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Natural and anthropogenic environmental oestrogens: the scientific basis for risk assessment*

Comparative reproductive physiology of non-mammalian species

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Abstract: This chapter aims to emphasise the ways in which reproductive physiology in fish, amphibians, reptiles and birds differs from that in mammals. Although reproductive physiology has shown considerable adaptation through evolution, many aspects, including the biosynthesis and structure of steroid hormones, and to some extent their functions, have shown a remarkable degree of conservation. Recent concern about endocrine disruption has focused on natural or synthetic chemicals interacting with gonadal steroid receptors to agonise or antagonise the actions of the natural ligands. Gonadal steroids are involved in most aspects of reproduction, from sexual differentiation during embryonic development to gamete maturation and sexual behaviour, in all vertebrates. Physiologically significant endocrine disruption has the potential to compromise reproduction in many ways.

INTRODUCTION

Assessing the effects or potential effects of endocrine disrupting chemicals on wildlife requires an understanding of endocrine function and a knowledge of how this varies amongst different groups. The vertebrates evolved 400 million years ago from an aquatic ancestor and now comprise about 42 000 species occupying almost every habitat on the planet. This divergence has necessitated considerable adaptation, and whilst variation is apparent in reproductive physiology, the broad structure and function of the hypothalamo-pituitary-gonad axis is conserved. In all vertebrates, secretion of a decapeptide gonadotrophin-releasing hormone (GnRH) from the hypothalamus stimulates gonadotrophs in the anterior pituitary gland to secrete gonadotrophic peptide hormones. These regulate gonadal and gamete maturation and stimulate synthesis of the gonadal steroid hormones, which have a wide variety of functions.

This chapter provides a brief comparative description of endocrine function in the major groups of non-mammalian vertebrates. For a more complete comprehensive review of vertebrate endocrinology, see ref. 1. The aim of this chapter is to highlight the ways in which reproductive physiology varies throughout the vertebrates from the situation in mammals, particularly humans and laboratory rodents, as described in pp. 1633–1646. It is divided into three parts. The first deals with the morphology of the reproductive system, the second with the structure of the reproductive hormones, and the final part describes how the whole system functions to result in successful reproduction. For brevity, citations are restricted to recent endocrine discoveries rather than longer established morphological details.

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STRUCTURE OF THE REPRODUCTIVE SYSTEM

Anatomy of the hypothalamus and pituitary gland

Within the hypothalamus, there are a number of different nuclei, collections of nerve cells usually devoted to controlling one particular function. These different nuclei have gradually become more clearly differentiated through vertebrate evolution, but most of the nuclei, or homologous structures, have been identified in the majority of groups. There are several forms of vertebrate GnRH, and cells containing some of these different forms may be distributed widely throughout the brain. However, cells containing the form(s) thought to have a physiological gonadotrophin releasing function tend to be present mainly in the pre-optic nuclei (refs 2–4). As in mammals, these cells are thought to have migrated from the olfactory placode during ontogeny (ref. 5).

In many vertebrates, the more primitive cartilaginous fish as well as amphibians and reptiles, the hypothalamo-hypophysial arrangement is similar to that in mammals. The fibres of the neurosecretory neurones terminate in the median eminence and neurohormones pass to the secretory cells of the anterior pituitary via a hypothalamo-hypophysial portal system. The hypothalamo-hypophysial axis of birds functions in the same manner but the anatomy is slightly different in that there is no pituitary stalk; the anterior pituitary is anatomically separated from the median eminence so there is no connection other than the portal system. In teleosts there is no true median eminence. Fibres from neurosecretory neurones follow tracts directly to the anterior pituitary so that GnRH and other neurohormones reach the pituitary secretory cells without passing through a portal system.

Sex determination and sexual differentiation

The hypothalamus and pituitary are essentially similar in males and females, but the structure and function of the gonads and accessory sex organs are, of course, sexually dimorphic and this results from sexual differentiation early in development. There are several mechanisms of sex determination. In birds and amphibians, and some reptiles and fish, sex is determined genetically (genotypic sex determination), as it is in mammals. In mammals and most anuran amphibians, it is the male that is heterogametic (XY) and the female that is homogametic (XX). In birds and most urodele amphibians, it is the male which is homogametic (ZZ) and females which are heterogametic (ZW). The homogametic sex is the default sex. In the absence of gonadal steroids, this sex develops normally. In the heterogametic sex, gonadal steroids are required for appropriate sexual differentiation.

The embryos of all vertebrates appear, at first, to develop in an identical fashion, whether they are destined to become male or female. The genital ridges, the rudiments of the testes and ovaries, are initially indistinguishable and are associated with two sets of ducts, the Wolffian ducts, which are the potential male reproductive tracts, and the Müllerian ducts, the potential female tracts. In male mammals, the gene responsible for male development is termed *Sry* and is found on the Y chromosome. Expression of *Sry* in the undifferentiated genital ridges causes differentiation of the genital ridges into testes. These produce Müllerian-inhibiting substance (MIS, also known as anti-Müllerian hormone, AMH) which causes the Müllerian ducts to regress, and testosterone, which stabilises the Wolffian ducts. MIS also inhibits P450 aromatase ($P450_{arom}$), the enzyme which synthesises oestrogen from testosterone. In the absence of MIS and testosterone, female development occurs. Transfection of the *Sry* gene gives rise to testicular development in chromosomal females.

In birds, the heterozygote is female and it would therefore seem unlikely that the sex-determining system is identical to that in mammals. Perhaps unsurprisingly therefore, attempts to detect expression of an *Sry* homologue in the differentiating genital ridge of birds have been unsuccessful (ref. 6). Another gene, *Sox9*, may be important (ref. 7). The gonads of genetically male embryos are larger than those of genetically female embryos, and in both sexes the left gonads are larger than the right gonads and contain a germinal epithelium which has the potential to differentiate into an ovarian cortex under the influence of oestrogen (ref. 8). Later, female embryos become bilaterally asymmetrical and the left gonad outgrows the other and develops into an ovary. The left Müllerian duct develops into the oviduct and shell gland, the right one remains rudimentary. The mechanisms of sexual differentiation in birds remain unclear although MIS, $P450_{arom}$ and gonadal steroids are all known to have roles. As in mammals, MIS causes

regression of the Müllerian ducts and may inhibit the activity of $P450_{arom}$ in males. Expression of $P450_{arom}$ is an essential step in sexual differentiation of females (ref. 9) because oestrogens control differentiation into a female; in the absence of oestrogens, development is masculine, although both oviducts are retained. In the embryonic male gonad there is expression of $P450_{17\alpha}$, an enzyme involved in testosterone synthesis, but little expression of $P450_{arom}$, so there is no oestrogen production. Consequently, it is possible to cause phenotypic sex reversal from male to female by exposing bird embryos to oestrogens (ref. 10). Exposure of genetically male embryos to diethylstilboestrol results in the left gonad resembling an ovary and a reduction in size of the right gonad (ref. 11). Conversely, aromatase inhibitors induce reversal from female to male phenotype, to the extent of genetic females with paired sperm producing testes and a fully developed left oviduct (refs 12, 13). Males treated with aromatase inhibitors are unaffected. Sex differentiation also occurs in the brain, to programme sexual behaviour; early administration of oestrogen to males prevents the development of masculine behaviour.

Many vertebrates have no distinct sex chromosomes and sex is determined environmentally. In most reptiles, temperature during early development determines sex. A high temperature results in young developing all as one sex, and a low temperature, all as the opposite sex. In turtles, although sex determination is temperature-dependent, there is a genetic component; a gene important in sex determination (*Sox9*) has been cloned (ref. 14) and it is expressed differently at different incubation temperatures (ref. 15). As in birds, endogenous oestrogen may be important in female differentiation; if oestrogens are high during the thermosensitive period, ovaries differentiate, if oestrogen synthesis is low, testes differentiate. Treatment with aromatase inhibitors prevents ovarian differentiation. When eggs are incubated at the pivotal temperature, both males and females are obtained (as opposed to all intersex), but differentiation of the gonad is delayed compared to both lower temperatures (which results in 100% males) and higher temperatures (which results in 100% females). This can be related to the activity of $P450_{arom}$ (ref. 16). At the pivotal temperature, the gonads can develop as ovotestes. In some of these individuals, gonadal $P450_{arom}$ activity is slightly higher, it increases during development, and these differentiate into ovaries with $P450_{arom}$ activity the same as in animals incubated at a higher temperature. In ovotestes which eventually develop as testes, $P450_{arom}$ activity is slightly lower and decreases, so that at hatching it is the same as in animals incubated at a lower temperature. Thus, an intermediate temperature results, ultimately, in equal numbers of both sexes rather than an intersex condition. Temperature sex determination may be mediated by $P450_{arom}$ (ref. 17).

A number of studies have shown that amphibian sex determination is controlled genetically even though most species lack distinct sex chromosomes and no sex-determining genes have been described. The few studies suggesting temperature dependent sex determination have been conducted at temperatures outside of the range normally experienced by the species under study, and these effects probably do not occur under natural conditions (ref. 18). Sex differentiation can be altered by treatment with a variety of exogenous steroid hormones, but the involvement of endogenous gonadal steroids in sex differentiation is unknown.

Higher vertebrates are dioecious; sex is clearly defined early in development, either genetically or by temperature. Fish exhibit a broader range of possibilities. Fish may be dioecious or hermaphroditic. Amongst hermaphrodites, there is simultaneous hermaphroditism and sequential hermaphroditism. The latter can be protandry (a male changing to function as a female) or protogyny. Amongst dioecious species, most show genotypic sex determination. In some species the male is heterogametic (XY) and in others the females is heterogametic (ZW) (refs 19, 20). In both groups there are species where sex determination is entirely genetic, in other species, often closely related species, phenotypic sex is altered by temperature (refs 21–24). Early exposure to oestrogens alters phenotypic sex ratio in favour of females (ref. 25).

Gonadal structure and function

In most vertebrates, as in mammals, the gonads, Wolffian ducts and Müllerian ducts of early embryos are undifferentiated. In males, the gonads develop as testes, the Müllerian ducts regress and the Wolffian ducts develop into paired sperm ducts. In females, the Müllerian ducts develop into oviducts.

Testis

Testicular morphology amongst vertebrates can be classified into two broad types. In most fish and urodele amphibians, the testes are composed of lobules. Each lobule is composed of cellular cysts, and each cyst contains Sertoli cells. All of the cells within a cyst, and all of the cysts within a lobule, are normally at the same stage of spermatogenesis. In some species of fish, a continuous process of spermatogenesis and sperm release occurs throughout the year, but in the majority of species, especially those in temperate and high latitudes, the reproductive cycle is seasonal. Spermatogenesis is active over a period of a few months, at the end of which all spermatozoa are stored in the testes and may remain there for several months more before spermiation and sperm release occurs. At spermiation, the more posterior lobules, which are the most mature, are emptied of sperm and degenerate. New lobules then develop towards the anterior of the testis. Both lobule boundary cells and Sertoli cells are sites of steroid hormone biosynthesis. There is no true interstitial tissue. The size of fish testes at maturity varies widely. For example, in some species of Tilapia they may form only 0.2% of body mass, whereas for trout they may be 10%.

Testicular morphology in anuran amphibians, reptiles and birds is broadly similar to that in mammals. The testes consist of convoluted seminiferous tubules lined with germinal epithelium and interspersed with interstitial tissue. The interstitial cells are steroidogenic and show seasonal changes associated with androgen production. There are many Sertoli cells and these too are steroidogenic. In birds the onset of spermatogenesis results in particularly rapid testicular growth. In non-tropical species, testicular mass increases several hundred-fold at the beginning of the breeding season, up to typically 3–4% of body mass. In tropical birds, mature testis mass is less and there is less seasonal variation.

Ovary

The ovaries of most fish are hollow and lined with germinal epithelium. They consist of masses of follicles supported by stroma. The follicles initially consist of the oocyte surrounded by an epithelial layer which later develops to form the granulosa, and which is responsible for yolk deposition. Stroma near the follicles develops into a theca. The granulosa and theca are steroidogenic. There are three patterns of oocyte maturation shown by fish, and these are related to their reproductive strategies. Species which spawn frequently have asynchronous ovaries containing oocytes at all stages of maturation. Species which spawn just once each year have group-synchronous ovaries with at least two populations of oocytes, and species which spawn only once during their life-time have synchronous ovaries where all oocytes are at the same maturational stage. In species which spawn only once, e.g. Pacific salmon, most of the body reserves are converted into ovarian tissue and spawning is followed shortly afterwards by death. Even in species which spawn every year, e.g. flatfish, the ovarian mass before spawning can exceed 30% of body mass. The ovary is continuous with the oviduct, which, in fish is not derived from the Müllerian duct and so is not homologous to the oviducts of other vertebrates. Eggs are discharged from the ovary into the oviduct and hence to the exterior. Fish do develop *corpora lutea* after ovulation but, unlike mammals, an endocrine function for them has not been established.

Amphibian ovarian structure and function is similar to that of fish. After breeding, the ovary contains young follicles which become the subsequent crop of mature oocytes, numerous cell nests that become the young follicles of the next vitellogenic period, and primary germ cells that give rise to new cell nests for future generations of oocytes. This progression may take several years. The ovarian granulosa and thecal cells are steroidogenic but the interstitial tissue is not. As in fish, *corpora lutea* develop after ovulation. In oviparous species, these show steroidogenic activity, but an endocrine function for this has not been established. In viviparous species, the *corpora lutea* can convert pregnenolone to progesterone and they are required for successful gestation. Furthermore, development of new oocytes may be arrested before the degeneration of post-ovulatory *corpora lutea* suggesting that progesterone may inhibit gonadotrophin secretion. Oestrogens are indirectly involved in the actions of progesterone because the progesterone receptor is often oestrogen-dependent.

Reptiles also have paired hollow ovaries. Developing oocytes are surrounded by granulosa cells and these are the major source of oestrogens. Unlike fish and amphibians, the theca of reptiles becomes differentiated into a theca interna and theca externa. *Corpora lutea* develop after ovulation and

synthesise progesterone (refs 26, 27). Progesterone levels increase at about the time of ovulation, and high levels are maintained in viviparous species throughout gestation. Most reptiles produce several eggs simultaneously from each ovary, in others, a single egg is produced by each ovary in turn, and in some species, eggs are provided by one ovary in one season and the other in the next season.

In most species of birds, only the left ovary and oviduct develop; the right ovary remains rudimentary, possibly as an adaptation to flight. As in mammals, the full complement of oocytes is complete early in development (before hatching) and most of these undergo subsequent atresia (ref. 28). The primary follicle consists of an oocyte surrounded by granulosa cells. As the follicles grow, thecal layers are added and the follicles become vascularized. Both the granulosa and theca are steroidogenic. Follicular development progresses rapidly immediately before breeding. The larger follicles bulge from the ovarian surface and a hierarchy of follicular sizes develops as yolk is laid down in these larger follicles (ref. 29). The largest of the follicles ruptures, the egg passes down the oviduct into the shell gland (a specialised region of the oviduct) where a layer of albumin is laid around it, followed by the shell and finally, pigmentation. The egg is normally laid 24h after ovulation. Most birds lay one egg every day until the clutch is complete, but larger birds may lay one egg only every two days. After ovulation, the largest remaining follicle then develops rapidly and is the next to rupture. *Corpora lutea* are not found in birds.

REPRODUCTIVE HORMONES

GnRH

GnRH is the primary physiological regulator of the cascade of hormones responsible for reproductive function in all vertebrates. This function, and the peptide structure, were first identified in mammals. Since then, at least ten variants of the mammalian peptide sequence have been isolated, all of them decapeptides (ref. 30).

	1	2	3	4	5	6	7	8	9	10
Mammalian	E	H	W	S	Y	G	L	R	P	G-NH_2
Chicken I	E	H	W	S	Y	G	L	**Q**	P	G-NH_2
Sea Bream	E	H	W	S	Y	G	L	**S**	P	G-NH_2
Salmon	E	H	W	S	Y	G	**W**	**L**	P	G-NH_2
Catfish	E	H	W	S	**H**	G	L	**N**	P	G-NH_2
Dogfish	E	H	W	S	**H**	G	**W**	**L**	P	G-NH_2
Chicken II	E	H	W	S	**H**	G	**W**	**Y**	P	G-NH_2
Lamprey III	E	H	W	S	**H**	**D**	**W**	**K**	P	G-NH_2
Lamprey I	E	H	**Y**	S	**L**	**E**	**W**	**K**	P	G-NH_2
Tunicate I	E	H	W	S	**D**	**Y**	**F**	**K**	P	G-NH_2
Tunicate II	E	H	W	S	**L**	**C**	**H**	**A**	P	G-NH_2

Differences from the mammalian form are shown in bold. Positions 1, 2, 4, 9 and 10 are completely conserved. Positions 3 and 6 are conserved except for lamprey. Position 8 is highly variable.

Although reproductive function in all vertebrates is ultimately controlled by one or more forms of GnRH, not all forms of GnRH have a physiological role in reproduction. In birds, experimental treatment with both chicken GnRH-I (cGnRH-I) and cGnRH-II stimulates gonadotrophin release, but only cGnRH-I is present in the median eminence and so can have a gonadotrophin releasing function. cGnRH-II is found in extra-hypothalamic areas of the brain and may act as a neuromodulator or neurotransmitter. Since its discovery in chickens, cGnRH-II has been found in many species. It is the most ubiquitous form in vertebrates and so the description ‘chicken’ is no longer appropriate. It is now referred to as Type II GnRH (ref. 30). Type I GnRH, mammalian GnRH and related variants, are less conserved than Type II. It is these forms that act predominantly as hypophysiotrophic peptides regulating pituitary gonadotrophin secretion. In addition to stimulating gonadotrophin secretion in fish, salmon and dogfish GnRH also

stimulate growth hormone secretion (refs 31, 32). In mammals, GnRH also has a paracrine role within the gonads, and this may also be true in fish (refs 33, 34).

Gonadotrophins

In response to GnRH stimulation, the anterior pituitary gland of higher vertebrates secretes two gonadotrophic hormones, follicle-stimulating hormone (FSH) and luteinizing hormone (LH). These are glycoproteins, closely related to each other and to another pituitary hormone, thyroid-stimulating hormone (TSH). All three hormones consist of an α subunit and a β subunit. The $\alpha\beta$ dimer forms after transcription to form the active hormone. The α subunit is common to all three hormones; specificity is conferred by the β subunit. FSH and LH have been identified in mammals, birds, reptiles and amphibians. In fish, although there are two types of chemically distinct gonadotrophins (GTH-I and GTH-II), and both comprise α and β subunits (ref. 35), their identities to FSH and LH remain to be established. Both GTH-I and GTH-II show equal potencies in stimulating steroidogenesis *in vitro*, however GTH-I appears to be FSH-like and controls spermatogenesis and oogenesis while GTH-II appears to be LH-like and controls the final stages of gamete maturation, ovulation and spermiation.

During the course of evolution, structural changes have occurred both in the gonadotrophins and in their receptors. LH is much more species specific than FSH and only binds to LH receptors of closely related species. In mammals and birds, FSH receptors bind only FSH, and LH receptors bind only LH. In lower vertebrates there is less specificity. In reptiles, receptors which bind mammalian FSH occur on both tubular and interstitial cells which suggests that FSH and LH receptors may be identical. This also appears to be true for amphibians. However, despite the lack of specific receptors, FSH and LH induce different actions on the amphibian testis. In fish, the receptor which binds both GTH-I and GTH-II (GTH-R I) is localised in the theca and the granulosa in the ovary, whereas the receptor which specifically binds GTH-II (GTH-R II) is localised only in the granulosa (ref. 36).

Other pituitary hormones

Prolactin is a single chain peptide, related to growth hormone, and secreted by the anterior pituitary. It was first discovered and named as a consequence of its role in stimulating milk production in mammary glands of mammals and the crop sac of pigeons. It has a wide variety of functions including osmoregulation, metabolic effects, integumental effects and reproductive effects. The latter include inducing gonadal regression, inhibiting steroidogenesis, and stimulating incubation and parental behaviour. Unlike mammals, in which prolactin secretion is under inhibitory control from the hypothalamus, in birds it is under stimulatory control. The prolactin-releasing hormone is vasoactive intestinal polypeptide (VIP).

Somatolactin is a peptide hormone related to prolactin which has been identified in the anterior pituitary of fish. Secretion is stimulated by GnRH and apparently by testosterone (ref. 37). Its function remains uncertain, although elevated levels during the latter stages of oocyte maturation suggest a role in reproduction (ref. 38).

Steroids

Synthesis

Most concern about endocrine disruption has focused on natural or synthetic chemicals acting as agonists or antagonists of androgens or oestrogens. The basic synthetic pathways for these steroid hormones are conserved throughout all vertebrates. These are described in detail in 1.2, and so will be dealt with only briefly here. The first step in biosynthesis is the side chain cleavage of cholesterol to form pregnenolone, followed by conversion to progesterone. This latter reaction is catalysed by 3β-hydroxy-Δ^5-steroid dehydrogenase (3β-HSD). Consequently, detecting 3β-HSD activity in a tissue is a clear indication that the tissue is steroidogenic. The main source of sex steroid hormones is the gonad although adrenal (interrenal) tissue is often an additional source of androgens. The brain too is steroidogenic; not only is it

a site for aromatisation of androgens to oestrogens, essential for the behavioural actions of steroids, it is also a source of progesterone. In eutherian mammals, the placenta also synthesises progesterone and oestrogens.

The major androgens amongst vertebrates are testosterone, androstenedione, androsterone, dehydroepiandrosterone (DHEA), 5α-dihydrotestosterone (DHT) and 11-ketotestosterone. Although androgens are considered typical of males, females frequently produce significant amounts which may be physiologically important (ref. 39). The major oestrogens are oestradiol-17β, oestriol and oestrone. In fish, additional steroids are synthesised which are important in oocyte maturation.

Metabolism and excretion

The concentrations of steroid hormones in the circulation depend on the rate of synthesis, the amount of plasma-binding protein, and the rate of metabolism and excretion. Most steroid metabolism occurs in the liver. This can involve reduction of side chains followed by conjugation to form glucuronides (androgens and oestrogens) or sulphates (oestrogens) (ref. 40). These conjugates are water soluble and do not bind to serum proteins, so they are readily excreted through the kidneys.

Cytochrome P450s

Cytochrome P450s are haem containing oxidative enzymes normally occurring in smooth endoplasmic reticulum and mitochondria. They can be classified into two groups, with important roles in reproductive physiology and toxicology respectively (ref. 41). The first group contains forms where the substrates are endogenous compounds, and these are important in steroid hormone synthesis. For example, $P450_{scc}$ cleaves the side chain from cholesterol and $P450_{aro}$ aromatises testosterone to oestradiol. The other group contains forms which metabolise xenobiotic compounds. These P450s are inducible, i.e. the presence of substrate xenobiotic compounds increase the activity of the enzyme. These forms are potentially important in endocrine disruption because some of them are also concerned with steroid hormone metabolism. This provides a mechanism by which xenobiotic compounds could affect steroid hormone dynamics (ref. 42).

REPRODUCTIVE FUNCTION

Puberty and breeding seasons

The young of all vertebrates need to achieve a certain degree of somatic maturation before gonadal maturation can begin (puberty). In many species, puberty is attained within the first year of life, in others it may take many years, depending on growth patterns. The physiology underlying puberty remains unclear. In mammals, one hormone which may have a role in puberty is leptin. This is produced by fat cells and so provides an animal with a measure of its body condition. However, leptin has not yet been positively identified in non-mammalian species.

In humans, primates, and some domesticated mammals, individuals reach puberty and then remain reproductively active more or less continuously until senescence. This pattern of reproduction is rare. The vast majority of vertebrates live in environments which change seasonally and so natural selection has resulted in the evolution of breeding seasons. Some species adopt an even more extreme strategy. In Pacific salmon and the Atlantic eel, breeding is not just a once a year event, it is a once in a lifetime event.

Seasonal patterns in GnRH secretion

Gonadal maturity is ultimately dependent on the rate of secretion of GnRH and this changes seasonally in response to environmental cues. In female mammals, the breeding season consists of a sequence of oestrus cycles (unless or until she becomes pregnant). During the rest of the year the female is in anoestrus. In male mammals, the testes mature at the beginning of the breeding season and remain active until they regress at the end of the breeding season. The difference between the breeding and non-

breeding situation is entirely due to differences in the pattern of GnRH secretion; there is no evidence for a change in the pituitary sensitivity to GnRH stimulation. Secretion of GnRH in mammals is pulsatile. During the breeding season, GnRH pulses occur frequently; outside the breeding season they are infrequent but the amplitude of each pulse is no less than of those during the breeding season. There is no change in the hypothalamic content of GnRH so day-length controls seasonality by controlling secretion of GnRH rather than its synthesis (refs 43, 44). In birds, the seasonal changes in reproductive physiology are more profound than in mammals. In most birds, gonadal maturation is stimulated by increasing day-length during in spring. This may be due to an affect of day-length on GnRH secretion as in mammals. However, prolonged exposure to long days causes spontaneous gonadal regression and this is associated with a marked decrease in hypothalamic GnRH content. So there are seasonal changes in both GnRH secretion and GnRH synthesis (ref. 45). Little is known about seasonal changes in GnRH dynamics in lower vertebrates although temperature, as well as photoperiod, is important (ref. 46).

Control of gonadal function

Fish

GnRH stimulates GTH synthesis and secretion. During early gonadal development in goldfish, both GTH-I β and GTH-II β gene expression increase in the pituitary gland, whereas in salmonids there is evidence that GTH-I β gene expression precedes that of GTH-II β (ref. 47). In goldfish, basal GTH-II β gene expression can be stimulated by androgens and oestrogens independently, whereas GnRH-induced GTH-II β gene expression can be augmented by oestrogens but is inhibited by androgens (ref. 48). In salmonids, gonadal steroids inhibit GTH-I secretion but not GTH-II (ref. 49) except in spermiating males, where steroids do inhibit GTH-II secretion (ref. 50).

Gamete production is regulated by the binding of GTH-I and GTH-II to gonadal receptors and the subsequent effects on steroid hormone synthesis (refs 36, 51). GTH-I and GTH-II stimulate steroid synthesis with similar potencies during the early stages of spermatogenesis and vitellogenesis. Both hormones appear to bind to the same receptor (GTH-RI) in the testis and ovary. The major steroid synthesised by the ovarian follicles at this time is oestradiol-17β. During the final stages of gamete maturation, the potency of GTH-II exceeds that of GTH-I in stimulating steroid synthesis and this coincides with the appearance of a second receptor (GTH-RII) which binds GTH-II specifically. Steroid synthesis in the ovary switches from oestradiol to 17α,20β-dihydroxy-4-pregnen-3-one (17-20-P), the steroid which is responsible for the final stages of oocyte maturation and ovulation, 17α,20β,21-trihydroxy-4-pregnen-3-one and other progestagens, significant amounts of which are sulphated (ref. 52, 53, 54). Deoxycorticosterone is also synthesised in the ovary. The major androgens produced in male fish are testosterone and 11-ketotestosterone. However, the ovaries also synthesise these two androgens. Circulating levels of 11-ketotestosterone are normally higher in males than females, but testosterone in females is often higher than in males (ref. 55). 11-ketotestosterone is more effective in stimulating secondary sexual characters, reproductive behaviour and spermatogenesis.

Amphibia

Little is known of the functional control of amphibian reproduction. Seasonal cycles in brain levels of mGnRH (ref. 56) and in both gonadotrophins have been shown in both sexes (ref. 57). In females, the gonadotrophins do not appear to be required for development of pre-vitellogenic follicles, but they are required later, during vitellogenesis. A distinction between the roles of LH and FSH is less clear than in higher vertebrates. Although LH stimulates ovarian and testicular steroidogenesis, there is evidence that FSH does also. In females, high FSH and LH coincide with high oestradiol and plasma vitellogenin. Vitellogenic follicles secrete oestradiol and mature follicles secrete progesterone. The amphibian ovary also produces oestrone, testosterone, DHT and deoxycorticosterone. As in higher vertebrates, ovulation is controlled by LH and progesterone. In males high LH coincides with high androgen levels before breeding.

Reptiles

There is also a paucity of information on reproductive function in reptiles. Secretion of GnRH in response to day-length and temperature is presumed to control gonadotrophin secretion, but the roles of each gonadotrophin are unclear. There have been few studies of seasonal changes in gonadal steroids. In alligators, oestradiol is elevated during ovarian maturation and testosterone is high during late vitellogenesis (ref. 58). Progesterone may have a role in ovulation; levels are high during ovulation and decline after oviposition (ref. 26). In viviparous species, prolactin remains high through gestation. Some species of reptiles show parental care but the hormonal basis of this is not known.

Birds

Seasonal changes in gonadotrophin and gonadal steroid secretion have been studied in many species of free-living birds, but the changes specifically associated with ovulation have been restricted to domestic fowl. Synthesis of cGnRH-I begins during autumn, resulting in a slight but physiologically significant increase in gonadotrophin secretion and consequent increases in testosterone and 5α-DHT in males and oestradiol in females. Although levels remain low in comparison to those during breeding, they are important in driving sexual behaviours such as song, territoriality and courtship. During spring, gonadotrophin secretion increases rapidly leading to marked gonadal maturation. FSH receptor levels are high in granulosa, thecal and stromal cells of pre-hierarchical follicles, suggesting a role in initiating granulosa cell differentiation at the time when follicles recruited into pre-ovulatory hierarchy, but decline approaching ovulation (ref. 59). LH receptors are not found in pre-hierarchical follicles, but increase during follicular development after recruitment (ref. 60). As in mammals, inhibin is produced in the granulosa cells and this specifically inhibits FSH secretion (ref. 61). Young follicles produce testosterone and oestradiol. As follicular development progresses, steroid synthesis switches to progesterone, and the increase in progesterone leads to the pre-ovulatory surge in LH.

Although the major steroid produced by the testis is testosterone, recently it has been shown that the germ cells of male birds, and the sperm, are able to synthesise oestrogen and that the epididymal region of the testis contains oestrogen receptors (ref. 62). This suggests a role for oestrogen in sperm maturation and presents another potential target for endocrine disruption.

Once a clutch of eggs is complete, reproductive physiology switches from a sexual mode to a parental mode. In some species both sexes share parental care, in others it is only the female, and in a few species it is the male which is entirely responsible for care. Prolactin is important in this process. Prolactin secretion, like the gonadotrophins, is under photoperiodic control, but longer day-lengths are required so that the seasonal peak in prolactin occurs later than that of the gonadotrophins. Oestradiol enhances prolactin secretion and the presence of eggs provides a further stimulus. Prolactin and oestradiol act synergistically to cause development of the brood patch, a defeathered and highly vascularized part of the abdomen which allows heat transfer to the eggs. The first parental behaviour after the eggs are laid, is incubation. Broody behaviour is stimulated by prolactin and possibly also by progesterone. This role of progesterone is mediated by progesterone receptors within the central nervous system, and these are dependent on oestrogen. In some species, the young hatch in an advanced state of development, capable of feeding for themselves. In such (precocial) species, parental prolactin levels decrease at hatch. In other species, the young are dependent on their parents to provide food. In these (altricial) species, parental prolactin remains high until the young become independent. In the pigeons and doves, the young are fed on a specialised secretion from the crop-sac (crop milk). This is analogous to lactation in mammals and, as in mammals, is dependent on prolactin.

Some species raise only one brood of young each year. In others, more than one brood is raised. In such species, once one brood is fledged, prolactin decreases permitting resumption of sexual behaviour and reproductive function, until another clutch of eggs is laid. At the end of the breeding season however, GnRH synthesis ceases and the whole reproductive system regresses.

Function of gonadal steroids

Androgens and oestrogens have a wide variety of morphological, physiological and behavioural roles, many of which have been mentioned already. They are involved in sexual differentiation, but their role depends on which sex is homogametic. In species where the female is heterogametic, oestrogens are crucial for female development. In species where the male is heterogametic, suppression of oestrogen synthesis is important, and early exposure to xeno-oestrogens could result in inappropriate development. Moreover, the P450 enzymes which metabolise steroid hormones differ between the sexes, and it is the steroid hormones themselves which induce the appropriate forms of enzymes for their own metabolism, during development (ref. 63). Early exposure to xeno-oestrogens could therefore affect steroid hormone profiles later in life as a consequence of effects on metabolism.

As well as primary sexual differentiation, the gonadal steroids are responsible for development of the appropriate accessory sex organs, brain differentiation and the secondary sexual characters. The secondary sexual characters are not directly involved in the formation or delivery of gametes, but may be important in pre-nuptial or nuptial events such as display or copulation. Some sexually dimorphic features, such as body size or plumage, are fully developed by puberty and then remain fixed. Others are renewed each breeding season.

In seasonally breeding vertebrates, the roles of gonadal steroids change seasonally. Circulating levels of steroids increase early in the breeding season in response to increasing gonadotrophin secretion, but they indirectly control their own secretion by modulating gonadotrophin secretion through actions at the level of the hypothalamus and the pituitary. A primary function of gonadal steroids is to provide the required hormonal environment for maturation of the gametes within the gonads. In addition, increased levels induce renewed hypertrophy of the accessory sex organs, the oviducts and sperm ducts, and the redevelopment of secondary sexual characters. Such characters may include colour change in fish, nuptial tubercles on the snouts of cyprinid fish, the development of nuptial pads and brightly coloured crests in amphibia, fan-like structures around the necks of some reptiles and bill colour in birds.

Behaviour

Successful reproduction often requires sexual behaviour. In fish this may be rudimentary, sufficient only to ensure juxtaposition of sperm and eggs. In higher vertebrates it is more involved. In birds, many months of complex behavioural interactions may be required before ovulation and copulation can occur. Sexual behaviour is dependent on gonadal steroid hormones in two ways. Firstly, sexual behaviour is dependent on sexual differentiation of the central nervous system early in development, and this is controlled by steroid hormones in a similar way to somatic differentiation, and secondly, sexual behaviour is directly stimulated by steroid hormones.

In mammals, sexual differentiation of the central nervous system is dependent on the actions of testosterone or its metabolites (DHT or oestradiol, depending on the tissue). In the absence of testosterone, feminine development occurs. For example, treatment of developing females with oestradiol masculinises neural differentiation. In birds, in which females are heterogametic and masculinity is the default situation, treatment of male embryos with oestrogen prevents them from fully developing male copulatory behaviour. Blocking the synthesis of oestrogens in female embryos causes them to develop male copulatory behaviour. However, development of the full behavioural repertoire may be more complex. For example, differentiation of the neural circuitry required for song production may be the reverse. Treatment of female embryos with testosterone or its metabolites masculinises the song system.

In adult birds, male behaviours, such as territoriality, aggression, display, song and copulation are stimulated by testosterone. They occur at the appropriate time of year because testosterone is elevated then. For example, song is controlled by specific vocal centres within the brain. The size and development of these ‘song centres’ changes seasonally in response to testosterone. However, such behaviours are not driven directly by testosterone; the testosterone has to be aromatised to oestradiol within the brain. Aromatase activity is intimately involved in reproductive behaviour, certainly in mammals and birds, and probably other vertebrates (ref. 64). In birds, the brightly coloured plumage of many male birds, which is often used in sexual display, is the result of steroids. Because female birds are homogametic, it is the drab female plumage which is imposed by oestrogens. Ovariectomy results in the

development of the bright male default plumage. Behaviour may also be affected by pheromones. Many mammals secrete pheromones, which stimulate or inhibit reproductive activity in conspecifics. Such secretions are steroid dependent. Fish also secrete pheromones (ref. 65), and in their case the pheromones themselves may be steroids (ref. 66).

Sexual behaviour culminates in the fertilisation of the eggs. In many species, particularly amongst lower vertebrates, this may represent the end of reproductive activity. In other species however, particularly amongst higher vertebrates, sophisticated parental behaviour may follow fertilisation to ensure that the offspring are successfully reared. A key hormone in parental behaviour is prolactin. During parental behaviour, further sexual behaviour and further production of gametes, is inappropriate. One important action of prolactin therefore is to suppress gonadal function and the synthesis of steroid hormones.

Vitellogenin

A major function of oestradiol is vitellogenin synthesis. In all non-mammalian vertebrates, nutrition for the developing embryo is provided by the mother in the form of yolk, the major constituent of which is vitellogenin. This is synthesised in the liver, following stimulation by oestradiol. In fish and reptiles, this is oestradiol-induction is potentiated by growth hormone, but not prolactin (ref. 67). In birds (ref. 68) and amphibians (ref. 69), there is evidence that growth hormone or prolactin can induce vitellogenin production in the absence of oestradiol. Oestradiol induces vitellogenin synthesis in both male and female livers when administered *in vivo*. Vitellogenin is transported in the blood to the ovary where gonadotrophin stimulates micropinocytosis of vitellogenin by oocytes. Circulating vitellogenin binds free calcium ions, resulting in elevated total plasma calcium levels through release of calcium from storage sites, and this elevated calcium is important for shell formation in reptiles and birds. Oestrogens also stimulate the secretion of albumin around the yolk of the egg, followed by the secretion of the shell.

CONCLUSIONS

Gonadal steroid hormones have a wide variety of roles throughout life, in all vertebrates, from the earliest stages of development to reproduction during adulthood. They are concerned with sex determination and with sexual differentiation during development of the primary and secondary sex organs, the secondary sexual characters and the central nervous system, and they induce the enzymes involved in their own metabolism. In adults, they are involved in the seasonal hypertrophy of the reproductive organs and secondary sexual characters, they drive sexual behaviour, provide the appropriate milieu for the gametes and regulate their own synthesis by negative feedback. Significant disturbance of steroid hormone profiles therefore has the potential to disrupt normal physiology at a number of levels. However, in adulthood, endogenous steroid levels are controlled by homeostatic mechanisms operating through effects on synthesis and metabolism, and the changes induced by steroids have a degree of plasticity. In contrast, organisms in the early stages of development have little control over their endogenous steroids, and the changes induced by steroids remain permanent. Clearly, the potential for endocrine disruption is greatest during the early stages of an animal's life history.

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