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| **Supplementary table 1. History that traces advancement in knowledge about TSD** | | |
| **The Pioneers** | | |
| **Reference** | **Turtle** | **Contribution** |
| [Charnier, 1966] | Rainbow Lizard*, Agama agama* | Pioneer in the field.  Discovered that biased sex ratios relate to incubation temperature |
| [Pieau, 1971, 1972**]** | Greek tortoise*, Testudo graeca*  European pond, *Emys orbicularis* | Pioneer in the field.  Correlated the effect of steroids on temperature sex determination. |
| [Yntema, 1976] | Snapping turtle*, Chelydra serpentina* | Pioneer in the field.  Documented influence of incubation temperature on hatchling sex ratio. |
| **Studies about gonadal differentiation in TSD species** | | |
| [Yntema and Mrosovsky, 1982] | Loggerhead sea turtle*, Caretta caretta* | Determined that at 32 °C, all embryos formed ovaries and at 28°C, all embryos formed testes. Defined 30°C as the Pivotal Temperature (PT). |
| [Mrosovsky et al., 1985] | Leatherback sea turtle, *Dermochelys coriacea* | Demonstrated that eggs incubated below 29 °C resulted in phenotypic males.  The cortex is not as well-differentiated at hatching. |
| [Merchant-Larios et al., 1989; Merchant-Larios and Villalpando, 1990] | Olive ridley sea turtle, *Lepidochelys olivacea* | Documented migration of primordial germ cells, as well as gonadal morphogenesis at male-promoting temperature (MPT) and female-promoting temperature (FPT), *in vivo* and *in vitro*. |
| [Wibbels et al., 1991b, a] | Red-eared slider turtle,  *Trachemys scripta* | Described chronology of gonadal differentiation and synergy between temperature and estrogen levels. |
| **Analyzing the role of estradiol and aromatase in ovarian differentiation** | | |
| [Pieau, 1970, 1974] | *Testudo graeca*  *Emys orbicularis* | Demonstrated that aromatase participates in ovarian differentiation and that exogenous estrogens promote the feminization of gonads, at MPT.  Discovered that estradiol induces various degrees of gonad, resulting in ovotestis or ovary.  Showed that this is dependent on dose and embryonic stage at the time of administration. |
| [Gutzke and Bull, 1986; Gutzke and Chymiy, 1988] | Painted turtle, *Chrysemys picta.*  *Chelydra serpentina* |
| [Bull et al., 1988] | *Softshell, Trionyx spiniferus|* |
| [Crews et al., 1989] | *Chelydra serpentina* |
| [Merchant-Larios et al., 1997] | *Lepidochelys olivacea* | Demonstrated that estradiol treatments at MPT alter gonadal development, forming tiny ovaries |
| [Wibbels et al., 1993] | *Trachemys scripta* | Showed that 17β-estradiol and FPT induce medullar cord regression, prior to ovarian cortical thickening. |
| [Desvages and Pieau, 1991] | *Emys orbicularis* | Demonstrated activity on the part of steroidogenic enzymes: 3βHSD, 5α-reductase, and aromatase. |
| [Dorizzi et al., 1991; Desvages and Pieau, 1992; Desvages et al., 1993] | *Emys orbicularis* and the sea turtle *Dermochelys coreacea* | Documented gonadal aromatase activity and that fadrozole and letrozole induce various grades of gonadal masculinization. |
| [Dorizzi et al., 1994; Richard-Mercier et al., 1995; Dorizzi et al., 1996] | *Emys orbicularis* | Showed that antiestrogens or aromatase inhibitors promote testicular differentiation at FPT, and that after temperature-sensitive period (TSP), letrozole still induces different degrees of masculinization at FPT. The ovary retains male potential after TSP. |
| [Crews and Bergeron, 1994; Wibbels and Crews, 1994] | *Trachemys scripta* | Showed that fadrozol (aromatase inhibitor) induces male sex determination at FPT. |
| [Rhen and Lang, 1994] | *Chelydra serpentina* | Showed that fadrozole increases the percentage of males, at pivotal temperature. |
| [Barske and Capel, 2010] | *Trachemys scripta* | Showed that estrogen suppresses Sox9 and that aromatase inhibitors maintain Sox9 expression in medullary cords and form ovotestes. |
| [Matsumoto et al., 2013b] | *Trachemys scripta* | Showed that estradiol reverts the expression pattern of male candidate genes at MPT. Treatment with testosterone + aromatase inhibitor at FPT reverts the female pattern of candidate sex-determining genes. |
| [Bieser and Wibbels, 2014] | *Trachemys scripta* | Documented chronology of expression profiles of *Dmrt1*, *Sox9*, *Amh*, *Lhx9* and *Foxl2* in embryonic gonad treated with letrozole and estrogen. Treatment with estradiol reduce gonad size. |
| [Díaz-Hernández et al., 2015]  [Díaz-Hernández et al., 2017] | *Lepidochelys olivacea*  *Lepidochelys olivacea* | Documented that estradiol reduces cell proliferation of gonadal cells and produces hypoplastic ovaries and also induces precocious expression of *Foxl2* at MPT.  Treatments with fadrozole and fulvestrant showed that cell proliferation and male differentiation of the medullary cords is dependent on estradiol, whereas the cortex was unresponsive. |
| **Analyzing the role of androgens, the develop gonad** | | |
| [Wibbels et al., 1992; Wibbels and Crews, 1995] | *Trachemys scripta* | Showed that testosterone induces variable gonadal feminization, whereas non aromatizable DHT has no effect. |
| [Rhen and Lang, 1994; Rhen and Schroeder, 2010] | *Chelydra serpentina* | Showed that DHT has a feminizing effect at pivotal and feminizing temperatures, whereas the effect of flutamide is ambiguous, as both masculinizing and feminizing effects depend on temperature. |
| [Rhen et al., 2007] | *Chelydra serpentina* | Demonstrated that *androgen receptor* (Ar) expression increases with temperature change from MPT to FPT, during TSP. |
| [Schroeder and Rhen, 2019] | *Chelydra serpentina* | Demonstrated that DHT and flutamide induce ovary development  and that androgens and Ar participate in ovary determination |
| **The sex determination gene candidates** | | |
| [Jeyasuria et al., 1994] | Diamondblack terrapin, *Malaclemys terrapian* | Reported a partial cDNA sequence of *aromatase* |
| [Jeyasuria and Place, 1997] | *Malaclemys* terrapian | Showed that *aromatase* upregulation in the brain precedes gonadal sex differentiation. |
| [Bergeron et al., 1998] | *Trachemys scripta* | Demonstrated that *estrogen receptor* (*Er*) is expressed at FPT and MPT prior to gonadal differentiation. However, *Ar* is higher at FPT than MPT, at the beginning of TSP. |
| [Spotila et al., 1998] | *Trachemys scripta* | Identified partial sequence of *Wt1* and *Sox9*. *Sox9* is expressed in undifferentiated gonads and remains expressed in the testis. |
| [Moreno-Mendoza et al., 1999] | *Lepidochelys olivacea* | Revealed expression of Sox9 protein by immunofluorescence. Sox9 protein was detected in the nuclei of medullary cords of bipotential and male gonads. Sox9 was not detected in ovarian committed gonads. |
| [Kettlewell et al., 2000] | *Trachemys scripta* | Used RT-PCR to demonstrate expression of *Dmrt1* and *in situ* hybridization. Showed that *Dmrt1* is higher at MPT than FPT, during TSP. |
| [Torres-Maldonado et al., 2001] | *Lepidochelys olivacea* | Showed that *Sox9* is downregulated in gonads that switch from MPT to FPT. |
| [Moreno-Mendoza et al., 2001] | *Lepidochelys olivacea* | Demonstrated regulation of Sox9 protein in cultured gonads that change temperature during TSP. |
| [Torres Maldonado et al., 2002] | *Lepidochelys olivacea* | Showed that in early bipotential gonads, *Dmrt1* expression is higher than *Sox9* at MPT, than it is at FPT. |
| [Loffler et al., 2003] | *Trachemys scripta* | Showed that during TSP, expression of *Foxl2* is higher at FPT than it is at MPT. |
| [Rhen et al., 2007] | *Chelydra serpentina* | Identified expression of candidate sex-determining genes: *Dmrt1*, *Sox9*, *Ar*, *Foxl2*, *Dax1*, *Fgf9*, *aromatase* estrogen receptor (*Er*) and *Sf1.* |
| [Shoemaker et al., 2007a] | *Trachemys scripta* | Defined expression levels of *Dmrt1, MIS*, and *Sox*9, using qPCR and *in situ* hybridization. Demonstrated that *Dmrt1* expression is dimorphic before and after sex determination and that *Sox9* levels are similar at both temperatures in bipotential gonads. Also that expression of Dmrt1 is upregulated at MPT. |
| [Shoemaker et al., 2007b] | *Trachemys scripta* | Demonstrated that expression pattern of *Foxl2* is ovarian specific. Wnt4 is related to the formation of the ovary and testis. *Sox9* showed a pattern associated with testis formation. Amh was higher at MPT than at FPT, at all stages analyzed. |
| [Ramsey et al., 2007] | *Trachemys scripta* | Used q RT-PCR and *in situ* hybridization to identify *Sf1* and *aromatase* expression*.* They also showed that *Sf1* and *aromatase* were differentially expressed during sex determination. |
| [Smith et al., 2008] | *Trachemys scripta* | Showed that *Rspo1* is upregulated at FPT but remains low at MPT. At FPT it remains high throughout ovary differentiation. *Rspo1* expression is sensitive to temperature variation. |
| [Merchant-Larios et al., 2010] | *Lepidochelys olivacea* | Revealed expression of *Sox9* and *Amh* during TSP by *in situ* hybridization and immunofluorescence. |
| [Díaz-Hernández et al., 2012] | *Lepidochelys olivacea* | Demonstrated that the mesothelial cell domain of Sox9-expressing cells precedes the formation of the genital ridges. |
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| [Valenzuela et al., 2013] | *Chrysemys picta* | This review defined expression patterns of *Wt1*, *Sf1*, *Sox9*, *aromatase,* and *Dax1*  during gonad development using trunks, adrenal-kidney-gonad complex, or gonad of painted turtles incubated at constant FPT and MPT. |
| [Sifuentes-Romero et al., 2013] | *Lepidochelys olivacea* | Using RNAi to silence *Sox9* in cultured gonads, they deduced that Sox9 regulates *Amh* expression. |
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| [Schroeder et al., 2016] | *Chelydra serpentina* | Discovered that SNP in Cirbp protein loci is associated with gonadal sex determination and differentiation. |
| [Tang et al., 2017] | Chinese pond turtle (Mauremys reevesii) | Defined complete coding sequence and expression patterns of *Cyp19a1*, *Rspo1*, *Foxl2*, *Sf1* *Dmrt1*, and *Sox9*. Likewise showed that at C*yp19a1*, *Rspo 1* and *Foxl2* are expressed preferentially at FPT, during TSP.  Showed that *Dmrt1* is higher at MPT than FPT at the beginning of TSP and that *Sox9* manifests dimorphic expression until the end of TSP |
| [Guo and Rhen, 2017] | *Chelydra serpentina* | The authors cloned the proximal promoter and coding sequence of *Foxl2*, in frame with mCherry to produce a fluorescent reporter, in order to determine whether androgens regulate transcription of *Foxl2*.  DHT did not modify the expression of tFoxl2-mCherry. |
| [Ge et al., 2017] | *Trachemys scripta* | Authors identified loss- and gain-of-function and epigenetic studies to *Dmrt1*. They showed that *Dmrt1* manifests a dimorphic pattern, prior to sex differentiation. |
| [Diaz-Hernandez et al., 2019] | *Lepidochelys olivacea* | Used qTR-PCR and immunofluorescence to analyze the Spatiotemporal pattern of expression of Dmrt1 and Foxl2.  *Dmrt1* mRNA and protein showed a dimorphic pattern in early (stage 24), as opposed to *Foxl2* mRNA in late (stage 25) bipotential gonads. Sox9 showed dimorphic expression remaining in testes from stage 25 onwards. |
| [Mizoguchi and Valenzuela, 2020] | *Chrysemys picta* | Reported the first non-canonical Dmrt1 isoform, lacks exons 2 and 3. Dmrt1 canonical (contains all five exons). Isoforms showed dimorphic expression (male-biased) after the onset of sex determination |
| **Epigenetic mechanisms controlling TSD** | | |
| [Matsumoto et al., 2013a] | *Trachemys scripta* | Authors studied the methylation pattern of the *aromatase* gene.  The DNA methylation level of *Cyp19a1* promoter was higher at MPT than FPT and this pattern changed in response to temperature variation. They explained that aromatase is expressed strictly in relation to FPT. |
| [Matsumoto et al., 2016] | Red-eared slider turtle  *Trachemys scripta* | Authors described the changes in DNA methylation of *aromatase* that occur with temperature change and H3K4me3 histone changes. |
| [Venegas et al., 2016] | *Lepidochelys olivacea* | Authors described global DNA Methylation patterns in gonads at MPT and FPT. |
| [Ge et al., 2017] | Red-eared slider turtle  *Trachemys scripta* | Authors demonstrated that the DNA methylation level of *Dmrt1* manifested dimorphism from stage 25, as well as response to temperature changes. |
| [Radhakrishnan et al., 2017; Radhakrishnan et al., 2018] | *Chysemys picta*  *Chysemys picta* and *Apalone spinifera* (with genotypic sex determination) | Authors reported differences in genome-wide DNA methylation in the gonads, at FPT and MPT of 3-month-old hatchling.  Authors analyzed the embryonic gonadal expression of:  genome-wide epigenetic, genes, ncRNA, methylation, ubiquitination, phosphorylation, and small RNA |
| [Ge et al., 2018] | *Trachemys scripta* | Authors showed that the histone H3 Lysine 27 (H3K27) demethylase KDM6B was differentially expressed at FPT and MPT.  They also performed tests for gain and loss of function:  knockdown assay of Kdm6b at MPT induces female descendants. While overexpression of *Dmrt1* rescues the reversal phenotype.  *Kdm6b* responds to estrogens and aromatase inhibitor.  Kdm6b promotes the expression of *Dmrt1* at MPT to demethylate H3K27 in the promoter. |
| [Weber et al., 2020] | *Trachemys scripta* | Authors showed that higher calcium levels at 31°C (FPT) promote Stat3 phosphorylation, which represses *Kdm6b* transcription, a required activator for *Dmrt1* expression. |
| **TRANCRIPTOMES** |  |  |
| [Czerwinski et al., 2016] | *Trachemys scripta* | Authors undertook a transcriptome-wide analysis at MPT and FPT.  The steroidogenic enzymes and brain showed sexually dimorphic expression, prior to gonad formation. |
| [Radhakrishnan et al., 2017] | *Chysemys picta* and *Apalone spinifera*  (with genotypic sex determination) | Authors reported temperature-specific gonadal transcriptomes, corroborated by PCR. |

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