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SEX REVERSAL IN ANIMAL KINGDOM : SPECIAL EMPHASIS ON ROLE OF STEROID HORMONES

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ABSTRACT

Sexual fate is no longer seen as an irreversible deterministic switch set during early embryonic development but as an ongoing battle for primacy between male and female developmental trajectories. That sexual fate is not final and must be actively maintained via continuous suppression of the opposing sexual network creates the potential for flexibility into adulthood. In many Animals, sexuality is not only extremely plastic, but sex change is a usual and adaptive part of the life cycle. Sequential hermaphrodites begin life as one sex, changing sometime later to the other, and include species capable of protandrous (male- to-female), protogynous (female-to-male), or serial (bidirectional) sex change. Natural sex change involves coordinated transformations across multiple biological systems, including behavioural, anatomical, neuroendocrine, and molecular axes. This can be caused by human pollutants, including herbicides, which can act as estrogen promoters or inhibitors, which would respectively increase or decrease the number of female offspring, through controlling aromatase. This review project paper highlights the biological processes underlying this amazing transformation, Gonadal restructuring among germinal and somatic cell interaction, Neuroendocrine control of sex change and Stress response and role of Steroid hormones-balance between 17beta-estradiol & 11- ketotestosterone in sex changes, Endocrine disruption on Changing of Sexual Behaviour, which remains poorly understood, but where new genomic technologies are significantly advancing our understanding of how sex change is initiated and progressed at Hormonal and molecular levels. Knowledge of how a usually committed developmental process remains plastic in sequentially hermaphroditic fishes, Amphibians and Birds are relevant to understanding the evolution and functioning of sexual developmental systems in vertebrates generally, as well as pathologies of sexual development in mammals, some time observed in nematodes and Gastropodes also.

Keywords- sex change, hormones, hermaphrodism

WHAT IS SEX & SEX REVERSAL

In the early stage, the Gonad's have undifferentiated tissue which can differentiate into both testis and ovary. Genetic sex is the balance between Androgens and Estrogens production which directs sexual differentiation and Gonodal Sex Development by the formation of testis and ovary. Streiods Hormones are responsible for natural differentiation of testis and ovary in the two sexes. 17beta-estradiol and 11ketotestosterone are principle oestrogen and androgen that promote ovarian or testicular function respectively. With dysfunction of these steroid hormones sex reversal occur naturally.

Sex reversal is the phenomenon whereby organisms developing at sex-specific conditions such as temperatures or karyotypes hatch the opposite sex. This can be caused by human pollutants, including herbicides, which can act as estrogen promoters or inhibitors, which would respectively increase or decrease the number of female offspring, through controlling aromatase. This also can be done artificially with the treatment by male hormones, androgens (include testosterone an androgenic C 19 steroid) and female hormone, estrogens. Estrogens are C 18 steroids.

Sex-reversal is the change of primary sex (gonadal sex) and secondary sex characteristics and to another sex during adulthood, occurs in many fish species and is triggered by social or environmental conditions. This is an extreme example of phenotypic

plasticity – the ability of animal to change its form due to a cue from the environment. Its' requires considerable changes to both the reproductive system (testis or ovary) and changes to secondary sex characteristics (such as pigment, body shape).

In Fish Biology sex reversal has many advantages in fish farming but the great disadvantages is that such hormones treated fish cannot be used for human consumption and due to government regulation on the use of chemical on food fish. The advantage is that in male fishes typically grow faster than female.

Experimental Discovery

►Yamamoto (1953-1955) showed the importance of Sex Reversal for studying Sex Ditermination mechanism in Fishes - Lebestes and Oryzias.

He used androsterone for transfer female (XX) into functional male (XX). He also reported that the sex of male can be reversed as functional female phenotypic with XY genotype by treatment with female hormones.

►Shelton (1982) made experiment in 1977, 1978, 1979, according to his experiments he obtained mature sex reversal male (XX – male) and the successfully grown 8000 to 10,000 presumed monosex fish.

DIFFERENT EVENTS OF SEX - REVERSAL

Sequential hermaphroditism

Sequential hermaphroditism (called dichogamy in botany) is a type of hermaphroditism that occurs in many fish, gastropods, and plants. It occurs when the individual changes sex at some point in its life. In particular, a sequential hermaphrodite produces eggs (female gametes) and sperm (male gametes) at different stages in life. Species that can undergo these changes from one sex to another do so as a normal event within their reproductive cycle that is usually cued by either social structure or the achievement of a certain age or size.

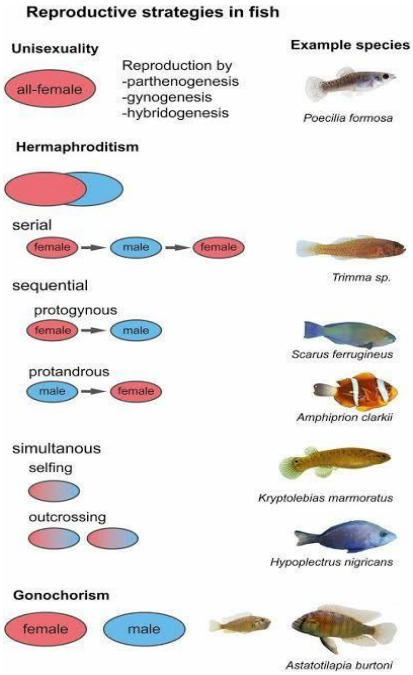


Fig:- Sequential Hermaphrodism

i) **Protandry:-** In general, protandrous hermaphrodites are animals that develop as males, but can later reproduce as females. However, protandry features a spectrum of different forms, which are characterized by the overlap between male and female reproductive function throughout an organism's lifetime:

1. Protandrous sequential hermaphroditism: Early reproduction as a pure male and later reproduction as a pure female.

2. Protandrous hermaphroditism with overlap: Early reproduction as a pure male and later reproduction as a pure female with an intervening overlap between both male and female reproduction.

3. Protandrous simultaneous hermaphroditism: Early pure male reproduction and later reproduction in both sexes.

A strongly female-biased reproductive size advantage should favour the evolution of protandrous sex change.

Example :-

>> Protandrous fishes include clownfish. Clownfish have a very structured society. In the Amphiprion percula species, there are zero to four individuals excluded from breeding and a breeding pair living in a sea anemone.

Fig:- Gonadal Sex changes of Clown Fish

ii) **Protogyny** :-

Protogynous hermaphrodites are animals that are born female and at some point in their lifespan change sex to male. Protogyny is a more common form of sequential hermaphroditism, especially when compared to protandry.As the animal ages, it shifts sex to become a male animal based on internal or external triggers. Unlike females, male fecundity increases greatly with age, and it is hypothesized that it is more selectively advantageous to be a male when an organism's body is larger and more experienced.This advantage may cause certain species to be protogynous hermaphrodites as the sex change to male allows for an increased chance of survival.

The Reproductive success by male size advantage and breeding with multiple Female should drive the evolution of protogynous sex change.

Example :- Protogyny is ubiquitous in highly social wrasses (Labridae), of which the bluehead wrasse (Thalassoma bifasciatum) is an especially well-studied model. Bluehead wrasse are small, polygamous reef fish with a lek-like mating system, whereby dominant males defend spawning sites to which females have high fidelity [Warner and Schultz, 1996]. Loss of a dominant (terminal phase, TP) male stimulates sex change in (typically) the largest female of a social group and involves dramatic changes in behaviour, anatomy, and colouration [Warner and Swearer, 1991]. Although most juvenile bluehead wrasse develop as females, some develop directly as small, female-mimic (initial phase, IP) males, which employ a ‘sneaker' mating tactic [Semsar and Godwin,1993]. IP male development is also under social control: more IP males develop on high-density reefs where TP males can less-effectively monopolise mating opportunities. In these and other diandric fish, it remains unknown whether the same or different molecular pathways are involved in sex determination and differentiation of IP and TP males.

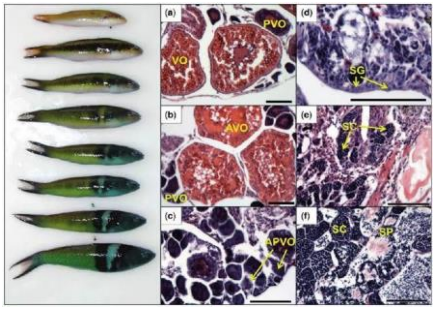


Fig:- Morphological and gonadal sex change in the bluehead wrasse. Left: morphological changes from female (top) to TP male (bottom) (images by J. Godwin). Right: six gonadal sex-change stages from ovary to functional testis (images by M. Lamm and J. Godwin).

(a) Stage 1: mature ovary with healthy vitellogenic oocytes (VO) and pre-vitellogenic oocytes (PVO); (b) Stage 2: atretic vitellogenic oocytes (AVO) with degraded zona pellucida ; (c) Stage 3: atretic previtellogenic oocytes (APVO); (d) Stage 4: proliferation of presumed spermatogonia (SG) and Leydig cells; (e) Stage 5: onset of spermatogenesis, indicated by spermatocytes (SC) in spermatocysts; (f) Stage 6: presence of mature, tailed sperm (SP). Based on classification by Nakamura et al. (1989). Scale bar: 50 m.

Ultimate causes

The ultimate cause of a biological event determines how the event makes organisms better adapted to their environment, and thus why evolution by natural selection has produced that event. While a large number of ultimate causes of hermaphroditism have been proposed, the two causes most relevant to sequential hermaphroditism are the size-advantage model and protection against inbreeding.

Size-advantage model

The size-advantage model states that individuals of a given sex reproduce more effectively if they are a certain size or age. To create selection for sequential hermaphroditism, small individuals must have higher reproductive fitness as one sex and larger individuals must have higher reproductive fitness as the opposite sex. For example, eggs are larger than sperm, thus larger individuals are able to make more eggs, so individuals could maximize their reproductive potential by beginning life as male and then turning female upon achieving a certain size.

Protection against inbreeding

Sequential hermaphroditism can also protect against inbreeding in populations of organisms that have low enough motility and/or are sparsely distributed enough that there is a considerable risk of siblings encountering each other after reaching sexual maturity, and interbreeding. If siblings are all the same or similar ages, and if they all begin life as one sex and then transition to the other sex at about the same age, then siblings are highly likely to be the same sex at any given time. This should dramatically reduce the likelihood of inbreeding. Both protandry and protogyny are known to help prevent inbreeding in plants,and many examples of sequential hermaphroditism attributable to inbreeding prevention have been identified in a wide variety of animals.

Proximate causes

The proximate cause of a biological event concerns the molecular and physiological mechanisms that produce the event. Many studies have focused on the proximate causes of sequential hermaphroditism, which may be caused by various hormonal and enzyme changes in organisms. The role of aromatase has been widely studied in this area. Aromatase is an enzyme that controls the androgen/estrogen ratio in animals by catalyzing the conversion of testosterone into oestradiol, which is irreversible. It has been discovered that the aromatase pathway mediates sex change in both directions in organisms. Many studies also involve understanding the effect of aromatase inhibitors on sex change.

iii) **Bidirectional Sex Change**

Bidirectional hermaphrodites have the capacity for sex change in either direction, potentially repeatedly during their lifetime. Most reports are for species formerly thought to be protogynous. Sex-changed males may revert back to female should they find themselves competing with a larger male (e.g., Okinawa pygmy goby). Natural bidirectional sex change has not been reported for any otherwise protandrous species.

GONADAL CHANGES : CO-RELATING WITH SEX-REVERSAL

Detailed histological descriptions of gonadal sex change have been made for numerous species representative of protandrous [Godwin, 1994; reviewed by Lee et al., 2001], protogynous [Nakamura et al., 1989; Lo Nostro et al., 2003; Muncaster et al., 2013; reviewed by Liu et al., 2016], and bidirectional [Sunobe et al., 2005; reviewed by Cole, 2010, 2011; Kuwamura et al., 2015] hermaphrodites. In many sequential hermaphrodites, recognisable tissues of both sexes are present in the gonad prior to sex change, whereas in others, reproductive tissues are completely replaced by those of the secondary sex.

Gonadal restructuring is complete in protogynous wrasses, where no testicular tissues are detectable in the ovary prior to sex change, and secondary male testes have only a remnant ovarian lumen and lamellae structure as evidence of their former function [Warner and Robertson, 1975]. Ovarian follicle atresia and oocyte degeneration heralds the onset of sex change, followed by proliferation of spermatogonia and Leydig cells in the peripheral ovarian lamellae.

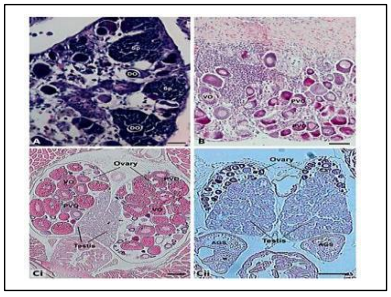


Fig:- Gonadal sex changes

A. A transitional bluehead wrasse (Thalassoma bifasciatum) gonad in the later stages of Protogynous sex change

B. The ventral portion of a transitional barramudi (Lates calcarifer) gonad in the later stages of protandrous sex change

C. The paired ovotestis of female-phase(i) and male-phase (ii) individual of the Bidirectional sex-changing goby, Trimma kudoi.

CELLULAR ORIGINS DURING COMPLETE GONADAL RESTRUCTURING

In protogynous hermaphrodites, early male tissues are usually first observed at the periphery of the ovarian lamellae in the vicinity of the germinal epithelium [Lo Nostro et al., 2003]. However, it is difficult to establish whether proliferating spermatogonia arise from a dormant.

Current evidence suggests that in protogynous species, testicular construction begins with bipotential germ (gonia) and somatic (epithelial) cells residing within the ovarian germinal epithelium [Lo Nostro et al., 2003]. At the initiation of sex change in freshwater swamp eel (Synbranchus marmoratus), gonial cells that formerly produced oocytes become spermatogonia, enter meiosis, and produce sperm [Lo Nostro et al., 2003]. Epithelial cells associated with these early spermatogonia become Sertoli cells, which previously differentiated as granulosa cells in the female ovary [Lo Nostro et al., 2003].

Knowledge of how germinal and somatic cells interact in transitioning gonads to potentially influence sexual fate in a reciprocal manner will be key to understanding precisely how a gonad of one sexual phenotype can be re- engineered into that of the opposite sex.

ENDOCRINE REGULATION OF SEX CHANGE

Steroid Balance Controls Sexual Fate

The balance between gonadal oestrogen and androgen production directs sexual differentiation and gonadal development. In teleosts, 17β-estradiol (E2) and 11-ketotestosterone (11-KT) are the principal oestrogen and androgen that promote ovarian or testicular function, respectively. The relationship between them is especially close, as production of either E2 or 11-KT depends on the bioconversion of testosterone (T) via the aromatase (cyp19a1a) or the 11β- hydroxylase (cyp11b)/11β-hydroxysteroid dehydrogenase (11β-HSD, coded by hsd11b2) pathways, respectively [see Guiguen et al., 2010]. Therefore, relative expression of these opposing pathways in the gonad determines the sex steroid balance and ultimately controls gonadal fate.

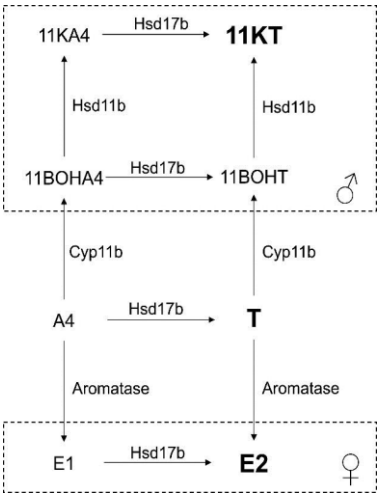


Fig:- Steroid Balance Controls Sexual Fate

A precipitous drop in plasma E2 precedes ovarian degeneration and protogynous sex change and is followed by a gradual increase in 11-KT production at the onset of spermatogenesis [Nakamura et al., 1989; Bhandari et al., 2003, 2006]. The opposite trend characterises protandrous sex change, whereby plasma E2 increases after 11-KT levels fall [Godwin and Thomas, 1993; Lee et al., 2001]. In bidirectional gobies, E2 follows this sexually dimorphic pattern, but 11-KT does not [Kroon et al., 2003, 2009]. The role of 11-KT in gobies is unclear as there is no apparent association between concentrations of this steroid and gonadal development [e.g., Kroon et al., 2009]. Low 11-KT concentrations in gobies have been proposed to reflect the lack of secondary male characteristics in these species and to facilitate rapid switching between sexual phenotypes. Exogenous manipulation of sex steroids causes masculinisation or feminisation in fish [e.g., Chang et al., 1995; reviewed by Devlin and Nagahama, 2002; Higa et al., 2003; Yeh et al., 2003; Budd et al., 2015]. Application of non-aromatisable androgens downregulates the aromatase pathway in female fish, leading to sex change. Aromatase inhibitors (AI) disrupt ovarian E2 production in protogynous, protandrous [Lee et al., 2001; Nakamura et al., 2015], and bidirectional hermaphrodites [Kroon et al., 2005]. Although AI treatment leads to complete sex change in protogynous species, rescue is possible through the co-administration of E2. Sex steroids clearly regulate gonadal fate, a molecular switch is required to sustain the shift in hormone production and maintain sex change.

NEUROENDOCRINE CONTROL OF SEX CHANGE

The oestrogen-androgen balance is ultimately regulated through the hypothalamic-pituitary-gonadal (HPG) axis and its interaction with neighbouring axes (fig. 4). Gonadotropin releasing hormone (GnRH), released in pulses from the hypothalamus, stimulates the pituitary to produce and release the gonadotropins (GtHs) follicle stimulating hormone (FSH) and luteinising hormone (LH) into circulation. GtHs directly regulate gonadal steroidogenesis via receptor-mediated stimulation of ovarian follicle cells or somatic Leydig cells in the testis [reviewed by Devlin and Nagahama, 2002].

Manipulating GnRH or GtH signalling can induce partial or complete sex change in protogynous (e.g., honeycomb grouper, rainbow wrasse, Reinboth and Bnrusle-Sicard [1997]) and protandrous hermaphrodites [Lee et al., 2001]. Expression of GtH subunits and their receptors (LHR, FSHR) also fluctuates across sex change in protogynous [e.g., Kobayashi et al., 2010a], protandrous [e.g., An et al., 2009, 2010], and bidirectional species. However, the precise roles of GnRH and GtH signalling in controlling sex change is difficult to model as patterns are inconsistent even between closely related species, e.g., contradictory patterns of expression for GtH receptors are observed in protogynous grouper [Alam et al., 2010; Hu et al., 2011] and may reflect species-specific gonadotropin functioning in teleosts.

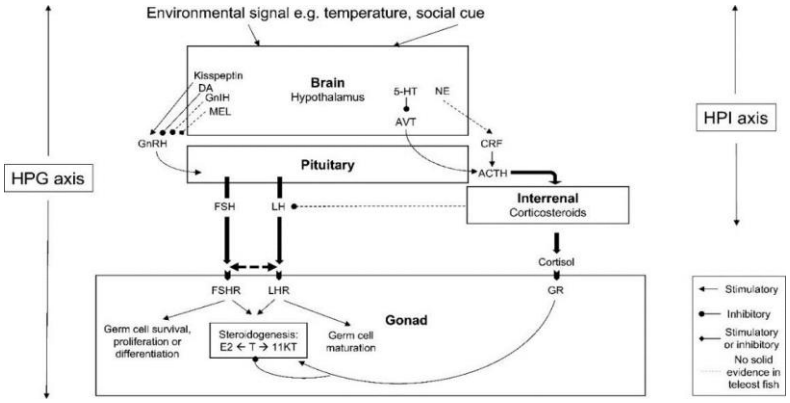


Fig:- Neuroendocrine Control of Sex

SEX CHANGE AND THE STRESS RESPONSE

The stress response, regulated through the hypothalamic-pituitary-interrenal (HPI) axis, modulates processes central to major life-history transitions, including changes in behaviour, metabolism, and growth [Solomon-Lane et al., 2013]. Through the actions of corticotropic releasing hormone (CRH) and glucocorticoid steroids (GCs), the HPI axis responds to environmental stressors, with potentially significant effects on gonadal fate. That these factors apparently also mediate agonistic behaviour and social status information implicates the HPI axis in linking social status to sex change in sequential hermaphrodites [Solomon-Lane et al., 2013].

Elevated temperatures and other environmental stressors cause gonadal masculinisation in various gonochoristic fishes via increased cortisol levels causing downregulation of aromatase and activation of androgen pathways [reviewed by Fernandino et al., 2013]. Cortisol is thought to stimulate 11β-HSD expression, which catalyses the production of both 11-KT and cortisone, the deactivated metabolite of cortisol [Fernandino et al., 2012, 2013]. There is accumulating evidence that, by influencing steroidogenic gene expression and communicating environmental and social status information along the HPI axis, cortisol plays a role in regulating natural sex change in sequential hermaphrodites [Solomon-Lane et al., 2013]. A transient spike in serum cortisol has been recorded during protandrous sex change in cinnamon clownfish and during protogynous sex change in bluebanded goby [Solomon-Lane et al., 2013], experiencing a ‘permissive' social environment. Furthermore, long-term cortisol administration was recently shown to promote protogynous sex change in three-spot wrasse [Nozu and Nakamura, 2015]. Therefore, the cortisol pathway may interface between the HPI and HPG axes to promote sex change in sequential hermaphrodites.

MOLECULAR REGULATION OF SEX CHANGE: SWITCHES, TRIGGERS, AND ANTAGONISTIC NETWORKS

Antagonistic sex-specific gene networks maintain sexual fate in fishes by promoting either an oestrogenic or androgenic environment. In females (left), cyp19a1a expression generates aromatase, which converts testosterone (T) to estradiol (E2) and maintains the autoregulatory feed-forward loop that sustains high oestrogen levels to support ovarian function [Guiguen et al., 2010]. Within this loop, the transcription factors Foxl2 and Sf1 interact to upregulate cyp19a1a expression [Wang et al., 2009], which is also controlled by gonadotropins like FSH through the synthesis of cAMP [Guiguen et al., 2010]. An oestrogenic environment reinforces female-specific gene expression while suppressing male-promoting genes. In males (right), cyp19a1a expression and aromatase production is suppressed such that androgenesis prevails and supports testicular function and male-specific gene expression. Androgens have been reported to both inhibit and activate amh (+/−) expression [Pfennig et al., 2015]. Dmrt1 suppresses cyp19a1a promoter activity directly as well as indirectly via its antagonistic relationship with Foxl2 (see text). Dax1 may also negatively regulate cyp19a1a expression through its suppression of sf1 and foxl2 expression [Wang et al., 2009]

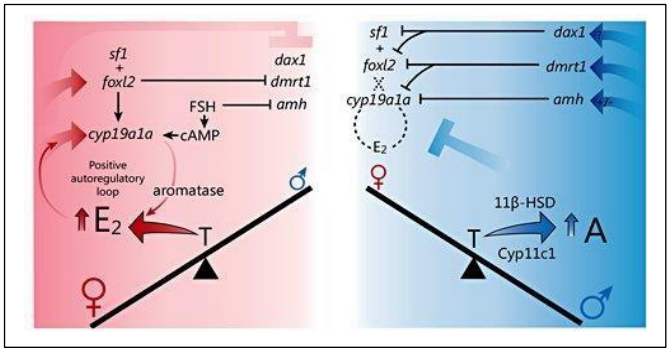


Fig:- Molecular Regulation of Sex Change

In fishes, 2 widely conserved components of the molecular machinery essential for vertebrate sexual development are the genes dmrt1 (doublesex and mab-3 related transcription factor 1) and cyp19a1a/b (coding for gonadal/brain aromatase that catalyses conversion of androgens to oestrogens). Each is indispensable within male- and female-promoting networks, respectively. In females, cyp19a1a expression maintains an auto-regulatory loop that sustains the high oestrogen environment necessary for ovarian function [Guiguen et al., 2010]. In males, Dmrt1 is a critical transcriptional regulator activating male- promoting genes (e.g., sox9, sox8) while suppressing ovarian pathways (foxl2 and rspo1/wnt/β-catenin signalling) [Herpin and Schartl 2011]. Dmrt1 interacts antagonistically with the female-specific transcription factor Foxl2 to influence cyp19a1a expression and control oestrogen production and gonadal fate in teleosts. Under- or overexpression of either foxl2 or dmrt1 induces sexual cell fate reprogramming and gonadal sex reversal in mice and fish [Uhlenhaut et al., 2009]. In gonochoristic and sex-changing fishes, cyp19a1a, foxl2, and dmrt1 expression is consistently sex-specific, depending on which gonadal phenotype is developing [Xia et al., 2007; Alam et al., 2008]. Expression of these genes also responds predictably to hormonal manipulations: dmrt1 is upregulated and cyp19a1a and foxl2 are downregulated in fish given androgens, aromatase blockers, or oestrogen antagonists, whereas oestrogen treatments have the opposite effect [reviewed in Guiguen et al., 2010; Herpin and Schartl, 2011]. Therefore, a molecular and endocrine feedback loop becomes apparent through which dmrt1 and cyp19a1a regulate the androgen/oestrogen balance to control sexual fate in fish. Evidence that cyp19a1a, dmrt1, and amh (anti-Müllerian hormone) expression respond to external environmental fluctuations, most notably in fishes where rearing temperature influences sex ratios [Fernandino et al., 2008; Guiguen et al., 2010; Pfennig et al., 2015], raises the possibility that these and other key sex genes may be similarly sensitive to environmental cues that initiate sex change in sequential hermaphrodites.

EPIGENETIC MECHANISMS

Epigenetic regulation of gene expression is critical during development and is a mechanism through which environmental cues can be translated into plastic phenotypic responses [Bossdorf et al., 2008]. Epigenetic modifications to DNA (e.g., methylation of cytosine bases) and histones (e.g., acetylation) regulate gene expression by reversibly altering the availability of genes, or specific exons, to transcription and typically inhibit and promote transcription, respectively [Duncan et al., 2014].

There is now good evidence that epigenetic factors regulate sexual fate, including sex reversal, by adjusting the responsiveness of male- or female-

promoting gene networks to activation. DNA methylation has been linked to environmentally sensitive sex reversal in several fishes.

Species and sexual system	Gene/s assayed	Epigenetic mechanism	Gonadal gene expression	Reference
European sea bass *(Dicentrarchus labrax)* XY GSD, high-temperature ♀-to-♂ sex reversal	*cyp19a1a*	DNA methylation	↓with sex reversal to ♂	Navarro-Martin et al. [2011]
Japanese flounder *(Paralichthys olivaceus)* XY GSD, high-temperature ♀-to-♂ sex reversal	*cyp19a1a* *dmrt1*	DNA methylation DNA methylation	↓testis vs. ovary ↓ovary vs. testis	Wen et al. [2014]
Half-smooth tongue sole *(Cynoglossus semilaevis)* ZW GSD, high-temperature ♀-to-♂ sex reversal	♀-pathway (e.g., *cyp19a1a*, *figla*) ♂-pathway (e.g., *gsdf*, *amh*, *dmrt1*)	DNA methylation DNA methylation	↓ZZ/ZW testes vs. ovary ↓ovary vs. ZZ/ZW testes	Shao et al. [2014]
Ricefield eel *(Monopterus albus)* protogynous hermaphrodite	*cyp19a1a* *cyp19a1a*	DNA methylation histone acetylation	↓testis vs. ovary ↓with sex change to ♂ ↑testis vs. ovary	Zhang et al. [2013]

GSD = Genotypic sex determination.

Fig:- Different Epigenetic Factors

AFFECT OF ENDOCRINE DISRUPTION ON CHANGING OF SEXUAL BEHAVIOUR – LEADS TO SEX REVERSAL

Endocrine disruption has not been predicted from toxicity tests but discovered by accident, as for example by Aubin-Horth et al. (2009), who found that chemicals were released from Laboratory plastics that interfered with the estrogen receptor of cell cultures. Many of tge substance that have been discovered to elicit endocrine disrupting potential fit neither into the categories of mutagens nor acute toxicants, so their threat to the health of wildlife had been underestimated until recently. The large number of studies on the estrogenic potential of sewage treatment plant effluents was initiated as a consequences of the accidental observation of feminized fishes in the UK. Effects of chemicals and effluents on fish in their natural habitat include disturbed gonadal maturation, impaired gonad development (intersex & abnormal development of gonad duct), vitelogenin induction, disturbed steroid metabolism and abnormal development of secondary sex characteristics. An endocrine disrupter is defined as an exogenous substance or mixture that alters function of the endocrine system and consequently cause adverse health effects in an intact organism or its progeny. It exerts its effects through interference with the synthesis, storage/release, transport, metabolism, binding, action or elimination on natural hormones that are responsible for the regulation of homeostasis and the regulation of development processes. Exposure to endocrine disrupting substances in nature is mostly chronic and the effects of exposure may be latent and sometimes not observable for years after exposure. Among substances found to have such effects are the classical chlorinated Organic pesticides like DDT and lindane.

Disruption of sex determination and sexual development

When males of the Japanese medaka, a species in which spontaneous intersexuality probably does not occur, were exposed to β-HCH (hexachlorocyclohexane, an isomer of the insecticide lindane: γ-HCH) they developed intersex. In the same species, injection of DDT into embryonic yolk induced functional male-to-female sex reversal, i.e. genetic males developed ovaries and gave rise to viable larvae (Feng, Reik, 2000). Nonylphenol and Bisphenol A are also caused intersex and sex reversal from male to female.

Vitellogenin – the biomarker for feminization in fish

Vitellogenin is the precursor protein for the production of yolk proteins. It is produced by the liver under the stimulation from estrogens and released into the blood stream from where it is incorporated into the oocytes. Both male and female fish, as well as immature juveniles have hepatic estrogen receptors, but only the liver of female fish will normally be exposed to estrogens. Estrogenic xenobiotics can also act on the hepatic receptors to induce the synthesis of vitellogenin. In the UK, rainbow trout exposed to sewage effluent were found with elevated vitellogenin levels. It can be indicative of a disruption in sexual differentiation.Vitellogenin induction is in many cases found in combination with the observation of intersex, which does not necessarily mean they are mediated via the same mechanism. For example, male roach with intersex (oviduct and ovo-testis) in the UK had also increased vitellogenin levels (Ghiselin et al. 1969) Vitellogenin induction in the fathead minnow was correlated

with developmental and reproductive impairment and kidney failure (Erisman et al. 2012) and inhibited spermatogenesis. Vitellogenin is the by far most widely used biochemical marker for feminization in fish.

Spiggin – an androgen-responsive marker protein

Spiggin is an androgen-induced glue protein produced by the kidneys of male sticklebacks during the breeding season (Black, Reavis, 2004). In order to cope with the spiggin production, the epithelial cells of kidney tubuli become highly hypertrophied, which results in an enlargement of the whole kidney. While female sticklebacks do not produce spiggin under normal conditions it has recently been demonstrated that the kidneys of female sticklebacks will produce spiggin when exposed to androgens in the water (Budd et al. 2015).

Fig:- Male sticklebacks fish

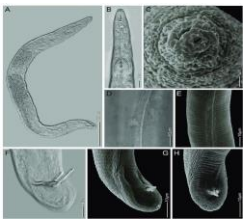
SEX REVERSAL IN INVERTEBRATES

Sex reversal in Nematodes

A number of free-living nematodes are capable of sex reversal—if the sex ratio in a given population is not optimal or if environmental conditions are not ideal, the ratio of males to females can be altered (Avise 2009).

Fig:- *Tylenchulus semipenetrans*

Fig:-Light Microscopy & SEM of *Meloidogynea africana* males (A) habitus of dwarf sex-reversal female, (B) anterior body in lateral view, (C) cephalic region, en face view, (D,E) mid-body lateral field, (F,G,H) tail.



Sex reversal in Gastropods

• In the slipper-shell snails (Crepidula), which are rather sessile, all the young are males; their subsequent sex, however, is determined by their nearest neighbour. They remain males as long as they are near a female but change into females if isolated or placed near another male.

Fig:- Plant Parasitic Nematodes

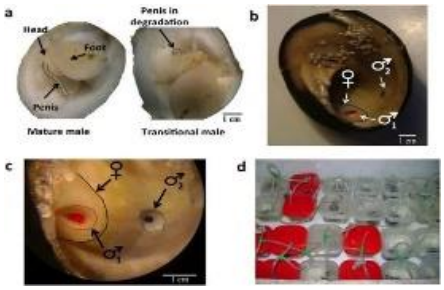


Fig:- (a) Photographs of C. coquimbensis showing anatomical details of individuals at the male stage and transitional stage (changing from male to female). (b) One female and two males (individuals with red and blue spots) inside of a host shell of the marine gastropod Tegula sp. (c) A close up of the individuals inside the host shell. (d) Plastic boxes used to maintain the experimental aggregations of C. coquimbensis.

• Protandric marine gastropod Crepidula coquimbensis and plant parasitic nematodes has been discussed to be an intermediate stage of sex reversal of male into female. Four possible reasons for the sex reversal in Gastropods:

(1) gene mutation,

(2) unusual numerical relationship between autosomes and sex chromosomes during fertilization,

(3) effect of female sex hormone secreted by adult females alongwith the mating attractant, and

(4) presence or absence of andrgenic hormone in males. The possible effect of female sex hormone on the synthesis of macromolecules which might play a role in sex reversal.

SEX REVERSAL IN VERTEBRATES

Sex reversal in some fishes by following ways

1. Sex-reversal occurring in nature is observed in some species of goby fish. These fish get around as a group of females (harem) with a single dominant male. With the loss of the male from group, one of the adult female fish (usually the largest) undergoes sex reversal to become the male of the group .

Fig:- Goby Fish

Fig:- Zebra Fish

2. ndergo sex-reversal induced by ce as water temperature or the presence of aromatase inhibitors.

In a new article by Dranow et al., they examined the role of oocytes in maintaining the female sex phenotype in zebrafish. To determine if loss of oocytes from adult female zebrafish could cause sex-reversal – they used two methods:-

i) One method was to make use of a mutant animal that undergoes germ cell loss later in adulthood – nanos 3 null mutants.

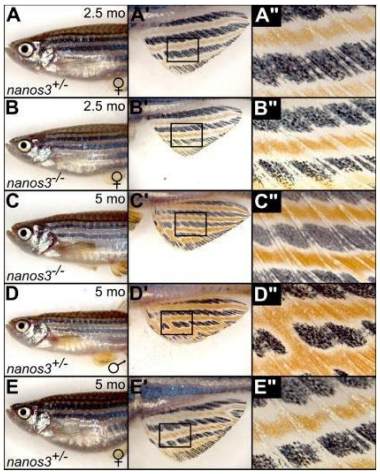


Fig:- nanos3 mutant of Zebrafish sex changes

ii) The second method they used was a transgenic approach to induce germ cell loss after exposed to a certain drug, metronidazole (Mtz).

3. Masculinization of genotypic females of three species of tilapia, Sarotherodon (Tilapia) mossambicus, Sarotherodon (Tilapia) nilotica, Sarotherodon (Tilapia) zilli and Sarotherodon (Tilapia) aurea , has been achieved by feeding methyltestosterone and ethynyltestosterone in the diet to fry, and similarly monosex female tilapia have been produced by treatment with estrone, ethynylestradiol and stilbesterol (Cong, Ran 2013) While steroid administration is capable of reversing sex in tilapia, the percentage of fishes showing sex reversal is highly variable.

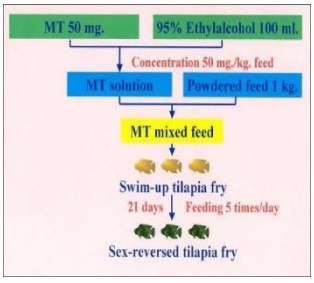


Fig:- Sex reversal Tilapia fry

Fig:- *Rainbow trout*

4. The sex of gynogenetic rainbow trout was reversed to produce XX males by using two steroids, 17α-methyltestosterone (MT) and 11 β- hydroxyandrostenedione (OHA).

Fig:- *Labroides dimidiatus*

Social control of sex reversal in a coral-reef fish:- Males of Labroides dimidiatus control the process of sex reversal within social groups. Each group consists of a male with a harem of females, among which larger individuals dominate smaller ones. The male in each harem suppresses the tendency of the females to change sex by actively dominating them. Death of the male releases this suppression and the dominant female of the harem changes sex immediately (Broquet et al *2015)*

Sex reversal in Amphibian Frog

Amphibians such as frogs lay somewhere in the middle. They're mainly influenced by genetics, but the environment also plays a role. In the laboratory, certain pollutants like synthetic estrogens and herbicides have been shown to induce genetically male frogs to develop outwardly as females.

ex- Some green frogs (Rana clamitans) can reverse their sex even into suburban ponds in the U.S.

Fig- Rana clamitans

Unnoticed sex reversal in amphibians due to artificial estrogen from pills

Hormonally active substances may contribute to global amphibian decline. Some compounds, for example from pharmaceuticals, occur in biologically relevant concentrations in freshwater ecosystems, and thus can affect the hormonal system and the sexual development of animals. Researchers from the IGB and the University of Wroclaw have compared the effects of the pill estrogen ethinylestradiol (EE2) in three amphibian species. The study, published in the journal Scientific Reports, shows that EE2 can lead to a complete feminization of genetic males. Without molecular establishment of the genetic sex, this has remained partly unnoticed.

Gonadal Hormone Synthesis and Sex Reversal in Birds

Sexual differentiation in birds is controlled genetically as in mammals, although the sex chromosomes are different. Males have a ZZ sex chromosome constitution, while females are ZW. Gene(s) on the sex chromosomes must initiate gonadal sex differentiation during embryonic life, inducing paired testes in ZZ individuals and unilateral ovaries in ZW individuals. The traditional view of avian sexual differentiation aligns with that expounded for other vertebrates; upon sexual differentiation, the gonads secrete sex steroid hormones that masculinise or feminise the rest of the body. However, recent studies on naturally occurring or experimentally induced avian sex reversal suggest a significant role for direct genetic factors, in addition to sex hormones, in regulating sexual differentiation of the soma in birds.

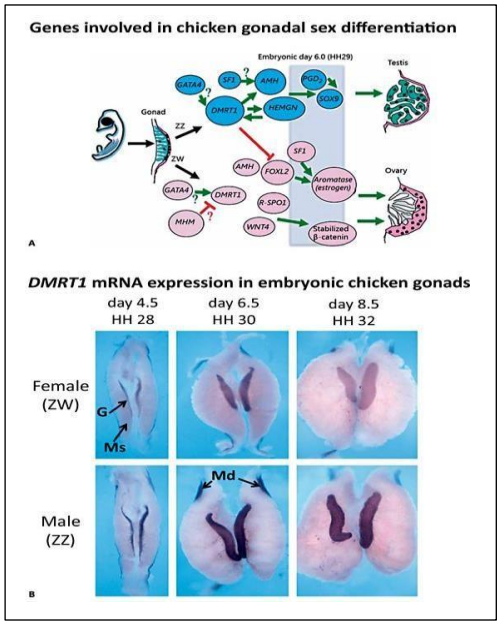


Fig:- Gonadal Sex changes in Chicken

In the chicken embryo, oestrogen synthesis by the gonad is female-specific. Expression of the rate-limiting enzyme in oestrogen synthesis, P450 aromatase, occurs only in female gonads at the onset of embryonic gonadal sex differentiation. Oestrogen-receptor alpha is expressed in the outer cortex, and to a lesser extent in the underlying medulla, mediating the effects of local oestrogen production. Injection of aromatase blockers such as fadrozole into chicken eggs prior to the onset of gonadal sex differentiation induces development of testes in ZW embryos [Elbrecht and Smith, 1992]. In most studies, these birds can develop as phenotypic males, with male sexual dimorphisms that in some cases persist beyond hatching, characterised by well- developed testes, spurs, comb, and wattle [Elbrecht and Smith, 1992; Burke and Henry, 1999; Vaillant et al., 2001]. In some cases, gonads can revert to ovotestits or ovarian structures, which may reflect different degrees of aromatase suppression in different instances [reviewed in Vaillant et al., 2001]. The ZW male chickens produced by aromatase enzyme inhibition at embryonic stages can exhibit spermatogenesis as adults [Elbrecht and Smith, 1992]. Conversely, oestrogens injected into eggs prior to gonadal sex differentiation can feminise male gonads, characterized by a thickened gonadal cortex and fragmented medulla. Partial sex reversal (partial feminisation) of embryonic male chicken gonads following RNAi-mediated knockdown of gonadal DMRT1 expression.

Sex reversal in Mammals

1. Male-to-female sex reversal in mice lacking Fibroblast growth factor 9 (Fgf9), demonstrating a novel role for FGF signaling in testicular embryogenesis and SRY, triggers testes development in the early embryo to becomes male.

2. Mutation of Dmrt1, Wt1, Foxl2, Wnt4 and Sox9 induce Sex reversal in mammals.

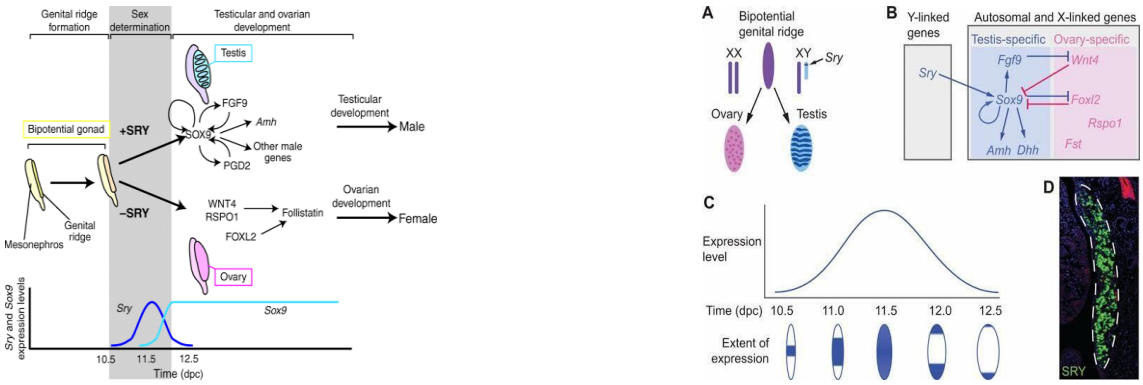


Fig:- Mammalian Sex reversal Genes

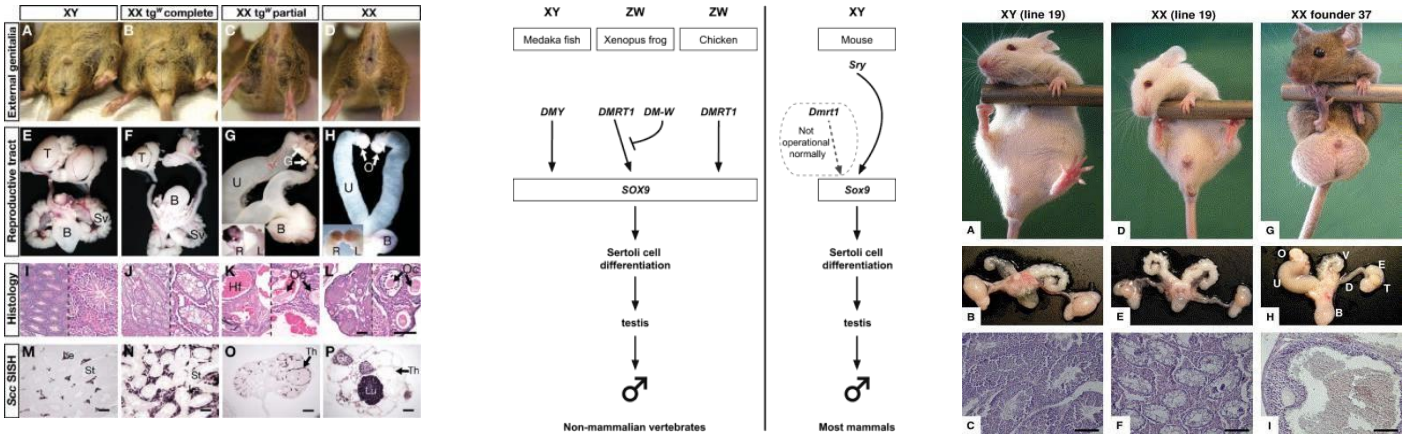


Fig:- Sex reversal in Mice

ADVANTAGES OF MONOSEX CULTURE OR SEX REVERSAL

1. Monosex culture is a solution to overpopulation caused by high fecundity.

2. Monosex culture has advantage when one of the sex have superior produ•ction traits, better growth rate or higher marketing value.

3. Sex reversal has many advantages in Fish farming.

BIOLOGICAL EFFECTS OF SEX REVERSAL

- Steroid hormone treatment increase mortality in a fish population.
- Androgen treatment during sex reversal enhance growth rate in several species of Cyprinds and Cichilds.
- High dose or long duration of Steroid Hormone treatment inhibits normal gonadal development.
- High dose of Androgen treatment sometimes led to production of females in Lieu of males (Paradoxical Sex Reversal).

TEMPERATURE INDUCED SEX REVERSAL

Thermal liability of Sex Differentiation was demonstrated in some teleosts.

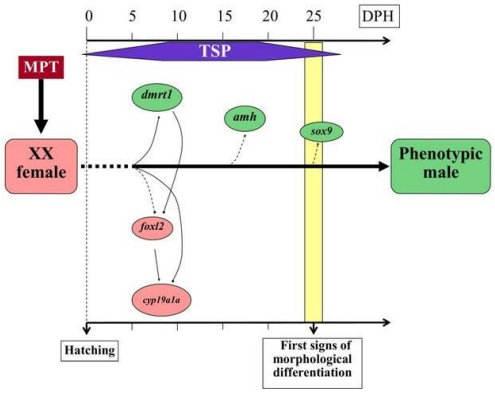
100 % females were obtained in the atherinid fish when juveniles were transferred to colder temperature of 13 – 19 °C.

100 % males were obtained when they were transferred in a warmer temperature of 29°C.

Temperature induced sex reversal may prove more advantageous than hormonal approaches because it will be easier to practice.

Fig:- Putative pathway of temperature-induced sex reversal of XX females forwards phenotypic males taking Nile tilapia as an example.

MPT(male producing temperature), (TSP-Thermosensitive period of Gonad), DPH(Day post hatching)



SEX REVERSAL INDUCED BY ARTIFICIALLY

Androgens and estrogens are considered to be the natural sex- inducers. It has been known that treatment with steroid hormone can cause a change in the gonadal sex in fish.

Paradoxical feminization :-

When treating fish with testosterone (T) or MT either at high doses or over extended periods, often a paradoxical feminizing effect of these androgens can be observed.

The female grass carp or the fishes which are homogamatic are treated with male hormone, methyl-testosterone MT.

MT causing sex reversal of female fish to phenotypic male with sex genotype as

XX. This sex reversed fish is phenotypic male but genotypically female (XX) and when such fishes are crossed with normal females (XX) should produce only XX progeny.

Sensitive period :-

many gonochorist fish species are particularly susceptible to the action of exogenous steroids or environmental factors that influence the direction of sexual differentiation during a certain sensitive period.

CONCLUSION

Various pictures are given in this project paper from different sources to emerge of how transformations across a variety of biological systems integrate to initiate and progress natural sex change with imbalance of Steroids Hormones. Sex change is clearly initiated in the brain, but where, by what, and how this is communicated to induce gonadal sex change and Epigenetic factors remain areas of significant opacity. Re-direction of gonadal fate begins when expression of critical sex-maintenance genes (e.g., cyp19a1a in protogynous and dmrt1 in protandrous species), neuroendocrine control through HPG axis, cellular origins during Complete Gonadal Restructuring, Steroid balance is interrupted, causing a cascaded collapse of the prevailing expression landscape, endocrine environment, and gonadal anatomy. Once antagonistic suppression of the opposing sexual network is lifted, establishment of a new sex-specific expression and endocrine environment drives gonadal development towards the secondary sex. A multi-layered trigger mechanism is likely at the head of this cascade, ensuring that sex change proceeds only under specific circumstances. Recent research already implicates several factors that could act in consort to silence cyp19a1a expression in the initiating stages of protogynous sex change (e.g., cortisol, DNA methylation, Amh). Size-advantage model & protection against inbreeding of Ultimate cause and Proximate cause induce evolutionary significance in Sex reversal. However, current models are largely based on affect of correlations in the timing of Endocrine disruption changes sexual behaviour, and experimental validation is required to disentangle cause from effect before the functioning of these mechanisms can be fully appreciated. Also critical will be a deeper understanding of how complex neurological systems in the brain crosstalk with the stress (HPI) and reproductive (HPG) axes to translate external environmental signals into internal physiological responses. Expression of GtH subunits and their receptors (LHR, FSHR) also fluctuates across sex change in protogynous, protandrous species.

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