the northern crested newt (*Triturus cristatus*), the proportion of males increases when the ambient temperature increases, and a decrease of temperature leads to an excess of females. Thus, in amphibians, an increase or decrease of the ambient temperature leads to a modification of the normal development of the gonads and sex determination. Here, sex-determining genes are not the decisive factor in determining sex. A number of experiments have shown that atrazine and some other pesticides that affect the endocrine system affect the formation of sex in frogs. As a result, males are changed to females. Exogenous steroids (introduced from the outside) are also changing the sex in amphibians [20]. The unexplainable mechanism of sex determination in the rice frog species was introduced. Amphibians bearing a novel sex determining mechanism are yet to be identified [17].

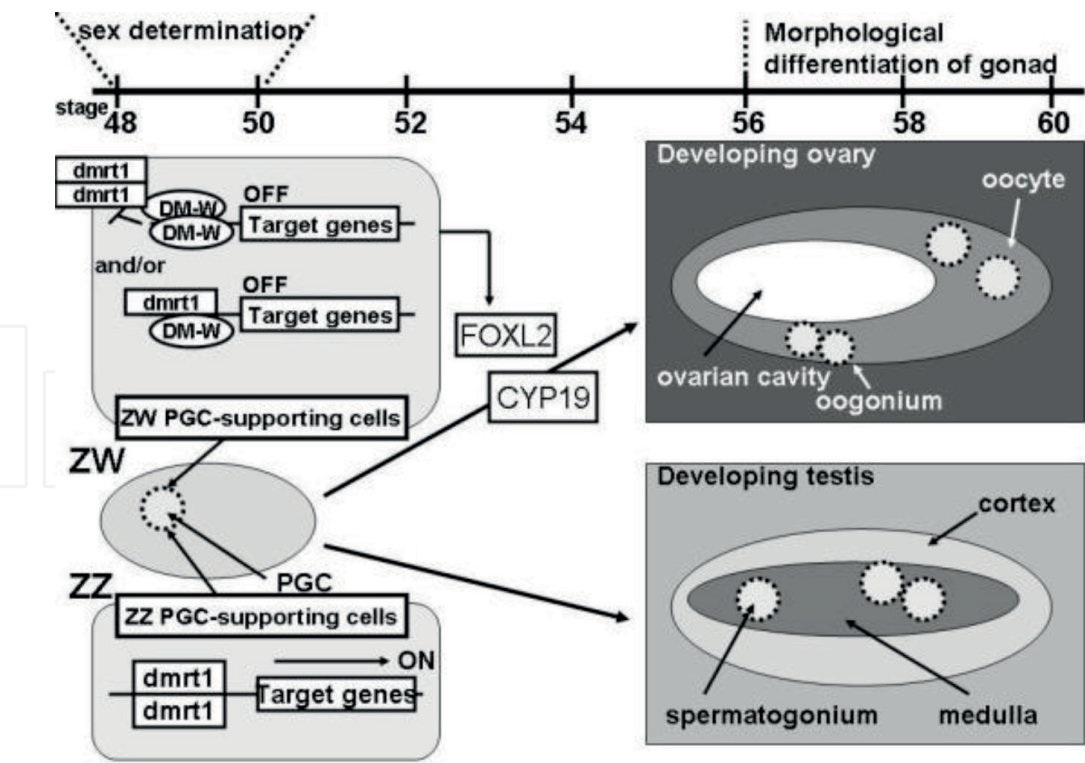


Figure 4. Model of ZZ/ZW-sex determined system and the formation of the ovary from *Xenopus laevis* (adopted from Liu et al. [18]).

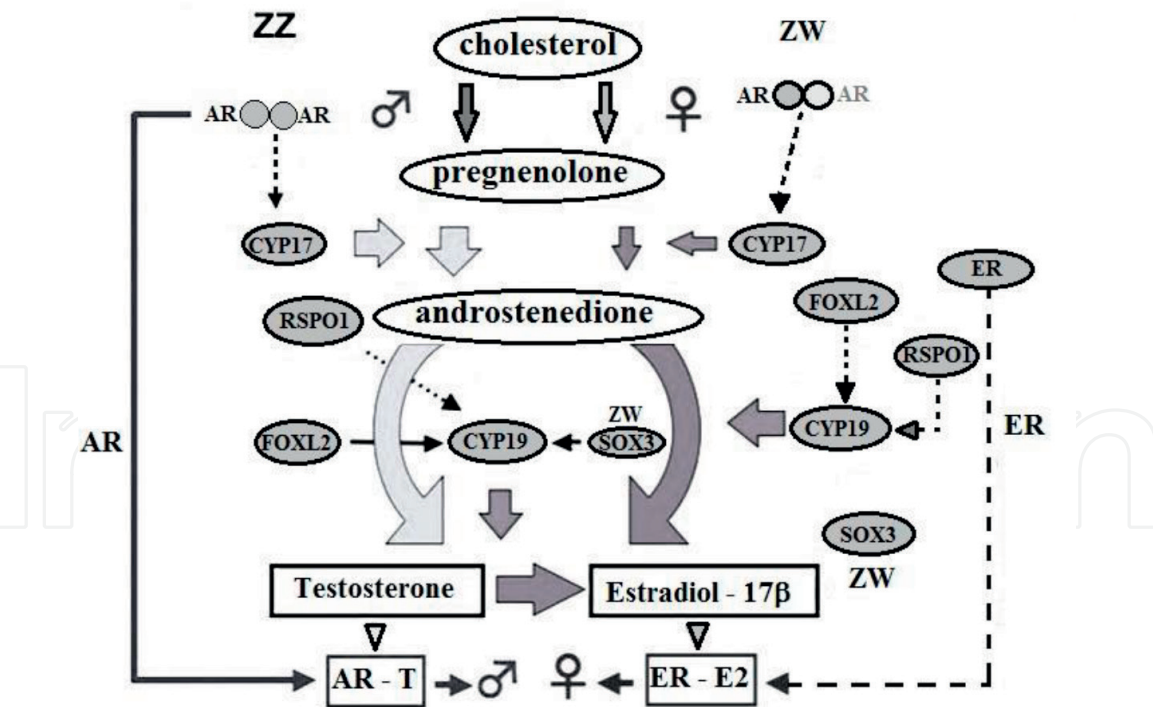


Figure 5. The role of steroid hormones in sex determination from *Rana rugosa*. At the stage of sex determination in the undifferentiated gonads of males, testosterone is synthesized at the same time females synthesize estradiol-17β. Letters ZZ, ZW indicate sex chromosomes. AR-T and ER-E2 represent complex androgen receptor (AR) to testosterone, and respectively, estrogen receptor and estradiol-17β (adopted from Nakamura [23]).

The dominant hypothesis of sex determination for amphibians is proposed in relation to the *Rana rugosa*. In the Japanese wrinkled frog (*Rana rugosa*), four populations are described, in one of which (the northern population) females are heterogametic. Assume that sex determining genes really do not need to determine the sex of

amphibians, as well as the presence of the transcription factor, localized in the X or W chromosomes, influencing the feminization of vertebrates with TSD or GSD systems of sex determination. In males, if there is a specific mechanism of sex determination, it is likely that it supports the regulation of steroid hormones in undifferentiated gonads through the inhibition of *cyp19* gene transcription for the formation of the ovaries. In the scheme of **Figure 5**, a possible role of steroid hormones in sex determination is shown for *Rana rugosa* [21]. According to the next experimental data, complete female-to-male sex reversal occurred in the AR-Tg-transgenic ZW female frogs when a low dosage of T was supplied in the rearing water of tadpoles. In the sex reversed testes, the expression of *dmrt1*, *ar*, and *cyp17* genes required for masculinization was significantly upregulated. Next, AR-knockdown (KD) ZW female frogs were produced by the CRISPR/Cas9 system. Interestingly, no sex-reversal was observed in AR-KD ZW female frogs when the gonads were treated with dosages of T high enough to induce complete female-to-male sex-reversal, even in wild type frogs. In the AR-KD ZW female gonads, the expression of genes required for masculinization was not up-regulated. These results indicate that AR together with androgens can be a male sex-determinant in an amphibian species [22, 23].

4. Sex in reptiles: determination of sex under the influence of temperature

Sex determination by environmental factors is mainly known in reptiles. The most well studied temperature sex determination (TSD) is occurring in three of the five main taxonomic groups of reptiles: turtles, crocodiles, and lizards, but it is not found in snakes. The adaptive significance of such sex determination mechanism is shown. During early embryonic development of gonad, epithelial cells are divided and unite in the epidermal strip of mesonephros mesenchyme. Further, during the so-called temperature-dependent period under the level of endogenous estrogen, such strip forms seminiferous tubules with Sertoli cell epithelium or gaps with squamous epithelium. The mechanism of this sex determination is poorly understood. Obviously, it is found in species with undifferentiated Y chromosome. The transition from the female promoting temperature (FPT) to male promoting temperature (MPT) is carried out in a temperature-period (TSP), during the so-called “window” of vulnerability [24].

In some species of reptiles, GSD is not fixed for life, and the original gender may change during development without changing the genotype. This phenomenon is known as environmental sex reversal (ESR) and observed also in insects, fish, and amphibians [25] (**Figure 6**).

In reptiles, there is an “open” sex determination program that is different from a “closed” program, characteristic of birds and mammals. It is believed that in this case, the gender depends on the ratio of estrogens and androgens during sexual differentiation of the gonads. The temperature of incubation may change the activity of genes encoding aromatase, estrogen receptor, and reductase. It is not excluded that different taxonomic groups of animals with TSD have different mechanisms of regulation of sex. There may be temperature-sensitive genes *sox9* and *dax1* (fresh-water turtles—*Emydidae*) and genes *sox9*, *sf1*, and *wt1* (*Testudinidae*). In mammals, this mechanism is not valid, because the Y chromosome has genes that inhibit the aromatase enzyme.

For Mississippi alligator (*Alligator mississippiensis*), pond slider turtle (*Trachemys scripta*), and olive ridley (*Lepidochelys olivacea*, from the family of sea turtles), the expression level of the gene *dmrt1* was higher during the incubation of embryos at a temperature that contributed to the emergence of males. In reptiles and in particular

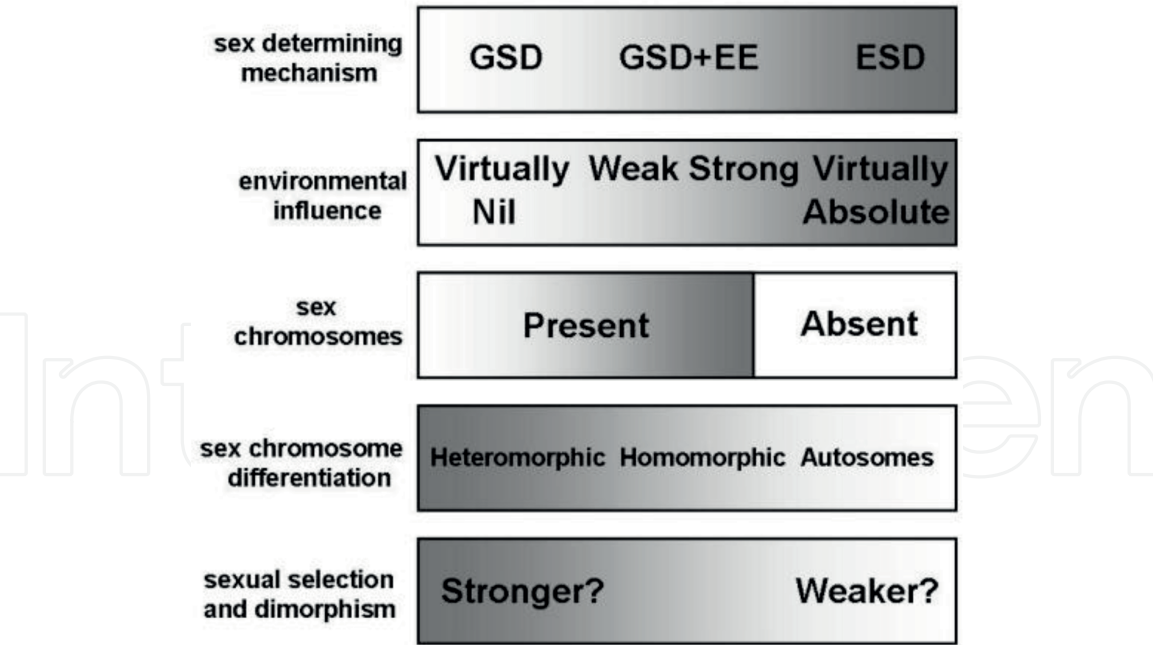


Figure 6.
The continuum of sex determination. Distribution mechanisms from GSD to ESD, including intermediate system to overcome genetic sex determination with environmental factors (GSD + EE) (adopted from Valenzuela et al. [26]).

Trachemys scripta elegans, a large amount of the KDM6B product is observed at a temperature favorable for males (MPT) and activates the expression of the *dmrt1* gene, and its reduction represses the expression of *dmrt1* and promotes the appearance of females. The latter is associated with H3K27 trimethylation. KDM6B is a member of the Jumonji gene family. It is believed that such genes are somehow regulated. One such regulator—*cirbp* (cold-inducible RNA binding protein)—has recently been described in the turtle *Chelydra serpentina* [27]. It managed to detect differences in the structure of *dmrt1*-gene in 34 species of reptiles with temperature and genetic mechanisms of sex determination, affecting sequence in exon 2 near DM-binding domain. In species with TSD, threonine occurs at position 54 (T54) and serine at position 57 (S57), while in species with a genetic sex determination mechanism, serine is observed in the S54-S57 position. This is obviously only the discovery of the molecular differences in sex determining gene when changing the mechanism of sex determination [28]. The discovery of the triploid male (ZZW) in the colubrid snake testifies to the absence of a particular role of the B chromosome in the determination of sex in this species [29].

Sex reversal has not yet been demonstrated in nature for any amniote, although it occurs in fish and rarely in amphibians. There is only one report about sex change in reptiles in the wild (Australian bearded dragon (*Pogona vitticeps*)) and the use of animals with inverse sex in order to experimentally induce a rapid transition from GSD to ESD. Controlled mating of normal males to sex-reversed females produces a viable and fertile offspring whose phenotypic sex is determined solely by temperature (temperature-dependent sex determination). The W sex chromosome is eliminated from this lineage in the first generation which indicates its specific role in genetic sex [30].

5. Sex determination in bird

In birds, estrogens play an important role in sex determination. They regulate expression of key sex determining genes during the first 3 days of embryonic

development and further. At the same time, the set of sex chromosomes is equally important. Embryos with two Z chromosomes in birds develop as males, and those with ZW chromosomes develop as females. At present, two hypotheses on sex determination in birds compete. One of these hypotheses considers the number of Z chromosomes as a key sex determining factor, while the other hypothesis supposes the presence in W chromosome of the key gene controlling ovarian development or suppressing the appearance of testes. The presence in Z chromosome of a strong candidate gene for sex determination (DMRT1 gene) supports the dose scheme. **Figure 7** presents a hypothetical scheme of genetic control of primary sex differentiation in *Gallus gallus*. The gonad appears on the 3.5th day (stage 22) as thickening on the surface of mesonephros. It consists of the epithelial layer of somatic and germ cells and medullary cordate layer (epithelial cords), which is mixed with mesenchymal cells. On the 6.5th day (30th stage), the first sex determining genes are activated. In the modern scheme of the genetic control of sex determination in birds (practically within the dose scheme), an epigenetic mechanism for switching off the single allele of avian key sex determining *dmrt1* gene in females through hypermethylation and using noncoding MHM RNA came into sharp focus (**Figure 8**) [30–34]. Synthetic aromatase inhibitors (an enzyme catalyzing the synthesis of estrogens) can induce steady female → male sex inversion. In this case, the left gonad becomes an ovotestis, or a testis, and the right gonad becomes a testis. Injection of aromatase inhibitors *in ovo* in most experiments was carried out on the third or fourth day of incubation. At the same time, in experimental males, injection of estradiol results in reversible feminization of the gonads [35, 36]. Unfortunately, the genetic and

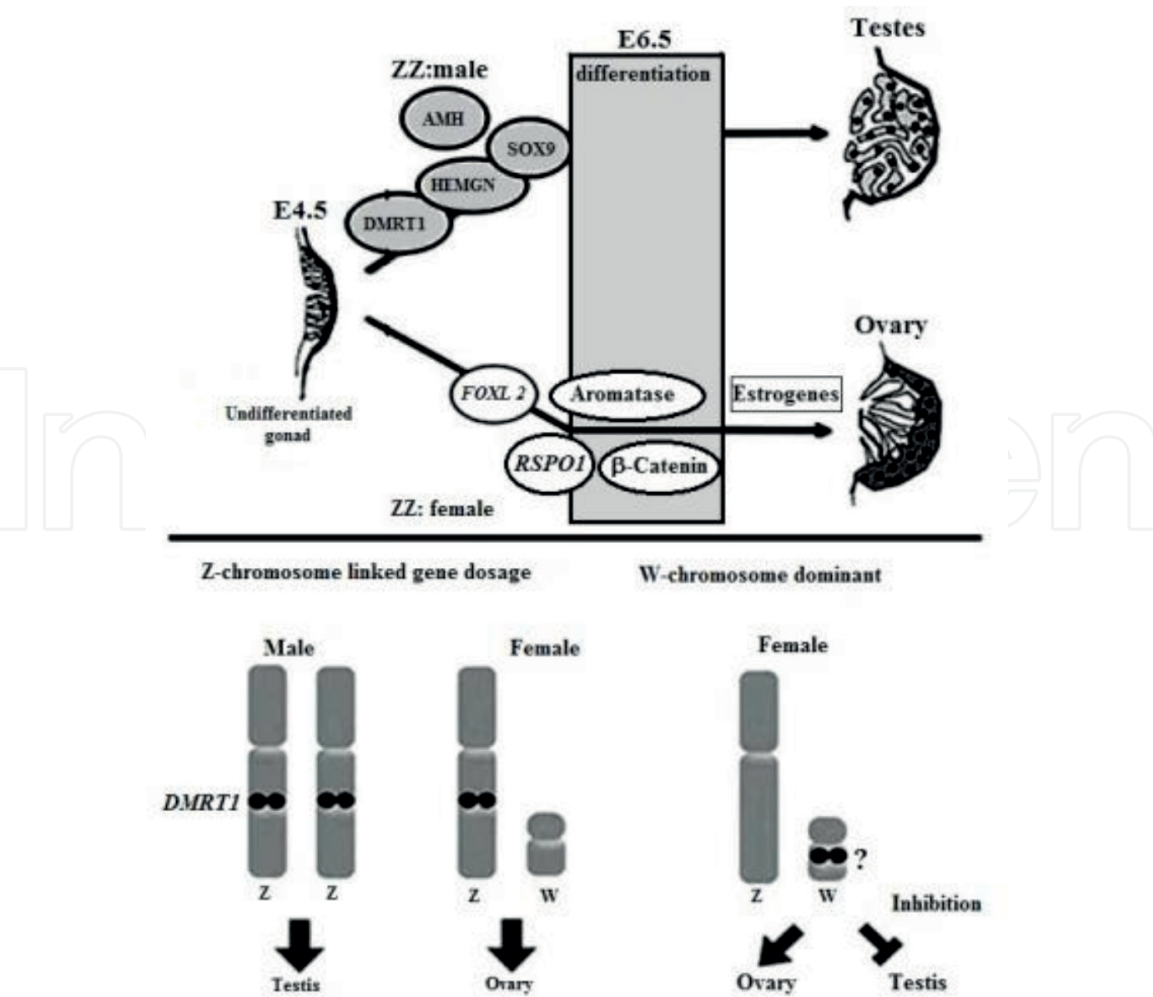


Figure 7.
Possible models of primary sex determination in birds by the example of *Gallus gallus* (adopted from Kuroiwa [33]).

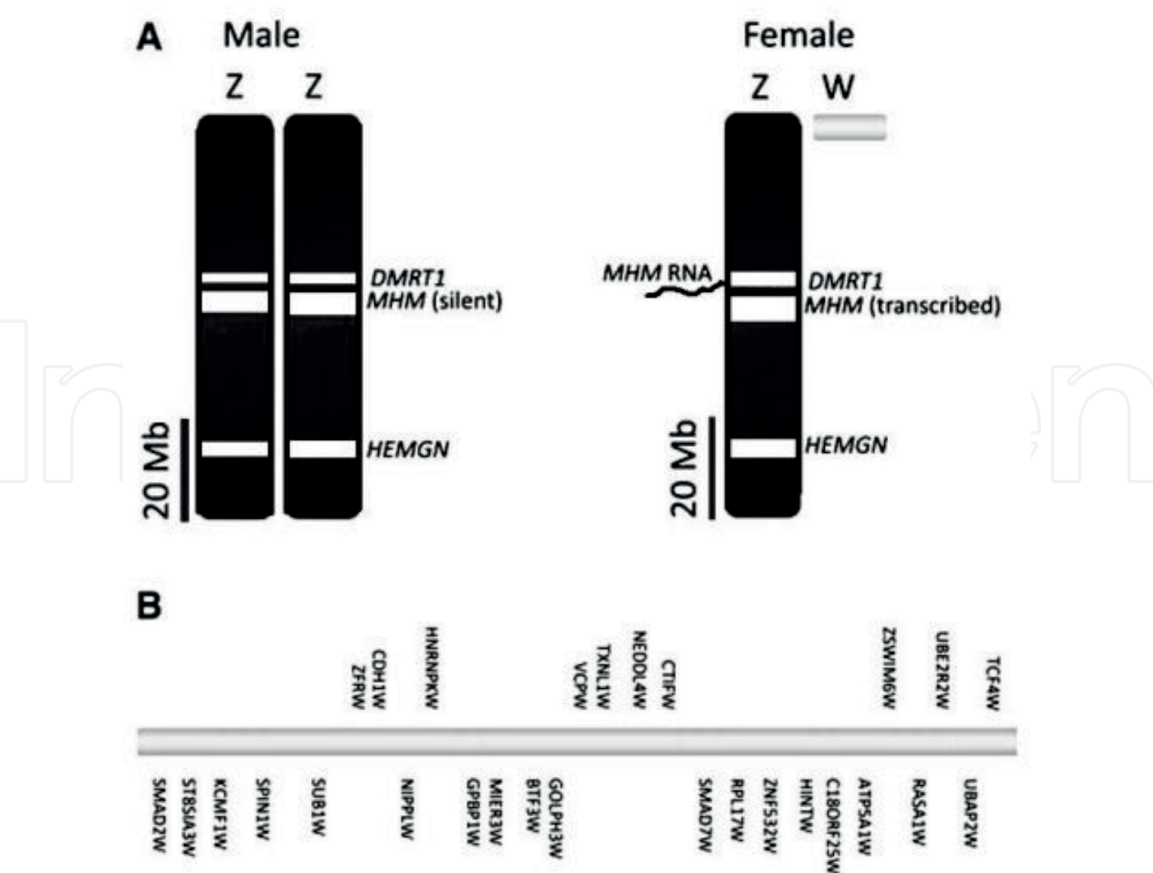


Figure 8. The large Z chromosome (82.3 Mb) is drawn to scale next to the degenerate W chromosome (7 Mb). (A) Male (ZZ) have two copies of DMRT1 and HEMGN, while the female (ZW) only has one. The MHM locus is transcribed from the single Z in the female and may play a role in local dosage and epigenetic regulation of DMRT1 in the female. (B) Location and orientation of the 28 protein coding genes that are located on the W chromosome (adopted from Hirst et al. [34]).

hormonal status of individuals with sex inversion was not investigated. The two enzymes required for the synthesis of estrogen, aromatase, and 17-beta-hydroxysteroid dehydrogenase (17 β -HSD) are synthesized only in ovarian medullary cords at the onset of morphological differentiation. It is suggested that the earliest expression of aromatase in birds is detected only on the fifth day of embryonic development. It is worth mentioning that the appearance of aromatase was recently demonstrated as early as in the maternal body, upon oogenesis in the theca layer of early follicles [37]. The data obtained make it possible to suggest earlier appearance of aromatase and estrogens in female gonadogenesis than that follows from the classical scheme of primary sex determination in *Gallus gallus* [38].

In birds, sex determination depends on sex hormones and sex-hormone-specific receptors. Estrogen receptors are also important in this process. In a recent study, the gonads and endocrine profile of a gynadromorphic chicken were described. It had male features on the right and female features on the left. At sexual maturity, the gonads of this bird were largely testicular. The right gonad was a testis, with SOX9⁺Sertoli cells, DMRT1⁺ germ cells, and active spermatogenesis. According to histology, the left gonad was primarily testicular, but with a few number of peripheral aromatase follicles. The gynandromorph had low levels of serum 17 β -estradiol (39 pmol/L). In contrast, the gynandromorph had very elevated levels of serum testosterone (41.3 nmol/L). Despite the elevated testosterone, the bird was female on one side of the body. The right male side was almost entirely ZZ (96%), whereas those from the left female side were a mixture of male (77% ZZ) and female (23% ZW) cells. It had a low percentage of ZW cells on the female side, but still

had female sex-linked feathering, smaller muscle mass, smaller leg and spur, and smaller wattle. This indicates that sexually dimorphic structures such as the wattle, spur, and feathering must be at least partly independent of sex steroid effects. Even a small percentage of ZW cells appear sufficient to support female-type sexual differentiation [39–41]. Studies of chimeric embryos also support the hypothesis that avian sexual differentiation is largely, or partly, cell autonomous, involving direct genetic factors and steroid hormones.

6. Conclusion

So, estrogens and androgens play important roles in sexual differentiation and reproduction, particularly in the development and expression of male and female sexual characteristics. These effects are principally mediated by the estrogen and androgen receptors (ESRs and ARs), which belong to superfamily of the nuclear receptors [42]. The nature of the relationship between sex hormones and gender determining genes and the patterns of their interaction remains unclear. For some amphibians, the absence of appropriate genes and the replacement by control factors of steroid hormones and receptors are postulated. For birds, we can assume a special role of heteromorphism of sex chromosome and the presence of a specific interaction of the W and Z chromosomes. In this regard, we should mention the phenomenon of detection of specific chromosomes (germ line restricted chromosomes, GRS) found only in the germ cells of songbirds.

In mammals, aromatase is expressed later in embryonic development and the gonadal sex is formed independently of sex hormones and differentiation can occur in the absence of steroidogenesis. For mammals, two-step primary sex determination is typical. At the first stage, its determination is carried out by the *sry* gene. At the second one, sex hormones are synthesized in gonads and genetic endocrine regulation of sex development is maintained. It raises questions about the sensitivity to androgens and estrogens of sex determination in fish, amphibians, reptiles, and birds. The functional role of the emerging chromosome heteromorphism is not clear. It is believed that the realization of the phenomenon of sex reversal is different in nonmammal vertebrates and mammals. It is intended to introduce a special term for nonmammal's sex change [43].

So, determination of gonadal development in vertebrates like testis or ovary was initially controlled mainly by sex hormones (fish and amphibians). Later, various sex determining genes were involved in this process. The system was quite plastic and was able to respond to changes in external conditions (reptiles). The appearance of heteromorphic sex chromosomes (birds) has led to the emergence of some specific W chromosomal signal, which provides estrogen control of the development of a heterogametic sex. In mammals, the control of the primary determination of sex (the appearance of the gonad) becomes purely genetic, and the role of sex hormones is reduced to the differentiation of testis or ovaries.

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