Pituitary multi-hormone cells in mammals and fish: history, origin, and roles

Romain Fontaine*, Muhammad Rahmad Royan, Christiaan Henkel, Kjetil Hodne, Eirill Ager-Wick, Finn-Arne Weltzien

Department of Preclinical Sciences and Pathology, Faculty of Veterinary Medicine, Norwegian University of Life Sciences, Ås, Norway

A R T I C L E   I N F O

Keywords: Pituitary Endocrine Hormone Plasticity Fish Teleost Mammals Multi-hormonal cell Proliferation Transdifferentiation Hypophysis

A B S T R A C T

The vertebrate pituitary is a dynamic organ, capable of adapting its hormone secretion to different physiological demands. In this context, endocrinologists have debated for the past 40 years if endocrine cells are mono- or multi-hormonal. Since its establishment, the dominant “one cell, one hormone” model has been continuously challenged. In mammals, the use of advanced multi-staining approaches, sensitive gene expression techniques, and the analysis of tumor tissues have helped to quickly demonstrate the existence of pituitary multi-hormone cells. In fishes however, only recent advances in imaging and transcriptomics have enabled the identification of such cells. In this review, we first describe the history of the discovery of cells producing multiple hormones in mammals and fishes. We discuss the technical limitations that have led to uncertainties and debates. Then, we present the current knowledge and hypotheses regarding their origin and biological role, which provides a comprehensive review of pituitary plasticity.

1. Introduction

The pituitary is a key endocrine gland located below the hypothalamic region of the brain. It plays a central role in the regulation of major physiological processes in all vertebrates, including growth, reproduction, stress, and homeostasis. Information is integrated and transmitted from the brain to peripheral organs by secreting a multitude of peptide hormones into the blood.

The pituitary develops from the ectodermal Rathke’s pouch (Schlosser, 2017), although in fish a small number of pituitary endocrine cells have been found with endodermal origins (Fabian et al., 2020). It is composed of two main parts: the anterior (adenohypophysis) and the posterior pituitary (neurohypophysis). These parts have different developmental origins and contain different cell types. The neurohypophysis originates from a down-growth of the diencephalon. In mammals, it is mainly composed of nerve terminals of oxytocin- and vasopressin-secreting neuroendocrine cells, originating from the preoptic-hypothalamic region of the brain. In teleosts, these are replaced by their homologues, isotocin and vasotocin (Kulczykowska, 2007). By contrast, the adenohypophysis originates from the ectodermal placodes at the anterior neural ridge which invaginates (forming Rathke’s pouch) and subsequently separates (Larkin and Ansorge, 2000). The adenohypophysis contains several endocrine cell types producing different peptide hormones and a few non-endocrine cell populations, including progenitor/stem cells, endothelial cells, and folliculo-stellate cells (FS-cells) (Denef, 2008; Gleiberman et al., 2008). The adenohypophysitis is histologically divided according to the size and shape of the cells and their nuclei, as well as the stainability of their secreting granules (Van Oordt and Peute, 1983). Three parts are identified: the pars intermedia (PI), the pars distalis (PD), and the pars tuberalis (PT), the latter present in mammals but not in teleosts (Fig. 1). In fishes, the PD is further divided in the rostral pars distalis (RDP, the most anterior part) and the proximal pars distalis (PPD, the median part of the pituitary).

Anterior pituitary hormones include two gonadotropins (follicle-stimulating- and luteinizing hormone, FSH and LH), thyrotropin (TSH), prolactin (PRL), and growth hormone (GH). They also include melanocyte-stimulating hormone (α-MSH) and adrenocorticotropin (ACTH), which are both encoded by the POMC gene. In addition, somatolactin (SL), a hormone closely related to GH and PRL, is produced in the fish pituitary but not in other vertebrate groups (Kaneko, 1996). LH, FSH and TSH are heterodimeric proteins. They are produced in the fish pituitary but not in other vertebrate groups (Kaneko, 1996). LH, FSH and TSH are heterodimeric proteins. They share one of their subunits, the alpha-subunit (Gp), and each have a specific subunit

* Corresponding authors.
E-mail addresses: romain.fontaine@nmbu.no (R. Fontaine), finn-arne.weltzien@nmbu.no (F.-A. Weltzien).

https://doi.org/10.1016/j.yfrne.2022.101018
Received 24 May 2022; Received in revised form 10 July 2022; Accepted 18 July 2022
Available online 20 July 2022
0091-3022/© 2022 The Author(s). Published by Elsevier Inc. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).
pitsuitary progenitor cells and the phenotypic plasticity of mature
certain number of questions regarding the differentiation mechanisms of
life of an animal and/or with pathological conditions. However, the
multi-hormone cells (i.e. whether they produce one or several hor-
important question of whether pituitary endocrine cells are mono- or
2.1. Mammals
Here, we review the studies in mammals and fish challenging the
uncertainties and debates. We also present the current hypotheses on the
in fact, studies on the origin of new endocrine cells have raised a
certain number of questions regarding the differentiation mechanisms of
pituitary progenitor cells and the phenotypic plasticity of mature endocrine cells. The first dogma was that pituitary endocrine cells were
one cell, one hormone”
However, over the last 40 years, several studies have challenged this concept. Here, we review the studies in mammals and fish challenging the important question of whether pituitary endocrine cells are mono- or multi-hormone cells (i.e. whether they produce one or several hormones). We discuss the technical limitations that have led to uncertainties and debates. We also present the current hypotheses on the origin and roles of multi-hormone cells which provides a comprehensive review of pituitary plasticity.

2. Historical discovery of the existence of multi-hormone cells

2.1. Mammals

Researchers started to challenge the mono-hormone cell concept four decades ago with the discovery that gonadotropes can produce both LH and FSH in mammals (Moriarty, 1975, 1976). Since then, it has become clearer that other pituitary endocrine cells can produce a combination of several hormones. Indeed, soon after, ACTH and FSH were found in the same cells in adult rat pituitaries (Moriarty and Garner, 1977). This was quickly followed by the demonstration of rat pituitary cells containing both gonadotropins (FSH and LH) together with ACTH, which appeared to be more abundant during development than in adults (Childs et al., 1982).

The investigation of pituitary tumors in humans contributed to gain knowledge regarding the multi-hormonal competence of pituitary endocrine cells. The morphological study of mixed GH/PRL human prolactinoma revealed the existence of mammosomatotropes (also named lactosomatotropes or somatolactotropes), producing both GH and PRL (Horvath et al., 1983). Similarly, in pituitary adenomas from patients with active acromegaly, GH was found with Gp (the subunit common to LH, FSH and TSH) (Beck-Peccoz et al., 1985). In human pituitary prolactinoma, PRL was observed together with both gonadotropins and FSH alone, and also in cells producing ACTH (Newman et al. 1989). While tumors are known to induce modifications in the cell program that can easily change the ability of the cell to produce more than one hormone, some of these findings were later confirmed in nontumorous tissues. For instance, following their discovery in neoplastic tissues, mammosomatotropes were described in dissociated cells from the normal rat pituitary (Frawley et al., 1985) and then observed in vivo (Lloyd et al., 1987) as reviewed by (Frawley and Boockfor, 1991). These observations suggest that bi-hormone cells are part of the set of tools that the normal pituitary uses to adjust hormone production according to the demand.

While most of these findings were based on single immune labeling on serial sections or combining the labeling with morphometric markers, the development of dual staining techniques has enabled the identification of more hormone combinations. TSH was found with PRL in cells from rat pituitaries (Losinski et al., 1989). A small fraction of pituitary cells contained both ACTH and TSH in rat pituitary cell cultures (Childs et al., 1989). GH was also found with TSH in pituitary cells from rats treated with propyl-thiouracil (PTU, a medication to treat hyperthyroidism) (Horvath et al., 1990). The combination of in situ hybridization for Lhb or Fshb with immunocytochemistry for GH revealed the existence of GH/Lhb and GH/Fshb cells in the rat pituitary (Childs et al., 1994). However, co-labeling with immunostaining could in theory result from internalization of one or two hormones, and thus does not prove hormone synthesis as suggested previously (for review (Childs, 1991)). Internalization of ligand-receptor complexes via endocytosis (Pastan and Willingham, 1981) has also been shown to occur for

Fig. 1. Schema of a parasagittal section from the adult pituitary in rodents (mammals) and teleosts, showing the organization.
peptide hormone receptor complexes (Segaloff and Ascoli, 1988). This is, for instance, the case in rat gonadotropes for gonadotropin-releasing hormone (GnRH, produced in the hypothalamus) and its receptors (Schwartz and Hazum, 1987). Some pituitary cells express receptors for other pituitary hormones (e.g. LH receptors in FSH cells in the mice pituitary (Wen et al., 2010)). Therefore, the detection of internalized pituitary hormones in pituitary cells by immunostaining, which could lead to false identification of multi-hormone cells, cannot be completely excluded. Caution should also be taken when investigating mRNA transcripts as hormone-encoding mRNA are frequently observed in cells without the associated proteins (reviewed in Seuntjens et al., 2002a), suggesting strong post-transcriptional control of hormone production.

The discovery of multi-hormone cells has often been questioned due to technical flaws, such as the possible cross reaction between antibodies. Indeed, certain hormones are highly similar as shown by the cross reaction between PRL and GH (Nicoll et al., 1986). The fact that TSH, FSH and LH share an alpha subunit also resulted in complications. These similarities between proteins made the production of specific antibodies and labeling difficult. However, other techniques have enabled the confirmation of these findings, and further investigation of multi-hormone cells. These techniques include the detection of the expression of multiple hormone-encoding genes within the same cell. For instance, RT-PCR has allowed detection of multiple hormone-encoding transcripts in a single cell with high sensitivity (Kumazaki et al., 1994). Fluorescence-activated cell sorting (FACS) has been used to separate individual cells, allowing for lysis of and cDNA synthesis from single cells (Gaynor et al., 1996). Cytoplasm collection from single cells was made possible using the patch clamp technique, in which a seal is made between the cell membrane and a glass pipette before the cytoplasm is aspirated and released in a separate tube for cDNA synthesis and subsequent gene expression analysis (Hodne et al., 2010; Chiang, 1998; Dixon et al., 2000). Using these methods, pituitary cells expressing two, three, and even more pituitary hormone mRNAs in various combinations were found in the rat (Roudabehi et al., 1999) and mouse pituitary (Seuntjens et al., 2002b). These include cells co-expressing Gh/Prl/Tshb, Gh/Prl/Tshb/Lhb, Gh/Prl/Tshb/Pomc or Gh/Prl/Tshb/Pomc/Lhb (for review, (Seuntjens et al., 2002a) and (Hauspie et al., 2003)). However, single-cell expression techniques have also raised questions as contamination can result in false positives (Hodne et al., 2010). Indeed, because as much as 30% of the mRNA content in a hormone-producing pituitary cell codes for a particular hormone (Ager-Wick et al., 2013), we found that relatively rough treatment during a tissue dissociation process results in occasional acute lysis of cells, with the consequence that the mRNA from the lysed cells contaminates the sampled cells.

Transgenic lines where fluorescent reporter proteins are produced in specific cells have also been used to investigate the existence of multi-hormone cells in the pituitary. For instance, a mouse transgenic line expressing the yellow fluorescent protein in lactotropes has been used to study the appearance of mammosomatotropes during pregnancy and lactation (Castriaco et al., 2010). This study reported almost no mammosomatotropes in the mouse pituitary during these life stages. This contrasts with a previous study in the rat pituitary (Frawley et al., 1985). While these inconsistent findings may reflect differences between rats and mice, they could also reveal differences in the specificity of the technique used. Indeed, identification of hormone producing cells by immunolabeling is dependent on hormone quantity and synthesis. In contrast, the use of well-validated transgenic lines with fluorescent reporter proteins increases the reliability of identification of such cells. However, certain of these lines sometimes provide an incomplete labeling (not all cells are fluorescent). Thus, some limitations in result interpretation may arise because the labeled cells do not always represent the whole population (Castriaco et al., 2010).

Today multi-hormone cells are still investigated in mammals using different techniques. For instance, a recent study in the normal adult human pituitary confirmed the presence of cells co-producing two hormones (PRL/ACTH; PRL/TSH; PRL/FSH; PRL/LH; GH/ACTH; GH/TSH; GH/FSH; GH/LH, and TSH/ACTH; TSH/FSH; TSH/LH) using multiple combinations of double immunolabeling (Mitrofanova et al., 2017). Most recently, the development of single cell transcriptomics has allowed for the identification of a Pou1f1(Pit1)-expressing cell population characterized by a unique multi-hormonal gene expression profile including the expression of Prl, Gh, Tshb, Fshb, Lhb and Pomc in the adult mouse pituitary (Ho et al., 2020). Interestingly, the authors report that the level of expression for the hormone-encoding genes are as high in the multi-hormone cluster as in their respective mono-hormone clusters. These observations clearly suggest the existence of multi-hormone cells in the mice pituitary. However, a previous study using the same approach did not reveal the existence of such a multi-hormonal cluster in the male mice pituitary (Cheung et al., 2018). In addition, such cells were reported neither in the rat (Fletcher et al., 2019) nor human (Zhang et al., 2020) pituitary. In addition, Pou1f1 (Pit1) is a known transcription factor for GH, PRL and TSH cells but not for LH, FSH, ACTH and MSH cells (for review, see (Tahara et al., 2006; Sanno et al., 2001; Kelberman et al., 2009b; Larkin and Anson, 2000)). While the existence of bi- and tri-hormone producing cells have been clearly validated in mammals, whether this multi-hormonal cluster is species-specific, or an artefact (maybe due to the high background induced by the very high level of expression of all hormone-encoding genes) remains to be seen. This could be determined by obtaining more pituitary single cell transcriptomes and further analysis of the existing ones.

### 2.2. Teleost fish

The earliest work on teleost pituitary cells dates back to 1908, when Herring (Herring, 1908) conducted a histological study on Atlantic cod (Gadus morhua) and presented its pituitary organization with some basic chemical cell characteristics. Two decades later, histophysiological studies characterized the granularity of stickleback pituitary cells (Bock, 1928). Three decades later, Legait (Legait and Legait, 1958) described the ultrastructure of glandular cells in carp and trout pituitaries, and that was followed by several immuno-physiological studies that identified endocrine cell types in the pituitary of many teleost species.

During the early stage of research, at least six different endocrine cell types were identified in the teleost pituitary: lactotropes (Prl), corticotropes (Acth), somatotropes (Gh), thyrotropes (Tsh), gonadotropes (Fsh and Lh), and melanotropes (Msh) (Nagahama, 1973). Somatolactotropes (Sl), which are found only in fish, were discovered much later (Kaneko, 1996; Schreibman et al., 2015). The first experiments to determine the hormones produced by teleost pituitary cells led to the assumption that each cell produces one specific hormone in fish. Today, this is still commonly accepted in teleosts. Indeed, the pituitary endocrine cell types in teleosts have been mainly described in distinct areas, leading to the concept that they are arranged in zones (Ball and Baker, 1969; Schreibman et al., 2015) through the entire lifespan in fishes (Weltzien et al., 2004; Pogoda and Hammerschmidt, 2007). This is somewhat different from mammals. Indeed, in these animals the endocrine cell populations are spatially discrete during embryogenesis and more mosaically distributed (Voss and Rosenfeld, 1992) but organized into structural and functional networks in adults (Le Tissier et al., 2012; Santiago-Andres et al., 2021). Based on the zonation, lactotropes and corticotropes are localized in the RPD, while gonadotropes, somatotropes, and thyrotropes are found in the PPD. In the PI, somatolactotropes are found alongside melanotropes (Fig. 2). This pattern has been observed in all teleost species investigated so far (Table 1) and can be appreciated in the online 3D atlases of the medaka pituitary that we recently developed (Royer et al., 2021).

Although gonadotropes are recognized as one cell type in teleost fish species as in mammals, findings have shown that these cells are divided into two distinct populations responsible for Fsh and Lh production. Indeed, since the findings from Olivereau and colleagues (Olivereau, 1976) in which two different gonadotrope cell populations were
observed in rainbow and brown trout, many studies have reported this duality in other teleost species, such as Atlantic croaker (Copeland and Thomas, 1993), gilthead seabream (Elizur et al., 1995), Mediterranean yellowtail (García-Hernández et al., 1997), bonito (Koide et al., 1993), tuna (Okada et al., 1994), chum salmon (Suzuki et al., 1988), red seabream (Tanaka et al., 1993), common carp (Van Der Kraak et al., 1992), rainbow trout (Naito et al., 1991), and Atlantic salmon (Nozaki et al., 1990). While the duality of gonadotrope cells in the teleost pituitary appears to confirm that one endocrine cell type is indeed responsible for producing one hormone, some gonadotrope cells producing both Fsh and Lh were found in Mediterranean yellowtail (García-Hernández et al., 2002) and cells expressing both fshb and lhb were observed in the pituitary of zebrafish (Golan et al., 2014), European hake (Candelma et al., 2017), and Japanese medaka (Fontaine et al., 2020a; Royan et al., 2021). These observations suggest that some gonadotropes can produce both gonadotropins in teleosts.

A number of studies have also shown co-staining between Prl, Gh and Sl (Honji et al., 2013; Parhar et al., 1998; Naito et al., 1983; Weltzien et al., 2003; García-Hernández et al., 1996), and between Sl and Lh (Batten et al., 1975; Batten, 1986; Margolis-Kazan et al., 1981; Camacho et al., 2020). These observations could suggest the existence of multi-hormone cells in the fish pituitary. However, to a higher extent than in mammals, questions have been raised as to whether the so-called bi-hormone cells are truly cells producing more than one hormone as several studies used non-species-specific antibodies which might have caused cross-reactivity. For instance, cross-reactivity of antibodies against antigens in both thyrotropes and gonadotropes (Batten, 1986; Quesada et al., 1988; Yan and Thomas, 1991) occurred likely due to the glycoprotein-α subunit that these proteins share.

Nevertheless, other techniques looking at transcript location, which use specific probes, succeeded in demonstrating the existence of bi-hormone cells in the teleost pituitary. These observations suggest that some gonadotropes can produce both gonadotropins in teleosts.

| Species                                | Reference                                      |
|----------------------------------------|------------------------------------------------|
| White seabream (Diplodus sargus)       | Segura-Noguera et al., 2000                    |
| Japanese medaka (Oryzias latipes)      | Aoki and Umeura, 1970; Royan et al., 2021      |
| Nile tilapia (Oreochromis niloticus)   | Kasper et al., 2006                           |
| Greater weever fish (Trachinus draco)  | Sánchez Cáls et al., 2003                     |
| Cichlasoma dimerus (Percomorm, Cichlidae) | Pandolfi et al., 2009                        |
| Fourspine sculpin (Cottus kisku)       | Mukai and Oota, 1995                         |
| Atlantic croaker (Micropogonias undulatus) | Yan and Thomas, 1991                      |
| Spotted seatrout (Cynoscion nebulosus)  | Yan and Thomas, 1991                         |
| Red drum (Sciaenops ocellatus)         | Yan and Thomas, 1991                         |
| Cardinal tetra (Parachthodon axelrodi) | Camacho et al., 2020                         |
| Bloodfin tetra (Aphyocharax anisitsi)  | Camacho et al., 2020                         |
| Gilt-head seabream (Sparus aurata)     | Quesada et al., 1988                         |
| Atlantic halibut (Hippoglossus)        | Weltzien et al., 2003                        |
| hippocoglossus                         |                                                |
| Sailfin molly (Poecilia latipinna)     | Batten et al., 1975; Batten, 1986             |
| Sadle zeane (Thalassoma duperrey)      | Parhar et al., 1998                          |
| Mediterranean yellowtail (Seriola dumerilii) | García-Hernández et al., 1996, 2002          |

Fig. 2. Schematic illustration of the distribution of endocrine cell populations in a midsagittal section from a teleost pituitary. Adapted from Royan et al. (2021)
prl (Ager-Wick, 2014). These results suggest that gonadotropes may produce other hormones. However, the observations made from FACSorted cells could not be supported by the scRNA-seq approach. Indeed, no cluster of multi-hormone cells was described in zebrafish or Japanese medaka (Fabian et al., 2020; Siddique et al., 2021). In medaka, we mostly found bi-hormone cells (the majority being cells co-expressing tshb/lhb, fshb/tshb, and lhb/fshb) and only very few cells expressing three hormone-encoding genes (mostly a combination of lhb/fshb/tshb) (Royan et al., 2021). Therefore, the recent findings suggest that cells expressing many hormone-encoding genes do not exist in the teleost pituitary. However, this should be investigated further considering the high diversity among teleosts. The pituitary single cell transcriptome should be obtained from more species; more accessible scRNA-seq, as well as the novel spatial transcriptomic technique, will certainly help achieve this aim.

3. Origin of multi-hormone cells

Although multi-hormone cells are difficult to study, several studies have started to provide clues on their origin and biological role. The processes by which they arise are not fully determined as several mechanisms seem to co-exist. These include: (1) the differentiation of existing immature progenitor cells with multipotent properties (able to differentiate into cells of various types). Along their differentiation, it is thought that progenitor cells might enter a temporary stage in which they are undecided, thus producing more than one hormone-encoding mRNA at the same time (Fauquier et al., 2008; Chen et al., 2013b).

(2) the transdifferentiation (also termed phenotypic conversion) of existing mature hormone-producing cells from one cell type to another (Horvath et al., 1990). This phenomenon could involve the existence of a transitional cell stage in which the cell produces the two hormones. (3) that certain cells have a plastic multi-hormonal phenotype. Often dormant, their transcriptional activity can be activated by neuroendocrine or endocrine signals, allowing the production of one or several hormones according to the biological need without really changing identity. This would, in theory, allow them to respond to multiple signals received simultaneously, and potentially produce several hormones at the same time (Fig. 3).

3.1. Differentiating multipotent progenitor

In mammals, the pituitary cells are classified into three cell lineages: the GH/PRL/TSH cell lineage, the ACTH/MSH lineage and the LH/FSH lineage (Fig. 4B). Several mammalian studies have shown that multipotent stem cells within the pituitary can differentiate into cells of various types (for review (Andoniadou, 2016)), including all hormone-producing cells (for review (Camilletti et al., 2021)). These multipotent stem cells generate new cells in the developing postnatal pituitary, while mostly remaining quiescent in homeostatic conditions in adulthood (Cox et al., 2017). However, they show signs of activation during processes of increased cell remodeling in the gland, including maturation at neonatal age, adaptation to physiological demands, and regeneration upon injury, as well as in pathological conditions (e.g. tumors) (Cox et al., 2017).

**Fig. 3.** Potential origin of multi-hormone cells: (A) differentiation of multipotent immature progenitor cells, (B) transdifferentiation of existing mature hormone-producing cells (phenotypic transformation from one differentiated endocrine cell type to another) (C) permanent multi-hormone cells with a plastic phenotype, adapting hormone production according to input signals. They often express several receptors for different neuroendocrine factors and can thus respond to different stimuli (they are named multi-responsive cells).
Pomc gene expression in corticotropes and melanotropes. Pit1-dependent lineage includes thyrotropes, somatotropes and lactotropes, while SF1 and GATA2

The multipotent progenitor cell marker SOX2 (Fauquier et al., 2008; Kelberman et al., 2009a) was used to locate multipotent progenitor cells in mammals. In the adult mouse (Fauquier et al., 2008) and rat (Chen et al., 2013a) pituitary, SOX2-positive cells are mostly located at the border of the neurohypophysis and adenohypophysis with a few cells distributed in the adenohypophysis (Fig. 4A). In teleosts, we described Sox2-immunoreactive cells in the medaka pituitary (Fontaine et al., 2019). Interestingly, they are located in the dorsal part of the pituitary at the junction between the neurohypophysis and adenohypophysis with few cells spread in the PPD, as in mammals. Other groups have described Sox2 cells in the fish brain (Okuda et al., 2006; Alunni et al., 2010; Germana et al., 2011; Diotel et al., 2013) and retina (Lust and Wittbrodt, 2018), sometimes linked to other pluripotency-associated markers and proliferative capacities. However, the clear multipotency competence of pituitary Sox2 cells remains to be demonstrated in fish. Cell lineage experiments are thus needed to determine whether pituitary stem cells are multipotent in teleosts and to identify the transcription factors necessary for pituitary progenitor cell differentiation, which remains mainly unknown in teleosts (for review (Weltzien et al., 2014).

Whether bi- or multi-hormone cells are really differentiating progenitors remains uncertain for now. As the pool of endocrine pituitary cells constantly replenishes, intermediate cell forms are constantly present in the anterior pituitary. Some research groups proposed that, during their differentiation, progenitor cells might enter a temporary stage in which they are still undetermined, thus producing more than one hormone-encoding mRNA at the same time (Fauquier et al., 2008; Chen et al., 2013b) (Fig. 3A). This hypothesis is supported by the frequent observation of cells with mRNA encoding multiple hormones, but lacking the proteins (reviewed in (Seuntjens et al., 2002a)). This could be supported by a recent single cell transcriptomic work in the adult mouse pituitary demonstrating the existence of a cell cluster expressing all hormone-encoding genes (Ho et al., 2020). However, as discussed above, such a population has not been reported in any other species studied and might be the result of the high background induced by the high expression of hormone-encoding genes. Additionally, in this study, the authors found Pomc (which encodes ACTH and MSH) and Pouf1 (also named Pit1), which are believed to belong to two different cell lineages, expressed in the same cells (Fig. 4B). Lineage studies in pituitary organoids, also named pituispheres (Fauquier et al., 2008; Cox et al., 2019; Zhou et al., 2022), will certainly be important tools in understanding the differentiation of progenitor cells and allow us to investigate whether some pituitary progenitor cells are multihormonal.

The role of these adult progenitor cells is also under investigation. Gonadectomy and adrenalectomy were shown to increase mitotic activity in pituitary non-endocrine cells, leading to an increase of gonadotropes and corticotropes (Nolan and Levy, 2006b; Ibrahim et al., 1986). It was later shown that part of these activated mitotic cells were in fact SOX2-progenitor cells (Rizzoti et al., 2013). The other mitotic cells appeared to be the gonadotropes and corticotropes, although they proliferate at a rate much lower than that of the SOX2-progenitor cells (Rizzoti et al., 2013; Nolan and Levy, 2006b). Therefore, adult pituitary stem cells seem to make a significant contribution to the pool of new endocrine cells generated in response to physiological demand following gonadectomy or adrenal ablation. Similarly, SOX2-progenitor cells showed increased mitotic activity following ablation of GH cells in the mouse pituitary, suggesting a role of stem cells in regenerative competence of the pituitary (Fu and Vankelecom, 2012). However, another study in adult mice showed that depletion of SOX2-progenitor cells does not affect adult endocrine cell homeostasis and remodeling (Roose et al., 2017). Another study in the adult mouse pituitary showed that ablation

Fig. 4. A. Location of stem (SOX2/Sox2-positive) cells in parasagittal sections of adult pituitaries from mammals (top) and fish (bottom). Anterior to the left and dorsal to the top. B. Pituitary cell lineage in mammals. The development and determination of pituitary precursors into mature secretory cells results from a temporally-regulated cascade of homeodomain transcription factors of which several have been identified. These include among others, Tpit, Ptx1, Prop1, Pit1, SF1 (also named Nr5a1), NeuroD1, GATA2 (for review, see (Tahara et al., 2000; Sanno et al., 2001; Kelberman et al., 2009b; Larkin and Ansorge, 2000; Zhu et al., 2007; Davis et al., 2016)). All endocrine cells of the intermediate and anterior lobe originate from Prop1 positive progenitors (Davis et al., 2016). NeuroD1 and T-pit regulate POMC gene expression in corticotropes and melanotropes. Pit1-dependent lineage includes thyrotropes, somatotropes and lactotropes, while SF1 and GATA2 are needed for gonadotropes to differentiate. Finally, GATA2 is essential for the differentiation of TSH cells but not GH or PRL cells in the Pit1 lineage. In fish, only a limited number of studies were performed, but they suggest that the differentiation path of fish pituitary endocrine cells might be highly comparable to mammals. This is, for instance, supported by medaka FACS-sorted Lh gonadotropes, in which a high correlation between lhb expression and Sf1 (nr5a1) was observed (Ager-Wick, 2014).
of dividing corticotropes led to a reduction of the corticotrope population, suggesting that adult progenitor cells do not participate in proliferation of corticotropes in adult animals (Langlais et al., 2013), and thus might not play a major role in pituitary plasticity. The involvement of adult progenitor cells and whether they are multi-hormonal at some point of their differentiation path thus remains to be demonstrated. In contrast, studies in mammals support the hypothesis that during early development progenitor cells can become multi-hormones. This is, for instance, supported by the observation of bi-hormonal FSH/FSH cells in mice, with one subgroup expressing steroidogenic factor 1 (SF1, a transcriptional factor for the gonadotrope lineage (Childs and Neill, 2006)), suggesting that SF1-expressing cells will develop into gonadotropes whereas the others will become thyrotropes (Wen et al., 2010).

While in mammals, detection of hormones belonging to different lineages within the same cells has been observed in multiple studies and in several species (see part 2.1), this seems to be more limited in fishes. Studies reported the presence of corticotropes and gonadotropes, which belong respectively to the TPIT and Pit1 lineages in mammals, in the same area within the PPD of several teleost species (Garcia-Ayala et al., 1997; Quezada et al., 1988; Margolis-Kazan et al., 1981). However, no study reported double labeling for Acth and Fsh or Lh so far. Our work in the medaka pituitary only identified very few cells co-expressing pomc with lhb or fshb in the scRNA-seq data from the male fish (not observed in the female) and they could not be observed in the tissue using multicolor fluorescent in situ hybridization (Royan et al., 2021). In contrast, we demonstrated the presence of thyrogonadotropes in the teleost pituitary using both in situ hybridization and scRNA-seq (Royan et al., 2021). As gonadotropes and thyrotropes both depend on GATA2 transcription factor in mammals (Charles et al., 2006; Dasen et al., 1999), these observations suggest that some fish cells can also express multiple hormones if they belong to the same lineage.

In mice, LH-expressing cells appears slightly before FSH-expressing cells during development (Japón et al., 1994), suggesting that gonadotropes first appear as mono-hormone cells. Soon after, when FSH is detected, it is mostly located in the same cells as LH, suggesting that mammalian gonadotropes quickly become bi-hormonal, even if the authors do not exclude that mono-hormonal gonadotropes continue to exist. Similarly, we demonstrated that early during development in medaka, gonadotropes arise as either Lh or Fsh cells (Fontaine et al., 2020a). These observations support the idea that among the gonadotropic adenomas (Ikeda et al., 1995; Egensperger et al., 2001), suggesting species dependency or differences in technique specificity. Somatotropes where also shown to transdifferentiate into thyrotropes in hypothryoids (Horvath et al., 1999) and humans (Vidal et al., 2000a; Alunni et al., 2010; Horvath et al., 1999; Childs et al., 1981; Vidal et al., 2000b; Radian et al., 2003). All three above-mentioned cell types belong to the Pit1 derived cell lineage (Sornsom et al., 1996). Cells belonging to different lineages have also been shown to transdifferentiate in mammals. For instance, GnRH induces the transdifferentiation of somatotropes (Pit1-derived cell lineage) into somatogonadotropes (Slf1-derived cell lineage for gonadotropes) in rat pituitary cell culture (Childs and Unabia, 1997). Transdifferentiation of gonadotropes into corticotropes (Tpit/NeuroD1-derived cell lineage) was described to occur rarely in gonadotropic adenomas (Ikeda et al., 1995; Egensperger et al., 2001), and can be induced by diethylstilbestrol treatment (a nonsteroidal estrogen medication used, for instance, in the treatment of menopausal and postmenopausal disorders) in male mice (Shukwu et al., 2006). Such phenomena have never been described in teleosts but that may be due to a lack of tools to investigate these processes in fish. However, we recently reported cells expressing hormone-encoding genes belonging to different lineages in adult medaka (sl with lhb, prl with pomc) (Royan et al., 2021), suggesting that this remains a possibility in fish also.

While many of the studies investigating this phenomenon have used immunostaining approaches and reverse hemolytic plaque assays, performed in fetal and neonatal life stages or following ablation techniques (Frawley and Boockfor, 1999), the use and development of transgenic lines with fluorescent reporter proteins expressed in endocrine cells have also helped in understanding the role transdifferentiation plays in specific physiological stages (Castrique et al., 2010). These lines are powerful as they allow for the study of the dynamic of a hormone production. Nevertheless, while such lines have been widely used to study brain cells, they have been used much less to study pituitary endocrine cell plasticity. We believe that together with pituitary organoids and time series transcriptomics, such lines would be useful tools to further investigate the phenotypic conversion of endocrine cells both in mammals and fish.

### 3.2. Transdifferentiation (or phenotypic conversion) of pituitary endocrine cells

Transdifferentiation, the phenotypic transformation from one differentiated endocrine cell type to another (Horvath et al., 1990) (Fig. 3B), is also thought to lead to a cell stage in which both hormones could be expressed together during a transitory period. In several cases, pituitary cells have indeed been observed to change phenotype, thereby changing their secretory capacity and becoming at least temporarily multi-hormonal.

In most cases, observations of transdifferentiation have been between cell types belonging to the same lineage. This may be because they share an important part of their differentiation pathway, and a phenotypic change could thus be achieved by a change in only a few transcription factors. For instance, gonadotropes in mammals can change between the typical bi-hormonal phenotype (producing both FSH and LH) and a mono-hormonal phenotype as shown in sheep, where bi-hormonal gonadotropes can switch to a LH-only phenotype (Taragnat et al., 1998). In contrast, mono-hormonal FSH cells may switch to the bi-hormonal phenotype during puberty in the rhesus monkey (Meeran et al., 2003). This is also the case in teleosts as we demonstrated in medaka, where Fsh cells produced lhb in vivo (Fontaine et al., 2020a), a phenomenon that could be enhanced by GnRH stimulation. Thus, we speculate that the presence of bi-hormonal gonadotrope cells in the pituitary of several teleost species (Golan et al., 2014; Candelma et al., 2017; Fontaine et al., 2020b; Royan et al., 2021) might be due to the phenotypic plasticity of gonadotropes.

More complex, but still cell lineage specific (Borrelli et al., 1989), somatotropes have been found to reversibly transdifferentiate into lactotropes in rats (Porter et al., 1991; Inoue and Sakai, 1991; Kakeya et al., 2000; Vidal et al., 2001). Interestingly, a study in mice showed that this phenomenon was rather rare in these animals (Castrique et al., 2010), suggesting species dependency or differences in technique specificity. Somatotropes where also shown to transdifferentiate into thyrotropes in hypothryoids (Horvath et al., 1999) and humans (Vidal et al., 2000a; Alunni et al., 2010; Horvath et al., 1999; Childs et al., 1981; Vidal et al., 2000b; Radian et al., 2003). All three above-mentioned cell types belong to the Pit1 derived cell lineage (Sornsom et al., 1996). Cells belonging to different lineages have also been shown to transdifferentiate in mammals. For instance, GnRH induces the transdifferentiation of somatotropes (Pit1-derived cell lineage) into somatogonadotropes (Slf1-derived cell lineage for gonadotropes) in rat pituitary cell culture (Childs and Unabia, 1997). Transdifferentiation of gonadotropes into corticotropes (Tpit/NeuroD1-derived cell lineage) was described to occur rarely in gonadotropic adenomas (Ikeda et al., 1995; Egensperger et al., 2001), and can be induced by diethylstilbestrol treatment (a nonsteroidal estrogen medication used, for instance, in the treatment of menopausal and postmenopausal disorders) in male mice (Shukwu et al., 2006). Such phenomena have never been described in teleosts but that may be due to a lack of tools to investigate these processes in fish. However, we recently reported cells expressing hormone-encoding genes belonging to different lineages in adult medaka (sl with lhb, prl with pomc) (Royan et al., 2021), suggesting that this remains a possibility in fish also.

### 3.3. Plastic multi-hormone-multi-responsive cells

Another hypothesis, which is difficult to distinguish from the transdifferentiation process, is that some pituitary cells differentiate into permanent multi-hormone cells, which can regulate hormone production according to demand (Fig. 3C). This hypothesis does not really
agree with the cell lineage concept. However, studies in the rodent anterior pituitary identified differentiated pituitary cells that could respond to multiple neuroendocrine factors that are known to regulate different cell types (named paradoxical secretion). Indeed, a large population of endocrine cells was shown to possess multiple hypothalamic releasing hormone receptors in the rodent pituitary (Kashara et al., 1994; Villalobos et al., 1997). For instance, GHRH receptors were found in gonadotropes (Childs et al., 1999), and GnRH receptors are transiently expressed in somatotropes allowing GH secretion by GnRH stimulation (Childs, 2000). Stimulation with different neuroendocrine factors also evoked PRL paradoxical secretion (Villalobos et al., 1997; Villalobos et al., 2004b). These cells were therefore named multi-responsive cells. Multi-hormone and multi-responsive cells were identified within all six anterior pituitary endocrine cell types in dispersed mice pituitary cell cultures (Nunez et al., 2003). While paradoxical secretion is common in cells from pituitary tumors (Matsukura et al., 1977; Marinis et al., 1990; Barlier et al., 1997), they have also been reported in cells from normal pituitaries, both in vivo and in vitro (Amsterdam et al., 1982; Harvey, 1990).

These multi-responsive cells thus have the capacity to contribute to the functional plasticity of the pituitary by the generation of several hormones, adapting the production of each according to the demand. However, their exact role in the plastic response of the pituitary remains unclear. Indeed, hormone production in response to thyrotropin-releasing hormone was much weaker in multi-hormonal than in mono-hormonal thyrotropes, suggesting a limitation in the role of paradoxical secretion under physiological conditions (Villalobos et al., 2004a). In teleosts, proofs are lacking regarding the existence of such permanent multi-hormonal/multi-responsive pituitary cells. In a study with juvenile African catfish, Lh gonadotrope number increased in the steroid-treated group compared to control, despite no observed proliferation of Lh gonadotropes (Cavaco et al., 2001). The authors therefore speculated that quiescent gonadotropes might play role in increasing the number of Lh cells. Whether these were dormant Lh cells only, or endocrine cells already producing other types of hormones is not known, but these observations open up the possibility that such phenomena could also exist in fish.

Yet, technical limitations might affect our interpretation of pituitary endocrine cell plasticity events. We demonstrated that medaka pituitary endocrine cells can change phenotype following dissociation (Fontaine et al., 2020a). While this work demonstrates the plastic properties of pituitary gonadotrope cells in fish, it also revealed that some observations made in vitro may not be physiologically relevant. In this study, Fsh cells in vitro did not respond to GnRh stimuli 24 h following dissociation, while they did 48 h after dissociation. We also showed that Fsh cells do not express GnRh receptors in vivo, supporting the absence of response observed in the first place. We therefore believe that by dissociating the cells, an unknown factor was removed, allowing the Fsh cells to start producing GnRh receptors later, which then allowed them to respond to GnRh stimuli and produce Lh. Thus, the change in environment changed the cell phenotype, which might or might not occur in vivo. Therefore, more studies are needed to determine the in vivo capacity of pituitary endocrine cells to change phenotype or to produce different hormones. The combination of the recent imaging approaches to study live in vivo pituitary cells (Hoa et al., 2019) and the use of transgenic lines expressing different fluorescent reporter proteins for hormone-encoding genes would greatly help to answer these questions.

### 4. Possible biological role of this plasticity and of multi-hormone cells

The production or secretion of two hormones or more by a cell is often related to particular physiological or pathological conditions for which the production of a significant amount of a specific hormone is needed rapidly. For instance, the percentage of bi-hormonal gonadotrope cells increases during the pre-ovulatory period in ewe (Molter-Gerard et al., 2000) and in rats (for review, (Childs, 1995)), when large amounts of gonadotropins are needed. Hypothyroidy induces the transdifferentiation of somatotropes into thyrotropes in humans (Vidal et al., 2000a). Somatotropes were described to become lactotropes during pregnancy and lactation in rats (Porter et al., 1990) and humans (Stefaneanu et al., 1992). The number of cells producing LH and FSH together with GH increases in female rats during proestrus, possibly to assist gonadal development (Childs et al., 1994; Childs et al., 2000). Indeed, estrogens (Porter et al., 1992) and GHRH (Stefaneanu et al., 1998) stimulate the transdifferentiation of somatotropes into mammomammatropes. Another example is the number of cells co-producing TSH and ACTH which increases when rat pituitary cells are stimulated with arginine vasopressin (Childs et al., 1989), and in vivo when exposing the animals to a cold stress condition (Childs, 1992).

In fish, the role of these multi-hormone cells remains unknown. The lack of multi-labeling studies throughout the life cycle of the fish, prevents associations of the presence of such cells with any particular physiological status. Studies investigating the effect of a quick change in physiological condition on pituitary endocrine cell population and multi-hormone cells would certainly help determine the role this multi-hormone cell plasticity plays in fish. This could be achieved, for instance, by modifying important environmental factors (e.g. photoperiod, which has been shown to control the reproductive status in medaka (Koger et al., 1999)) or by removing other endocrine organs (e.g. gonadectomy which suddenly removes sex steroid feedback (Royan et al., 2020)). These should induce a rapid change in hormone production and thus potentially increase the number of these multi-hormone gonadotropes in fish.

While the pituitary needs to frequently adapt its hormone production and thus the number of cells producing a hormone, producing new cells every time they are needed would be time and energy consuming for the tissue. Additionally, if the pituitary produced new cells every time they are required, it follows that these cells would also be removed when their presence is not required anymore. However, a significant rate of apoptosis has never been reported in the pituitary in any normal physiological conditions. Therefore, phenotypic plasticity appears to be a good alternative for efficient adaptation of pituitary hormone production. Indeed, phenotypic conversion or recruitment of dormant cells allows, with a lower energy cost, to quickly adapt the amount of a specific hormone’s production.

However, some limitations may apply. If the cellular signaling pathways for two or more neuroendocrine signals share many molecular components, activation by one of them might trigger the production of the wrong hormone. In the human pituitary, PRL and ACTH were found in different secretory granules within the same cells (Newman et al., 1989). Also, in rat pituitary bi-hormonal GH/TSH cells, GH was found in secretory granules while TSH was in the diluted rough endoplasmic reticulum (Horvath et al., 1990), suggesting that the two hormones have different secretion mechanisms and that the cells might not release the two hormones simultaneously. In contrast, GH and PRL were found within the same secretory granules in human mammomammatropes (Bassetti et al., 1986), suggesting shared secretory mechanisms between these two hormones. This is also the case in the human pituitary where GH and Gpa (the subunit common to LH, FSH and TSH) (Beck-Peccoz et al., 1985), or PRL and LH/FSH (Newman et al., 1989) were found in the same secretory granules. Thus, upon stimulation, both hormones will be released at the same time. This could explain why on some occasions, the emergence of new-hormone endocrine cells might be required. These new cells could originate from proliferation of mature endocrine cells as previously reported in both mammal and fish gonadotropes (for review, (Fontaine et al., 2020)), or by the proliferation and differentiation of progenitor cells as reported in mammals (Nolan et al., 1998; Nolan and Levy, 2006a).

A lot of work remains to be done on the regulation of the pituitary endocrine cell types. Indeed, many knowledge gaps remain on how hormone production (hormone mRNA and protein synthesis) is
regulated. Deeper investigation of the promoter region of hormone encoding-genes would certainly help us to better understand hormone synthesis regulation and to determine whether several hormone-encoding genes share some regulatory elements. Likewise, DNA methylation and chromatin organization should be investigated now that whole genome sequencing methods exist to study epigenetics. Chromatin organization has for instance been shown to play a major role in determining how gonadotropin gene promoters are activated (Melamed et al., 2018). In addition to hormone production, the regulation of pituitary endocrine cell proliferation (either from stem cell, phenotypic change, or endocrine cell mitosis) is still far from being characterized well in both mammals and fish. Fortunately, the expansion of the genetic toolbox allowing the development of new transgenic lines in which the expression of hormone-encoding genes can be tracked by reporter proteins, novel imaging techniques such as biphoton microscopy, and new transcriptomic approaches such as scRNA-seq, will help address these gaps.

5. Conclusion

As discussed above, a lot remains to be investigated regarding pituitary multi-hormone cells in mammals and even more in fish. It seems that mammalian cells are more capable of producing multiple hormones than fish. In general, fish seem less plastic regarding phenotypic conversion which, as suggested previously, might be due to fewer studies addressing this question and a lack of tools. Another explanation could be if fish selected a different strategy through evolution which makes them more capable of increasing the number of cells producing a specific hormone by regulating their proliferation as many stem cells and high proliferative activity of mature endocrine cell seem to remain in adult fish. Therefore, mammals and fish appear to be complementary models to study pituitary endocrine cell plasticity. Studying both will certainly help us to identify the conserved and varied mechanisms allowing us to understand the common and differential evolutionary paths taken by these animals.

Author Contributions

RF drafted the outline. RF, MRR, CH and KH wrote the manuscript with the input from all other authors.

Funding

This study was funded by the Norwegian University of Life Sciences and the Norwegian Research Council grants No. 251307, 255601, and 248828.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We thank Lauren Glose for the grammar and English proofreading.

References

Ager-Wick, E., 2014. Transcriptome profiling of teleost pituitaries. Thesis published by Norwegian University of Life Sciences (Oslo).
Ager-Wick, E., Dirks, R.P., Burgerhout, E., Nourizadeh-Lilabbadi, R., de Wijze, D.L., Spaink, H.P., van den Thillart, G.E.F.M., Tsukamoto, K., Dubour, S., Wietzen, F.-A., Henkel, C.V., 2013. The pituitary gland of the European eel reveals massive expression of genes involved in the melanocortin system. PLoS ONE 8, e77396.
Ager-Wick, E., Maugars, G., von Krogh, K., Fontaine, R., Siddique, K., Nourizadeh-Lilabbadi, R., Wietzen, F.-A., Henkel, C., 2021. Ectopic expression of lipid homeostasis genes in rare cells of the teleost pituitary gland, bioRxiv. 2021.06.11.448009. https://doi.org/10.1101/2021.06.11.448009.
Alunni, A., Heremel, J.M, Heuré, A., Bourrat, F., Jemen, F., Joly, J.S., 2010. Evidence for neural stem cells in the medaka optic tectum proliferation zones. Dev. Neurobiol. 70 (10), 963-711. doi: 10.1002/dneu.20799. PMID: 20506557.
Amsterdam, Jay D., Winokur, A., Lucki, I., Snyder, P., Harris, R.L., Caroff, S., Rickels, K., 1982. Growth hormone, prolactin and thyrotropin responses to gonadotropin-releasing hormone in depressed patients and healthy volunteers. In: 177-94. Netherlands: Elsevier Science.
Andoniadou, Cynthia, L., 2016. Pituitary Stem Cells During Normal Physiology and Disease. In: (Springer International Publishing).
Aoki, K., Umeura, H., 1970. Cell types in the pituitary of the medaka (oryzias latipes). Endocrinologia Japonica 17, 45-55.
Ball, J.N., Baker, B.L., 1969. 1 - The Pituitary Gland: Anatomy and Histophysiology. Fish Physiology (Academic Press) .
Children, G.V., Unahia, G., Wu, P., 2000. Differential expression of growth hormone messenger ribonuclease acid by somatotropes and gonadotropes in male and cycling females. Endocrinology 141, 1560-1570.

Childs, G.V., Westlund, K.N., Unahia, G., 1989. Characterization of anterior pituitary target cells for arginine vasopressin: including cells that secrete adrenocorticotropin, thyrotropin-beta, and both hormones. Endocrinology 125, 554-559.

Childs, G.V., Unahia, G., Collins, T., 1999. Expression of gonadotropin and prolactin antigens by GHIR target cells from male and female rats. J. Endocrinol. 162, 177-187.

Childs, Gwen V., Neill, J.D., 2006. Gonadotropins and lactotropes. Neill JD, Physiology of female reproduct: Elsevier.

Childs, G.V., 1995. Division of Labor among Gonadotropes. Vitamins & Hormones (Academic Press).

Collins, S.T., P.A., 1993. Isolation of gonadotropin subunits and evidence for two distinct gonadotropins in Atlantic croaker (Micropogonas undulatus). Gen. Comp. Endocrinol. 91, 115–125.

Cox, B., Laporte, E., Vennekens, A., Kobayashi, H., Nys, C., Van Zundert, L., Uji-i, H., Dubbel, A.V., Beck, B., Roose, H., Boretto, M., Vankelecom, H., 2019. Orgnoids from a pituitary as a novel research model toward pituitary stem cell exploration. J. Endocrinol. 240, 287-308.

Cox, B., Roose, H., Vennekens, A., Vankelecom, H., 2017. Pituitary stem cell regulation: who is pulling the strings? J. Endocrinol. 234, R15-R158.

Dass, J.E., O’Connell, S.M., Flynn, S.E., Treier, M., Gleiberman, A.S., Zet, D.P., Hoshochond, F., Aggarwal, A.K., Rosenfeld, M.G., 1999. Reciprocal interactions of Pit1 and GATA2 mediate signaling gradient-induced determination of pituitary cell types. Cell 97, 585-593.

Davis, S.W., Keiser, J.L., Perez-Miller, M.S., Schade, V., Camper, S.A., 2016. All hormone-producing cell types of the pituitary intermediate and anterior lobes derive from Prop1-expressing progenitors. Endocrinology 157, 1385-1396.

Dee, K., V.G., Lee, J.E., Lee, H., B. 1992. Properties of common carp gonadotropin I and gonadotropin II. Gen. Comp. Endocrinol. 85, 217-229.

Denef, C., 2008. Paracrine: the story of 30 years of cellular pituitary crosstalk. J Neuroendocrinol. 20, 1-70.

Dietel, N., Vaillant, C., Haapasalo, M., Mironov, S., Fostier, A., Gauguen, M-M., Anglade, L., Kah, O., Pellegrini, E., 2013. Effects of estradiol in adult neurogenesis and brain repair in zebras. Horm. Behav. 63, 193-207.

Dixon, A.K., Richardson, P.J., Pinnock, R.D., Lee, K., 2000. Gene-expression analysis at the single-cell level. Trends Biotechnol. 18, 555-70.

Egensperger, R., Scheithauer, B.W., Horvath, E., Kovacs, K., Giannini, C., Young, W.F., 2010. Lineage analysis reveals an endodermal contribution to the vertebrate pituitary gland. Science 327, 577-80.

Elizur, A., Zmora, N., Rosenfeld, H., Meiri, I., Hassin, S., Gordin, H., Zohar, Y., 1996. Mammosomatotropes: presence and functions in normal and neoplastic pituitary tissue. Endocr. Rev. 12, 337.

Emancipator, E., Drubbel, A.V., Beck, B., Roose, H., Boretto, M., Vankelecom, H., 2019. Orgnoids from a pituitary as a novel research model toward pituitary stem cell exploration. J. Endocrinol. 234, R15-R158.

Elzir, A., Mora, N., Rosenfeld, H., Meiri, I., Hassin, S., Gordin, H., Zohar, Y., 1996. Gonadotropins beta-GtHI and beta-GtHII from the pituitary. Science 370, 463.

Fabian, P., Tseng, K.C., Smeeton, J., Lancman, J.J., Dong, P.D.S., Cerny, R., Crump, J.G., 1999. Characterization of anterior pituitary cells. Front. Endocrinol. 10.

Falk, M., Aspenson, P., Elizur, A., Zmora, N., Rosenfeld, H., Meiri, I., Hassin, S., Gordin, H., Zohar, Y., 1996. Gonadotropins beta-GtHI and beta-GtHII from the gilthead seabream, Sparus aurata. Gen Comp Endocrinol. 102(1), 39-46. doi:10.1006/genc.1996.0044. PMID: 8603007.

Fauquier, T., Rizzotti, K., Dattani, M., Lovell-Badge, R., Robinson, I.C., 2008. SOX2-expressing progenitor cells generate all the major cell types of the adult mouse pituitary gland. Proc. Natl Acad. Sci. USA 105, 2907-2912.

Fletcher, P.A., S.J., Blackhall, J.B., R., Bell, M.R., Smith, M.B., A., Schoen, S.L., Stojiljkovic, S.S., S., 2010. Cell type- and sex-dependent transcriptome profiles of rat anterior pituitary cells. J. Endocrinol. 10.

Fontaine, R., Aeger-Wick, E., Hodne, K., Weltzien, F.-A., 2019. Plasticity of Lh cells caused – 21 – 21 – 21

Fontaine, R., Ciani, E., Haug, T.M., Hodne, K., Weltzien, F.A., 2019. Erenumab in: Proc Natl Acad Sci U S A 2008 Aug;105(17):11023-11028. doi: 10.1073/pnas.0809644105. Epub 2008 Aug 26. Pmcid: PMC2556820.

Go, O., Lafont, C., Fontanaud, P., Guillou, A., Kenev, K., Ii R, M., Lafont, C., Fontanaud, P., Guillou, A., Kemkem, Y., Kineman, R.D., Luque, R.M., 2008. Cell-type transcriptomic analysis of adult male mouse pituitary reveals sexual dimorphism and physiologic demand-induced cellular plasticity. Proc. Cell 11, 565-583.

Hoeh, H, B, Haug, T.-M., Weltzien, F.-A., 2010. Single-cell qPCR on dispersed primary pituitary cells-an optimized protocol. BMC Mol. Biol. 11, 82.

Honji, R.M., N., Yokota, R., Tsukuba, S., Shimizu, B., Borella, M.I., Moreira, R.G., 2013. Immunohistochemical study of pituitary cells in wild and captive Salminus hilarii Characiformes: Characidae fishes during the annual reproductive cycle. SpringerPlus 2, 460.

Horvath, E., Lloyd, R.V., Kovacs, K., 1990. Propylthiouracil-induced hypothyroidism results in reversible transdifferentiation of somatotrophs into thyrotrophs. Endocrinology. 1986 Aug;119(2):629-37. doi: 10.1210/endo-119-2-1379.

Honji, R.M., Yokota, R., Tsukuba, S., Shimizu, B., Borella, M.I., Moreira, R.G., 2013. Immunohistochemical study of pituitary cells in wild and captive Salminus hilarii Characiformes: Characidae fishes during the annual reproductive cycle. SpringerPlus 2, 460.

Horvath, E., Kovacs, K., Killinger, D.W., Smyth, H.S., Weiss, M.H., Ertzin, C., 1983. Mammosomatotrop cell adenoma of the human pituitary: a morphologic entity. Vircchows Arch A Pathological Anatomy Histopathol. 398, 277-289.

Ibrahim, S., Moussa, M., Childs, G. Morphometric studies of rat anterior pituitary cells after gonadectomy: correlation of changes in gonadotropes with the serum levels of gonadotropins. Endocrinology. 1986 Aug;119(2):629-37. doi: 10.1210/endo-119-2-1396.

Ishikawa, K., Inoue, K., Sakai, T., 1991. Conversion of growth hormone-secreting cells into prolactin-secreting cells in the pituitary of male rats. Endocrinology. 1986 Aug;119(2):629-37. doi: 10.1210/endo-119-2-1396.

Ishikawa, K., Inoue, K., Sakai, T., 1991. Conversion of growth hormone-secreting cells into prolactin-secreting cells in the pituitary of male rats. Endocrinology. 1986 Aug;119(2):629-37. doi: 10.1210/endo-119-2-1396.
Shukuwa, K., Izumi, S.-I., Hishikawa, Y., Ejima, K., Inoue, S., Muramatsu, M., Ouchi, Y., Kitaoka, T., Koji, T., 2006. Diethylstilbestrol increases the density of prolactin cells in male mouse pituitary by inducing proliferation of prolactin cells and transdifferentiation of gonadotropins. Histochem. Cell Biol. 126, 111–123.

Siddique, K., Ager-Wick, E., Fontaine, R., Weltzien, F.A., Henkel, C.V., 2021. Characterization of hormone-producing cell types in the teleost teleost pituitary gland using single-cell RNA-seq. Sci Data 8(1), 279. doi: 10.1038/s41597-021-01058-8. PMID: 34711832; PMCID: PMC8555774.

Soroom, M.W., Wei, W.-l., Dassen, J.S., Flynn, S.E., Norman, D.J., O’Connell, S.M., Guzkowsky, I., Carriere, C., Ryan, A.K., Miller, A.P., Zuo, L., Gleiberman, A.S., Andersen, B., Beamer, W.G., Rosenfeld, M.G., 1996. Pituitary lineage determination by the Prophet of Pit-1 homeodomain factor defective in Ames dwarfism. Nature 384, 327–333.

Stefaneanu, L., Kovacs, K., Horvath, E., Asa, S.L., Losinski, N.E., Billestrup, N., Price, J., Vale, W., 1989. Adenosine/physiophyal changes in mice transgenic for human growth hormone-releasing factor: a histological, immunocytochemical, and electron microscopic investigation. Endocrinology 125, 2710–2718.

Stefaneanu, L., Kovacs, K., Lloyd, R.V., Scheithauer, B.W., Young, W.F., Sano, T., Jin, L., 1992. Pituitary lactotrophs and somatotrophs in pregnancy: a correlative in situ hybridization and immunocytochemical study. Virchows Arch B 62, 291.

Suzuki, K., Kawachi, H., Nagahama, Y., 1988. Isolation and characterization of two distinct gonadotropins from chum salmon pituitary glands. Gen. Comp. Endocrinol. 71, 292–301.

Tahara, S., Kurotani, R., Sanno, N., Takumi, I., Yoshimura, S., Osamura, R.Y., Teramoto, A., 2000. Expression of pituitary homeo box 1 (Ptx1) in human non-neoplastic pituitary and pituitary adenomas. Mod Pathol 13, 1108–1114.

Tanaka, H., Kagawa, H., Okawara, K., Hirose, K., 1993. Purification of gonadotropins (PmGTH I and II) from red seabream (Pagrus major) and development of a homologous radioimmunoassay for PmGTH II. Fish Physiol. Biochem. 10, 409–418.

Teramoto, A., 2000. Expression of pituitary homeo box 1 (Ptx1) in human non-neoplastic pituitary and pituitary adenomas. Mod Pathol 13, 1108–1114.

Vale, W., 1989. Adenohypophysial changes in mice transgenic for human growth hormone-releasing factor: a histological, immunocytochemical, and electron microscopic investigation. Endocrinology 125, 2710–2718.

Weltzien, F.A., Andresson, E., Andersen, O., Shalchian-Tabrizi, K., Norberg, B., 2004. The brain-pituitary-gonad axis in male teleosts, with special emphasis on flatfish (Pleuronectiformes). Comp. Biochem. Physiol. A Mol. Integr. Physiol. 137, 447–477.

Weltzien, F.A., Hildahl, J., Hodne, K., Okubo, K., Haug, T.M., 2014. Embryonic development of gonadotrope cells and gonadotrophic hormones – Lessons from model fish. Mol. Cell. Endocrinol. 385, 18–27.

Weltzien, F.A., Norberg, B., Helvik, J.V., Andersen, Ø., Swanson, P., Andersson, E., 2003. Identification and localization of eight distinct hormone-producing cell types in the pituitary of male Atlantic halibut (Hippoglossus hippoglossus L.). Comp. Biochem. Physiol. A: Mol. Integr. Physiol. 134, 315–327.

Wen, S., Ai, W., Alim, Z., Boehm, U., 2010. Embryonic gonadotropin-releasing hormone signaling is necessary for maturation of the male reproductive axis. Proc. Natl. Acad. Sci. 107, 16372–16377.

Yan, H.Y., Thomas, P., 1991. Histochemical and immunocytochemical identification of the pituitary cell types in three sciaenid fishes: Atlantic croaker (Micropogonias undulatus), spotted seatrout (Cynoscion nebulosus), and red drum (Sciaenops ocellatus). Gen. Comp. Endocrinol. 84, 389–400.

Zhang S, Cui Y, Ma X, Yong J, Yan L, Yang M, Ren J, Tang F, Wen L, Qiao J. Single-cell transcriptomics identifies divergent developmental lineage trajectories during human pituitary development. Nat Commun. 2020 Oct 19;11(1):2575. doi: 10.1038/s41467-020-19012-4. PMID: 33077725; PMCID: PMC7572359.

Zhou, Y.u., Wilson, R.R.A., Udaiyar, A., McLemore, J., Sadri-Ardekani, H., Criswell, T., 2021. Pituitary Lineage Differentiation from Human Induced Pluripotent Stem Cells in 2D and 3D Cultures. Stem Cells Dev. 31, 239–249.