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Human Embryonic Stem Cells: A Model System for Delineating the Molecular Basis of Human Embryogenesis and Aging-related Diseases

Craig S. Atwood and Sivan Vadakkadath Meethal
University of Wisconsin, Departments of Medicine and Neurological Surgery and Geriatric Research, Education and Clinical Center, Veterans Administration Hospital, Madison, WI 53705
U.S.A.

1. Introduction

The recent development of techniques to culture human embryonic stem cells (hESC) has allowed the study of reproductive (pregnancy) hormones involved in the growth and development of the early embryo. Until the advent of hESC culture techniques, no model system existed that could readily assess the requirement for pregnancy hormones in the growth and development of the human embryo. Hormonal manipulation of developing embryoes *in utero* was technically cumbersome and complicated by competing *in vivo* maternal hormonal signals. This chapter describes recent experimental studies, utilizing hESC, aimed at identifying physiologically relevant signals that promote cell division, differentiation and apoptosis during early embryogenesis and summarizes our current knowledge of how reproductive hormones direct growth and development during embryogenesis. It also describes the potential for using hESC, embryoid bodies (EBs) and neuroectodermal rosettes to gain insights into how reproductive endocrine dyscrasia associated with menopause/andropause drives aberrant cell cycle signalling mechanisms leading to age-related diseases including neurodegeneration and associated cognitive decline.

2. Human embryogenesis

2.1 Hormonal regulation of pregnancy

Embryogenesis is a complex coordinated series of molecular and cellular changes that takes place within a well-defined internal environment. Human embryogenesis is orchestrated by a complex array of endocrine signals that commences with conception, is followed by the growth and development of the zygote into a blastocyst, its implantation into the endometrium, and the subsequent growth and development of the blastocyst into the neonate. Following conception, the developing embryo (zygote/morula/blastocyst) has 7-14 days in which to produce sufficient human chorionic gonadotropin (hCG), and subsequently progesterone (P_4; from both embryonic and corpus luteal sources), to allow implantation and halt degradation and discharging of the endometrium (menstruation) (Gupta, et al. 2007). The upregulation of hCG and P_4 not only allows for the maintenance of

the endometrium, blastocyst attachment and synctiotrophoblast proliferation into the endometrium (Larson et al. 2003; Licht et al. 2001; Pepe and Albrecht 1995), but also prevents ovulation and prepares the immune, metabolic and psychological systems of the mother for pregnancy.

P_4 production is an *absolute* requirement for the maintenance of pregnancy (Larson et al. 2003). Indeed, administration of RU-486 (mifepristone), an anti-P_4 and anti-glucocorticosteroid agent to humans is used for the medical termination of pregnancies of up to 49 days gestation (up to 63 days gestation in Britain and Sweden), and in combination with prostaglandin E1, for termination of pregnancies between 13 and 24 weeks gestation (Fiala and Gemzel-Danielsson 2006). Inhibition of P_4 signaling using RU-486, a P_4 receptor (PR) competitive antagonist, results in endometrial decidual degeneration, trophoblast detachment and decreased syncytiotrophoblast production of hCG which in turn decreases P_4 production by the corpus luteum. In addition, RU-486 induces cervical softening and dilatation, release of endogenous prostaglandins and an increase in the sensitivity of the myometrium to the contractile effects of prostaglandins leading to the expulsion of the embryo/fetus (Gemzell-Danielsson et al. 2006).

Despite our understanding of the endocrinology of pregnancy, the lack of an appropriate model system limited experimentally our ability to answer fundamental questions such as what endocrine/paracrine/juxtacrine/autocrine factors 1) regulate embryonic cell division, 2) regulate cell migration, 3) specify differentiation into particular lineages, and 4) regulate apoptosis, during early embryogenesis.

2.2 Human embryonic stem cells: A model system for understanding the cellular and molecular mechanisms regulating early human embryogenesis

Thompson and colleagues first isolated pluripotent hESC lines from surplus embryos donated by individuals undergoing infertility treatment (Thomson et al. 1998). Inner mass cells isolated from these embryos were allowed to develop to the blastocyst stage and then passaged in defined media (to maintain pluripotency) to increase cell numbers. Five diploid cell lines (H1, H7, H9, H13 and H14) were obtained from 14 blastocysts. These cells are Oct-4, SSEA1, SSEA-3, SSEA-4, TRA 1-60, TRA 1-81 and alkaline phosphatase positive.

hESC derived from the inner cell mass of the blastocyst can be differentiated into EBs which resemble the early post-implantation embryo (blastocyst containing all 3 germ layers) (O'Shea 1999). hESC also can be differentiated into columnar neuroectodermal cells and mimics *in vivo* neuroectodermal development in terms of timing and morphology (Li and Zhang 2006). *In vitro*, hESC differentiate into primitive neuroectodermal (or neural precursor) cells at around day 10 and then neuroectodermal cells that exhibit neural tube-like rosettes in 14–17 days of differentiation in a chemically defined neural induction media (**Fig. 1**; Gallego et al., 2010; Zhang et al. 2001). These structures are predominantly composed of neuroectodermal cells akin to those that form the neural tube and are neural precursor cells/neural stem cells that can be further differentiated into different neural lineages. Considering hESC are equivalent to a 5-6 days embryo, development of the neuroectoderm *in vitro* takes about 18-20 days, the time window when the neural tube forms in a human embryo (Muller and O'Rahilly 1985; Zhang 2003). The ability to manipulate the hormonal milieu of cultured hESC, embryroid bodies or neuroectodermal rosettes during this time period, either positively or negatively, allows for the identification of signaling pathways involved in cell division, differentiation and apoptosis during embryogenesis.

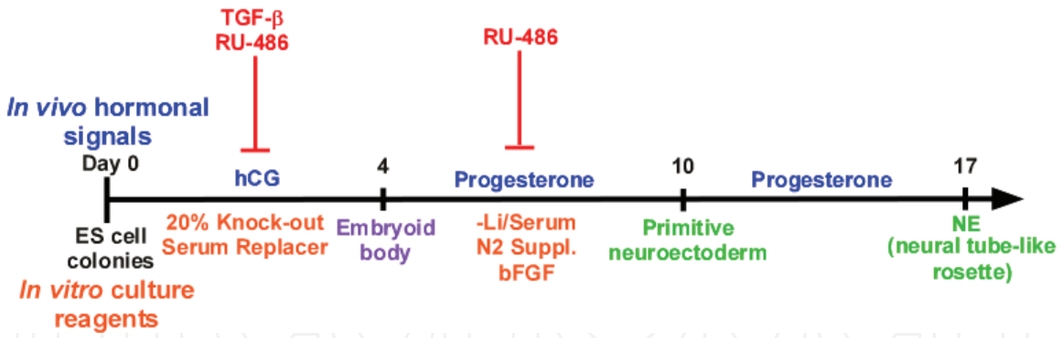


Fig. 1. Time course for the induction of embryoid bodies and neuroectodermal rosettes by hCG and P_4. hESC can be differentiated into primitive blastocyst-like structures (embryoid bodies (EBs) that contain the 3 germ layers) and then into primitive neuroectodermal cells (NE; or neural precursor cells) at ~day 10 and then NE that exhibit neural tube-like rosettes in 14–17 days of differentiation (Okabe et al. 1996; Zhang et al. 2001). Above Line: Physiological hormones secreted by trophoblasts demonstrated to induce cell division (hCG) during EB formation and cell differentiation (P_4) during neuroectodermal rosette formation. Below Line: Current unspecified medias used to generate EBs and neuroectodermal rosettes. These processes are negatively regulated by TGF-β signaling and by blocking signaling via PR

2.3 Trophoblastic hormone secretion during early embryogenesis

Zygotic division into a blastocyst establishes the extra-embryonic tissues (trophoblast layer or outer cell mass) and hypoblast (extraembryonic endoderm) that support the embryonic epiblast (inner cell mass) early in embryogenesis (Gilbert 2003). Trophoblasts secrete an array of hormones (Cemerikic et al. 1994; DiPirro and Kristal 2004; Gallego et al. 2009, 2010; Pidoux et al. 2007; Zhuang and Li 1991) including P_4, endorphins, hCG, 17β-estradiol (E_2) and gonadotropin-releasing hormone (GnRH) (**Fig. 2**). The dramatic elevation in the production of hCG by the trophoblastic layer of the blastocyst during the early embryonic stage (from 5 to ≥1000 mIU/mL in the maternal serum; Braunstein 1976; Pidoux et al. 2007) signals both the corpus lutea and trophoblast (Golos, et al. 2006) to synthesize and secrete P_4 (Bukovsky et al. 1995; Carr et al. 1982; Casper and Yen 1979; Duncan et al. 1996; Richardson and Masson 1985; Strauss et al. 2000). Trophoblastic secretion of these hormones appears to occur during the migration of the blastocyst through the fallopian tube and its implantation into the endometrium and subsequently from the placental tissues during later stages of embryogenesis. This is most clearly demonstrated by the elevation in maternal hCG during the early embryonic period.

The secretion of P_4, endorphins, hCG, E_2 and GnRH by trophoblasts that lie adjacent to the embryoblast in the blastocyst suggests that these hormones may *directly* signal the growth and development of the embryoblast. Evidence supporting this notion includes the presence of placental opioid-enhancing factor in amniotic fluid and placenta, and that the ingestion of placenta potentiates delta- and kappa-opioid antinociception (DiPirro and Kristal 2004). Likewise, trophoblastic and corpa luteal production of hCG/P_4 is markedly elevated post-conception and is obligatory for the maintenance of pregnancy (Larson et al. 2003). An autocrine/paracrine role for hCG secreted from invasive extravillous cytotrophoblasts (Handschuh et al. 2007) in the induction of neoangiogenesis during endometrial vascularization has previously been proposed (Licht et al. 2001). hCG signaling

via full-length LH/hCG receptors (LHCGR) on trophoblasts has been shown to modulate differentiation of the trophoblasts for subsequent villus projection and placentation.
Given the close spatial localization of trophoblasts to the embryoblast, and the availability of hESC that can be cultured continuously, i.e. embryoblast-derived stem cells, it has become possible to explore trophoblastic hormone function in the development of the embryo.

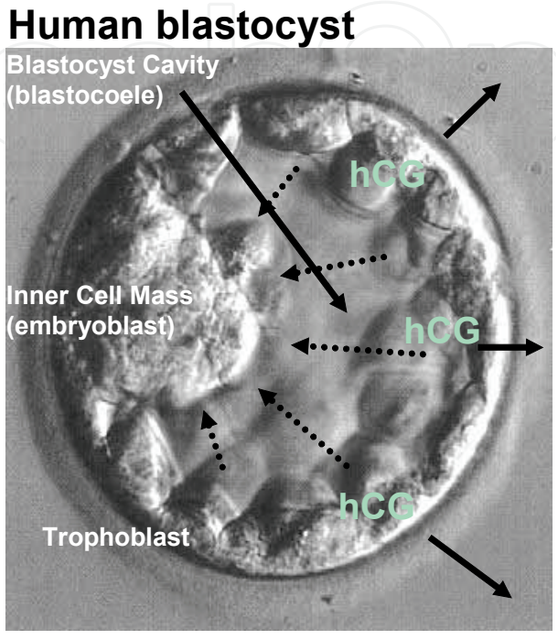


Fig. 2. Trophoblastic hCG secretion from the human blastocyst. Illustration of hCG autocrine (dotted line) and paracrine/endocrine (full line) secretion from the trophopblastic layer of a human blastocyst. Modified from: Muckle, C, Feinberg, E, Glob. libr. women's med., (ISSN: 1756-2228) 2008; DOI 10.3843/GLOWM.10002

3. Trophoblastic hormones and early human embryogenesis

3.1 Regulation of blastulation by human chorionic gonadotropin, progesterone and opioids

The first evidence for a function of any trophoblastic hormone in the regulation of human embryogenesis was ironically demonstrated by the finding that hCG induces the expression of the adhesion and neuritogenic protein amyloid-β precursor protein (AβPP; Porayette et al. 2007), a protein normally associated with the neurodegenerative pathology of Alzheimer's disease (AD; Hardy and Selkoe 2002). AβPP expression was detected at the transcriptional and translational levels (Porayette et al. 2007, 2009). These results indicated a critical molecular signaling link between the hormonal environment of pregnancy and the expression of AβPP in hESC that was suggestive of an important function for this protein during early human embryogenesis prior to the formation of neural precursor cells (Porayette et al. 2007).
That hCG could induce changes in epiblast protein expression was subsequently supported by the finding that hESCs express mRNA and protein for the full-length mature LHCGR (Gallego et al. 2008, 2010). That LHCGR is expressed on hESC implicates hCG as an

important signaling molecule in the growth and development of the embryo. LHCGR expression did not alter upon differentiation into EBs (structures that resemble early post-implantation embryos containing all three germ layers) (O'Shea 1999), or into neuroectodermal rosettes, which consist of >90% columnar neural precursor cells (NPC) and are the *in vitro* equivalent of a rudimentary neural tube (Gallego et al. 2008, 2010; Li and Zhang 2006). The comparable level of LHCGR expression between the different cell lineages was suggestive of 1) the existence of a tight regulatory system for the maintenance of hCG signaling during embryonic stem cell division and differentiation, and 2) a basal requirement for LH/hCG signaling during this early stage of embryogenesis. Indeed, hCG signaling via its hESC receptor was found to be essential for the proliferation of hESC (**Fig. 1**); inhibition of LHCGR signaling with P-antisense oligonucleotides suppressed hESC proliferation, as did a specific blocking antibody against the extracellular activation site of LHCGR, an effect that was reversed by treatment with hCG (Gallego et al. 2008, 2010). These data are supported by the known proliferative properties of (hyperglycosylated) hCG, which has been demonstrated to act as an autocrine factor on extravillous invasive cytotrophoblast cells to initiate and control invasion as occurs at implantation of pregnancy and the establishment of hemochorial placentation, and malignancy as occurs in invasive hydatidiform mole and choriocarcinoma (Cole 2009).

In addition to its cell cycle signaling activity, signaling of hCG via the hESC receptor rapidly upregulated steroidogenic acute regulatory protein (StAR)-mediated cholesterol transport and the synthesis of P_4, a neurogenic steroid (**Fig. 3**; Brewer et al. 1993; Wang et al. 2005). StAR, a key rate-limiting step in the production of sex steroids in reproductive tissues, was detected in hESC at both mRNA and protein (37-kDa, 30-kDa and 20-kDa variants) levels. hCG treatment dose-dependently suppressed the expression of these StAR variants, while P_4 treatment decreased the truncation of the 37-kDa to the 30/32-kDa variants of StAR, indicative of decreased cholesterol transport across the mitochondrial membrane for steroidogenesis (Epstein and Orme-Johnson 1991; Krueger and Orme-Johnson 1983; Pon et al. 1986; Stocco 2001; Stocco and Chen 1991; Yamazaki et al. 2006). Importantly, hCG treatment markedly increased P_4 secretion 15-fold, indicating that embryoblast-derived hESC already possess the machinery to transport cholesterol and synthesize sex steroids. Together, these findings indicate negative feedback pathways exist for the regulation of hCG/LH signaling and mitochondrial cholesterol uptake for the synthesis of sex steroids in hESC and differentiating lineages.

hESC and EBs express P_4 receptor A (PR-A; Gallego et al. 2010; Hong et al. 2004; Sauter et al. 2005) implying P_4 signals hESC differentiation. The requirement for P_4 in the differentiation of hESC into EBs was confirmed by the finding that RU-486, a PR competitive antagonist (Fiala 2006), potently inhibited the differentiation of hESC into EBs (**Fig. 1**). RU-486 treated colonies failed to form normal cystic structures after 10 days in culture, and instead formed solid irregular spheres that were ~20% the size of normal spheroidal EBs (Gallego et al. 2008, 2009, 2010).

Trophoblastic production of endorphins (Zhuang and Li 1991) also is crucial for embryogenesis (Gallego et al. 2009). Treatment of hESC colonies with the delta opioid receptor selective antagonist ICI 174,864 (Corbett et al. 1984; Paterson et al. 1984) inhibits the formation of the EB cystic structure, and instead forms non-spherical structures ~40% the size of normal spheroidal EBs. The mechanism by which opioid signaling promotes blastulation is unclear, however delta opioid antagonists may function to inhibit

embryogenesis by regulating hCG release (Cemerikic et al. 1992) required for P_4 production. Thus, the tight regulation of hCG signaling, and sex steroid and opioid synthesis and signaling, is required to coordinate hESC proliferation and differentiation during gastrulation.

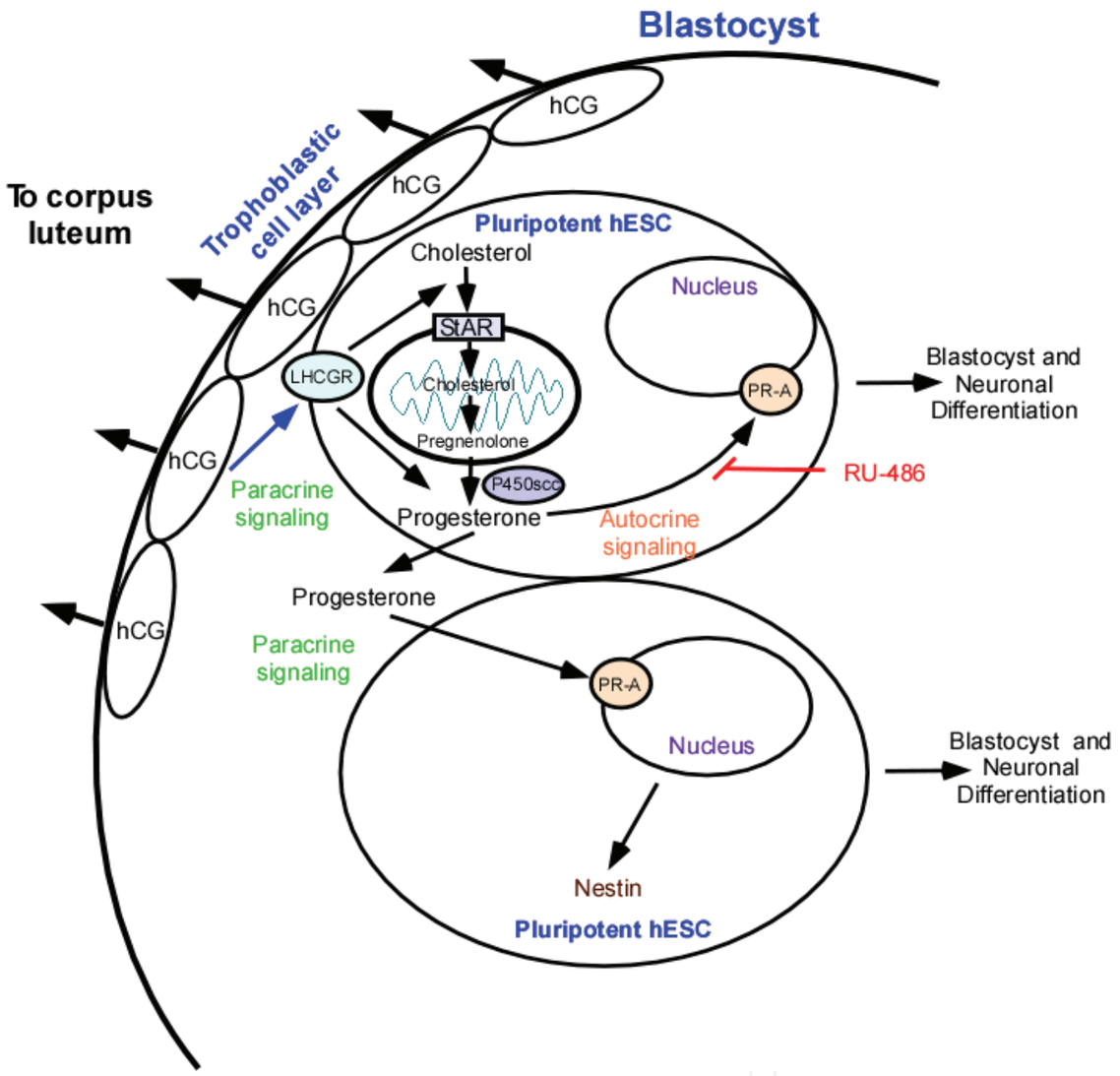


Fig. 3. Model of the autocrine and paracrine pathways regulating blastulation and neurulation. Putative autocrine and endocrine signalling pathways involved in cell proliferation, steroidogenesis and differentiation of the blastocyst and primitive neural tube. For details see Gallego et al., (2010)

3.2 Regulation of neurulation by human chorionic gonadotropin and progesterone

In addition to the obligatory signaling of P_4 for gastrulation, P_4 signaling also is required for the specification of NPCs from hESC (**Fig. 1**; Gallego et al. 2008, 2009, 2010). hCG treatment suppresses expression of the pluripotent marker Oct-3/4, suggesting hCG, or steroid production initiated by hCG signaling, could direct lineage commitment (Gallego et al. 2008,

2010). In the presence of P_4, hESC colonies differentiate into spherical structures containing a minimum of three neuroectodermal rosettes inside of the cavity, while hESC colonies treated without P_4 or with RU-486 failed to form rosettes with columnar neuroectodermal cells after 17 days in culture (Gallego et al. 2008, 2009, 2010). Morphological changes were more severe in the absence of P_4 than with RU-486. P_4, and to a lesser extend E_2, were found to increase the expression of nestin, an early marker of NPC formation, in hESC. RU-486 completely suppressed nestin expression. Interestingly, 'E_2 priming' is required for induction of PR expression in other tissues (Atwood et al. 2000; Mylonas et al. 2007). Thus, the increase in nestin expression with E_2 treatment may reflect increased PR expression together with endogenous P_4 signaling, and explain the current requirement for serum priming of hESC colonies in the preparation of neuroectodermal rosettes.

Interestingly, hESCs default towards a primitive neural stem cell fate if maintained for any length of time in culture (Munoz-Sanjuan and Brivanlou 2002). Since hCG treatment induces nestin expression in hESC, endogenous gonadotropin production by hESC or trophoblastic cells (Golos et al. 2006) may be sufficient for NPC formation, thereby explaining the intrinsic hormonal signals regulating the 'default pathway' of hESC differentiation into neuronal lineages (Munoz-Sanjuan and Brivanlou 2002).

These results suggest that trophoblastic hCG production adjacent to the embryoblast is required not only for trophoblast steroidogenesis and attachment of the blastocyst to the uterine wall (Wahabi et al. 2007), but also for signaling normal proliferation and differentiation of the epiblast (**Fig. 3**). hCG-induced P_4 synthesis therefore has, in addition to its role in uterine decidualization for the implantation and maintenance of pregnancy, an obligatory role prior to the formation of neural precursor cells, as well as an inductive role in the directed differentiation and specification of the first neuronal cell types (organogenesis) and the formation of the neural tube. While the structural importance of P_4 and alloprogesterone has previously been recognized by its early synthesis (by at least day 13) within the developing rat central nervous system (Pomata et al. 2000), these results demonstrate an early (within the first 7 days) and absolute requirement for P_4 during human blastulation and neurulation. In this respect, it has been shown that P_4 is necessary and sufficient (in Neurobasal media) for the maintenance and differentiation of primary hippocampal/cortical/striatal neurons *in vitro* (Brewer et al. 1993). That P_4 is the hormone regulating these key events is perhaps not surprising given its location high in the steroidogenic synthetic pathway; P_4 is the first steroid synthesized from pregnenolone, the precursor to all other steroids.

Opioid signaling also is required for neuroectodermal rosette formation since ICI 174,864 (Corbett et al. 1984; Paterson et al. 1984) inhibits normal neuroectodermal rosette formation and nestin expression (Gallego et al. 2009, 2010). Previous data has implicated P_4 as acting in the arcuate nucleus and anteroventral periventricular nucleus through beta-endorphin and dynorphin B neurons to affect preoptic area GnRH neurons and gonadotropin secretion (Dufourny et al. 2005; Gu and Simerly 1994). Thus, delta opioid receptor signaling is required both for normal human blastulation and neurulation, but it remains to be determined if there is crosstalk between opioid signaling and the regulation of GnRH/gonadotropin secretion.

Previous studies have demonstrated the importance of P_4 and related steroids as neurotrophic agents that promote adult neurogenesis, neuronal survival and neuroprotection (Brewer et al. 1993; Ciriza et al. 2004; Cutler et al. 2006; Guo et al. 2006;

Mauch et al. 2001; Schumacher et al. 2003, 2004; VanLandingham et al. 2006; Wang et al. 2005). Clinical studies supporting the neurotrophic actions of P_4 administration are demonstrated by the decrease in mortality rate and improved outcome following acute traumatic brain injury in humans (Wright et al. 2007).

Dependent upon the timing of administration during pregnancy, suppression of P_4 signaling with RU-486 (Fiala and Gemzel-Danielsson 2006) aside from intrauterine disruptive functions (decidual breakdown and trophoblast detachment) also will disrupt time-sensitive developmental processes. The requirement of P_4 during cavitation processes indicates the structural influence of these molecular pathways on the developing embryo within the first 7 days, but also on the formation of the neural tube at around day 17-19, which will influence future neural connectivity. The relative binding affinity of RU-486 for the PR is twice that of P_4 (Heikinheimo et al. 2003), and is used at a dose of 200-600 mg for the termination of pregancies (Fiala 2006) (this equates to ~6-19 μM, equivalent to that used in Gallego et al. 2008, 2009, 2010). Thus, the abortifacient effects of RU-486 in blocking PR signaling also extend to blocking blastulation and neurulation and the normal growth and development of the embryo.

3.3 Regulation of organogenesis by human chorionic gonadotropin and progesterone

Aside from the induction of blastulation and neurulation early in embryogenesis, hCG/LH and P_4 signaling may play a role in the development of other tissues (LH is the adult hCG homolog with 83 % homology and binds the same receptor - LHCGR). LHCGR and PR have been identified on numerous reproductive and non-reproductive tissues (Ascoli et al. 2002; Bouchard 1999; Bukovsky et al. 2003; Mulac-Jericevic and Conneely 2004). With regard hCG/LH, the free glycoprotein α-subunit of gonadotropins has been shown to stimulate differentiation of prolactin cells in the pituitary (Avsian-Kretchmer and Hsueh 2004) and endometrial stromal cell decidualization in the placenta (Blithe et al. 1991). Although it has not been demonstrated if hCG/LH has a developmental function during organogenesis, hyperglycosylated hCGβ has potent cell growth and invasion properties as observed in early pregnancy, gestational choriocarcinoma and testicular cancers (Cole 2009). Interestingly, in the adult brain, subcutaneous administration of LH has been shown to induce neurogenesis in the hippocampus of the adult mouse (Mak et al. 2007), while in sheep there is evidence that GnRH directly, or indirectly via LH, induces neurogenesis in the hippocampus (Hawken et al. 2009). hCG also is known to promote angiogenesis by inducing the up-regulation of vascular endothelial growth factor (Berndt et al. 2006; Licht et al. 2002; Zygmunt et al. 2002) and P_4 (Rogers et al. 2009).

The potential for P_4 to regulate organogenesis has been reported during puberty and adulthood, where P_4 is obligatory for the development of the tertiary ducts on the mammary gland, and the functional differentiation of the lobuloalveolar system from the lobular buds (Atwood et al. 2000). In the adult, P_4 and related metabolites have been demonstrated to regulate bone formation (Prior 1990), promote angiogenesis and arteriogenesis (Rogers et al. 2009), promote formation of the placenta and promote neurogenesis by increasing rat neuroprogenitor cell proliferation and human neural stem cell proliferation (Wang et al. 2005). While our knowledge of hCG/LH and P_4 during organogenesis is rudimentary at this point, the above evidence indicates these hormones likely play important functions in many tissues during organogenesis.

4. Utilizing human embryonic stem cells as a model system to understand the molecular basis of aging-related diseases

It is becoming clear that the hormonal mechanisms that regulate the coordinated division and differentiation of cells early in life become dysregulated later in life following menopause and during andropause (which starts at ~ 30 years of age; see Vadakkadath Meethal et al. 2005; Atwood and Bowen 2011), and that these dysregulated hormonal mechanisms drive aberrant re-entry of cells into the cell cycle and their abortive death leading to tissue compromise and ultimately disease (Atwood et al. 2005).

The reproductive endocrine dyscrasia associated with menopause and andropause are intimately associated with disease (Bowen and Atwood 2004; Atwood and Bowen, 2011). The decline in sex steroid production by the gonads following menopause and during andropause leads to a loss of hypothalamic feedback inhibition that stimulates GnRH and gonadotropin production (Carr 1998). In addition, the decrease in gonadal inhibin production at this time (Reichlin 1998) results in decreased activin receptor inhibition, and together with the increase in bioavailable activin (Gray et al. 2002) leads to a further increase in the secretion of GnRH and gonadotropins (MacConell et al. 1999; Schwall et al. 1988; Weiss et al. 1993). Thus, the lack of negative feedback from the ovary (P_4, E_2 and inhibin) is responsible for the unopposed and marked elevations in the secretion of GnRH and gonadotropins with ovarian and testicular senescence (Atwood et al. 2005; Chakravarti et al. 1976; Neaves et al. 1984; Reame et al. 1996; Schmidt et al. 1996).

The concentration of brain sex steroids, including P_4, is a mixture of peripherally derived sex steroids, converted peripheral steroids, and neuro-sex steroids. The contribution of peripheral sex steroids to total brain sex steroids is unknown, but post-reproductive declines in peripheral P_4 would be expected to impact brain P_4 concentrations. While elevations in GnRH and gonadotropin concentrations might promote brain neurosteroid production (see Vadakkadath Meethal et al. 2009), including P_4, it is not known if this is sufficient to counter the loss of peripherally-derived sex steroids. The consequences of these hormonal alterations are discussed below in the context of AD.

4.1 Alzheimer's disease

Dementia accounts for 3% of deaths in the USA (CDC National Vital Statistics Report, 2009) although by the age of 85 ~45% of the population has some form of dementia. AD accounts for ~70% of all dementia cases and is characterized neurologically by age-related progressive memory loss, impairments in behavior, language, and visuo-spatial skills (Atwood et al. 2005; Vadakkadath Meethal et al. 2005).

Unlike development, where mitogenic and differentiative signaling are both elevated, senescence is associated with elevated mitogenic (i.e. gonadotropins, GnRH) signaling but decreased differentiative signaling (i.e. sex steroids).

The age-related loss of P_4 is of particular importance given the differentiative properties of this steroid described above. Also of importance are the aging-related elevations in serum GnRH, FSH and LH, especially given the known proliferative properties of hCG/LH (Cole 2009; Gallego et al. 2008, 2010). In this regard, LH/hCG is known to have powerful mitogenic properties in certain reproductive tissues (Davies et al. 1999; Harris et al. 2002; Horiuchi et al. 2000; Sriraman et al. 2001; Webber and Sokoloff 1981), the brain (Mak et al., 2007; Hawken et al., 2009) and are frequently expressed by tumor cells (Krichevsky et al.

1995; Whitfield and Kourides 1985; Yokotani et al. 1997 and reviewed in Cole 2009). Thus, these multiple changes in hormonal signaling with menopause/andropause, i.e. increased mitogenic signaling but decreased differentiation signaling (*dyotic signaling*) might be expected to impact normal cell cycle dynamics. Indeed, accumulating evidence suggests there is a reactivation of the cell cycle with aging (see Bowen and Atwood 2004) as has been demonstrated for post-mitotic pyramidal neurons of the brains of aged individuals with AD (Herrup and Yang 2007; Raina et al. 2000). This data includes 1) the ectopic expression of cell cycle proteins in those regions of the brain affected by AD, but not in areas unaffected by AD pathology or in control brains, 2) chromosomal replication (endoreduplication) in differentiated AD neurons, demonstrating entry into S-phase of the cell cycle, 3) elevated cytoplasmic mitochondrial DNA and Cox-1 expression, suggestive of *de novo* mitochondrion synthesis, and 4) upregulated growth factor signal transduction pathways. Importantly, the spatio-temporal expression of sex hormone receptors throughout the brain is in those areas of AD neurodegeneration. Other parallels between embryonic neurogenesis and adult neurodegeneration include the expression of AβPP, secretases and tau, together with the processing of AβPP either towards the amyloidogenic or non-amyloidogenic pathways, and the phosphorylation of tau (Porayette et al. 2009) (Atwood and Porayette, unpublished data). Similarly, the fetal brain has been reported to display a number of biochemical similarities to the AD brain, namely the presence of Aβ and AβPP (Arai et al. 1997; Takashima et al. 1990), presenilin-1 expression (Berezovska et al. 1997) and hyper-phosphorylated tau (Goedert et al. 1993). The phosphorylation of tau is a mitogenic-associated event that normally occurs during metaphase of neuronal division, and is observed during differentiation of neurons in the fetal brain (Goedert et al. 1993; Liu, et al. 2004).

This increased developmental protein expression in the AD brain suggests reactivation of the cell cycle in differentiated neurons of the AD brain (Herrup and Yang 2007) and explains the majority of the biochemical and pathological features associated with the disease (Atwood et al. 2005; Meethal et al. 2005). Two recent studies support this claim. Forced cell cycle activation in terminally differentiated neurons via conditional expression of the simian virus 40 large T antigen (oncogene) forms Aβ deposits and tau pathology in the mouse cortex (Park et al. 2007). Similarly, forced cell cycle activation in primary neurons is accompanied by tau phosphorylation (McShea et al. 2007). These data suggest that AD neuropathology is a result of an imbalance in cell cycle regulation in the adult brain.

4.2 Amyloid-β precursor protein and neurogenesis

That amyloidogenic pathways are involved in neurogenesis has recently been reported by a number of workers (Calafiore et al. 2006; Heo et al. 2007; Liu et al. 2004; Lopez-Toledano and Shelanski 2004). In this context, an increase in neurogenesis has been reported in young transgenic mice overexpressing human mutant AβPP (Jin et al. 2004; Lopez-Toledano and Shelanski 2007). Moreover, the overexpression of wild-type or FAD mutant AβPP, which promotes Aβ generation (Citron et al. 1997), also has been shown to promote the re-entry of primary neurons into the cell cycle, as demonstrated by the induction of DNA synthesis and cell cycle markers (McPhie et al. 2003). Not surprisingly, AβPP has structural similarity to growth factors (Trapp and Hauer 1994) and modulates several important neurotrophic functions, including neuritogenesis, synaptogenesis, and synaptic plasticity (Gralle and Ferreira 2007).

4.3 Hormonal regulation of neurogenesis via modulation of amyloid-β precursor protein metabolism

In hESC, the differential processing of AβPP via secretase enzymes regulates the proliferation and differentiation of hESC; processing towards the amyloidogenic pathway is associated with cell proliferation, processing towards the non-amyloidogenic pathway is associated with cell specification and differentiation. Specifically, P_4 induces processing of AβPP towards the production of the soluble AβPPα in hESC (Porayette et al. 2009), which has known differentiative properties (Milward et al. 1992). Similarly, E_2 has been shown to stimulate the processing of AβPP by the nonamyloidogenic α-secretory pathway and reduces cellular Aβ production in both nonneuronal (Jaffe et al. 1994) and neuronal cultures (Greenfield et al. 2002; Manthey et al. 2001; Xu et al. 1998). Conversely, the loss of sex steroids and elevation in gonadotropins following ovariectomy has been shown to increase Aβ generation in non-transgenic animals (Petanceska et al. 2000). Importantly, we have demonstrated that LH promotes the processing of AβPP towards the amyloidogenic pathway *in vitro*, while suppression of serum LH in mice using GnRH agonists decreases the concentration of brain Aβ1-40 and Aβ1-42, the 2 major variants that deposit in the AD brain (Bowen et al. 2004). It is therefore plausible that the interaction of these hormonal pathways on the modulation of the processing of AβPP may regulate cell cycle events throughout life, with dyotic signaling by these hormones leading to the reactivation of the cell cycle in differentiated neurons of the AD brain (Herrup and Yang 2007). This aberrant, albeit unsuccessful, re-entry of neurons into the cell cycle leads to synapse contraction and neuron death (see Atwood et al. 2005; Herrup et al. 2004; Raina et al. 2000 for reviews). In addition to the loss of neurons following the reactivation of the cell cycle in differentiated neurons, it is possible that dyotic signaling prevents normal neurogenesis from resident neural stem cells, thereby preventing replacement of neurons. Further studies are required to determine whether post-reproductive levels of GnRH/gonadotropins are sufficient to induce neurosteroidogenesis in neural stem cells and neuronal cell types, and whether the level of post-reproductive neurosteroid synthesis dictates normal or dyotic signaling in these cell types.

5. Conclusion

The advent of the human embryonic stem cell era has allowed experimental determination of the physiological hormone requirements for early embryogenesis. In this respect, although progestagens are often considered primarily reproductive hormones with maternal influences, it is now clear that progestagens and hCG are essential for the growth and development of the embryo as well as the normal health of the brain throughout life. Paracrine/juxtacrine signaling of hCG (and opioids) for mobilization of cholesterol for P_4 production by the epiblast/synctiotrophoblast following conception is obligatory for human blastulation and neurulation (**Fig. 3**). This paracrine/juxtacrine signaling by extraembryonic tissues is the commencement of trophic support by placental tissues in the growth and development of the human embryo. The identification of these hormones that regulate cell proliferation and differentiation of hESC *in vitro* will help direct the development of medias that most closely reflect their *in utero* environment for *in vitro* culture of these cells and their differentiation towards various cell lineages. Such medias could be used to further delineate the molecular basis of embryogenesis and organogenesis, as well as establish *ex utero* embryonic and fetal cultures.

The above discussion also indicates how hESC can provide insight into neurodegenerative disease (and likely many other aging-related diseases). While appropriate gonadotropin/ GnRH and P_4 signaling is necessary for normal growth and development during embryogenesis, fetal life and childhood, and for the maintenance of brain health during adult reproductive life, the unopposed elevations in GnRH/gonadotropins with the loss of sex steroids following menopause/andropause appears to lead to dysregulation of cell cycle events (Bowen and Atwood 2004).

It will be possible in the future to use hESC cells as a model for understanding how endocrine, paracrine and autocrine signals regulate cell cycle progression, from entry to exit, and how dysregulated signaling leads to entry but no exit from the cell cycle, i.e. leading to endoreduplication such as is apparent in the pyramidal neurons of the AD brain. Thus, hESC are a useful cell model system for examining questions related to early embryonic neurogenesis, adult neuroregeneration and neurodegeneration.

6. References

Arai Y, Suzuki A, Mizuguchi M & Takashima S 1997 Developmental and aging changes in the expression of amyloid precursor protein in Down syndrome brains. *Brain Dev* 19 290-294.

Ascoli M, Fanelli F & Segaloff DL 2002 The lutropin/choriogonadotropin receptor, a 2002 perspective. *Endocr Rev* 23 141-174.

Atwood C & Bowen R 2011 The Reproductive-Cell Cycle Theory of Aging: An Update. *Experimental Gerontology* In press.

Atwood CS, Hovey RC, Glover JP, Chepko G, Ginsburg E, Robison WG & Vonderhaar BK 2000 Progesterone induces side-branching of the ductal epithelium in the mammary glands of peripubertal mice. *J Endocrinol* 167 39-52.

Atwood CS, Meethal SV, Liu T, Wilson AC, Gallego M, Smith MA & Bowen RL 2005 Dysregulation of the hypothalamic-pituitary-gonadal axis with menopause and andropause promotes neurodegenerative senescence. *J Neuropathol Exp Neurol* 64 93-103.

Avsian-Kretchmer O & Hsueh AJ 2004 Comparative genomic analysis of the eight-membered ring cystine knot-containing bone morphogenetic protein antagonists. *Mol Endocrinol* 18 1-12.

Berezovska O, Xia MQ, Page K, Wasco W, Tanzi RE & Hyman BT 1997 Developmental regulation of presenilin mRNA expression parallels notch expression. *J Neuropathol Exp Neurol* 56 40-44.

Berndt S, Perrier d'Hauterive S, Blacher S, Pequeux C, Lorquet S, Munaut C, Applanat M, Herve MA, Lamande N, Corvol P, et al. 2006 Angiogenic activity of human chorionic gonadotropin through LH receptor activation on endothelial and epithelial cells of the endometrium. *Faseb J* 20 2630-2632.

Blithe DL, Richards RG & Skarulis MC 1991 Free alpha molecules from pregnancy stimulate secretion of prolactin from human decidual cells: a novel function for free alpha in pregnancy. *Endocrinology* 129 2257-2259.

Bouchard P 1999 Progesterone and the progesterone receptor. *J Reprod Med* 44 153-157.

Bowen RL & Atwood CS 2004 Living and dying for sex. A theory of aging based on the modulation of cell cycle signaling by reproductive hormones. *Gerontology* 50 265-290.

Bowen RL, Verdile G, Liu T, Parlow AF, Perry G, Smith MA, Martins RN & Atwood CS 2004 Luteinizing hormone, a reproductive regulator that modulates the processing of amyloid-beta precursor protein and amyloid-beta deposition. *J Biol Chem* 279 20539-20545.

Braunstein GD, Rasor J., Danzer H. 1976 Serum human chorionic gonadotropin levels throughout normal pregnancy. *Am J Obstet Gynecol* 126 678.

Brewer GJ, Torricelli JR, Evege EK & Price PJ 1993 Optimized survival of hippocampal neurons in B27-supplemented Neurobasal, a new serum-free medium combination. *J Neurosci Res* 35 567-576.

Bukovsky A, Caudle MR, Keenan JA, Wimalasena J, Upadhyaya NB & Van Meter SE 1995 Is corpus luteum regression an immune-mediated event? Localization of immune system components and luteinizing hormone receptor in human corpora lutea. *Biol Reprod* 53 1373-1384.

Bukovsky A, Indrapichate K, Fujiwara H, Cekanova M, Ayala ME, Dominguez R, Caudle MR, Wimalsena J, Elder RF, Copas P, et al. 2003 Multiple luteinizing hormone receptor (LHR) protein variants, interspecies reactivity of anti-LHR mAb clone 3B5, subcellular localization of LHR in human placenta, pelvic floor and brain, and possible role for LHR in the development of abnormal pregnancy, pelvic floor disorders and Alzheimer's disease. *Reprod Biol Endocrinol* 1 46.

Calafiore M, Battaglia G, Zappala A, Trovato-Salinaro E, Caraci F, Caruso M, Vancheri C, Sortino MA, Nicoletti F & Copani A 2006 Progenitor cells from the adult mouse brain acquire a neuronal phenotype in response to beta-amyloid. *Neurobiol Aging* 27 606-613.

Carr BR 1998 Williams Textbook of Endocrinology. pp 751-817. Ed FD Wilson JD, Kronenberg HM, Larsen PR. Philadelphia PA: WB Saunders Co.

Carr BR, MacDonald PC & Simpson ER 1982 The role of lipoproteins in the regulation of progesterone secretion by the human corpus luteum. *Fertil Steril* 38 303-311.

Casper RF & Yen SS 1979 Induction of luteolysis in the human with a long-acting analog of luteinizing hormone-releasing factor. *Science* 205 408-410.

Cemerikic B, Schabbing R & Ahmed MS 1992 Selectivity and potency of opioid peptides in regulating human chorionic gonadotropin release from term trophoblast tissue. *Peptides* 13 897-903.

Cemerikic B, Zamah R & Ahmed MS 1994 Opioids regulation of human chorionic gonadotropin release from trophoblast tissue is mediated by gonadotropin releasing hormone. *J Pharmacol Exp Ther* 268 971-977.

Chakravarti S, Collins WP, Forecast JD, Newton JR, Oram DH & Studd JW 1976 Hormonal profiles after the menopause. *Br. Med. J.* 2 784-787.

Ciriza I, Carrero P, Azcoitia I, Lundeen SG & Garcia-Segura LM 2004 Selective estrogen receptor modulators protect hippocampal neurons from kainic acid excitotoxicity: differences with the effect of estradiol. *J Neurobiol* 61 209-221.

Citron M, Westaway D, Xia W, Carlson G, Diehl T, Levesque G, Johnson-Wood K, Lee M, Seubert P, Davis A, et al. 1997 Mutant presenilins of Alzheimer's disease increase production of 42-residue amyloid beta-protein in both transfected cells and transgenic mice. *Nat Med* 3 67-72.

Cole LA 2009 New discoveries on the biology and detection of human chorionic gonadotropin. *Reprod Biol Endocrinol* 7 8.

Corbett AD, Gillan MG, Kosterlitz HW, McKnight AT, Paterson SJ & Robson LE 1984 Selectivities of opioid peptide analogues as agonists and antagonists at the delta-receptor. *Br J Pharmacol* 83 271-279.

Cutler SM, VanLandingham JW, Murphy AZ & Stein DG 2006 Slow-release and injected progesterone treatments enhance acute recovery after traumatic brain injury. *Pharmacol Biochem Behav* 84 420-428.

Davies BR, Finnigan DS, Smith SK & Ponder BAAogsponmoseGE 1999 Administration of gonadotropins stimulates proliferation of normal mouse ovarian surface epithelium. *Gynecol. Endocrinol.* 13 75-81.

DiPirro JM & Kristal MB 2004 Placenta ingestion by rats enhances delta- and kappa-opioid antinociception, but suppresses mu-opioid antinociception. *Brain Res* 1014 22-33.

Dufourny L, Caraty A, Clarke IJ, Robinson JE & Skinner DC 2005 Progesterone-receptive beta-endorphin and dynorphin B neurons in the arcuate nucleus project to regions of high gonadotropin-releasing hormone neuron density in the ovine preoptic area. *Neuroendocrinology* 81 139-149.

Duncan WC, McNeilly AS, Fraser HM & Illingworth PJ 1996 Luteinizing hormone receptor in the human corpus luteum: lack of down-regulation during maternal recognition of pregnancy. *Hum Reprod* 11 2291-2297.

Epstein LF & Orme-Johnson NR 1991 Regulation of steroid hormone biosynthesis. Identification of precursors of a phosphoprotein targeted to the mitochondrion in stimulated rat adrenal cortex cells. *J Biol Chem* 266 19739-19745.

Fiala C 2006 Exelgyn, (February 2006). "Mifegyne UK Summary of Product Characteristics (SPC)" Retrieved on 9-9-08; http://emc.medicines.org.uk/emc/assets/c/html/displaydoc.asp?documentid=617.

Fiala C & Gemzel-Danielsson K 2006 Review of medical abortion using mifepristone in combination with a prostaglandin analogue. *Contraception* 74 66-86.

Gallego MJ, Porayette P, Kaltcheva M, Bowen RL, Vadakkadath Meethal S & Atwood CS 2008 Trophoblastic hormones direct early human embryogenesis. *Available from Nature Precedings* http://hdl.handle.net/10101/npre.2008.2671.1.

Gallego MJ, Porayette P, Kaltcheva M, Bowen RL, Vadakkadath Meethal S & Atwood CS 2010 The pregnancy hormones hCG and progesterone induce human embryonic stem cell proliferation and differentiation into neuroectodermal rosettes. *Stem Cell Research and Therapy* 1:28.

Gallego MJ, Porayette P, Kaltcheva MM, Meethal SV & Atwood CS 2009 Opioid and progesterone signaling is obligatory for early human embryogenesis. *Stem Cells Dev* 18 737-740.

Gemzell-Danielsson K, Bygdeman M & Aronsson A 2006 Studies on uterine contractility following mifepristone and various routes of misoprostol. *Contraception* 74 31-35.

Gilbert S 2003 *Developmental Biology*. Sunderland: Sinauer Associates.

Goedert M, Jakes R, Crowther RA, Six J, Lubke U, Vandermeeren M, Cras P, Trojanowski JQ & Lee VM 1993 The abnormal phosphorylation of tau protein at Ser-202 in Alzheimer disease recapitulates phosphorylation during development. *Proc Natl Acad Sci U S A* 90 5066-5070.

Golos TG, Pollastrini LM & Gerami-Naini B 2006 Human embryonic stem cells as a model for trophoblast differentiation. *Semin Reprod Med* 24 314-321.

Gralle M & Ferreira ST 2007 Structure and functions of the human amyloid precursor protein: the whole is more than the sum of its parts. *Prog Neurobiol* 82 11-32.

Gray PC, Bilizikjian LM & Vale W 2002 Antagonism of activin by inhibin and inhibin receptors: a functional role for betaglycan. *Mol. Cell. Endocrinol.* 188 254-260.

Greenfield JP, Leung LW, Cai D, Kaasik K, Gross RS, Rodriguez-Boulan E, Greengard P & Xu H 2002 Estrogen lowers Alzheimer beta-amyloid generation by stimulating trans-Golgi network vesicle biogenesis. *J Biol Chem* 277 12128-12136.

Gu G & Simerly RB 1994 Hormonal regulation of opioid peptide neurons in the anteroventral periventricular nucleus. *Horm Behav* 28 503-511.

Guo Q, Sayeed I, Baronne LM, Hoffman SW, Guennoun R & Stein DG 2006 Progesterone administration modulates AQP4 expression and edema after traumatic brain injury in male rats. *Exp Neurol* 198 469-478.

Gupta AK, Holzgreve W & Hahn S 2007 Decrease in lipid levels of syncytiotrophoblast micro-particles reduced their potential to inhibit endothelial cell proliferation. *Arch Gynecol Obstet*.

Handschuh K, Guibourdenche J, Tsatsaris V, Guesnon M, Laurendeau I, Evain-Brion D & Fournier T 2007 Human chorionic gonadotropin expression in human trophoblasts from early placenta: comparative study between villous and extravillous trophoblastic cells. *Placenta* 28 175-184.

Hardy J & Selkoe DJ 2002 The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science* 297 353-356.

Harris D, Bonfil D, Chuderland D, Kraus S, Seger R & Naor Z 2002 Activation of MAPK cascades by GnRH: ERK and Jun N-terminal kinase are involved in basal and GnRH-stimulated activity of the glycoprotein hormone LHbeta-subunit promoter. *Endocrinology* 143 1018-1025.

Hawken PA, Jorre TJ, Rodger J, Esmaili T, Blache D & Martin GB 2009 Rapid induction of cell proliferation in the adult female ungulate brain (Ovis aries) associated with activation of the reproductive axis by exposure to unfamiliar males. *Biol Reprod* 80 1146-1151.

Heikinheimo O, Kekkonen R & Lahteenmaki P 2003 The pharmacokinetics of mifepristone in humans reveal insights into differential mechanisms of antiprogestin action. *Contraception* 68 421-426.

Heo C, Chang KA, Choi HS, Kim HS, Kim S, Liew H, Kim JA, Yu E, Ma J & Suh YH 2007 Effects of the monomeric, oligomeric, and fibrillar Abeta42 peptides on the proliferation and differentiation of adult neural stem cells from subventricular zone. *J Neurochem* 102 493-500.

Herrup K, Neve R, Ackerman SL & Copani A 2004 Divide and die: cell cycle events as triggers of nerve cell death. *J Neurosci* 24 9232-9239.

Herrup K & Yang Y 2007 Cell cycle regulation in the postmitotic neuron: oxymoron or new biology? *Nat Rev Neurosci* 8 368-378.

Hong SH, Nah HY, Lee YJ, Lee JW, Park JH, Kim SJ, Lee JB, Yoon HS & Kim CH 2004 Expression of estrogen receptor-alpha and -beta, glucocorticoid receptor, and progesterone receptor genes in human embryonic stem cells and embryoid bodies. *Mol Cells* 18 320-325.

Horiuchi A, Nikaido T, Yoshizawa T, Itoh K, Kobayashi Y, Toki T, Konishi I & Fujii S 2000 HCG promotes proliferation of uterine leiomyomal cells more strongly than that of myometrial smooth muscle cells in vitro. *Mol. Hum. Reprod.* 6 523-528.

Jaffe AB, Toran-Allerand CD, Greengard P & Gandy SE 1994 Estrogen regulates metabolism of Alzheimer amyloid beta precursor protein. *J Biol Chem* 269 13065-13068.

Jin K, Galvan V, Xie L, Mao XO, Gorostiza OF, Bredesen DE & Greenberg DA 2004 Enhanced neurogenesis in Alzheimer's disease transgenic (PDGF-APPSw,Ind) mice. *Proc Natl Acad Sci U S A* 101 13363-13367.

Krichevsky A, Campbell-Acevedo EA, Tong JY & Acevedo HF 1995 Immunological detection of membrane-associated human luteinizing hormone correlates with gene expression in cultured human cancer and fetal cells. *Endocrinology* 136 1034-1039.

Krueger RJ & Orme-Johnson NR 1983 Acute adrenocorticotropic hormone stimulation of adrenal corticosteroidogenesis. Discovery of a rapidly induced protein. *J Biol Chem* 258 10159-10167.

Larson P, Kronenberg H, Melmed S & Polonsky K 2003 *Williams Textbook of Endocrinology*. Philadelphia, PA: Saunders.

Li X & Zhang S 2006 In vitro differentiation of neural precursors from human embryonic stem cells. *Methods in Molecular Biology* 331 169-177.

Licht P, Russu V, Lehmeyer S, Moll J, Siebzehnrubl E & Wildt L 2002 Intrauterine microdialysis reveals cycle-dependent regulation of endometrial insulin-like growth factor binding protein-1 secretion by human chorionic gonadotropin. *Fertil Steril* 78 252-258.

Licht P, Russu V & Wildt L 2001 On the role of human chorionic gonadotropin (hCG) in the embryo-endometrial microenvironment: implications for differentiation and implantation. *Semin Reprod Med* 19 37-47.

Liu T, Perry G, Chan HW, Verdile G, Martins RN, Smith MA & Atwood CS 2004 Amyloid-beta-induced toxicity of primary neurons is dependent upon differentiation-associated increases in tau and cyclin-dependent kinase 5 expression. *J Neurochem* 88 554-563.

Lopez-Toledano MA & Shelanski ML 2004 Neurogenic effect of beta-amyloid peptide in the development of neural stem cells. *J Neurosci* 24 5439-5444.

Lopez-Toledano MA & Shelanski ML 2007 Increased Neurogenesis in Young Transgenic Mice Overexpressing Human APP_{Sw, Ind}. *J Alzheimers Dis* 12 229-240.

MacConell LA, Lawson MA, Mellon PL & Roberts VJ 1999 Activin A regulation of gonadotropin-releasing hormone synthesis and release in vitro. *Neuroendocrinology* 70 246-254.

Mak GK, Enwere EK, Gregg C, Pakarainen T, Poutanen M, Huhtaniemi I & Weiss S 2007 Male pheromone-stimulated neurogenesis in the adult female brain: possible role in mating behavior. *Nat Neurosci* 10 1003-1011.

Manthey D, Heck S, Engert S & Behl C 2001 Estrogen induces a rapid secretion of amyloid beta precursor protein via the mitogen-activated protein kinase pathway. *Eur J Biochem* 268 4285-4291.

Mauch DH, Nagler K, Schumacher S, Goritz C, Muller EC, Otto A & Pfrieger FW 2001 CNS synaptogenesis promoted by glia-derived cholesterol. *Science* 294 1354-1357.

McPhie DL, Coopersmith R, Hines-Peralta A, Chen Y, Ivins KJ, Manly SP, Kozlowski MR, Neve KA & Neve RL 2003 DNA synthesis and neuronal apoptosis caused by

familial Alzheimer disease mutants of the amyloid precursor protein are mediated by the p21 activated kinase PAK3. *J Neurosci* 23 6914-6927.

McShea A, Lee HG, Petersen RB, Casadesus G, Vincent I, Linford NJ, Funk JO, Shapiro RA & Smith MA 2007 Neuronal cell cycle re-entry mediates Alzheimer disease-type changes. *Biochim Biophys Acta* 1772 467-472.

Meethal SV, Liu T, Chan HW, Ginsburg E, Wilson AC, Gray DN, Bowen RL, Vonderhaar BK & Atwood CS 2009 Identification of a regulatory loop for the synthesis of neurosteroids: a steroidogenic acute regulatory protein-dependent mechanism involving hypothalamic-pituitary-gonadal axis receptors. *J Neurochem* 110 1014-1027.

Meethal SV, Smith MA, Bowen RL & Atwood CS 2005 The gonadotropin connection in Alzheimer's disease. *Endocrine* 26 317-326.

Milward EA, Papadopoulos R, Fuller SJ, Moir RD, Small D, Beyreuther K & Masters CL 1992 The amyloid protein precursor of Alzheimer's disease is a mediator of the effects of nerve growth factor on neurite outgrowth. *Neuron* 9 129-137.

Mulac-Jericevic B & Conneely OM 2004 Reproductive tissue selective actions of progesterone receptors. *Reproduction* 128 139-146.

Muller F & O'Rahilly R 1985 The first appearance of the neural tube and optic primordium in the human embryo at stage 10. *Anat Embryol (Berl)* 172 157-169.

Munoz-Sanjuan I & Brivanlou AH 2002 Neural induction, the default model and embryonic stem cells. *Nat Rev Neurosci* 3 271-280.

Mylonas I, Jeschke U, Shabani N, Kuhn C, Kunze S, Dian D, Friedl C, Kupka MS & Friese K 2007 Steroid receptors ERalpha, ERbeta, PR-A and PR-B are differentially expressed in normal and atrophic human endometrium. *Histol Histopathol* 22 169-176.

Neaves WB, Johnson L, Porter JC, Parker CR, Jr. & Petty CS 1984 Leydig cell numbers, daily sperm production, and serum gonadotropin levels in aging men. *J Clin Endocrinol Metab* 59 756-763.

O'Shea KS 1999 Embryonic stem cell models of development. *Anat Rec* 257 32-41.

Okabe S, Forsberg-Nilsson K, Spiro AC, Segal M & McKay RD 1996 Development of neuronal precursor cells and functional postmitotic neurons from embryonic stem cells in vitro. *Mech Dev* 59 89-102.

Park KH, Hallows JL, Chakrabarty P, Davies P & Vincent I 2007 Conditional neuronal simian virus 40 T antigen expression induces Alzheimer-like tau and amyloid pathology in mice. *J Neurosci* 27 2969-2978.

Paterson SJ, Corbett AD, Gillan MG, Kosterlitz HW, McKnight AT & Robson LE 1984 Radioligands for probing opioid receptors. *J Recept Res* 4 143-154.

Pepe GJ & Albrecht ED 1995 Actions of placental and fetal adrenal steroid hormones in primate pregnancy. *Endocr Rev* 16 608-648.

Petanceska SS, Nagy V, Frail D & Gandy S 2000 Ovariectomy and 17beta-estradiol modulate the levels of Alzheimer's amyloid beta peptides in brain. *Neurology* 54 2212-2217.

Pidoux G, Gerbaud P, Tsatsaris V, Marpeau O, Ferreira F, Meduri G, Guibourdenche J, Badet J, Evain-Brion D & Frendo JL 2007 Biochemical characterization and modulation of LH/CG-receptor during human trophoblast differentiation. *J Cell Physiol* 212 26-35.

Pomata PE, Colman-Lerner AA, Baranao JL & Fiszman ML 2000 In vivo evidences of early neurosteroid synthesis in the developing rat central nervous system and placenta. *Brain Res Dev Brain Res* 120 83-86.

Pon LA, Epstein LF & Orme-Johnson NR 1986 Acute cAMP stimulation in Leydig cells: rapid accumulation of a protein similar to that detected in adrenal cortex and corpus luteum. *Endocr Res* 12 429-446.

Porayette P, Gallego MJ, Kaltcheva MM, Bowen RL, Vadakkadath Meethal S & Atwood CS 2009 Differential processing of amyloid-beta precursor protein directs human embryonic stem cell proliferation and differentiation into neuronal precursor cells. *J Biol Chem* 284 23806-23817.

Porayette P, Gallego MJ, Kaltcheva MM, Meethal SV & Atwood CS 2007 Amyloid-beta precursor protein expression and modulation in human embryonic stem cells: a novel role for human chorionic gonadotropin. *Biochem Biophys Res Commun* 364 522-527.

Prior JC 1990 Progesterone as a bone-trophic hormone. *Endocr Rev* 11 386-398.

Raina AK, Zhu X, Rottkamp CA, Monteiro M, Takeda A & Smith MA 2000 Cyclin' toward dementia: cell cycle abnormalities and abortive oncogenesis in Alzheimer disease. *J Neurosci Res* 61 128-133.

Reame NE, Kelche RP, Beitins IZ, Yu MY, Zawacki CM & Padmanabhan V 1996 Age effects of follicle-stimulating hormone and pulsatile luteinizing hormone secretion across the menstrual cycle of premenopausal women. *J Clin Endocrinol Metab* 81 1512-1518.

Reichlin S 1998 Williams Textbook of Endocrinology. In *Williams Textbook of Endocrinology. 10 ed*, pp 165-248. Eds JD Wilson, DW Foster, HM Kronenberg & PR Larsen. Philadelphia PA: WB Saunders Co.

Richardson MC & Masson GM 1985 Lack of direct inhibitory action of oxytocin on progesterone production by dispersed cells from human corpus luteum. *J Endocrinol* 104 149-151.

Rogers PA, Donoghue JF, Walter LM & Girling JE 2009 Endometrial angiogenesis, vascular maturation, and lymphangiogenesis. *Reprod Sci* 16 147-151.

Sauter CN, McDermid RL, Weinberg AL, Greco TL, Xu X, Murdoch FE & Fritsch MK 2005 Differentiation of murine embryonic stem cells induces progesterone receptor gene expression. *Exp Cell Res* 311 251-264.

Schmidt PJ, Gindoff PR, Baron DA & Rubinow DR 1996 Basal and stimulated gonadotropin levels in the perimenopause. *Am J Obstet Gynecol* 175 643-650.

Schumacher A, Arnhold S, Addicks K & Doerfler W 2003 Staurosporine is a potent activator of neuronal, glial, and "CNS stem cell-like" neurosphere differentiation in murine embryonic stem cells. *Mol Cell Neurosci* 23 669-680.

Schumacher M, Guennoun R, Robert F, Carelli C, Gago N, Ghoumari A, Gonzalez Deniselle MC, Gonzalez SL, Ibanez C, Labombarda F, et al. 2004 Local synthesis and dual actions of progesterone in the nervous system: neuroprotection and myelination. *Growth Horm IGF Res* 14 Suppl A 18-33.

Schwall RH, Szonyi E, Mason AJ & Nikolics K 1988 Activin stimulates secretion of follicle-stimulating hormone from pituitary cells desensitized to gonadotropin-releasing hormone. *Biochem Biophys Res Commun* 151 1099-1104.

Sriraman V, Rao VS, Rajesh N, Vasan SS & Rao AJ 2001 A preliminary study on the possible role for luteinizing hormone in androgen independent growth of prostate. *Reprod Biomed Online* 3 6-13.

Stocco DM 2001 StAR protein and the regulation of steroid hormone biosynthesis. *Annu Rev Physiol* 63 193-213.

Stocco DM & Chen W 1991 Presence of identical mitochondrial proteins in unstimulated constitutive steroid-producing R2C rat Leydig tumor and stimulated nonconstitutive steroid-producing MA-10 mouse Leydig tumor cells. *Endocrinology* 128 1918-1926.

Strauss JF, 3rd, Christenson LK, Devoto L & Martinez F 2000 Providing progesterone for pregnancy: control of cholesterol flux to the side-chain cleavage system. *J Reprod Fertil Suppl* 55 3-12.

Takashima S, Kuruta H, Mito T, Nishizawa M, Kunishita T & Tabira T 1990 Developmental and aging changes in the expression patterns of beta-amyloid in the brains of normal and Down syndrome cases. *Brain Dev* 12 367-371.

Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS & Jones JM 1998 Embryonic stem cell lines derived from human blastocysts. *Science* 282 1145-1147.

Trapp BD & Hauer PE 1994 Amyloid precursor protein is enriched in radial glia: implications for neuronal development. *J Neurosci Res* 37 538-550.

Vadakkadath Meethal S, Atwood CS. (2005) The role of hypothalamic-pituitary-gonadal hormones in the normal structure and functioning of the brain. *Cell Mol Life Sci*. 62 257-70.

VanLandingham JW, Cutler SM, Virmani S, Hoffman SW, Covey DF, Krishnan K, Hammes SR, Jamnongjit M & Stein DG 2006 The enantiomer of progesterone acts as a molecular neuroprotectant after traumatic brain injury. *Neuropharmacology* 51 1078-1085.

Wahabi H, Abed Althagafi N & Elawad M 2007 Progestogen for treating threatened miscarriage. *Cochrane Database Syst Rev* CD005943.

Wang JM, Johnston PB, Ball BG & Brinton RD 2005 The neurosteroid allopregnanolone promotes proliferation of rodent and human neural progenitor cells and regulates cell-cycle gene and protein expression. *J Neurosci* 25 4706-4718.

Webber RJ & Sokoloff L 1981 In vitro culture of rabbit growth plate chondrocytes. 1. Age-dependence of response to fibroblast growth factor and "chondrocyte growth factor". *Growth* 45 252-268.

Weiss J, Crowley WF, Jr., Halvorson LM & Jameson JL 1993 Perifusion of rat pituitary cells with gonadotropin-releasing hormone, activin, and inhibin reveals distinct effects on gonadotropin gene expression and secretion. *Endocrinology* 132 2307-2311.

Whitfield GK & Kourides IA 1985 Expression of chorionic gonadotropin alpha- and beta-genes in normal and neoplastic human tissues: relationship to deoxyribonucleic acid structure. *Endocrinology* 117 231-236.

Wright DW, Kellermann AL, Hertzberg VS, Clark PL, Frankel M, Goldstein FC, Salomone JP, Dent LL, Harris OA, Ander DS, et al. 2007 ProTECT: a randomized clinical trial of progesterone for acute traumatic brain injury. *Ann Emerg Med* 49 391-402, 402 e391-392.

Xia X & Zhang SC 2009 Differentiation of neuroepithelia from human embryonic stem cells. *Methods Mol Biol* 549 51-58.

Xu H, Gouras GK, Greenfield JP, Vincent B, Naslund J, Mazzarelli L, Fried G, Jovanovic JN, Seeger M, Relkin NR, et al. 1998 Estrogen reduces neuronal generation of Alzheimer beta-amyloid peptides. *Nat Med* 4 447-451.

Yamazaki T, Matsuoka C, Gendou M, Izumi S, Zhao D, Artemenko I, Jefcoate CR & Kominami S 2006 Mitochondrial processing of bovine adrenal steroidogenic acute regulatory protein. *Biochim Biophys Acta* 1764 1561-1567.

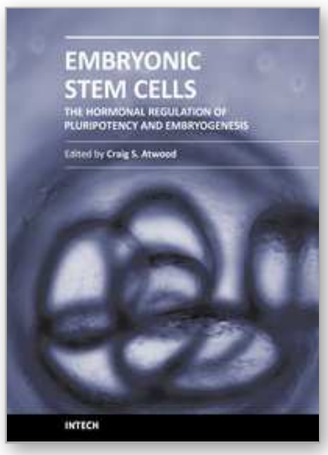
Yokotani T, Koizumi T, Taniguchi R, Nakagawa T, Isobe T, Yoshimura M, Tsubota N, Hasegawa K, Ohsawa N, Baba S, et al. 1997 Expression of alpha and beta genes of human chorionic gonadotropin in lung cancer. *Int J Cancer* 71 539-544.

Zhang SC 2003 Embryonic stem cells for neural replacement therapy: prospects and challenges. *J Hematother Stem Cell Res* 12 625-634.

Zhang SC, Wernig M, Duncan ID, Brustle O & Thomson JA 2001 In vitro differentiation of transplantable neural precursors from human embryonic stem cells. *Nat Biotechnol* 19 1129-1133.

Zhuang LZ & Li RH 1991 Study on reproductive endocrinology of human placenta (II)--Hormone secreting activity of cytotrophoblast cells. *Sci China B* 34 1092-1097.

Zygmunt M, Herr F, Keller-Schoenwetter S, Kunzi-Rapp K, Munstedt K, Rao CV, Lang U & Preissner KT 2002 Characterization of human chorionic gonadotropin as a novel angiogenic factor. *J Clin Endocrinol Metab* 87 5290-5296.



Embryonic Stem Cells: The Hormonal Regulation of Pluripotency and Embryogenesis

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Pluripotency is a prerequisite for the subsequent coordinated differentiation of embryonic stem cells into all tissues of the body. This book describes recent advances in our understanding of pluripotency and the hormonal regulation of embryonic stem cell differentiation into tissue types derived from the ectoderm, mesoderm and endoderm.

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Phone: +385 (51) 770 447
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Unit 405, Office Block, Hotel Equatorial Shanghai
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中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
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