A Review on Estrogen Synthesis, the Effects on Brain and Breast Development, and the Proceeding Effects on Transgender Hormone Replacement Therapy.

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Abstract

Hormone therapy has become an important part of gender affirming care. However, our current understanding of the specifics of what goes on in trans women's bodies is lacking. Here the research demonstrates how aromatase is a powerful enzyme crucial to the synthesis of Estradiol (E2). Estrogen signaling also has estrogen receptor dependent and receptor independent ways to signal and promote hormonal growth. What research also shows is that while our knowledge of breast development in cis women has been studied well through mouse models, our understanding of breast development in trans women is underdeveloped. This lack of understanding leaves much room for clinical trials to focus on large scale analysis of transgender individuals on a biochemical level.

1.0 Introduction

The current political discourse around trans people in 2026 can at best be described as "polarizing." Despite what some political movements might believe, trans people are both a small minority and have been around for millenia. There are a lot of misconceptions about trans people in regards to their brains and how their bodies process hormones. Rhetoric around sex and gender has led to stereotypical views and often misogynistic and transphobic views around the roles of women and the disregard of trans people.

The science around transgender people is very much lacking. For as long as societies have needed scapegoats, queer people, and specifically trans people have been key targets. Because of this the history around trans people has often been understudied historically, or been burned down as in the Nazi book burnings.

As trans people become a more spotlighted and scapegoated population, it becomes clear that more scientific writing is necessary to both learn more about transgender bodies and minds, and also to better educate those unaware of anything about the biochemistry and neurology around transgender people and transgender individuals. This paper's goal is to highlight the science around specifically transgender women (TW), their experience with gender affirming hormone therapy (GAHT) in regards to often desired physical changes, and how the current science validates the unique experiences undergone before and during GAHT.

2.0 An Overview of Estrogen Synthesis:

"Estrogen" as a term is in reference to three different forms, Estrone (E1), Estradiol (E2), and Estriol (E3). As estradiol is the primary component when discussing estrogen signaling during reproductive years and is core to hormone replacement therapy (HRT) for both menopausal women and TW, E2 will be the primary focus of this review article.

Estradiol is a steroidal product and is thus synthesized from cholesterol. This will be a brief overview of the pathway from cholesterol to androstenedione and testosterone before taking a more indepth look at what occurs at the E1 to E2 step. Here it is important to note that while many animals have uses for helping study and predict patterns in the human body, that differences are common and should be noted. Steroidogenesis is a process that most commonly occurs within the adrenals and gonads. The first step of synthesis taken by all steroidal compounds is a cholesterol side chain cleavage enzyme (SCC) here labeled CYP11A1 (Cytochrome P450scc). CYP11A1 performs a series of two monooxygenations to add two alcohol groups to cholesterol, then a cleavage of the side chain is performed. Here there are differences between steroidogenesis pathway in rats compared to humans, where CYP17A1 catalyzes both pregnenolone to DHEA and progesterone to to androstenedione , humans use CYP17A1 to catalyze specifically pregnenolone to DHEA and not progesterone to androstenedione. In cis human males with testes, androstenedione is converted to testosterone via 17β-Hydroxysteroid dehydrogenases 3 and 5(17β-HSD3, 5) and testosterone is transformed to dihydrotestosterone (DHT) via 5α-Reductase. In cis human females, production of E2 has many pathways to discuss with its most important enzyme being aromatase (65).

2.1 Aromatase Mechanism of Action

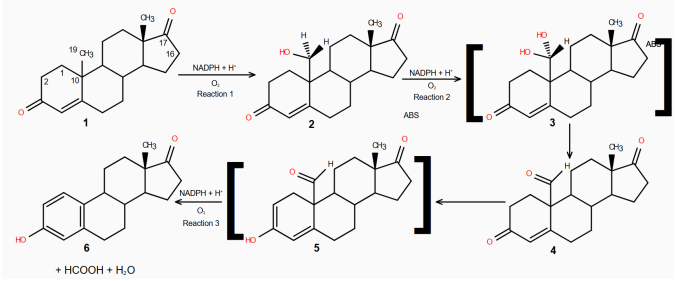

Figure 1: Scheme depicting the sequence of reactions done by the enzyme aromatase. Aromatization proceeds via a sequence of monooxygenase reactions. Adapted from Ref 46. Drawn using MolDraw

Aromatase (CYP19A1) is a crucial enzyme to the biosynthesis of estrogen focused on the aromatization of the A ring of androstenedione or testosterone to E1 and E2 respectively. The first two reaction steps are relatively unordinary for cytochrome P-450 where we see the 19Methyl carbon get oxygenated once to form an alcohol group, then

get oxygenized again to form a gem diol intermediate. After formation of the gem diol intermediate, the intermediate dehydrates into the aldehyde (4). The third step of this reaction has many questions regarding the reaction mechanism, but studies done with ^{18}O have helped to illuminate this process. The structure of aromatase has been found that protonated Asp 309 forms hydrogen bond interactions with the 3 carbon ketone group. This aids in both substrate binding and the subsequent 2,3 enolization double bond that forms. Akhtar also proposes that Ala 306 with participation of the hydroxyl of Thr 310 and possibly a catalytic water help carry out this step (46). Then as the heme group, the presence of the C19 aldehyde causes the peroxide dianion to form an adduct with our aldehyde. From the formation of this adduct, we see a homolytic cleavage to form an Fe^{III}–O• and an alkoxy radical. The C10-C19 bond is now homolytically cleaved to form formate and a radical carbon. The Fe^{III}–O• radical then causes a homolytic cleavage of the C1-H bond that forms the second carbon radical necessary to the final double bond to aromatize the A ring of estrogen. This mechanism helps us explain the presence of the third carbon in the released formate during reaction. An interesting note is that this would show that the catalytic site for aromatase would be catalyzing two different reactions in the same binding site.

Depending on current life state, the synthesis and metabolism of estrogen occurs in differing ratios, and different places. Prepubertal cis females and postmenopausal women are similar in this regard where the primary estrogen released is E1, a less potent estrogen, and primarily is produced in extragonadal sites including kidney, adipose tissue, skin, and brain. These estrogens in nonreproductive women are primarily for nonreproductive functions and are thus kept local, signaling via paracrine and intracrine signals. Estrogen synthesis also occurs in male testes and is an important part of gonadal development and spermatogenesis.

Estrogen synthesis in the ovaries is an important part of premenopausal women's reproductive system. The onset of puberty brings upon the release of many changes. Gonadotropin-releasing hormones trigger the release of luteinizing hormone (LH) and follicle stimulating hormone (FSH) (57). FSH here is key because it is a promoter of aromatase, signaling through cAMP via proximal promoter II (31). An interesting pathway here is that the thecal cells and granulosa cells of ovaries during folliculogenesis serve different purposes during estrogen synthesis, with thecal cells, the outer layer of ovarian follicle cells, being able to

convert cholesterol to androgens, but not androgens to estrogens, while granulosa cells, the inner layer of ovarian follicle cells, are unable to produce androgens, but can produce estrogens from androgens. Estrogens produced in the ovaries are then circulated around the body in an endocrinological fashion to reproductive and nonreproductive tissue.

2.2 Estrogen Receptors

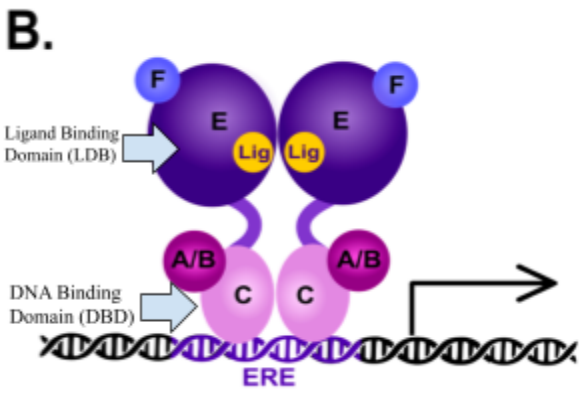

Figure 2: Drawing representing the binding of the estrogen receptor dimer to DNA. A/B represents the AF-1. C represents the DBD. E represents the LBD. F represents the AF-2. Adapted from Ref 59.

Estrogen Receptors are an important part of the estrogen signaling pathway. Estrogen receptors come in two main forms, Estrogen Receptor α (ERα) and Estrogen Receptor β (ERβ). Each of these receptors belong to a greater family called the Nuclear Receptor (NR) family, "a family of transcription factors that regulate numerous physiological processes such as metabolism, reproduction, inflammation, as well as the circadian rhythm" (66). Both ERα and ERβ consist of structurally and evolutionarily conserved domains, consisting of a central DNA binding domain (DBD), a ligand binding domain (LBD) found near near the C-Terminal of the DNA along with AF-2, and a sequence of variable length and pattern near the N-Terminal containing AF-1. Both estrogen receptors have equally high affinity for E2, and different from other NRs, they have a wide cavity, allowing other compounds homologous to E2 to bind to ERα and ERβ. Furthermore, when E2 binds to the LBD of either ERα or ERβ, both cause conformational changes that lead to the formation of homodimers. The presence of near identical homologs within the DBD and the LBD might suggest the potential absence of differentiation of between the two, studies done on ERα knockout mice show lack of ERα has an effect on "estrogen insensitivity (leading to hypoplasia) in the reproductive tract, hypergonadotropic hypergonadism, lack of pubertal mammary gland development, and excess adipose tissue" (41) in female mice. ERβ knockout mice did not experience these issues, but did experience "inefficient ovarian function and subfertility"

The ERα genome is a complicated genomic process that leads to a variety of isoforms of ERα with differing functions. One important factor of the ERα genome is the 5'-UTR, in which it has been

found to contain “seven leader exons and four internal exons in humans.” What is interestingly noted in Kenji Saito’s paper is that differing ERα transcripts containing different 5’-UTRs exons in different tissues produce the same ERα protein (ERα and ERα66). The current physiological function and significance of this is currently unknown, with Saito bringing up the idea that “multiple promoter usages can achieve context-dependent regulation of ERα expression and therefore robustness of ERα functions” (68).

There are currently a few agreed upon mechanisms on which estrogen signaling takes place. Direct genomic signaling, indirect genomic signaling, indirect nongenomic signaling, and ligand independent estrogen receptor signaling. Direct genomic signaling involves the binding of E2 and other agonists to the LBD of estrogen receptors and the estrogen receptor then binding to cellular DNA estrogen response elements (EREs) akin to enhancer regions to either promote or inhibit transcription and protein synthesis. Indirect genomic signaling is a similar mechanism to direct genomic signaling, involving the binding of E2 to the LBD of estrogen receptors, but in this mechanism, the estrogen receptor dimers bind to a transcription factor that then binds to the DNA.

3.0 Estrogen and the Brain

In the brain, neurons and astrocytes have the capacity to produce E2, while microglia and oligodendrocytes are unable to perform this task. One thing of note is that estrogen levels in the brain differ from that in the blood within cis women. Blood levels are typically between 20-400 pg/ml, while brain levels are reported between 0.08–0.19 ng/g (31). While there is some data within studies regarding blood hormone levels for transgender women, there is virtually zero data in any regards discussing brain estrogen levels in this regard. This is currently leaving a gap in our knowledge base where trans women are aware of their estrogen levels through blood work, but are currently unaware of estrogen levels in the brain and if that has an affect on gender dysphoria and other related issues.

The role of estrogen in the brain during prenatal development is interesting and not fully agreed upon yet. While research has shown in male rodents, the biosynthesis of estrogen takes place in sexually dimorphic regions of the brain via aromatase, via the use of knockout mice, we see that the elimination of CYP19A1 (aromatase gene) causes reduction of sexual characteristics in both male and female mice, and that elimination of androgen receptors (AR) can also cause reductions in sexual characteristics (55). The latter claim was shown by

treating mice with ERα negative genes with DHT, an androgen that cannot be transformed to estrogen. Mice lacking ERα but with DHT showed increased sexual characteristic behaviour, but not when AR are also removed. The lack of estrogenic effects on human female brains in the fetal phase is because ovaries do not produce estrogen during this stage of life. Human females see a small spike in estrogen concentration during the neonatal and infant phase that then phases out until the peripubertal, which then sees a massive spike in estrogen production via GSH.

While estrogen is most commonly thought of as interactive with reproductive organs, the role of estrogen and estrogen receptors within the brain can help us understand the differentiation occurring between male and female brains. ERα and ERβ are produced in varying amounts in the human brain within different regions and different times. A thing of note is that ERβ has been observed to have higher expression in the hippocampus and cerebral cortex, suggesting a potentially important role, the overall effect of which being synaptic plasticity, hippocampal function, and learning, in hippocampal neurons (8).

At a certain point in aging, all cisgender women will experience menopause, resulting in drastically lowered E2 levels and reliance on E1 as the primary estrogen (54). Some of the changes caused by menopause include loss of brain structures, changing sleep, and changing mood, though some fluctuations of brain size are common throughout the reproductive years of cisgender women's lives (64). One common treatment for menopausal women is HRT in nearly the same way that HRT is applied for cis women, to reintroduce E2 to help balance out the negative side effects of

3.1 Trans Women and the Brain

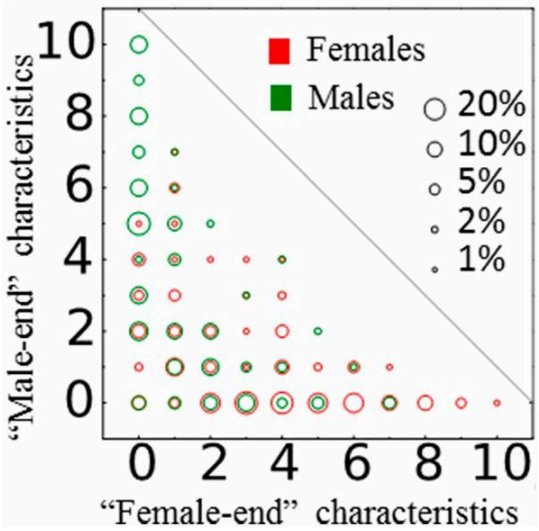

Figure 3: A bivariate scattergram depicting percentiles of people carrying certain amounts of "male-end" and "female-end" characteristics. The scattergram shows a lack of "internal consistency" which is a measure of whether brains that are male will have male characteristics consistently and vice-versa. Adapted from Ref 1

Sexual differences between human males and females have always been acknowledged, with human genitalia being the most clear example of this trend. In the past, however, when trying to analyze

the human brain, the approach has often been to look at the brain through a dimorphic lens, where distinct regions of the brain exhibit male characteristics or female characteristics. While there are differences between the brains of cis men and women, this dimorphic lens often obfuscates the reality of the human brain more closely resembling a “mosaic” of parts where individual brains contain male characteristics and female characteristics.

Although research speaking on transgender individuals brains is limited, and sometimes in opposition to what is generally agreed upon, there is good information to glean from neuroimaging comparing transgender individuals brains pre and post GAHT. Current large scale MRI analysis points to the idea that while TWs brains can be viewed as shifting between male brains and female brains, it is more apt to describe transgender individuals brains as a unique phenotype from male and female brains, falling in line with the idea of a gender mosaic discussed earlier (61, 63). For post hormone replacement therapy transgender women the distinction is made more clear, with trans women one year after starting homrone replacement therapy show lower brain matter, gray matter, and white matter, making their brains more closely in line with what is considered standard for cisgender women (62). While the mechanism underlying this change is currently unknown, one hypothesis states that the downregulation of testosterone caused by added synthetic E2 and antiandrogens downregulates the anabolic effects of testosterone and decreasing brain volume (62).

4.0 Breast Development in Cisgender Women

Estrogen plays a crucial factor when it comes to breast development in pubertal females. It is made clear the importance of ERα in this development via the use of knockout mice. When rodents categorized as ERα negative were normalized in the hormones of progesterone and estrogen, development of alveolar tissue was seen, but little to no ductal elongation had occurred. The effects of ERβ on breast development is more subtle, shown by the lack of difference of breast development from ERβ negative mice. Research on ERβ is currently lacking. The role of ERs and Progesterone receptors (PR) are a mechanism commonly seen in the nuclear receptor superfamily of binding to promoter regions, recruiting coactivators, and regulating the transcriptional machinery of the RNA polymerase II complex.

Breast development occurs in three life stages in cisgender women. The first stage being a hormone independent stage that occurs prenatally in both male and females, a pubertal hormone dependent phase, and a reproductive phase.

4.1 Embryonic Breast Development

The first stage is a phase of mammary development that occurs in cisgender men and women. This phase of mammary development is dependent on the mammary mesenchyme and is split into three phases, mammary line formation, mammary placode formation, and mammary bud formation. In mammary line formation, communication between epithelial cells and mesenchymal cells are key, mediated by Msx homeobox genes, bone morphogenetic proteins, and Wnt signaling (12, 24). Wnt signaling is an important part of all the embryonic mammary morphogenesis stages. Most studies on mammary morphogenesis are on mice, and as such I will use those same models in this discussion. In mice, the production of Wnt begins around day 10 Embryonic phase, where in the canonical Wnt/β-catenin pathway, Wnt proteins bind to specific sevenfold transmembrane receptor Frizzled protein (24-26). This signaling causes the β-catenin destruction complex to degrade, allowing β-catenin to bind to T-cell Factor (Tcf)/Lympoid enhancer factor (Lef1) which allows for transcription of target genes that promote target genes to promote mammary morphogenesis

The next step is the dorsal/ventral patterning occurring within the development of the mammary placode, regulated by the factor bone morphogenic protein 4 (BMP4) and T-Box transcription factor 3 (TBX3) along with fibroblast growth factors (FGF), with the end result being one pair of symmetrically localized placodes in humans. Both BMP4 and TBX3 have a regulatory effect on Wnt proteins, specifically Wnt10b, where BMP4 inhibits Wnt10b and TBX3 promotes Wnt10b. Both of these factors also have an antagonistic effect on each other; with the placement of BMP4 being primarily in the epithelial cells ventral of the body and TBX3 being this causes the development of placodes to occur primarily in the mesenchyme creating the dorsal/ventral patterning of this step (12, 28). FGF also plays a role here via regulating Wnt10b via its cognate receptor FGFR2b (12).

The last step that occurs in prenatal mammary development is development of the mammary placodes into a mammary bud, and then into a rudimentary duct. Three additional processes occur here, ductal lumen formation, creation of the nipple structure, and in mice, specification between male and female mammary glands (this third process occurs during puberty in humans) (12). Lumen formation can be simplified into four steps. Lumen initiation where the site of formation is decided. Apical membrane growth where membrane material is brought to the site to expand the luminal space. Maturation of the lumen, inflating the lumen to the

proper diameter. Finally, stabilization of the lumen, stabilizing the tubular structure and imparting properties to fulfil proper functions (29). Nipple generation involves “thickening of the epidermis, suppression of hair follicles, and generation of a nipple sheath from keratinocytes at the site where the primary duct connects to the skin surface” (12). Nipple generation is regulated by parathyroid hormone-related protein (PTHLH), and is important in formation of the nipple sheath via a paracrine signal.

4.2 Pubertal Breast Development

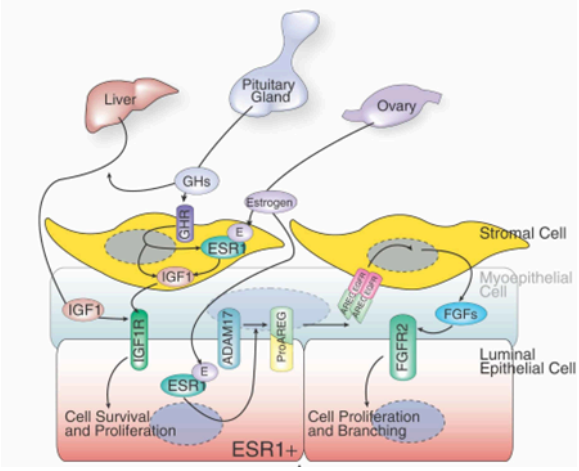

Figure 4: Schematized view of the different processes and layers occurring in the terminal end bud (TEB) cells during pubertal breast development. Adapted from Ref 12.

Between birth and puberty, the mammary glands are largely quiescent. At the onset of puberty, the rise of gonadotropins LH and FSH leads to secretion of estrogens and progesterones from the ovaries, and secretions of growth hormones (GH) and prolactin (PRL) from the pituitary gland, all of which have necessary functions towards ductal morphogenesis, with development of terminal end buds (TEB) being one of the goals (12, 30, 32).

The two key parts of breast tissue are the mammary glands themselves and the extracellular matrix (ECM). The breast tissue is made up of terminal end buds that consists of luminal epithelial cells, myoepithelial cells, and stromal cells. The ECM is made up of thin sheets of proteoglycans and glycoproteins whose purpose is to connect the stromal cells to the rest of the TEB to allow for communication and signaling (40). Many important enzymatic processes are occurring within the ECM, one of them being from matrix metalloproteinases (MMPs) and tissue inhibitors of MMPs (TIMPs). The ECM is a stable matrix which, when undisturbed, prevents TEB from elongating further. MMPs function in the ECM to degrade the matrix, then allowing the TEB to grow further into the adipose tissue. TIMPs are highly expressed in the epithelial cells in TEB, and their presence helps uphold ECM integrity and properly restricts TEBs by inhibiting MMPs (30). The final key structural element to discuss here is the P-cadherins and E-cadherins, which are capable of forming transmembrane

complexes with α and β catenins that form cell-cell adhesion that holds together the TEB (30).

Estrogen travels from the ovaries to the mammary glands via blood, where estrogen then binds to ERα. This hormone receptor complex can then interact with luminal epithelial cell DNA, where transcription of the amphiregulin (AREG) gene is promoted. The AREG gene leads to the translation of amphiregulin, which, after being cleaved by the transmembrane metalloproteinase enzyme ADAM 17, AREG interacts with epidermal growth factor receptor (EGFR) to promote cell growth. EGFR along with human epidermal growth factor 2 (HER2) form a heterodimer that is responsible for mammary gland signaling during puberty, while HER3 and HER4 are expressed at higher levels during pregnancy and lactation (30). Although it is not fully clear how exactly EGFR causes mammary gland development, one downstream pathway that almost certainly has an effect is the Ras/Erk pathway (33, 34). The estrogen signaling via EGFR is also important in the recruitment of leukocytes via cytokines and chemokines; The leukocytes' purpose in this case being to help clean up excess cells via apoptosis to produce a single layer of luminal epithelial and myoepithelial cells (35, 36). All together, the end product of estrogen signaling is ductal morphogenesis and TEB branching to help the mammary glands transition to their adult form until they fully throughout the fat pad.

Growth hormone signaling within the mammary gland occurs primarily through insulin growth factor 1 (IGF-1). IGF-1 production in the stromal cells has a positive feedback loop with E2 where E2 promotes the production of IGF-1 along with GH. IGF-1s role in mammary gland development is to promote TEB formation, though IGF-1 is not necessary when it comes to maintenance of TEB. During this time period, female breasts will undergo the five tanner stages, named so after the researchers Marshall and Tanner, refer to the physiological stages that humans undergo during puberty, starting at the peri-pubertal stage and ending post puberty (38).

One of the key negative regulatory factors that balances out the positive cell proliferation that occurs through growth hormone and estrogen signaling is from transforming growth factor β (TGFβ). TGFβ is secreted after binding to TGF-β propeptide (LAP) and TGFβ binding protein (LBTP) as the large latent complex (LLC) (39, 40). Secretion of TGFβ inhibits epithelial proliferation and stimulates ECM production, both important to stop ductal elongation from going past adipose tissue into the pectoralis major.

4.3 Adult Reproductive Breast Development

In order to prepare for lactation, the mammary glands must undergo gland maturation and alveologenesis. While progesterone and prolactin play a small part in pubertal breast development, progesterone's primary purpose here is when it comes to adult reproductive breast development (30). Pregnancy causes the secondary and tertiary branching of the mammary glands, which will eventually become distinct alveoli that are used as milk secreting lobules during lactation.

Progesterone is key to the formation of secondary and tertiary branches, and along with Prolactin, is an important player in the development of lactational competency (12). Progesterone interacts with the mammary gland primarily through two Isoforms of Progesterone Receptors, PGR_{IA} and PGR_{IB}. In mammary gland tissue, it has been shown that while PGR_{IA} negative mice did not show any differentiation from the standard, PGR_{IB} negative mice had significantly decreased sidebranching and alveologenesis, indicating that PGR_{IB} plays a significant role during pregnancy that PGR_{IA} does not (12, 44). The PGR receptors primary focus in this case is to produce tumor necrosis factor ligand superfamily, member 11 (TNFSF11), also known as RANKL. RANKL proteins bind to RANK receptors in the mammary progenitor cells to proliferate the basal luminal and myoepithelial cells towards secondary and tertiary branching (45). It is this element of breast development that leads many trans women to take oral progesterone in an attempt to aid breast development, but as will be discussed later, there is no strong evidence pointing towards this being the case.

Prolactin's primary purpose in this discussion is to make the mammary gland capable of producing milk, doing so through the JAK/STAT5 pathway. After binding to transmembrane prolactin receptors (PRLR), after which the JAK/STAT5 pathway causes the activation of milk protein genes, including casein β (Csn2) and whey acidic protein (WAP) via the Gamma interferon activation site (GAS) promoter elements (12, 40). Prolactin is also able to signal activation of RANKL through the same PRLR which causes similar effects from progesterone of secondary and tertiary branching and alveologenesis. Prolactin signaling is regulated by the integrins that are embedded within the ECM (12, 40). The talking between PRLR and integrins here is mediated signal regulatory protein alpha (SIRPA), a transmembrane glycoprotein, showing us that these pregnancy mammary gland developments are a highly regulated process (12).

4.4 Involution Post Pregnancy

Post pregnancy and when lactation stagnates, it is necessary for the mammary glands to

undergo an involution period. Involution is made up of two phases, a reversible phase apoptosis and alveolar cell detachment, followed by a second irreversible phase alveoli collapse and tissue remodeling (12, 59). During the first 48 hours of involution, if suckling were to restart, the mammary glands would go back to producing milk. After entering the second phase, it is not possible to have the mammary glands go back to producing milk. It was this process of involution that Nestle infamously used to cause dependency on their baby formula, ultimately leading to the deaths of over 10,000,000 infant lives between 1960 and 2015 (57).

In the first reversible phase of involution, one key protein group are the STAT proteins. STAT5 and STAT3 have reciprocal effects during lactation, with STAT5 promoting cell survival and STAT3 antagonizing STAT5 and promoting pro apoptotic pathways (12, 40, 59). The cytokine leukemia inhibitory factor (LIF) in this case acts as the activator of STAT3 via phosphorylation, after which STAT3 directly inhibits the phosphoinositide 3-kinase by upregulating the two inhibitory subunits p55α and p50α, which ultimately shuts off AKT, an important protein for cell survival (12, 58, 59). STAT3 can also cause apoptotic effects by upregulating IGF-1 binding protein 5 (IGFBP5), which causes the silencing of the proliferative effects of IGF-1 and leads to involution.

The second stage of involution is irreversible and is led by serine proteases and MMPs. During the second phase, MMPs are upregulated and TIMPs are downregulated, leading to an altered stromal and ECM environment (12, 59).The serine proteinases plasmin is produced the interactions between another plasminogen and Kallikrein 1 (KLK1). Plasmin is then responsible for epithelial cell apoptosis and adipocyte generation (12, 59). These proteins also promote adipogenesis and redifferentiation of adipocytes, which is an important step to fill in the space left by the apoptosis of myoepithelial cells.

5.0 Trans women and gender affirming hormone therapy

Within current literature available, it is evident that GAHT is critical to the overall health and wellbeing of TW. The earliest reports of GAHT come from Danish endocrinologist, Christian Hamburger, where in 1952 over the course of a 10 month period, 95 mgs of estradiol benzoate were injected intramuscularly and 13.9 mg of ethinyl estradiol was administered orally. What is interesting to note is that the initial treatment idea was to administer testosterone, but the patient adamantly refused (5).

The specifications of treatment for transgender individuals depends on the age of the recipient. When talking about transgender youth, it is typical to prescribe puberty blockers to prepubertal transgender children to stop effects that would cause potential harm to a child's mental health and to give physicians time to better assess a child's psychiatric point of view. While it is not conclusive, 16 is typically the age when transgender youth are switched from taking puberty blockers to hormonal therapy that matches the gender identity that individual aligns withf (23).

For transgender adult women, hormonal therapy usually includes two parts, the administration of estradiol and use of anti androgens.
Anti-androgens are orally taken medicines that are antagonists to testosterone, the two most common being spironolactone, also used to treat hormonal acne, and cyproterone acetate. Estradiol is administered via a few different means, either orally via 17beta-estradiol valerate, transdermally via patches or gels, or intramuscularly via estradiol valerate or estradiol cypionate. Some trans women will inject subcutaneously rather than intramuscularly, though there is no strong evidence on one method being more effective than the other in this case (68). While some trans women will take progestogens to aid breast development, evidence is inconclusive at best when comparing breast development with and without progestogens.

5.1 Perception and Uses of Progesterone as Gender Affirming Hormone Therapy

Table2. Results

	Standard GAHT group (n= 59 total)	Standard plus progesterone group (n= 29 total)	Difference *P* value
Breast development satisfaction at 6 mo, n (%)	6 mo (n= 56):	6 mo (n= 26):	0.004
Dissatisfied	6 (10.7)	0 (0)	
Neutral	39 (69.6)	12 (46.2)	
Satisfied	11 (19.6)	14 (53.8)	
Breast development satisfaction at 9 mo, n (%)	9 mo (n= 48):	9 mo (n= 14):	0.003
Dissatisfied	4 (8.3)	0 (0)	
Neutral	34 (70.8)	4 (28.6)	
Satisfied	10 (20.8)	10 (71.4)	

Table 1: From Bahr et al. Data covering two groups of individuals, one group undergoing standard GAHT (A form of Estradiol and an anti-androgen) and a second group undergoing GAHT plus progesterone. This was a retrospective cohort study asking patients about their satisfaction with breast development. Adapted from Ref 18.

Progesterone in cis women has a clear role in regards to breast development, surging during ovulation (12). This clear evidence of the effects of Progesterone on breast development has led many trans women and some professionals to hypothesize on the potential effects of progesterone as medication for GAHT and breast development. Bahr et al. (18) found in a retrospective cohort study that trans women were happier with their breast development at the six and nine month times when on progesterone

compared to without. Bahr et al. looks to refute a study from 1986 that shows that progesterone did not lead to breast development by pointing out how the sample size was small with only 19 patients, and that the progesterone prescribed then, medroxyprogesterone acetate is out of date and that micronized progesterones have a better effect. The data provided here is purely anecdotal however, and others studies have shown no correlation between progesterone and breast development, and no causative mechanism has been uncovered showing how this would occur specifically in TW (14, 19). Without hard data, it is hard to know for certain the reasons for anecdotal improvements from progesterone, but my speculation would make me think that the mythologization of progesterone within the trans women community might cause a placebo effect that creates the belief of better breast development, even if no physical changes occurred specifically because of progesterone. I support this speculation with the fact that the majority of the time, progesterone is taken with Ultimately, more research would be needed to make a definitive conclusion that rules out the anecdotal evidence entirely.

Changes in body composition is one of the big things that many trans women are looking to occur from GAHT. Prominently, breast development is a heavily anticipated aspect when it comes to the effects of GAHT. Currently, knowledge about the mechanism under which trans women undergo breast development is largely unknown (4). While it can be easy to believe that breast development would be similar, if not identical, in trans women as it is in cis women, until further research is done, that type of statement cannot be concluded. One of the things we do know is that breast tissue in trans women is histiologically indistinguishable from cis women breast tissue after hormone therapy (6). While this is true, another important fact is that trans women's breasts often stop development around tanner stage III, ending up smaller than the average breast size, and as some trans women would self describe as "conically shaped breasts," though whether the level of tuberous breast deformities in trans women is higher than in the cisgender population is currently not clear. What is clear is that further research regarding maximizing efficiency of breast development is crucial when it comes to increasing quality of life for TW. Many trans women seek breast augmentation surgery to combat gender dysphoria regarding dissatisfaction regarding their breasts, and improving general breast development results for TW will help many trans women forgo the perceived necessity of such surgeries.

Studying gynecomastia is a starting point when looking to study breast development in non-cis

women. Gynecomastia is the development of breast tissue in cisgender men, most typically due to hormonal imbalances where estrogen is at a higher level than is expected for cisgender men. This typically occurs in three life stages for men, either neonatally, during puberty, or during old age. This can be due to a number of reasons. Hypogonadism in men causes a lack of production of testosterone in men, leading to increases in LH and increases in aromatization. This is similar to what TW are trying to achieve, where the administration of estradiol leads to a negative feedback loop against the hypothalamic-pituitary axis causing lower expressions of testosterone as a desired effect. The estrogen uptake and testosterone suppression here is desired and leads to a positive mental health outcome for trans women. The main point here is that studying gynecomastia with intent can help us potentially understand transgender breast development.

Conclusion

Estrogen and estrogen receptors are a part of key hormonal activity that regulates both reproductive and neurological processes within the human body. TWs brains often are either resembling cis women's brains, or a unique phenotype even before administration of HRT. TWs bodies become more aligned with cis women's bodies then cis men's bodies after administration of E2 and an antiandrogen, though there is room for improvement of desired effects.

Much of the knowledge in regards to breast development for cisgender women is primarily from mouse models, and while mouse models have many upsides within scientific experimentation, to have a perfect model of human breast development requires a deeper look at human anatomy. While there are some studies different studies have different goals which leads to different criteria for recruitment of transgender individuals, leading to a messy, unclear view of the subject matter.

There is a lot of work to be done when it comes to understanding transgender bodies, minds, and overall health. An important aspect of gender identity that has been left out of this paper are nonbinary individuals, out of both a desire to focus specifically on trans women and transfeminine aligned people, and out of a lack of research out there about nonbinary individuals. Longitudinal studies for trans women based on neuroimaging have also never been done and will be a necessary step to better understanding trans health. It is important to acknowledge that while having a better understanding of the potential brain structures underlying transgender identities will help us better treat transgender patients, it is equally as important to recognize that in the current political landscape, it is

just as important to recognize trans people as the gender they identify as, rather than trying to use neuroimaging to definitively state someone's gender. There is a severe knowledge gap in how breast development occurs within trans women that is currently blocking the way towards better therapeutic options for TW. Studies on mice models might be a start, but the differences between mice and humans come into play here with mice differentiating between male and female mammary glands during the emybronic phase while human mammary glands differentiate during puberty. While larger studies on transgender individuals would most definitely help improve our understanding, performing these types of studies is currently very difficult in the United States due to intense amounts of transphobic rhetoric coming from the current administration.

Statement on AI use

AI tools were not used to generate scientific content. All scientific content, analysis, and conclusions were reviewed and revised by the author.

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