DOI: 10.21451/1984-3143-AR874

Anim. Reprod., v.13, n.3, p.313-333, Jul./Sept. 2016

The timing of puberty (oocyte quality and management)

A.H. Duittoz[1,4], Y. Tillet[1], D. Le Bourhis[2], L. Schibler[3]

[1]PRC, INRA, CNRS, IFCE, Université de Tours, Nouzilly, France.
[2]Allice, Department R&D, Monnaie, France.
[3]Allice, Département R&D, Paris, France.

Abstract

This review aims at giving an overview on the physiological events leading to puberty onset in mammals and more specifically in cattle. Puberty is an important developmental milestone in mammals involving numerous changes in various physiological regulations and behaviors. It is a physiological unique event integrating several important central regulations at the crossroad of adaptation to environment: reproductive axis, feeding behavior and nutritional controls, growth, seasonal rhythm and stress. Puberty onset is also an important economic parameter in replacement heifer program and in genomic selection (genomic bulls). The quest for advanced puberty onset should be carefully balanced by its impact on physiological parameters of the animal and its offspring. Thus one has to carefully consider each step leading to puberty onset and set up a strategy that will lead to early puberty without being detrimental in the long term. In this review, major contributions in the understanding of puberty process obtained in rodents, primates and farm animals such as sheep and cattle are discussed. In the first part we will detail the endocrine events leading to puberty onset with a special focus on the regulation of GnRH secretion. In the second part we will describe the neural mechanisms involved in silencing and reactivating the GnRH neuronal network. These central mechanisms are at the crossroad of the integration of environmental factors such as the nutritional status, the stress and the photoperiod that will be discussed in the third part. In the fourth part, we will discuss the genetic determinants of puberty onset and more particularly in humans, where several pathologies are associated with puberty delay or advance and in cattle where several groups have now identified genomic regions or gene networks associated with puberty traits. Last but not least, in the last part we will focus on the embryologist point of view, how to get good oocytes for in vitro fertilization and embryo development from younger animals.

Keywords: Glial-neuronal communication, GnRH, hypothalamus, neuroendocrinology, timing of puberty, transcriptional regulation

Introduction

Puberty is an important developmental milestone in mammals involving numerous changes in various physiological regulations and behaviors. It is a physiological unique event integrating several important central regulations at the crossroad of adaptation to environment: reproductive axis, feeding behavior and nutritional controls, growth, seasonal rhythm and stress.

Puberty, puberty onset, peri-pubertal, reproductive maturity what's the difference

Puberty onset results from a complex and integrated sequence of biological events leading to progressive maturation of sexual characteristics that ultimately lead to attainment of full reproductive capacity. This sequence is referred as the timing of puberty. Puberty timing in mammals is the result of evolution allowing females to attain ideal pelvic anatomy and size, complete growth and maximize skeletal mineralization, prior to the demands of pregnancy, lactation and offspring rearing.

Puberty is defined as the moment of the first emission of gametes, *ie* the first ovulation in females and the first spermatozoa entering the epididymis in males. Therefore puberty is expressed as a date or as an age. From this definition, it is obvious that puberty can be easily detected in females by detecting the first ovulation. However in males there is no non-invasive method to assess the presence of epididymal spermatozoa and it is usually defined according various physical and behavioral changes. Therefore puberty is very often studied through the modifications observed before and immediately following the first emission of gametes. In that case it is better to speak of peri-pubertal period. For example in females, breeders usually monitor the exterior signs of receptivity (age at first estrus). However one has to keep in mind that estrus behavior can exist without a proper ovulation and the reciprocal is also true: ovulation can occur without any sign of estrus behavior. For males, breeders look at the sexual behavior too: mounting behavior and erection. Here again, this behavior does not mean that is there any spermatozoa in the ejaculate.

The strict definition of puberty onset as the first emission of gametes does not mean that the animals are able to breed yet. They can produce and release gametes but reproduction is more than that. Females usually need a period of time after puberty onset to have regular ovarian cycles and to get their uterus capable of supporting a pregnancy. For males, the concentration of spermatozoa in the ejaculate should reach a certain threshold to give an adequate fertility; here again this can take some time after the puberty onset. Reproductive maturity is another phenomenon and the mechanisms leading to puberty onset are different from those leading to reproductive maturity.

[4]Corresponding author: duittoz@univ-tours.fr
Received: June 11, 2016
Accepted: July 25, 2016

Endocrine basis of puberty

Brief overview of the endocrine events across the estrus cycle

Post-pubertal females present estrus cycles, which is the reflection of the ovarian cyclicity. During the late follicular phase, the preovulatory follicles release high estradiol levels in the blood stream. The starting point of all endocrine events leading to ovarian cyclicity is the secretion of a neurohormone: the gonadotropin releasing hormone (GnRH). GnRH acts on the gonadotrope cells located in the anterior pituitary and promote the synthesis and release of both gonadotropins luteinizing hormone (LH) and follicle stimulating hormone (FSH). LH and FSH will act at the ovary level to promote the release of gonadal steroid estrogens and progestagens and to promote follicular growth. Granulosa cells and thecal cells collaborate to synthesize and release estrogens, among which 17-β-estradiol (E2) is the most prevalent estrogen in most species. The amount of estrogen released is dependent on the number of granulosa and thecal cells. Considering the growth of a sphere, the amount of estrogen that can be synthetized is proportional to the cubic of the follicle radius. Therefore the final growth of the dominant follicle is accompanied by a huge increase in E2 production. High E2 levels are responsible for the expression of estrus behavior. In most species studied, high E2 levels will also exert a positive feedback on GnRH secretion leading a large amount of GnRH release that causes a large amount of LH release: the pre-ovulatory GnRH and LH surges (see Fig. 1). LH surge occurs just before the ovulation and last several hours after, contributing to the luteinization of granulosa and thecal cells of the ovulated follicle. *De facto*, E2 levels drop and the positive feedback disappears, stopping its repressive action on GnRH secretion. In parallel progesterone (P4) secretion increases and exerts a negative feedback on GnRH secretion. High P4 levels have a positive action on E2 receptors (ERs) expression. Without P4-priming during the previous cycle, ERs expression is low and despite high E2 levels during the preovulatory phase, the estrus behavior, which is strongly dependent on ERα signaling, is poorly expressed. Once luteolysis occurs, P4 levels drop, the negative feedback is suppressed and GnRH secretion increases again leading to LH and FSH release and a new follicular phase starting.

Evolution of gonadotropin secretion in the pre-pubertal period

The key decisive event required for puberty to occur is an increase in pulsatile gonadotropin releasing hormone (GnRH) release from GnRH neurons leading to gonadotropins LH and FSH secretion. In mature adult, the GnRH is released in the portal veins in a pulsatile manner. GnRH secretion is difficult to assess. As a matter of fact, GnRH is released in capillaries within the ME that form portal veins along the pituitary stalk. From these portal veins, pituitary capillaries emerge and the GnRH is released in the intercellular space and reaches anterior pituitary cells. GnRH concentration in the portal veins varies between 4-100pg/ml (Caraty *et al*., 1982; Clarke and Cummins, 1982; Levine *et al*., 1982; Irvine and Alexander, 1987; Gazal *et al*., 1998), which gives a very small amount of GnRH for the small blood volume considered. Thus, the amount of GnRH that passes in the general circulation is very small; the concentration is well below the detection threshold of known hormonal assays. Moreover GnRH half-life is very short, a few minutes. Due to its small peptidic structure, circulating endopeptidases degrades rapidly the GnRH. Therefore to assess the GnRH secretion, blood should be punctured from pituitary portal vessels or from *canulae* inserted in the third ventricle (Gazal *et al*., 1998) and this can only be performed in large animals and requires invasive surgical procedures (Clarke and Cummins, 1982; Levine *et al*., 1982). An alternative is to follow LH secretion since it has been clearly demonstrated that a GnRH pulse precedes every LH pulse (Clarke and Cummins, 1982; Caraty *et al*., 1989).

In the female Rhesus monkey the early prepubertal period is characterized by an increase in pulsatile release with a concomitant increase in pulse frequency and pulse amplitude. In the midpubertal phase, only an increase in GnRH pulse amplitude is noticed and the global GnRH secretion is increased during the night (Watanabe and Terasawa, 1989). This is in contrast to ewes and heifers where the midpubertal period is characterized by an increase in LH pulse frequency associated with a decrease in pulse amplitude (Day *et al*., 1987). In heifers, the frequency of LH pulses is usually in a range of 2 to 4 pulses/24 h 100 to 50 days before puberty onset. The amplitude of LH pulses is high, reaching 6-8 ng/ml. From 50 days before to puberty onset, the frequency of LH pulses increased to reach 15-20 pulses/24 h and the mean amplitude of LH pulses decreased to values (<2 ng/ml; Day *et al*., 1987). Such increase in LH pulse frequency was also reported in female lambs (Claypool and Foster, 1990). In humans, this increase in pulsatile LH secretion is also observed but occurs during the night phase (Wu *et al*., 1996).

In spite of numerous physiological studies in model animals, little is known about the key events leading to GnRH neurons progressive activation at puberty onset. The scientific community admits that puberty onset is preceded by gradual changes in trans-synaptic and glial inputs to the GnRH neuronal network. The trans-synaptic changes consist of a coordinated increase in excitatory inputs and/or a reduction in inhibitory influences. Glial cells could also participate in regulating extracellular glutamate concentration, and in releasing growth factors and small diffusible molecules that directly or indirectly stimulate GnRH secretion. In addition to the classical excitatory glutamatergic neurons, kisspeptin signaling through GPR54 was discovered in 2005 as a powerful stimulator of GnRH release (Messager *et al*., 2005). Nevertheless, how these key events are triggered through environmental and nutritional factors is far from being understood.

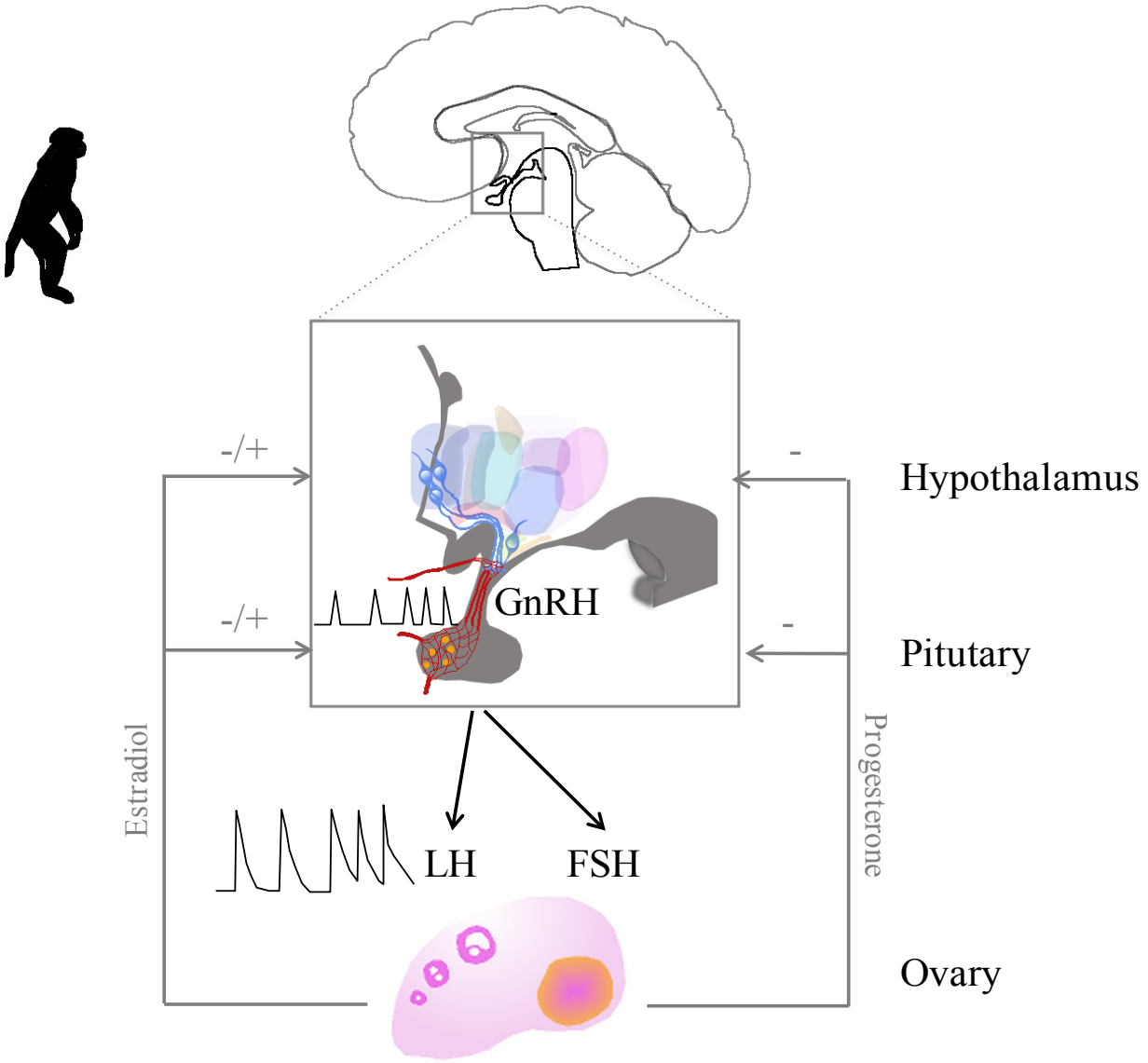

Figure 1. Schematic representation of the hypoyhalamic-hypophysis-ovary axis. GnRH neurons somas are mostly located in the preoptic area and send their axons towards the median eminence where GnRH is released in a pulsatile manner into capillaries. Median eminence capillaries merge to form the portal vessels on the ventral part of the anterior pituitary and give rise to pituitary capillaries. GnRH then can diffuse within the anterior pituitary and reach gonadotropic cells that release the gonadotropins: LH and FSH. LH and FSH will reach the general blood circulation and act on the ovaries to stimulate both oocyte and follicle growth and gonadal steroids secretion. Progesterone exerts an negative feedback at the pituitary and hypothalamic levels, estradiol at low concentration exerts a negative feedback at both pituitary and hypothalamic levels, but at high concentration (during estrus) it will have a positive feedback at both pituitary and hypothalamic levels.

GnRH control

The two modes of secretion

GnRH secretion is characterized by two modes of secretion: pulsatile and continuous (the surge). These two modes have been described in the pioneering work of Ernst Knobil in the rhesus monkey where he described a tonic and a phasic mode of LH secretion controlled by two different areas within the hypothalamus Preoptic area (POA) and mediobasal hypothalamus (MBH), respectively (Nakai *et al*., 1978). The pulsatile pattern of GnRH secretion was confirmed in the 80's when a trans-nasal surgical approach allowed the collection of blood from the portal vessels between the hypothalamus and the pituitary (Clarke and Cummins, 1982; Levine *et al*., 1982).

GnRH/LH secretion is pulsatile during the follicular and the luteal phases, the surge mode occurs during the pre-ovulatory period. In most species where GnRH and LH secretions have been monitored simultaneously, the LH secretion profile is a good estimate of the GnRH pulsatile secretion: a GnRH pulse always precedes one LH pulse. The frequency of pulsatile secretion varies across the estrus cycle. For example in the ewe, the follicular phase is characterized

by a high frequency ie 1 pulse *per* hour, and low amplitude of LH pulses, whereas the luteal phase is characterized by a low frequency ie 1 pulse *per* 6 h but high amplitude of LH pulses (Moenter *et al*., 1991). The GnRH pulse frequency is decoded by the GnRH receptor (GnRH-R) expressed by gonadotropic cells: high frequency favors the expression of the β-LH subunit whereas low frequency favors the expression of the β-FSH subunit (Bédécarrats and Kaiser, 2003; Thompson and Kaiser, 2014).

Anatomy of the GnRH neuronal network

The GnRH is a small peptide (10 amino-acids) issued from the processing of pre-pro-GnRH encoded by the *Gnrh1* gene. The pre-pro-GnRH is processed in GnRH neurons to give the GnRH and the GnRH-associated peptide (GAP), Both are packed in large dense core vesicles (LDCV) for further release (Clarke *et al*., 1987). The GnRH is synthetized and secreted by a specialized population of neurons: the GnRH neurons. In most mammals the GnRH neurons' somas are located in the POA with a few cell bodies located in the MBH and the axons project towards the median eminence at the bottom of the MBH. However in primates, the repartition is different with the majority of GnRH neurons' somas located in the MBH and just a few in the POA. Axonal projections are projected to the median eminence where GnRH is released in blood capillaries and transported in portal vessels to the capillaries network of the anterior pituitary where it will stimulate the expression and release of the gonadotropins FSH and LH.

Extracerebral embryonic origin of GnRH neurons

During embryogenesis, the GnRH neurons originate from the medial part of the nasal embryonic placode at early embryonic age 30 (E30) in sheep (Caldani *et al*., 1987; Caldani *et al*., 1995), E11.5 in mouse (Schwanzel-Fukuda and Pfaff, 1989; Wray *et al*., 1989), 6-7weeks of pregnancy in humans (Schwanzel-Fukuda *et al*., 1996). They then migrate along olfactory-, vomero-nasal- and terminal nerves to finally enter the forebrain through the cribiform plate. In the sheep, this phase of nasal migration is completed at E45, in the mouse at E13.5. Once in the brain they turn ventro-caudally to reach their final location in the POA/MBH. The phase of intra-cerebral migration is completed at E60 in the sheep and E16.5 in the mouse. Once settled, they grow their axonal projections toward the ME. This phase of axonal growth is terminated at E70 in sheep and E18.5 in the mouse. Once connected to the ME, it is believed that GnRH secretion occurs since it is correlated with the first observation of β-LH expression in the pituitary cells (Messaoud-Toumi *et al*., 1993). Primary cultures derived from embryonic nasal explants from E26 sheep embryos, E35 rhesus monkey embryos or E11.5 mouse embryos allow the development of functional secreting GnRH neurons that form a network in vitro. The GnRH secretion is pulsatile and the frequency is correlated to what it is observed in vivo according to each species considered (Duittoz and Batailler, 2000; Constantin *et al*., 2009). These *in vitro* approaches suggest that the pulsatility of secretion is an endogenous property of the GnRH network and that this property develops during the fetal life.

Functionality of the fetal GnRH neuronal network

Whether the pulsatile secretion develops in utero and plays a role in development has been studied particularly in the sheep species. Several groups have carried on a series of experiments on sheep fetuses. In chronically catheterized ovine fetuses, both LH and FSH exhibit a similar trend of peak values in midgestation (70-100 days) with a progressive decrease in plasma concentrations towards term (145 days; Sklar *et al*., 1981). Measurements from 55-60 days of gestation embryos gave low values of plasma LH and FSH concentrations. This pattern is similar to the one described in human fetuses with high concentration values of LH between 15-29 weeks of gestation (Kaplan and Grumbach, 1976; Clements *et al*., 2009). The ovine fetal pituitary gland has the capacity to respond to exogenous GnRH as early as 60 days of gestation with a maximal amplitude occurring during mid-gestation (Mueller *et al*., 1981). The pulsatile nature of LH fetal secretion was clearly assessed by serial blood sampling during a 4 h period in ovine fetuses at mid-gestation (Clark *et al*., 1984; Fig. 2). If we put in parallel the physiological maturation profiles of LH secretion and the development of the GnRH neuronal network we can clearly see the correlation between those events. Thus, once GnRH neuronal migration and axonal growth toward the median eminence is completed, the GnRH secretion can take place and induce the expression of gonadotropin subunits (Messaoud-Toumi *et al*., 1993). LH and FSH will act on the fetal gonad to stimulate gonadal steroid synthesis, and this is particular evident in male ovine fetuses where a spurt in testosterone secretion is detected at mid-gestation. This increase in testosterone in male fetuses or new born has been demonstrated in numerous mammals (Foster and Hileman, 2015; Plant *et al*., 2015; Prevot, 2015). In precocious mammals such as ovine and bovine species, the spurt occurs during the last third of gestation and is terminated at birth, whereas in altricial species such as rodents, the spurt occurs during the last days of pregnancy and during the first week post-natal (mice; Sisk and Foster, 2004). After this "mini puberty", the frequency of GnRH/LH secretion dramatically decreases and steroid levels drop; the infancy period is starting.

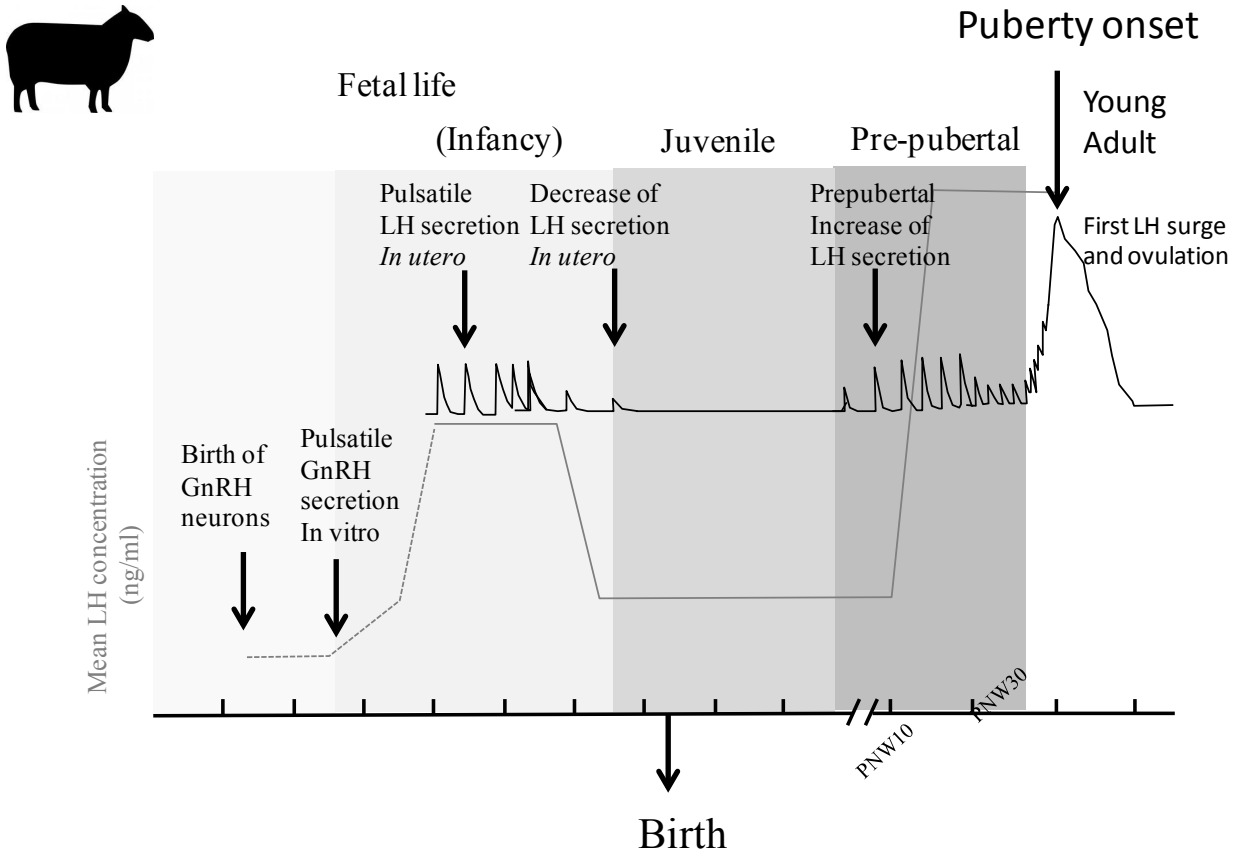

Figure 2. Schematic representation of the evolution of LH secretion from fetal life to adulthood in the ovine species. At 26 days of gestational age (G26), the first GnRH neurons are detected in the medial part of the nasal placode. From G26 to G35, GnRH neurons are born in the nasal placode and migrate along the nasal septum to reach the cribriform plate (intra nasal migration). From G35 to G45, GnRH neurons migrate into the brain and reach their final location in the preoptic area. From G45 to G60, GnRH neurons send axonal projections towards the external part of the median eminence (Caldani et al., 1995). At G60, the first expression of *LHB* is detected, suggesting that GnRH secretion is functional. From G80 to G120, LH is released in a pulsatile manner and contributes to the secretion of testosterone in male fetus and the sexualization of external genitalia and brain structures. From G120 to postnatal day 60 (PN60) there is virtually no LH secretion. From postnatal week 10 (PNW10) to PNW20, LH pulsatile secretion reappears with low frequency and high amplitude. From PNW20 to PNW30, the frequency of LH pulses increases and the amplitude decreases. The first preovulatory LH surge signs the onset of puberty.

Puberty: endocrine or brain revolution?

The pubertal transition involves both gonadal and behavioral maturation. The increase in the frequency of GnRH release and gonadotropins secretion progressively leads to the onset of gonadal functions: gametogenesis and steroid production. These steroids act in turn onto the brain to remodel neural circuits particularly those involved in sexual behaviors, but not only (Forger *et al*., 2015). In humans, several neurological or psychiatric diseases appear or are exacerbated at puberty (autism, schizophrenia, epilepsy, anorexia nervosa …).

Several decades of research have tempted to answer the question of the timing of the reactivation of GnRH secretion and the onset of puberty. As mentioned earlier, the hypothalamic-pituitary gonadal axis is functional during fetal/perinatal period, leading to the sexualization of external genitalia and specific regions of the nervous system. This activation is limited in time but offers a window of sensitivity to external factors such as endocrine disruptors (Parent *et al*., 2015; Hines *et al*., 2016).

Inhibitory mechanisms

Steroid-dependent mechanism

Early studies highlighted the role of the steroid negative feedback, the so-called "gonadostat" hypothesis (Frisch and Revelle, 1970). The "gonadostat" theory implies a higher sensitivity of GnRH neuronal network to the negative feedback of steroids: a steroid-dependent mechanism. In the prepubertal period, GnRH secretion is less sensitive to the negative feedback of gonadal steroids, the GnRH pulse frequency increases leading to gonadotropin secretion and gonadal activation. In the sheep species, early post-natal gonadectomy leads to immediately increased levels of gonadotropins as in the postpubertal period. Replacing

steroid gonadal hormones causes gonadotropins levels to go back to initial prepubertal values (Foster and Hileman, 2015). Similar findings were found in other mammals: hamster, ferret (Sisk and Foster, 2004). In heifers the negative feedback of estradiol declined as puberty approached (Day *et al*., 1987). However, in rat and rhesus monkey, the gonadostat theory is not sufficient to account for the low gonadotropins levels during infancy (Sisk and Foster, 2004). Interestingly, a steroid dependent mechanism exists at the end of the juvenile period of female rhesus monkey (Rapisarda *et al*., 1983).

Steroid independent mechanism

In rat and monkeys, after neonatal castration, gonadotropins levels remain low during the infantile period and increase progressively in the juvenile period to reach high levels as those expected at puberty. Such findings have been also reported in humans suffering from gonadal dysgenesis (Winter and Faiman, 2009). Although the precise neuronal target of gonadal steroid feedback is not clearly known, POA and the ArcN are involved in sensing estradiol negative feedback in gonadectomized prepubertal rats (Uenoyama *et al*., 2015). GnRH neurons, although located in the POA, are not considered as the primary target since they do not express the estrogen receptor α (ERα) albeit they do express ERβ (Hrabovszky *et al*., 2000; Herbison and Pape, 2001) but this later isoform does not seem to be involved in puberty onset. To account for this steroid-independent system, one assumption is that during infancy, inhibitory brain circuits block GnRH secretion; this break is released at puberty concomitantly with the onset of stimulatory brain circuits. GABA (γ-aminobutyiric acid) neurons are involved in the inhibition of GnRH neurons during juvenile period in several species. In the rhesus monkey, Terasawa's group showed the existence of a GABAergic break on gonadotropin secretion during the juvenile period. GABA level in the pituitary stalk (PS) and ME of juvenile monkeys is high but decreases during the peripubertal period (Mitsushima *et al*., 1994; Terasawa, 2005). The local infusion of GABA-A receptor antagonist bicuculline in the PS-ME of juvenile female rhesus monkey induces a rise in gonadotropins levels and the onset of ovarian cyclicity (Keen *et al*., 2011). The infusion of anti-sens mRNA encoding GAD67 (glutamic acid decarboxylase 67), a key enzyme for the synthesis of GABA, in juvenile rhesus monkey females triggered puberty onset with estrus cyclicity and ovulation (Kasuya *et al*., 1999; Fig. 3).

Both mechanisms co-exist to a different degree according to the species considered and also to the sex. One theoretical hypothesis would be that the steroid-independent mechanism provides a coarse regulation and will program the year (month) of puberty onset and the steroid-dependent mechanism will program the week/day when the first ovulation occurs.

Excitatory mechanisms

The pubertal reduction in GABAergic inhibition is accompanied by an increase in glutamate levels in the PS-ME, as well as an increase in the levels of the stimulatory neurotransmitters such as noradrenaline and Neuropeptide Y (NPY; Gore and Terasawa, 1991).

Neuropeptide Y

NPY is an appetite-stimulating neuropeptide and a neuromodulator of neuroendocrine functions. The interactions between NPY and neuroendocrine networks are complex and depend upon the sex and steroid environments. For example NPY is a potent stimulator of LH secretion in sex-steroid primed rats (Allen *et al*., 1985), whereas its intra-cerebroventricular (ICV) administration in gonadectomized rats inhibits LH release (McDonald *et al*., 1989). In the male Rhesus monkey, NPY exerts a negative effect on the GnRH pulse generator in prepubertal animals (Majdoubi *et al*., 2000). However in the female Rhesus monkey NPY release in the ME increases and is responsible for the observed increase in LH secretion at puberty onset (Gore *et al*., 1993). Two populations of NPY containing neurons have been described in the ArcN and the authors suggest that these two populations have distinct roles during the prepubertal period and at puberty onset (Majdoubi *et al*., 2000; Fig. 3). In the prepubertal ewe, NPY stimulates the expression of *Lhb* (β-LH subunit) in gonadotrope cells (Wańkowska and Polkowska, 2009). Neuroanatomical studies in prepubertal ewes demonstrate the presence of NPY inputs on Kp neurons in the ArcN (Polkowska *et al*., 2014) and on GnRH neurons in the POA (Norgren and Lehman, 1989; Tillet *et al*., 1989), thus suggesting two distinct pathways that can be involved in the stimulatory effect of NPY. The existence of 5 NPY receptors subtypes coupled to various signaling pathways and the existence of different hypothalamic and pituitary targets, can account for such opposite effects observed according to the sex, the steroid environment and the physiological state (Pralong, 2010).

Glutamate/NMDA

The excitatory amino acid glutamate and especially its NMDA subtype receptor are important components of the neural system that regulates sexual maturation. Multiple daily injections of NMDA agonists to immature rats (Smyth and Wilkinson, 1994) and monkeys (Urbanski and Ojeda, 1990) induce precocious puberty. On the contrary, administration of the non-competitive NMDA antagonist, MK801, delays puberty onset (Veneroni *et al*., 1990). GnRH neurons receive direct glutamatergic inputs and express NMDA and kainite receptors (Fig. 3). Hypothalamic glutamate contents increase during the prepubertal period and reach maximal values at puberty onset. Glutamate

receptors are ubiquitous in the CNS and they play important roles in many processes involving excitatory mechanisms, whether this increase in glutamatergic signaling is specific to puberty onset or whether it's a more general developmental process is not known (Parent *et al*., 2005).

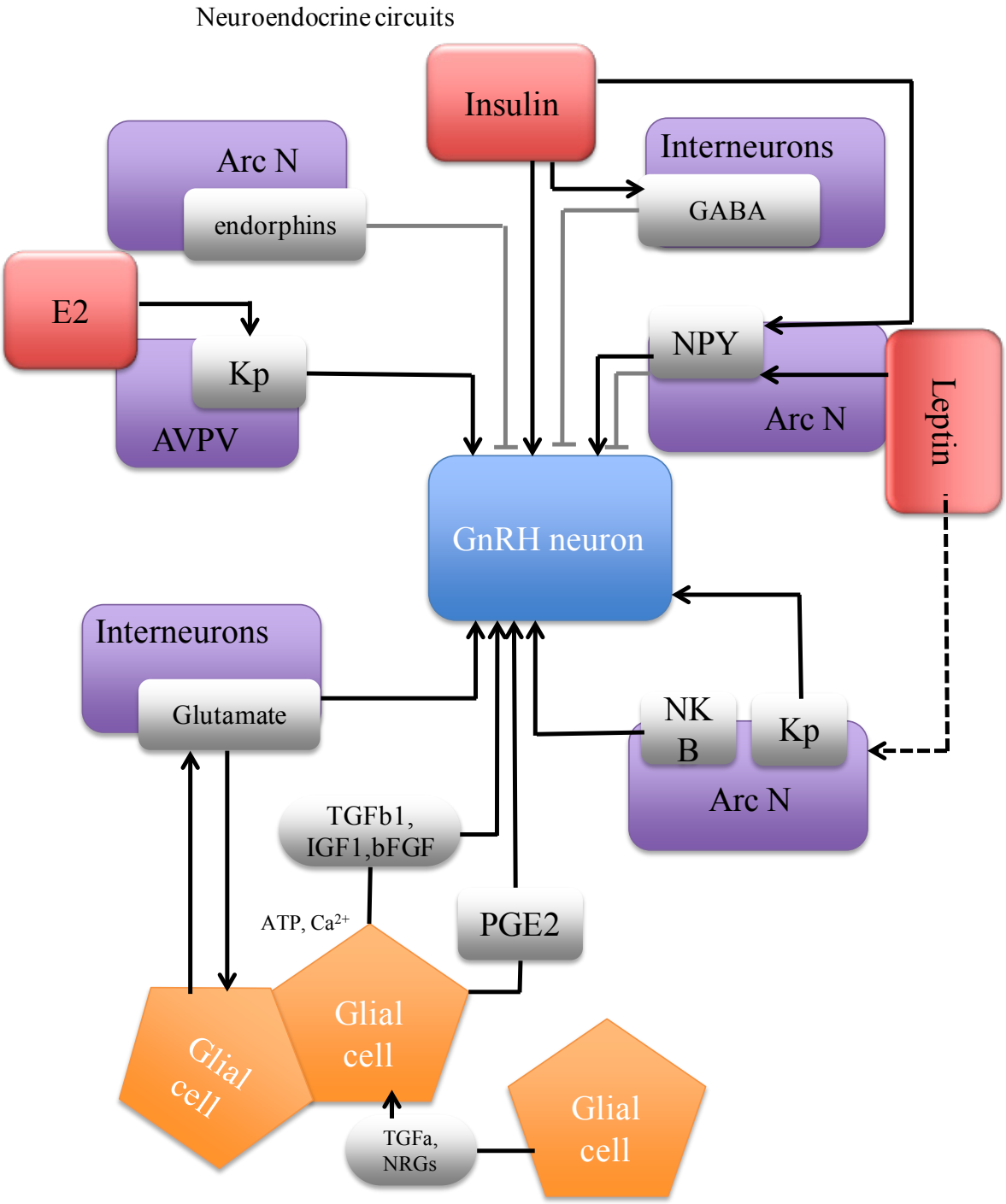

Figure 3. Schematic representation of neuroendocrine circuits. GnRH neurons receive inputs from Kp neurons located in the AVPV and ArcN, NKB neurons located in the ArcN , endorphin neurons located in the ArcN , GABA and glutamate from interneurons (grey boxes = neurotransmitters, purple boxes = neuroanatomical structure). Kp neurons, NPY neurons and GABA neurons are sensitive to E2, leptin and insulin respectively (hormones = red boxes). Glial cells (astrocytes and tanycytes, orange pentagons) in the microenvironment of GnRH neurons can release glutamate, growth factors such as TGFβ1, bFGF that stimulates the activity of GnRH neurons. Glial cells can also uptake glutamate from extracellular space. Glial cells also release neuregulins (NRGs) and TGFα, stimulating the release of PGE2 by neighboring glial cells, which stimulates GnRH neurons. Glial cells also release Ca^{2+}, ATP that regulate GnRH neurons' activity.

Kisspetin/GPR54

In 2004, a new key component was discovered, originally named metastin due to its anti-mitotic properties and now named Kisspeptin (Kp; Matsui *et al*., 2004; Seminara, 2005). *Kiss1* encodes a 54 amino-acids peptide Kp-54 (Kisspeptin-54) that cleaves into several shorter forms (Kp14, Kp13 and Kp10) forming the Kp family. Kp neurons strongly regulate the activity of GnRH neurons. Kp acts through a G-protein coupled receptor (GPCR): GPR54. Kp neurons are found in two distinct populations: ArcN and anteroventral periventricular nucleus (AVPV; Fig. 3). GnRH neurons express GPR54 and Kp fibers contact GnRH terminals in the ME. In humans, mutations in the GPR54 gene lead to an hyponadotropic hypogonadism (HH) characterized by a deficiency in pituitary secretion of gonadotropins which results in the impairment of pubertal maturation and of reproductive function (de Roux *et al*., 2003). Genetic models in rodents highlighted the central role of Kp/GPR54 system in the onset of puberty (Colledge and de Tassigny, 2009; Fig. 3). The Kp/GPR54 system is strongly regulated by metabolic factors and environmental factors, and could represent the central hub for decoding metabolic and environmental cues.

Glial regulation

When speaking of neuroendocrine regulations, most scientists focus on the roles played by neuronal circuits, neurotransmitters and neuromodulators and their cognate receptors. During the prepubertal period, although neuronal networks synaptically-connected to GnRH neurons govern the increase in GnRH secretion; glial cells contribute to the processes engaged through several mechanisms. Glial is a generic adjective to characterize several cell populations that are associated with GnRH neurons: astrocytes, tanycytes and olfactory unsheathing cells. For the sake of simplicity, we use the generic term, bearing in mind that different phenotypic cell types support it. One mechanism involves the production of growth factors acting on serine/threonine kinase receptors. Growth factors such as Transforming Growth Factor α (TGFα) and neuregulins acting on erbB receptors play a major role in glia-GnRH neurons communication. Activation of erbB receptors in glial cells associated with GnRH neurons, leads to the release of prostaglandin E2 (PGE2), which stimulates the electrical activity of GnRH neurons and the GnRH release (Prevot *et al*., 2003a, b, 2005; Ojeda *et al*., 2008). Other growth factors such as TGFβ, IGF1, bFGF are secreted by glial cells and regulate directly the activity of GnRH neurons (Ojeda *et al*., 2010; Fig. 3). Besides the secretion of growth factors, glial cells release small molecules such as calcium, glutamate and ATP that affect the GnRH neuronal activity. Glial cells can also uptake K^+ ions and glutamate that accumulate in the extracellular space during neuronal activity through glial specific dedicated transporters. These mechanisms are of major importance in regulating neuronal electrical activity and excitability. These mechanisms of regulation are tightly dependent upon the distance between the membrane of the glial and the synaptic cleft (Giaume *et al*., 2010).

Another mechanism that can affect glia-GnRH neurons interactions is the modulation of adhesiveness of glial cells onto GnRH neurons. Glial cells interact with GnRH neurons via homophilic interactions involving Neural Cell Adhesion Molecule (NCAM) and synaptic cell adhesion molecule (SynCAM1). In contrast, the poly-syalilated form of NCAM, PSA-NCAM, prevents hemophilic interactions between adjacent glial and GnRH neuronal cells. Heterophilic interactions also exist via the neuronal membrane protein contactin and the glial receptor like protein tyrosine phosphatase-b (Parent *et al*., 2007). These cell-to-cell interactions can trigger intracellular signaling cascades that can affect both glial and neuronal activities (Viguie *et al*., 2001; Parkash and Kaur, 2007; Sharif *et al*., 2013). Altering cell-to-cell communication through glial gap junctions or hemichannels decreases dramatically GnRH neuronal activity and GnRH secretion *in vitro* (Pinet-Charvet *et al*., 2015). Gap-junctions have previously been reported in the hypothalamus, particularly in the ArcN of female rats, where they are regulated by estrogen (Perez *et al*., 1990). Hypothalamic tanycytes, particularly the β-type which is closely associated with GnRH nerve terminals in the ME, express functional connexin-43 (Cx-43) hemichannels encoded by *Gja1*, which play a role in a glucose-sensing mechanism by releasing ATP (Orellana *et al*., 2012). The *Gja1* (Cx-43) promoting region contains AP1 and AP2 sites and a series of half palindromic estrogen response elements suggesting that Cx-43 (*Gja1*) expression can be directly regulated by estrogen levels (Yu *et al*., 1994). Taken altogether, these studies suggest that glial cells might exert a control of GnRH neuronal network as important as the classical transynaptic model.

Therefore, several layers of neuronal and glial components are involved in controlling the onset of puberty, increasing the complexity of the system. The most important question remains: what determines the timing of the inhibitory break removal and/or the timing on excitatory inputs onset?

Puberty: environmental cues

The timing of puberty is maybe the best example of the interaction between genotype and environment. Puberty is a physiological event integrating several important central regulations at the crossroad of adaptation to environment: reproductive axis, feeding behaviour and nutritional controls, growth, seasonal rhythm, corticotropic axis and stress.

Nutrition and metabolism

Nutritional factors have been considered for a long time as the key factor in puberty onset. In humans, until the mid-20th century, a gradual decline in age at menarche (first menstruation) has been reported in most industrialized populations. It is generally admitted that

this trend was due to gradual improvements in nutrition and healthcare (Sørensen *et al*., 2012) giving birth in the 70ies to the critical fat mass hypothesis according which, for a given species a critical fat mass is necessary for puberty onset. The link between nutrition and puberty onset was confirmed in numerous studies on laboratory animals, and also in farm animals. Adequate growth and adiposity are critical for the onset of puberty in mammals. Food restriction (Foster and Olster, 1984; Suttie *et al*., 1991) and excessive exercise during the juvenile period delay the onset of puberty (Manning and Bronson, 1989, 1991). The mechanism involved is the maintenance of the juvenile high sensitivity to the negative feedback sensitivity to gonadal steroids. In contrast, increased adiposity advance the onset of puberty (Kaplowitz *et al*., 2001; Rosales Nieto *et al*., 2014). This occurrence is associated with attenuation of estradiol negative feedback and increased pulsatile release of LH (Gasser, 2006). Therefore, nutritional cues interact with gonadal steroid feedback to time the onset of puberty in females. These findings led to the concept of nutritional programming of puberty in cattle. Age at puberty in cattle is indeed influenced by food intake, food composition and body weight (BW). It is usually admit that puberty occurs at 55-65% of adult BW, depending on the breed considered (Freetly *et al*., 2011). However, the cost of supplemental feeding to reach this target BW earlier is not always compensated by a sufficient improve in reproduction and calf production (Davis Rincker *et al*., 2011). The permissive nutritional signals for puberty onset are metabolic cues such as glucose, insulin and leptin for the most studied factors. These metabolic markers signal the brain that the somatic growth and energy stores are sufficient to sustain pregnancy and lactation without threating the mother and foetus' health. Interestingly, these factors are also important for males, although the mechanisms involved may differ. Since the discovery of the fat-signalling hormone leptin (Zhang *et al*., 1994), whose blood level is proportional to the amount of adipose tissue (Frederich *et al*., 1995), a great amount of research work has tried to demonstrate that leptin is a hormonal messenger signalling the metabolic state for initiating puberty and also for fertility. Studies performed in rodents suggested that leptin administration could advance the onset of female puberty (Ahima *et al*., 1997). Humans with leptin deficiency due to mutations in the leptin gene or in the leptin receptor, and mouse models with inactivated leptin gene or leptin receptor gene, are obese and do not undergo puberty (Chehab *et al*., 1996). However leptin administration in healthy juveniles does not advance puberty onset. In ewes (Henry *et al*., 2011) and cows (Amstalden *et al*., 2002) leptin administration does not affect the secretion of LH but leptin prevents fasting-induced reduction in LH pulsatility in prepuberal heifers (Maciel, 2004). In addition to leptin, other hormones such as insulin, or nutrients such as glucose, fatty acids and amino-acids have been shown to regulate GnRH neuronal activity in a direct manner or via a complex glial/neuronal network. Among the critical neuronal pathways, hypothalamic NPY/agouti-related protein (AgRP) and proopiomelanocortin (POMC) neurons located in the ArcN are considered as the two major pathways mediating nutritional cues. Theses neurons express the leptin receptor and target GnRH neurons, setting the physical pathway for the control of puberty onset. A small subpopulation of Kp neurons in the ArcN express LepR (Louis *et al*., 2011) and may constitute another target for nutritional regulation see Sánchez-Garrido and Tena-Sempere (2013) for a review. However selective ablation of LepR in Kiss1 expressing neurons does not alter puberty onset and fertility (Donato *et al*., 2011).

Taken altogether, these studies support a permissive role of leptin in the metabolic gating of pubertal maturation (Barash *et al*., 1996; Cheung *et al*., 1997).

Photoperiod

In photoperiodic species, puberty onset will depend on the timing of the birth. For example in the ovine species lambs born at the end of the winter or during spring time reach puberty at the next breeding season in autumn, a younger age that those born during autumn, reaching puberty at the following breeding season 10-12 months later. This delay in puberty in autumn-born ewe lambs is due to a prolonged hypersensitivity to the negative steroid feedback (Foster and Hileman, 2015). Similar findings were observed for photoperiodic short-lived animals such as Siberian hamsters where spring born individuals mature rapidly and breed during the summer whereas young born in lid to late summer have a delayed puberty the next spring (Butler *et al*., 2007). Exposing Holstein heifers to long day photoperiod enhance BW gain and hasten the onset of puberty (Rius *et al*., 2005), a result that has been observed also for the seasonal Murrah buffalo species (Roy *et al*., 2016). In photoperiodic species, the variation in food intake and metabolism is an adaptive physiological mechanism allowing the storage of energy resources in anticipation of the harsh days of winter. The immune response is also sensitive to photoperiod, short days photoperiod enhance immunological defenses. This seasonal plasticity of the immune system is highly conserved and is in opposite phase with the breeding season, one explanation would be that the energy cost of both activating reproduction and maintaining the immune function at its higher level is too high (Walton *et al*., 2011). In dairy cows, short days photoperiod improve mammary gland capacity, prolactin secretion and immune function (Dahl, 2008).

Stress and corticotropic axis

Prolonged or chronic stress results in the suppression of gonadotropin secretion and the inhibition of reproduction. Acute stress has variable effects (Tilbrook *et al*., 2000). Studies on adaptive response processes highlighted a positive link between childhood adversities with accelerated female reproductive development. Longer-term health costs are traded off for increased probability of reproducing before dying

via a process of accelerated reproductive maturation. Early adversity, early sexual maturation form the core component linking stress physiology with poor health later in life (Hochberg and Belsky, 2013).

Puberty: genetic determinants

While the timing of pubertal onset varies within and between different populations, it is a highly heritable trait, suggesting strong genetic determinants. Previous epidemiological studies estimate that 60-80% of the variation in pubertal onset is under genetic regulation (Parent *et al*., 2003; Gajdos *et al*., 2010). Abnormal pubertal timing affects up to 5% of adolescents and is associated with adverse health and psychosocial outcomes.

Genetic factors associated with delay of puberty in Humans

Idiopathic hypogonadotropic hypogonadism (IHH) is defined by absent or delayed sexual development, with puberty being either absent or incomplete by the age of 18 years. Deleterious mutations in genes coding for factors necessary for the migration of GnRH neurons lead to hypogonadotropic hypogonadism (IHH), which is the absence of puberty associated with low levels of gonadotropins and gonadal steroids. IHH is frequently accompanied by non-reproductive abnormalities such as anosmia (Kallmann's syndrome). In the Kallmann's syndrome, which associates IHH and anosmia, mutated genes encode for proteins involved in the development of GnRH neurons (Franco *et al*., 1991; Hardelin *et al*., 1992, 1991). The disruption of the migration of GnRH neurons causes them to stay into the nasal region or at the level of the cribriform plate, and they do not reach their final location in the hypothalamus. The Kallmann's syndrome is associated to mutations in *KAL1 , FGFR1* (Dodé *et al*., 2003)*, NELF* (Miura *et al*., 2004; Xu *et al*., 2011)*, PROKR2* (Dodé *et al*., 2006)*, FGF8* (Hardelin and Dodé, 2008)*, CHD7* (Kim *et al*., 2008)*,* and *WDR11* (Kim and Layman, 2011) genes encoding for anosmin, FGF receptor 1 (FGF-R1), NMDA receptor synaptonuclear signaling and neuronal migration factor (alias Nasal Embryonic Factor), prokinecitin receptor 2, FGF-8, chromodomain helicase binding protein 7, WD repeat domain 11, respectively (Fig. 4). In normosmic IHH (nIHH), the development of GnRH neurons is not affected but the functionality of the GnRH secretion is altered. n-IHH cases are associated with mutations in *GNRH* (Chevrier *et al*., 2011), *KISS1* (de Roux *et al*., 2003; Bianco *et al*., 2011), *DAX1* (Habiby *et al*., 1996; Merke *et al*., 1999), *GNRH1* (Bouligand *et al*., 2009; Chan *et al*., 2011), *LEPR*/*LEP* (Clement *et al*., 1998), PCSK1 (Jackson *et al*., 2003), *PROKR2/ PROK2* (Dodé *et al*., 2006), *SEMA3A/ SEMA7A* (Hanchate *et al*., 2012; Young *et al*., 2012), *TACR3/ TAC3* (Topaloglu *et al*., 2009; Topaloglu, 2010), *DMLX2* (Tata *et al*., 2014) genes encoding GnRH-R, GPR54, nuclear receptor 0B1, GnRH, Leptin-R, leptin, protein convertase subtilisin/kexin type 1, prokinecitin receptor 2, prokinecitin, neurokinin-B receptor, semaphorins-3a and -7a, neurokinin-B and Rab-connectin-3, respectively (Fig. 4). Most cases of IHH are sporadic, consistent with the affected individuals being infertile, but familial transmission has also been well described. Kindred analysis suggests that IHH is a wider spectrum of disease with individuals and relatives sharing an apparent common genotype but displaying a variety of reproductive or non-reproductive phenotypes. Oligogenicity could be one explanation for this phenotypic variation (Mitchell *et al*., 2011).

Oligogenic and complex genetic environmental interactions have now been identified, with physiological and environmental factors interacting in genetically susceptible individuals to alter their reproductive capacities.

Genetic factors associated with precocious puberty in Humans

Human precocious puberty is defined as the development of secondary sexual characteristics and elevated sexual hormones before 8 years of age in girls and 9 years of age in boys. There are two major forms of premature sexual maturation: inappropriate early activation of HPG axis that induces central precocious puberty (CPP) and peripheral precocious puberty (PPP) due to the increase of sex steroids with no activation of the HPG axis. Precocious puberty is highly deleterious since it will cause short stature, psychosocial problems and increase the risk of adulthood diseases.

Mutations in the *LHCGR* gene coding the LH receptor (LH-R) and leading to constitutive activation of the LH-R without ligand were the first mutations characterized in various family cases of peripheral precocious puberty limited to the male (Layman, 1999). These mutations affected only the male offspring and were without effect on the females. Recently cases of central precocious puberty have been associated with genetic variants affecting Kp signalling: mutation in the *KISS1* gene encoding Kp (Silveira *et al*., 2010; Mazaheri *et al*., 2015) or activating mutation of the *KISS1R* gene encoding GPR54 the Kp receptor (Teles *et al*., 2008; Silveira *et al*., 2010; Fig. 4). One of these mutations was present at heterozygous state in patient's mother and grandmother suggesting incomplete sex-dependent penetrance. Another possibility is that other genes could be involved in this phenotype evoking the oligogenicity concept in central precocious puberty as was well described for IHH (Mitchell *et al*., 2011).

Other cases of central precocious puberty are associated with mutations in the imprinted *MKRN3* gene encoding the makorin ring finger protein 3, a gene located in the imprinted Prader Willi syndrome region (Settas *et al*., 2014; Simon *et al*., 2015; Fig. 4). Data from Human cases and animal models suggests that *MKRN3* plays an inhibitory role in the reproductive axis and may represent a new pathway in pubertal regulation (Ong *et al*., 2009; Simon *et al*., 2015). MKNR3 is expressed ubiquitously.

Before 2000, clinical studies were individual case studies but now with the improvement of the

methods of sequencing of the genome, the increase of the capacities of calculation and the improvement of the algorithms, the studies of association of genomic data allow to find genetic variants associated to the age in the puberty. With this process, more than 100 loci involved in the susceptibility to precocious puberty have been discovered. Among them the *LIN28B* locus is one of the most significant (Ong *et al*., 2009; Elks *et al*., 2010; Fig. 4). *LIN28B* is a human homolog of *lin28* of *Caenorhabtidis elegans*, which was originally identified as a heterochronic regulator of developmental timing (Ambros and Horvitz, 1984) Deleterious mutations in *lin28* resulted in precocious larval to adult development and a partial transformation in sexual phenotype (Ambros, 2011). The Lin28 proteins are potent and specific post-transcriptional repressors of the biogenesis of let-7 miRNAs, which are time-specific expressed miRNAs that control developmental timing (Zhu *et al*., 2010).

A recent meta-analysis suggests that the variant allele carriers, especially people with heterozygote genotype for *ESR1* XbaI polymorphism and the wild allele for *ESR1 PvuII* polymorphism, are associated with precocious puberty susceptibility (Luo *et al*., 2015; Fig. 4).

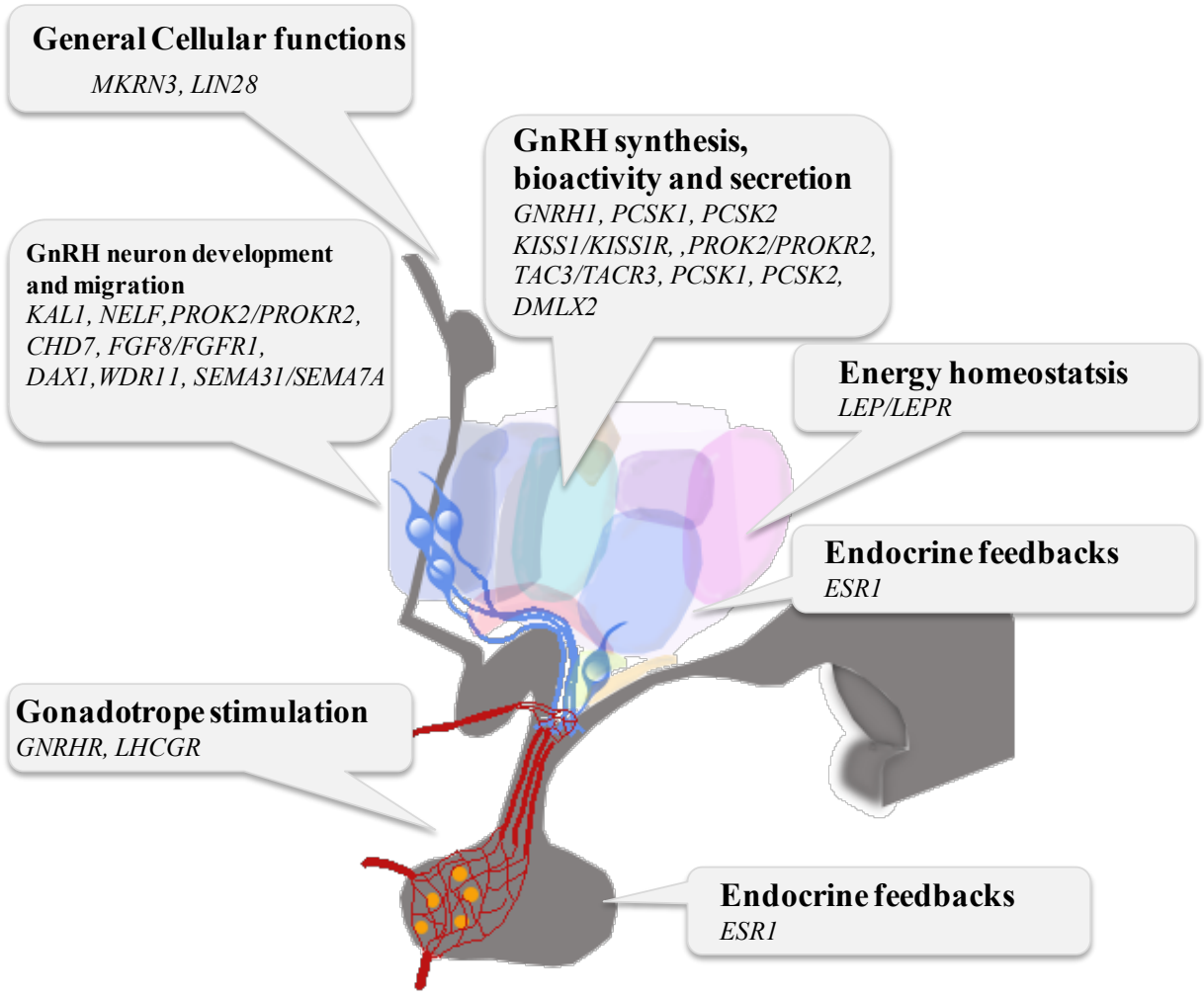

Figure 4. Genetic factors associated with pathological puberty delay or advance in humans. This figure summarizes how genetic factors associated with pathological conditions in humans are affecting cellular processes at hypothalamic and pituitary levels. From general cellular function GnRH neuron development and migration, GnRH synthesis, bioactivity and secretion, energy homeostasis, gonadotrope stimulation and endocrine feedbacks.

Genetic factors associated with age at puberty in cattle

Age at first calving usually varied between 24 and 36 months, according to cattle breeds and is considered a key factor in terms of profitability and efficiency in both dairy and beef cattle. Likewise, bull puberty also shows significant differences within and among breeds. In dairy cattle, age at first has continually decreased during the last decades. Improvement in nutrition and health have certainly contributed to an improve BW gain, but genetic selection for improved breeding and economic efficiency may also have indirectly impacted the onset of puberty (precocity; Mourits *et al*., 2000). Indeed, comparison of performances of 1970s and 1990s heifers from the same breed in New Zealand showed that modern heifers reached puberty at an earlier age than their predecessors, with a higher body weight than 20

years ago, meaning that mature size is different (Macdonald *et al*., 2007). As first calving at 24 months of age is becoming a common and general goal, one can safely assume that first-calving age will continue to decrease in the short term (Le Cozler *et al*., 2008).

Despite its economic importance, only a few studies have been conducted to identify genes and mutations associated with onset of puberty in either bulls or heifers. Most of these studies were done in beef cattle (mainly Angus), tropical breeds such as Brahman and Nelore cattle (*Bos indicus* cattle) and crosses which are reportedly older at puberty when compared with most *Bos taurus* breeds (Lunstra and Cundiff, 2003). Several parameters have been measured as a phenotype to study heifer puberty, from simple traits such as age at first service, age at first calving and age at first oestrus to more expensive and difficult to measure ones such as age at first *corpus luteum* (ultrasonography) or plasma progesterone concentration. For males, scrotal circumference, sperm quality (concentration, motility and morphology) as well as LH or IGF-1 circulating blood concentration have been monitored. One has to be aware that the nature of the quantitative puberty traits thus differs between studies. Moreover, their physiological meaning might be different than the strictly defined puberty onset. For example age at first oestrus does not mean age at puberty onset since oestrus behaviour is usually not present before the third oestrus cycle. Age at first calving is not age at first oestrus since the genital tract need several oestrus cycles to be fully developed in order to insure a full-length pregnancy.

Moderate to high heritability has been computed for heifer's age at puberty (0.2 to 0.48) and scrotal circumference (0.22 to 0.42; Vargas *et al*., 1998), suggesting that timing of puberty is likely to be a multigenic trait. Genetic correlations have also been observed between scrotal circumference or male IGF-1 blood concentration and heifer's age at puberty, suggesting that some common pathways may be involved in the two genders (Martinez-Velazquez and Gregory, 2003; Morris *et al*., 2010; Johnston *et al*., 2013).

Despite the multigenic nature of puberty onset, some major key player genes have been identified in humans, stimulating association studies in cattle, focused on some candidate genes. Polymorphisms in *GNRHR*, *LHR* and *IGF* were search for association with age of puberty in Angus male cattle (Lirón *et al*., 2012), showing significant association with one SNP located in IGF1. Likewise, polymorphisms in the *LHR*, *FSHR* and *GNRHR* were analysed in the Nellore breed, showing only association between *FSHR* and early puberty phenotype (Milazzotto *et al*., 2008). Furthermore, seven genes from the IGF1 pathway (*IGF1R, IGFBP2, IGFBP4, EIF2AK3, PIK3R1, GSK3B* and *IRS1*) were shown to be associated with heifer puberty in both Tropical Composite or Brahman breeds (Fortes *et al*., 2013). These findings support the hypothesis that IGF1 regulates arrival to puberty in male calves and also impact heifer puberty. In contrast to human and mouse, there are no evidences that genetic variation within *GNRH, LH* and its receptors could impact the regulation of pubertal timing in cattle. Based on their known effect on sexual precocity in mammals, 57 candidate genes related to lipid metabolism were also studied on a large panel of 1689 precocious and non-precocious Nellore heifers. Statistical analysis revealed that SNPs located within the *FABP4* and *PPP3CA* gene had a significant effect on sexual precocity (Dias *et al*., 2015).

Genome-wide association studies (GWAS) using microsatellites or SNPs have also been set up to identify QTL regions and highlight to new candidate genes. A search for markers associated with heifer's age at puberty and age at first calving in the Animal QTLdb (Hu *et al*., 2016) retrieves about 350 markers located within roughly 200 QTL regions, irrespective to breeds. Likewise, 10650 makers within 60 regions have been associated with male puberty, mainly on the X chromosome. Several candidate genes have been proposed starting from these regions and regulatory networks have been constructed (Fortes *et al*., 2010a, b, 2011, 2016). These findings suggest an enrichment of genes involved in axon guidance, cell adhesion, ErbB signaling, and glutamate activity, pathways that are known to affect pulsatile release of GnRH, which is necessary for the onset of puberty. In addition several TF were proposed as regulator of heifer's puberty, including *ESRRG, PPARG, HIVEP3, TOX, EYA1, NCOA2*, and *ZFHX4*. Combining GWAS and expression analysis in a multi-tissue omics also identified several key transcriptional regulators such as *PITX2, FOXA1, DACH2, PROP1, SIX6* … (Canovas *et al*., 2014). U6 spliceosomal RNA was also proposed as a positional candidate gene associated with age at first calving (Nascimento *et al*., 2016).

Interestingly, only a few common genes can be identified between genes located within QTL associated with either heifer's or bull puberty and genes already known in human to be involved in puberty onset: *HDAC8* and *NR0B1* may play a role in male puberty, whereas *CHST8, GABRA1, LEP* and *PROP1* may influence female puberty (Fig. 5). This finding suggests that cattle could provide new insight into the genetic basis of puberty in mammals. Consistent with the hypothesis of common pathways between genders, 16 common genes can be identified within heifer and bull QTL regions: *ARL2, CAPN1, CDC42EP2, DPF2, FRMD8, MRPL49, PARPBP, POLA2, SAC3D1, Slc22a20, SNX15, SPDYC, TIGD3 , TM7SF2 , VPS51, ZFPL1*.

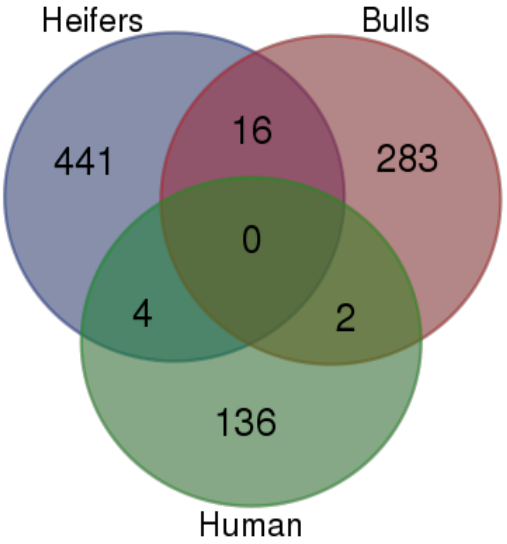

Figure 5. Only a few genes known to be associated with puberty onset in human are also located within cattle QTL regions. Cattle QTL regions were identified using the Animal QTLdb, taking into account "Age at first calving" for females and "Scrotal circumference" for males. Regions associated with "Age at puberty" were spread over female or male according to the experiment. QTL regions were defined as the critical mapping interval for linkage studies or a 500kb interval centered on the most significant marker for GWAS studies. Ensembl database was used to list genes located within these intervals and OMIM was used to establish a list of genes associated with puberty in Human. The Venn diagram presents the number of common genes between these lists, showing a limited number of common QTL and genes involved in bull's and heifer's puberty and only a few human candidate genes located within cattle QTL regions.

How to get good oocytes at younger age?

The overall goal of a replacement heifer program is to rear heifers to reach a desired age and body weight early so that they initiate puberty, establish pregnancy, and calve easily at a minimal cost. In addition to the investment needed to raise heifers from birth to calving, heifers that calve earlier spend a greater proportion of their life producing milk, and therefore returning profit to a dairy, whereas heifers that calve later spend more time in a non-productive period before initiation of lactation. The development of replacement heifers is a major economic investment for all beef and dairy operations. The costs associated with heifer development cannot be recovered if heifers do not conceive and remain productive in the herd; therefore, heifers need to conceive early in the breeding season or risk being culled. Breeders can use various levers to meet these objectives.

Advancing puberty

Feeding and photoperiod (ovine species) were the two main levers used by farmers to advance puberty. Young juvenile heifers fed with high-concentrate diet have a better weight gain and an advanced puberty onset compared to control heifers. The timing of this nutritional support is important, there is a developmental window during the early juvenile period (between 4-6.5 months) during which, high-concentrate diet will be effective on the timing of puberty onset. Feed restriction after this point will have little effect on the timing of puberty (Cardoso *et al*., 2015). One could imagine that the qualitative nutritional value and the timing of nutritional programming are of importance and should benefit from a research effort in this field.

Although this is not recommended by Europe, hormonal treatment can be used to advance puberty onset. Hormonal treatments are efficient to advance the first ovulation when administrated during the late juvenile period (8-10 months) in pre-pubertal heifers. They involve the administration of GnRH agonists or hCG (human chorionic gonatodotropin). The GnRH agonist Buserelin acetate is commonly used for oestrus synchronization or for treating post-partum anoestrus in adult females. Continuous infusion of GnRH or GnRH agonist (Deslorelin) using sub-cutaneaous implants or minipumps to 8-10 months' old heifers stimulate LH secretion and induce ovulation 30-48 h after the placement of the implant (Dodson *et al*., 1990; Grasselli *et al*., 1993). However luteinisation and the production of progesterone are not consistently observed and this may cause short luteal phases. The continuous exposure to GnRH or GnRH agonists induces the desensitization of the GnRH-R signalling. After GnRH agonist implants removal, the animals do not respond to exogenous GnRH treatment for 12 days (Bergfeld *et al*., 1996). For these reasons, hCG is usually preferred. hCG will mimic the effect of endogenous LH surge and stimulate the ovulation of the dominant follicle. The luteotropic effect of hCG guarantees the formation of a functional *corpus luteum* and will have a beneficial effect on the initiation of pregnancy. Its major side effect is that hCG is a human hormone and as such its repeated administration causes the development of an acquired immunity that impedes future treatments to be efficient (De Rensis *et al*., 2010; Dahlen *et al*., 2011). Both GnRH agonists- and hCG-based treatments rely on peptidic or proteic

substances that are not an environmental issue. In contrast to oestradiol- and progesterone- based hormonal treatments that have been used in the past in Europe or are still in use on the American, Asian and Australian continents. It would be interesting to test for Kp long life agonists that have been developed for the ovine species to see whether they could offer a more physiological activation of the central GnRH controlling system and thus avoiding the desensitization of GnRH-R signalling (Beltramo *et al*., 2015).

Collecting prepubertal oocytes

Another strategy is to overcome these problems by using *in vitro* production techniques and oocytes collection by Ovum Pick-Up (OPU) techniques. Despite the fact that large follicles are present before puberty, that good quality oocytes evaluated by the presence of compact cumulus can be collected by OPU, that the proportion of cleavages up to 8 cells after *in vitro* fertilization is correct, the rate of blastocysts obtained is low and their ability to produce successful pregnancy after embryo transfer is poor in comparison to data obtained from adult oocytes (Armstrong *et al*., 1992; Levesque and Sirard, 1994; Majerus *et al*., 1999; Landry *et al*., 2016). Ovarian stimulation using FSH can improve the rate of blastocyst formation, underlining the importance of hormonal environment to insure the oocyte competency to sustain development (Khatir *et al*., 1996). Different factors have been studied and sustain the cytoplasmic immaturity of prepubertal oocytes (Gandolfi *et al*., 1998; Oropeza *et al*., 2004; Bernal-Ulloa *et al*., 2016). Gene expression in blastocyst embryos relies mostly on post-transcriptional control of maternal transcripts accumulated during oocyte maturation. In calf oocytes, the expression of maternal transcripts differs from that of adult oocytes. Transcripts of PRDX2 and PRDX1 genes are in less quantities in oocytes collected from prepubertal animals in comparison to adult animals (Romar *et al*., 2011).

Conclusions

With the introduction of genomic selection 15 years ago, international agricultural politics have started to modify selection strategies, which now include puberty traits in order to advance puberty onset with the objective of reducing generation intervals. The selective pressure on onset of puberty will undoubtedly increase in a near future. Indeed, advances in molecular genetics have now made it possible to predict the total genetic value of animals by using genome-wide dense marker maps leading to the forthcoming of Genomic Selection (GS; Humblot *et al*., 2010). GS is of particular interest in cattle since the generation interval is long, artificial insemination bulls should be tested on their progeny before dissemination and some important traits such as fertility have a low heritability, due probably to a great sensitivity to environmental factors. Yearling bulls that have genomic breeding values information but lack phenotypic data on their daughters are often referred to as “genomic bulls”. There has been an immense shift among the AI companies toward the use of genomic bulls in the past 3 years. Some AI companies use almost all genomic bulls as sires of sons, whereas other companies use a combination of genomic bulls and progeny-tested bulls (Schefers and Weigel, 2012). Instead of waiting a minimum of 4.5 years to use progeny-tested bulls as sires of sons, AI companies could now use the best DNA-tested young bulls by roughly 1 year of age. Due to economical constrains, AI companies are now looking for animals having an advancement of their puberty.

It’s therefore of major importance to understand the link between these phenotypic changes, genetic determinants and environment. Indeed, GS de facto reduces the interval of generation and will speed up the selection process. This could be a great opportunity but may also increase the risk of disseminating unsuitable traits by lack of knowledge of their related pathways. Therefore, before implementing GS for QTL associated with puberty traits, it’s crucial to evaluate whether or not this selection process may affect other reproductive characteristics or reduce the robustness and increase vulnerability to environmental changes. There is clearly a need for basic research on factors that control puberty in order to improve heifer development and fertility (Perry, 2016) and address the question of robustness.

References

Ahima RS, Dushay J, Flier SN, Prabakaran D, Flier JS. 1997. Leptin accelerates the onset of puberty in normal female mice. *J Clin Invest*, 99:391-395.

Allen LG, Kalra PS, Crowley WR, Kalra SP. 1985. Comparison of the effects of neuropeptide Y and adrenergic transmitters on LH release and food intake in male rats. *Life Sci*, 37:617-623.

Ambros V, Horvitz HR. 1984. Heterochronic mutants of the nematode Caenorhabditis elegans. *Science*, 226:409-416.

Ambros V. 2011. MicroRNAs and developmental timing. *Curr Opin Genet Dev*, 21:511-517.

Amstalden M, Garcia MR, Stanko RL, Nizielski SE, Morrison CD, Keisler DH, Williams G. 2002. Central infusion of recombinant ovine leptin normalizes plasma insulin and stimulates a novel hypersecretion of luteinizing hormone after short-term fasting in mature beef cows. *Biol Reprod*, 66:1555-1561.

Armstrong DT, Holm P, Irvine B, Petersen BA, Stubbings RB, Mclean D, Stevens G, Seamark RF. 1992. Pregnancies and live birth from in vitro fertilization of calf oocytes collected by laparoscopic follicular aspiration. *Theriogenology*, 38:667-678.

Barash IA, Cheung CC, Weigle DS, Ren HP, Kabigting EB, Kuijper JL, Clifton DK, Steiner RA. 1996. Leptin is a metabolic signal to the reproductive system. *Endocrinology*, 137:3144-3147.

Beltramo M, Robert V, Galibert M, Madinier J-B, Marceau P, Dardente H, Decourt C, de Roux N, Lomet D, Delmas AF, Caraty A, Aucagne V. 2015. Rational design of triazololipopeptides analogs of kisspeptin inducing a long-lasting increase of

gonadotropins. *J Med Chem*, 58:3459-3470.
Bergfeld EG, D'Occhio MJ, Kinder JE. 1996. Pituitary function, ovarian follicular growth, and plasma concentrations of 17 beta-estradiol and progesterone in prepubertal heifers during and after treatment with the luteinizing hormone-releasing hormone agonist deslorelin. *Biol Reprod*, 54:776-782.
Bernal-Ulloa SM, Heinzmann J, Herrmann D, Hadeler K-G, Aldag P, Winkler S, Pache D, Baulain U, Lucas-Hahn A, Niemann H. 2016. Cyclic AMP affects oocyte maturation and embryo development in prepubertal and adult cattle. *PLoS One*, 11:e0150264.
Bédécarrats GY, Kaiser UB. 2003. Differential regulation of gonadotropin subunit gene promoter activity by pulsatile gonadotropin-releasing hormone (GnRH) in perifused L beta T2 cells: role of GnRH receptor concentration. *Endocrinology*, 144:1802-1811.
Bianco SDC, Vandepas L, Correa-Medina M, Gereben B, Mukherjee A, Kuohung W, Carroll R, Teles MG,. Latronico AC, Kaiser UB. 2011. KISS1R intracellular trafficking and degradation: effect of the Arg386Pro disease-associated mutation. *Endocrinology*, 152:1616-1626.
Bouligand J, Ghervan C, Tello JA, Brailly-Tabard S, Salenave S, Chanson P, Lombes M, Millar RP, Guiochon-Mantel A, Young J. 2009. Isolated familial hypogonadotropic hypogonadism and a GNRH1 mutation. *N Engl J Med*, 360:2742-2748.
Butler MP, Trumbull JJ, Turner KW, Zucker I. 2007. Timing of puberty and synchronization of seasonal rhythms by simulated natural photoperiods in female Siberian hamsters. *Am J Physiol Regul Integr Comp Physiol*, 293:R413-R420.
Caldani M, Batailler M, Jourdan F. 1987. The sheep terminal nerve: coexistence of LHRH- and AChE-containing neurons. *Neurosci Lett*, 83:221-226.
Caldani M, Antoine M, Batailler M, Duittoz AH. 1995. Ontogeny of GnRH systems. *J Reprod Fertil Suppl*, 49:147-162.
Cánovas A, Reverter A, DeAtley KL, Ashley RL, Colgrave ML, Fortes MRS, Islas-Trejo A, Lehnert S, Porto-Neto L, Rincón G, Silver GA, Snelling WM, Medrano JF, Thomas MG. 2014. Multi-tissue omics analyses reveal molecular regulatory networks for puberty in composite beef cattle. *PLoS One*, 9:e102551.
Caraty A, Orgeur P, Thiery JC. 1982. Demonstration of the pulsatile secretion of LH-RH into hypophysial portal blood of ewes using an original technic for multiple samples [in French]. *C R Seances Acad Sci III*, 295:103-106.
Caraty A, Locatelli A, Martin GB. 1989. Biphasic response in the secretion of gonadotrophin-releasing hormone in ovariectomized ewes injected with oestradiol. *J Endocrinol,* 123:375-382.
Cardoso RC, Alves BRC, Sharpton SM, Williams GL, Amstalden M. 2015. Nutritional programming of accelerated puberty in heifers: involvement of pro-opiomelanocortin neurones in the arcuate nucleus. *J Neuroendocrinol*,. 27:647-657.
Chan Y-M, Broder-Fingert S, Paraschos S, Lapatto R, Au M, Hughes V, Bianco SDC, Min L, Plummer L, Cerrato F, De Guillebon A, Wu I-H, Wahab F, Dwyer A, Kirsch S, Quinton R, Cheetham T, Ozata M, Ten S,. Chanoine J-P, Pitteloud N, Martin KA, Schiffmann R, Van der Kamp HJ, Nader S, Hall JE, Kaiser UB, Seminara SB. 2011. GnRH-deficient phenotypes in humans and mice with heterozygous variants in KISS1/Kiss1. *J Clin Endocrinol Metab*, 96:E1771-E1781.
Chehab FF, Lim ME, Lu R. 1996. Correction of the sterility defect in homozygous obese female mice by treatment with the human recombinant leptin. *Nat Genet*, 12:318-320.
Cheung CC, Thornton JE, Kuijper JL, Weigle DS, Clifton DK, Steiner RA. 1997. Leptin is a metabolic gate for the onset of puberty in the female rat. *Endocrinology*, 138:855-858.
Chevrier L, Guimiot F, de Roux N. 2011. GnRH receptor mutations in isolated gonadotropic deficiency. *Mol Cell Endocrinol*, 346:21-28.
Clark SJ, Ellis N, Styne DM, Gluckman PD, Kaplan SL, Grumbach MM. 1984. Hormone ontogeny in the ovine fetus. XVII. Demonstration of pulsatile luteinizing hormone secretion by the fetal pituitary gland. *Endocrinology*, 115:1774-1779.
Clarke IJ, Cummins JT. 1982. The temporal relationship between gonadotropin releasing hormone (GnRH) and luteinizing hormone (LH) secretion in ovariectomized ewes. *Endocrinology*, 111:1737-1739.
Clarke IJ, Cummins JT, Karsch FJ, Seeburg PH, Nikolics K. 1987. GnRH-associated peptide (GAP) is cosecreted with GnRH into the hypophyseal portal blood of ovariectomized sheep. *Biochem Biophys Res Commun*, 143:665-671.
Claypool LE, Foster DL. 1990. Sexual differentiation of the mechanism controlling pulsatile secretion of luteinizing hormone contributes to sexual differences in the timing of puberty in sheep. *Endocrinology*, 126:1206-1215.
Clement K, Vaisse C, Lahlou N, Cabrol S, Pelloux V, Cassuto D, Gourmelen M, Dina C, Chambaz J, Lacorte JM, Basdevant A, Bougneres P, Lebouc Y, Froguel P, Guy-Grand B. 1998. A mutation in the human leptin receptor gene causes obesity and pituitary dysfunction. *Nature*, 392:398-401.
Clements JA, Reyes FI, Winter JSD, Faiman C. 2009. Studies on Human Sexual Development. III. Fetal Pituitary and Serum, and Amniotic Fluid Concentrations of LH, CG, and FSH. *J Clin Endocrinol Metab*, 42:9-19.
Colledge WH, de Tassigny XD. 2009. Kisspeptin signaling in rodents: insights from knock-out mice. *Biol Reprod*, 81:75-76.
Constantin S, Caraty A, Wray S, Duittoz AH. 2009. Development of gonadotropin-releasing hormone-1 secretion in mouse nasal explants. *Endocrinology*, 150:3221-3227.
Dahl GE. 2008. Effects of short day photoperiod on prolactin signaling in dry cows: a common mechanism among tissues and environments? *J Anim Sci*, 86:10-14.
Dahlen CR, Marquezini GHL, Larson JE, Lamb GC. 2011. Human chorionic gonadotropin influences ovarian function and concentrations of progesterone in prepubertal Angus heifers. *J Anim Sci*, 89:2739-2749.
Davis Rincker LE, Vandehaar MJ, Wolf CA,

Liesman JS, Chapin LT, Weber Nielsen MS. 2011. Effect of intensified feeding of heifer calves on growth, pubertal age, calving age, milk yield, and economics. *J Anim Sci*, 94:3554-3567.

Day ML, Imakawa K, WolfPL, Kittok RJ, Kinder JE. 1987. Endocrine mechanisms of puberty in heifers. Role of hypothalamo-pituitary estradiol receptors in the negative feedback of estradiol on luteinizing hormone secretion. *Biol Reprod*, 37:1054-1065.

De Rensis F, López-Gatius F, García-Ispierto I, Techakumpu M. 2010. Clinical use of human chorionic gonadotropin in dairy cows: an update. *Theriogenology*, 73:1001-1008.

de Roux N, Genin E, Carel J-C, Matsuda F, Chaussain J-L., Milgrom E. 2003. Hypogonadotropic hypogonadism due to loss of function of the KiSS1-derived peptide receptor GPR54. *Proc Natl Acad Sci USA*, 100:10972-10976.

Dias M, Souza M, Takada L, Feitosa FLB, Costa RB, Diaz IDPS, Cardoso DF, Tonussi R L, Baldi F, Albuquerque LG, Oliveira HN. 2015. Study of lipid metabolism-related genes as candidate genes of sexual precocity in Nellore cattle. *Genet Mol Res*, 14:234-243.

Dodé C, Levilliers J, Dupont J-M, De Paepe A, Le Dû N, Soussi-Yanicostas N, Coimbra RS, Delmaghani S, Compain-Nouaille S, Baverel F, Pêcheux C, Le Tessier D, Cruaud C, Delpech M, Speleman F, Vermeulen S, Amalfitano A, Bachelot Y, Bouchard P, Cabrol S, Carel JC, Delemarre-van de Waal H, Goulet-Salmon B, Kottler M-L, Richard O, Sanchez-Franco F, Saura R, Young J, Petit C,. Hardelin J-P. 2003. Loss-of-function mutations in FGFR1 cause autosomal dominant Kallmann syndrome. *Nat Genet*, 33:463-465.

Dodé C, Teixeira L, Levilliers J, Fouveaut C, Bouchard P, Kottler M-L, Lespinasse J, Lienhardt-Roussie A, Mathieu M, Moerman A, Morgan G, Murat A, Toublanc J-E, Wolczynski S, Delpech M, Petit C, Young J, Hardelin J-P. 2006. Kallmann syndrome: Mutations in the genes encoding prokineticin-2 and prokineticin receptor-2. *PLoS Genet*, 2:1648-1652.

Dodson SE, McLeod BJ,. Lamming GE, Peters AR. 1990. Ovulatory responses to continuous administration of GnRH in nine-month-old prepubertal beef heifers. *Anim Reprod Sci*, 22:271-280.

Donato J, Cravo RM, Frazão R, Gautron L, Scott MM, Lachey J, Castro IA, Margatho LO, Lee S, Lee C, Richardson JA, Friedman J, Chua S, Coppari R, Zigman JM, Elmquist, JK, Elias CF. 2011. Leptin's effect on puberty in mice is relayed by the ventral premammillary nucleus and does not require signaling in Kiss1 neurons. *J Clin Invest*, 121:355-368.

Duittoz AH, Batailler M. 2000. Pulsatile GnRH secretion from primary cultures of sheep olfactory placode explants. *J Reprod Fertil*, 120:391-396.

Elks CE, Perry JRB, Sulem P, Chasman DI, Franceschini N, He C, Lunetta KL, Visser JA, Byrne EM, Cousminer DL, Gudbjartsson DF, Esko T, Feenstra B, Hottenga J-J, Koller DL, Kutalik Z, Lin P, Mangino M, Marongiu M, McArdle PF, Smith AV, Stolk L, van Wingerden SH, Zhao JH, Albrecht E, Corre T, Ingelsson E, Hayward C, Magnusson PKE, Smith EN, Ulivi S, Warrington NM, Zgaga L, Alavere H, Amin N, Aspelund T. Bandinelli S, Barroso I, Berenson GS, Bergmann S, Blackburn H, Boerwinkle E., Buring JE, Busonero F, Campbell H, Chanock SJ, Chen W, Cornelis MC, Couper D, Coviello AD, d'Adamo P, de Faire U, de Geus EJC, Deloukas P, Döring A, Smith GD, Easton DF, Eiriksdottir G, Emilsson V, Eriksson J, Ferrucci L, Folsom AR, Foroud T, Garcia M, Gasparini P, Geller F, Gieger C, GIANT Consortium, Gudnason V, Hall P, Hankinson S.E, Ferreli L, Heath AC, Hernandez DG, Hofman A, Hu FB, Illig T, Järvelin M-R, Johnson AD, Karasik D,. Khaw K-T, Kiel DP, Kilpeläinen TO, Kolcic I, Kraft P, Launer LJ, Laven JSE, Li S, Liu, J, Levy D, Martin NG, McArdle WL, Melbye M, Mooser V, Murray JC, Murray SS, Nalls MA, Navarro P, et al. 2010. Thirty new loci for age at menarche identified by a meta-analysis of genome-wide association studies. *Nat Genet*, 42:1077-1085.

Forger NG, de Vries GJ, Breedlove SM. 2015. Sexual differentiation of brain and behavior. *In*: Plant TM, J. Zeleznik AJ (Ed.). *Knobil and Neill's Physiology of Reproduction*. 4th ed. New York, NY: Academis Press. pp. 2109-2155.

Fortes MRS, Reverter A, Zhang Y, Collis E, Nagaraj Y, Johnoson NN, Barris W, Hawken RJ. 2010a. Association weight matrix for the genetic dissection of puberty in beef cattle. *Proc Natl Acad Sci USA*, 107:13642-13647.

Fortes MRS, Reverter A, Zhang Y, Collis E, Nagaraj SH, Johnsson NN, Prayaga KC, Barris W, Hawken RJ. 2010b. Association weight matrix for the genetic dissection of puberty in beef cattle. *Proc Natl Acad Sci USA*, 107:13642-13647.

Fortes MRS, Reverter A, Nagaraj SH, Zhang Y, Jonsson NN, Barris W, Lehnert S, Boe-Hansen GB, Hawken RJ. 2011. A single nucleotide polymorphism-derived regulatory gene network underlying puberty in 2 tropical breeds of beef cattle. *J Anim Sci*, 89:1669-1683.

Fortes MRS, Li Y, Collis E, Zhang Y, Hawken RJ. 2013. The IGF1 pathway genes and their association with age of puberty in cattle. *Anim Genet*, 44:91-95.

Fortes MRS, Nguyen LT, Porto Neto LR, Reverter A, Moore SS, Lehnert SA, Thomas M. 2016. Polymorphisms and genes associated with puberty in heifers. *Theriogenology*, 86: 333-339.

Foster DL, Olster DH. 1984. Effect of restricted nutrition on puberty in the lamb: patterns of tonic luteinizing hormone (LH) secretion and competency of the LH surge system. *Endocrinology*, 116:375-381.

Foster DL, Hileman SM. 2015. Puberty in the sheep. *In*: Plant TM, J. Zeleznik AJ (Ed.). *Knobil and Neill's Physiology of Reproduction*. 4th ed. New York, NY: Academis Press. pp. 1441-1485.

Franco B, Guioli S, Pragliola A, Incerti B, Bardoni B, Tonlorenzi R, Carrozzo R, Maestrini E, Pieretti M, Taillonmiller P, Brown CJ, Willard HF, Lawrence C, Persico MG, Camerino G, Ballabio A. 1991. A gene deleted in Kallmanns syndrome shares homology with neural cell-adhesion and axonal path-

finding molecules. *Nature*, 353:529-536.
Frederich RC, Haman A, Anderson S, Lollmann B, Lowell BB, Flier JS. 1995. Leptin levels reflect body lipid-content in mice - evidence for diet-induced resistance to leptin action. *Nat Med*, 1:1311-1314.
Freetly HC, Kuehn LA, Cundiff LV. 2011. Growth curves of crossbred cows sired by Hereford, Angus, Belgian Blue, Brahman, Boran, and Tuli bulls, and the fraction of mature body weight and height at puberty. *J Anim Sci*, 89:2373-2379.
Frisch RE, Revelle R. 1970. Height and weight at menarche and a hypothesis of critical body weights and adolescent events. *Science*, 169:397-399.
Gajdos ZKZ, Henderson KD, Hirschhorn JN, M. R. Palmert. 2010. Genetic determinants of pubertal timing in the general population. *Mol Cell Endocrinol*, 324:21-29.
Gandolfi F, Milanesi E, Pocar P, Luciano AM, Brevini T, Acocella F, Lauria A, Armstrong DT. 1998. Comparative analysis of calf and cow oocytes during in vitro maturation. *Mol Reprod Dev*, 49:168-175.
Gasser CL. 2006. Induction of precocious puberty in heifers III: Hastened reduction of estradiol negative feedback on secretion of luteinizing hormone. *J Anim Sci*, 84:2050-2056.
Gazal OS, Leshin LS, Stanko R, Thomas MG, Keisler DH, Anderson LL, Williams GL. 1998. Gonadotropin-releasing hormone secretion into third-ventricle cerebrospinal fluid of cattle: correspondence with the tonic and surge release of luteinizing hormone and its tonic inhibition by suckling and neuropeptide Y. *Biol Reprod*, 59:676-683.
Giaume C, Koulakoff A, Roux L, Holcman D, Rouach N. 2010. Astroglial networks: a step further in neuroglial and gliovascular interactions. *Nat Rev Neurosci*, 11:87-99.
Gore AC, Terasawa E. 1991. A role for norepinephrine in the control of puberty in the female rhesus-monkey, *Macaca-mulatta*. *Endocrinology*, 129:3009-3017.
Gore AC, Mitsushima D, Terasawa E. 1993. A possible role of neuropeptide-Y in the control of the onset of puberty in female rhesus-monkeys. *Neuroendocrinology*, 58:23-34.
Grasselli F, Baratta M, Tamanini C. 1993. Effects of a GnRH analogue (buserelin) infused via osmotic minipumps on pituitary and ovarian activity of prepubertal heifers. *Anim Reprod Sci*, 32:153-161.
Habiby RL, Boepple P, Nachtigall L, Sluss PM, Crowley WF Jr, Jameson JL. 1996. Adrenal hypoplasia congenita with hypogonadotropic hypogonadism: evidence that DAX-1 mutations lead to combined hypothalmic and pituitary defects in gonadotropin production. *J Clin Invest*, 98:1055-1062.
Hanchate NK, Giacobini P, Lhuillier P, Parkash J, Espy C, Fouveaut C, Leroy C, Baron S, Campagne C, Vanacker C, Collier F, Cruaud C, Meyer V, García-Piñero A, Dewailly D, Cortet-Rudelli C, Gersak K, Metz C, Chabrier G, Pugeat M, Young J, Hardelin J-P, Prevot V, Dodé C. 2012. SEMA3A, a gene involved in axonal pathfinding, is mutated in patients with Kallmann syndrome. *PLoS Genet*, 8:e1002896.
Hardelin JP, Levilliers J, Delcastillo I, Cohen-Salmon M, Legouis R, Blanchard S, Compain S, Bouloux P, Kirk J, Moraine C, Chaussain JL, Weissenbach J, Petit C. 1992. X-chromosome-linked Kallmann syndrome - stop mutations validate the candidate gene. *Proc Natl Acad Sci USA*, 89:8190-8194.
Hardelin JP, Dodé C. 2008. The complex genetics of Kallmann syndrome: KAL1, FGFR1, FGF8, PROKR2, PROK2, et al. *Sex Dev*, 2:181-193.
Henry BA, Goding JW, Alexander WS, Tilbrook AJ, Canny BJ, Dunshea F, Rao A, Mansell A, Clarke IJ. 2011. Central administration of leptin to ovariectomized ewes inhibits food intake without affecting the secretion of hormones from the pituitary gland: evidence for a dissociation of effects on appetite and neuroendocrine function. *Endocrinology*, 140:1175-1182.
Herbison AE, Pape JR. 2001. New evidence for estrogen receptors in gonadotropin-releasing hormone neurons. *Front Neuroendocrinol*, 22:292-308.
Hines M, Spencer D, Kung KT, Browne WV, Constantinescu M, Noorderhaven RM. 2016. The early postnatal period, mini-puberty, provides a window on the role of testosterone in human neurobehavioural development. *Curr Opin Neurobiol*, 38:69-73.
Hochberg Z, Belsky J. 2013. Evo-devo of human adolescence: beyond disease models of early puberty. *BMC Med*, 11:113.
Hrabovszky E, Shughrue PJ, Merchenthaler I, Hajszán T, Carpenter CD, Liposits Z, Petersen SL. 2000. Detection of estrogen receptor-beta messenger ribonucleic acid and 125I-estrogen binding sites in luteinizing hormone-releasing hormone neurons of the rat brain. *Endocrinology*, 141:3506-3509.
Hu Z-L, Park CA, Reecy JM. 2016. Developmental progress and current status of the Animal QTLdb. *Nucl Acids Res*, 44:D827-D833.
Humblot P. Le Bourhis D, Fritz S, Colleau JJ, Gonzalez C, Guyader Joly C, Malafosse A, Heyman Y, Amigues Y, Tissier M, Ponsart C. 2010. Reproductive technologies and genomic selection in cattle. *Vet Med Int*, 2010:1-8.
Irvine CH, Alexander SL. 1987. A novel technique for measuring hypothalamic and pituitary hormone secretion rates from collection of pituitary venous effluent in the normal horse. *J Endocrinol*, 113:183-192.
Jackson RS, Creemers JWM, Farooqi IS, Raffin-Sanson M-L, Varro A, Dockray GJ, Holst JJ, Brubaker PL, Corvol P, Polonsky KS, Ostrega D, Becker KL, Bertagna X, Hutton JC, White A, Dattani MT, Hussain K, Middleton SJ, Nicole TM, Milla PJ, Lindley KJ, O'Rahilly S. 2003. Small-intestinal dysfunction accompanies the complex endocrinopathy of human proprotein convertase 1 deficiency. *J Clin Invest*, 112:1550-1560.
Johnston DJ, Corbet NJ, Barwick SA, Wolcott ML, Holroyd. RG. 2013. Genetic correlations of young bull reproductive traits and heifer puberty traits with female reproductive performance in two tropical beef genotypes in northern Australian. *Anim Prod Sci*, 54:74-84.

Kaplan SL, Grumbach MM. 1976. The ontogenesis of human foetal hormones. II. Luteinizing hormone (LH) and follicle stimulating hormone (FSH). *Acta Endocrinol (Copenh)*, 81:808-829.

Kaplowitz PB, Slora EJ, Wasserman RC, Pedlow SE, Herman-Giddens ME. 2001. Earlier onset of puberty in girls: Relation to increased body mass index and race. *Pediatrics*, 108:347-353.

Kasuya E, Nyberg CL, Mogi K, Terasawa E. 1999. A role of gamma-amino butyric acid (GABA) and glutamate in control of puberty in female rhesus monkeys: Effect of an antisense oligodeoxynucleotide for GAD67 messenger ribonucleic acid and MK801 on luteinizing hormone-releasing hormone release. *Endocrinology*, 140:705-712.

Keen KL, Burich AJ, Mitsushima D, Kasuya E, Terasawa E. 2011. Effects of Pulsatile Infusion of the GABAA receptor blocker bicuculline on the onset of puberty in female rhesus monkeys. *Endocrinology*, 140:5257-5266.

Khatir H, Lonergan P, Carolan C, Mermillod P. 1996. Prepubertal bovine oocyte: A negative model for studying oocyte developmental competence. *Mol Reprod Dev*, 45:231-239.

Kim H-G, Kurth I, Lan F, Meliciani I, Wenzel W, Eom SH, Kang GB, Rosenberger G, Tekin M, Ozata M, Bick DP, Sherins RJ, Walker SL, Shi Y, Gusella JF, Layman LC. 2008. Mutations in CHD7, encoding a chromatin-remodeling protein, cause idiopathic hypogonadotropic hypogonadism and kallmann syndrome. *Am J Hum Genet*, 83:511-519.

Kim H-G, Layman LC. 2011. The role of CHD7 and the newly identified WDR11 gene in patients with idiopathic hypogonadotropic hypogonadism and Kallmann syndrome. *Mol Cell Endocrinol*, 346:74-83.

Landry DA, Bellefleur A-M, Labrecque R, Grand F-X, Vigneault C, Blondin P, Sirard M-A. 2016. Effect of cow age on the in vitro developmental competence of oocytes obtained after FSH stimulation and coasting treatments. *Theriogenology*, 86:1-7.

Layman LC. 1999. Mutations in human gonadotropin genes and their physiologic significance in puberty and reproduction. *Fertil Steril* ,71:201-218.

Le Cozler Y, Lollivier V, Lacasse P, Disenhaus C. 2008. Rearing strategy and optimizing first-calving targets in dairy heifers: a review. *Animal*, 2:1393-1404.

Levesque JT, Sirard MA. 1994. Proteins in oocytes from calves and adult cows before maturation - relationship with their development capacity. *Reprod Nutr Dev*, 34:133-139.

Levine JE, Pau KY, Ramirez VD, Jackson GL. 1982. Simultaneous measurement of luteinizing hormone-releasing hormone and luteinizing hormone release in unanesthetized, ovariectomized sheep. *Endocrinology*, 111:1449-1455.

Lirón JP, Prando AJ, Fernández ME, Ripoli MV, Rogberg-Muñoz A, Goszczynski DE, Posik, DM, Peral-García P, Baldo A, Giovambattista G. 2012. Association between GNRHR, LHR and IGF1 polymorphisms and timing of puberty in male Angus cattle. *BMC Genet*, 13:26.

Louis GW, Greenwald-Yarnell M, Phillips R, Coolen LM, Lehman MN, Myers MG. 2011. Molecular mapping of the neural pathways linking leptin to the neuroendocrine reproductive axis. *Endocrinology*, 152:2302-2310.

Lunstra DD, Cundiff LV. 2003. Growth and pubertal development in Brahman-, Boran-, Tuli-, Belgian Blue-, Hereford- and Angus-sired F1 bulls. *J Anim Sci*, 81:1414-1426.

Luo Y, Liu Q, Lei X, Wen Y, Yang Y-L., Zhang R, Hu M-Y. 2015. Association of estrogen receptor gene polymorphisms with human precocious puberty: a systematic review and meta-analysis. *Gynecol Endocrinol*, 31:516-521.

Macdonald KA, McNaughton LR, Verkerk GA, Penno JW, Burton LJ, Berry DP, Gore PJS, Lancaster JAS, Holmes CW. 2007. A comparison of three strains of holstein-friesian cows grazed on pasture: growth, development, and puberty. *J Dairy Sci*, 90:3993-4003.

Maciel M. 2004. Chronic administration of recombinant ovine leptin in growing beef heifers: effects on secretion of LH, metabolic hormones, and timing of puberty. *J Anim Sci*, 82:2930-2936.

Majdoubi El M, Sahu A, Ramaswamy S, Plant TM. 2000. Neuropeptide Y: A hypothalamic brake restraining the onset of puberty in primates. *Proc Natl Acad Sci USA*, 97:6179-6184.

Majerus V, De Roover R, Etienne D, Kaidi S, Massip A, Dessy F, Donnay I. 1999. Embryo production by ovum pick up in unstimulated calves before and after puberty. *Theriogenology*, 52:1169-1179.

Manning JM, Bronson FH. 1989. Effects of prolonged exercise on puberty and luteinizing hormone secretion in female rats. *Am J Physiol*, 257:R1359-64.

Manning JM, Bronson FH. 1991. Suppression of puberty in rats by exercise: effects on hormone levels and reversal with GnRH infusion. *Am J Physiol*, 260:R717-23.

Martinez-Velazquez G, Gregory KE. 2003. Genetic relationships between scrotal circumference and female reproductive traits. *J Anim Sci*, 81:395-401

Matsui H, Takatsu Y, Kumano S, Matsumoto H, Ohtaki T. 2004. Peripheral administration of metastin induces marked gonadotropin release and ovulation in the rat. *Biochem Biophys Res Commun*, 320:383-388.

Mazaheri A, Hashemipour M. Salehi M, Behnam M, Hovsepian S, Hassanzadeh A. 2015. Mutation of kisspeptin 1 gene in children with precocious puberty in Isfahan city. *Int J Prev Med*, 6:41.

McDonald JK, Lumpkin MD, DePaolo LV. 1989. Neuropeptide-Y suppresses pulsatile secretion of luteinizing hormone in ovariectomized rats: possible site of action. *Endocrinology*, 125:186-191.

Merke DP, Tajima T, Baron J, Cutler GB. 1999. Hypogonadotropic hypogonadism in a female caused by an X-linked recessive mutation in the DAX1 gene. *N Engl J Med*, 340:1248-1252.

Messager S, Chatzidaki EE, Ma D, Hendrick AG, Zahn D, Dixon J, Thresher RR, Malinge I, Lomet D, Carlton MBL, Colledge WH, Caraty A, Aparicio SAJR. 2005. Kisspeptin directly stimulates gonadotropin-releasing hormone release via G protein-

coupled receptor 54. *Proc Natl Acad Sci USA*, 102:1761-1766.

Messaoud-Toumi LH, Taragnat C, Durand P. 1993. Heterogeneity in the storage of gonadotropins in the ovine fetus and evidence for luteinizing hormone-follicle-stimulating hormone cells in the fetal pituitary. *Biol Reprod*, 48:1239-1245.

Milazzotto MP, Rahal P, Nichi M, Miranda-Neto T, Teixeira LA, Ferraz JBS, Eler JP, Campagnari F, Garcia JF. 2008. New molecular variants of hypothalamus-pituitary-gonad axis genes and their association with early puberty phenotype in Bos taurus indicus (Nellore). *Livest Sci*, 114, 274-279.

Mitchell A., Dwyer A, Pitteloud N, Quinton R. 2011. Genetic basis and variable phenotypic expression of Kallmann syndrome: towards a unifying theory. *Trends Endocrinol Metab*, 22:249-258.

Mitsushima D, Hei DL, Terasawa E. 1994. Gamma-aminobutyric-acid is an inhibitory neurotransmitter restricting the release of luteinizing-hormone-releasing hormone before the onset of puberty. *Proc Natl Acad Sci USA,* 91:395-399.

Miura K, Acierno JS, Seminara SB. 2004. Characterization of the human nasal embryonic LHRH factor gene, NELF, and a mutation screening among 65 patients with idiopathic hypogonadotropic hypogonadism (IHH). *J Hum Genet*, 49:265-268.

Moenter SM, Caraty A, Locatelli A, Karsch FJ. 1991. Pattern of gonadotropin-releasing hormone (GnRH) secretion leading up to ovulation in the ewe: existence of a preovulatory GnRH surge. *Endocrinology*, 129:1175-1182.

Morris CA, Wilson JA, Bennett GL, Cullen NG, Hickey SM, Hunter JC. 2010. Genetic parameters for growth, puberty, and beef cow reproductive traits in a puberty selection experiment. *NZ J Agric Res*, 43:83-91.

Mourits MC, Galligan DT, Dijkhuizen AA, Huirne RB. 2000. Optimization of dairy heifer management decisions based on production conditions of Pennsylvania. *J Dairy Sci*, 83:1989-1997.

Mueller PL, Sklar CA, Gluckman PD, Kaplan SL, Grumbach MM. 1981. Hormone ontogeny in the ovine fetus. IX. Luteinizing hormone and follicle-stimulating hormone response to luteinizing hormone-releasing factor in mid- and late gestation and in the neonate. *Endocrinology*, 108:881-886.

Nakai Y, Plant TM, Hess DL, Keogh E J, Knobil E. 1978. On the sites of the negative and positive feedback actions of estradiol in the control of gonadotropin secretion in the rhesus monkey. *Endocrinology*, 102:1008-1014.

Nascimento AV, Matos MC, Seno LO, Romero ARS, Garcia JF, Grisolia AB. 2016. Genome wide association study on early puberty in *Bos indicus*. *Genet Mol Res*, 15(1). doi: 10.4238/gmr.15017548.

Norgren RB, Lehman MN. 1989. A double-label pre-embedding immunoperoxidase technique for electron microscopy using diaminobenzidine and tetramethylbenzidine as markers. *J Histochem Cytochem,* 37:1283-1289.

Ojeda SR, Lomniczi A, Sandau US. 2008. Glial-gonadotrophin hormone (GnRH) neurone interactions in the median eminence and the control of GnRH secretion. *J Neuroendocrinol*, 20:732-742.

Ojeda SR, Lomniczi A, Sandau U. 2010. Contribution of glial-neuronal interactions to the neuroendocrine control of female puberty. *Eur J Neurosci*, 32:2003-2010.

Ong KK, Elks CE, Li S,. Zhao JH, Luan J, Andersen LB, Bingham SA, Brage S, Smith GD, Ekelund U, Gillson CJ, Glaser B, Golding J, Hardy R, Khaw K-T, Kuh D, Luben R, Marcus M, McGeehin M A, Ness AR, Northstone K, Ring SM, Rubin C, Sims MA, Song K, Strachan DP, Vollenweider P, Waeber G, Waterworth DM, Wong A, Deloukas P, Barroso I, Mooser V, Loos RJ, Wareham NJ. 2009. Genetic variation in LIN28B is associated with the timing of puberty. *Nat Genet*, 41:729-733.

Orellana JA, Sáez PJ, Cortés Campos C, Elizondo RJ, Shoji KF, Contreras Duarte S, Figueroa V. Velarde V, Jiang JX, Nualart F, Sáez JC, García MA. 2012. Glucose increases intracellular free Ca2+ in tanycytes via ATP released through connexin 43 hemichannels. *Glia*, 60:53-68.

Oropeza A, Wrenzycki C, Herrmann D, Hadeler K-G, Niemann H. 2004. Improvement of the developmental capacity of oocytes from prepubertal cattle by intraovarian insulin-like growth factor-I application. *Biol Reprod*, 70:1634-1643.

Parent A-S, Teilmann G, Juul A, Skakkebaek NE, Toppari J, Bourguignon JP. 2003. The timing of normal puberty and the age limits of sexual precocity: Variations around the world, secular trends, and changes after migration. *Endocr Rev*, 24:668-693.

Parent A-S, Matagne V, Bourguignon JP. 2005. Control of puberty by excitatory amino acid neurotransmitters and its clinical implications. *Endocrine*, 28:281-285.

Parent A-S, Mungenast AE, Lomniczi A, Sandau US, Peles E, Bosch MA, Rønnekleiv OK, Ojeda SR. 2007. A contactin-receptor-like protein tyrosine phosphatase beta complex mediates adhesive communication between astroglial cells and gonadotrophin-releasing hormone neurones. *J Neuroendocrinol*, 19:847-859.

Parent A-S, Franssen D, Fudvoye J, Gérard A, Bourguignon J-P. 2015. Developmental variations in environmental influences including endocrine disruptors on pubertal timing and neuroendocrine control: revision of human observations and mechanistic insight from rodents. *Front Neuroendocrinol*, 38:12-36.

Parkash J, Kaur G. 2007. Potential of PSA-NCAM in neuron-glial plasticity in the adult hypothalamus: role of noradrenergic and GABAergic neurotransmitters. *Brain Res Bull*, 74:317-328.

Perez J, Tranque PA, Naftolin F, Garcia-Segura LM. 1990. Gap junctions in the hypothalamic arcuate neurons of ovariectomized and estradiol-treated rats. *Neurosci Lett*, 108:17-21.

Perry GA. 2016. Factors affecting puberty in replacement beef heifers. *Theriogenology*, 86:373-378

Pinet-Charvet C, Geller S, Desroziers E, Ottogalli M, Lomet D, Georgelin C, Tillet Y, Franceschini I, Vaudin P, Duittoz A. 2015. GnRH episodic secretion is altered by pharmacological blockade of gap junctions:

possible involvement of Glial cells. *Endocrinology*, 157:304-322.

Plant TM, Terasawa E, Witchel SF. 2015. Puberty in non-human primates and man. *In*: Plant TM, J. Zeleznik AJ (Ed.). *Knobil and Neill's Physiology of Reproduction.* 4th ed. New York, NY: Academis Press. pp. 1487-1536.

Polkowska J, Picard S, Wankowska M, Cieslak M, Caraty A, Tillet Y. 2014. Localization of kisspeptin neurons in the hypothalamus of peripubertal female lambs; possible connection with gonadotrophin releasing hormone and neuropeptide Y neurons. *J Anim Feed Sci*, 23:139-148.

Pralong FP. 2010. Insulin and NPY pathways and the control of GnRH function and puberty onset. *Mol Cell Endocrinol*, 324:82-86.

Prevot V, Cornea A, Mungenast A, Smiley G, Ojeda SR. 2003a. Activation of erbB-1 signaling in tanycytes of the median eminence stimulates transforming growth factor beta1 release via prostaglandin E2 production and induces cell plasticity. *J Neurosci*, 23:10622-10632.

Prevot V, Rio C, Cho GJ, Lomniczi A, Heger S, Neville CM, Rosenthal NA, Ojeda SR, Corfas G. 2003b. Normal female sexual development requires neuregulin-erbB receptor signaling in hypothalamic astrocytes. *J Neurosci*, 23:230-239.

Prevot V, Lomniczi A, Corfas G, Ojeda SR. 2005. erbB-1 and erbB-4 receptors act in concert to facilitate female sexual development and mature reproductive function. *Endocrinology*, 146:1465-1472.

P**revot V**. 2015. Puberty in mice and rats. *In*: Plant TM, J. Zeleznik AJ (Ed.). *Knobil and Neill's Physiology of Reproduction.* 4th ed. New York, NY: Academis Press. . pp. 1395-1439.

Rapisarda JJ, Bergman KS, Steiner RA, Foster DL. 1983. Response to estradiol inhibition of tonic luteinizing-hormone secretion decreases during the final stage of puberty in the rhesus-monkey. *Endocrinology*, 112:1172-1179.

Rius AG, Connor EE, Capuco AV,. Kendall PE, Auchtung-Montgomery TL, Dahl GE. 2005. Long-day photoperiod that enhances puberty does not limit body growth in holstein heifers. *J Dairy Sci*, 88:4356-4365.

Romar R, De Santis T, Papillier P, Perreau C, Thelie A, Dell'Aquila ME, Mermillod P, Dalbies-Tran R. 2011. Expression of maternal transcripts during bovine oocyte in vitro maturation is affected by donor age. *Reprod Domest Anim*, 46:e23-e30.

Rosales Nieto CA, Thompson AN, Macleay CA, Briegel JR, Hedger MP, Ferguson MB, Martin GB. 2014. Relationships among body composition, circulating concentrations of leptin and follistatin, and the onset of puberty and fertility in young female sheep. *Anim Reprod Sci*, 151:148-156.

Roy AK, Singh M, Kumar P, Kumar BSB. 2016. Effect of extended photoperiod during winter on growth and onset of puberty in Murrah buffalo heifers. *Vet World*, 9:216-221.

Sánchez-Garrido MA, Tena-Sempere M. 2013. Metabolic control of puberty: roles of leptin and kisspeptins. *Horm Behav*. 64:187-194.

Schefers JM, Weigel KA. 2012. Genomic selection in dairy cattle: integration of DNA testing into breeding programs. *Anim Front*, 2:4-9.

Schwanzel-Fukuda M, Pfaff DW. 1989. Origin of luteinizing hormone-releasing hormone neurons. *Nature*, 338:161-164.

Schwanzel-Fukuda M, Crossin KL, Pfaff DW, Bouloux, PM, Hardelin JP, Petit C. 1996. Migration of luteinizing hormone-releasing hormone (LHRH) neurons in early human embryos. *J Comp Neurol*, 366:547-557.

Seminara SB. 2005. Metastin and its G protein-coupled receptor, GPR54: Critical pathway modulating GnRH secretion. *Front Neuroendocrinol*, 26:131-138.

Settas N, Dacou-Voutetakis C, Karantza M, Kanaka-Gantenbein C, Chrousos GP, Voutetakis A. 2014. Central precocious puberty in a girl and early puberty in her brother caused by a novel mutation in the MKRN3 gene. *J Clin Endocrinol Metab*, 99:E647-51.

Sharif A, Baroncini M, Prevot V. 2013. Role of glia in the regulation of gonadotropin-releasing hormone neuronal activity and secretion. *Neuroendocrinology*, 98:1-15.

Silveira LFG, Trarbach EB, Latronico AC. 2010. Genetics basis for GnRH-dependent pubertal disorders in humans. *Mol Cell Endocrinol*, 324:30-38.

Simon D, Ba I, Mekhail N, Ecosse E, Paulsen A, Zenaty D, Houang M, Jesuran-Perelroizan M, De Filippo G, Salerno M, Simonin G, Reynaud R, Carel JC, Léger J, de Roux N. 2015. Mutations in the maternally imprinted gene MKRN3 are common in familial central precocious puberty. *Eur J Endocrinol*, 174:EJE-15-0488-8.

Sisk CL, Foster DL. 2004. The neural basis of puberty and adolescence. *Nat Neurosci*, 7:1040-1047.

Sklar CA, Mueller PL, Gluckman PD, Kaplan SL, Rudolph AM, Grumbach MM. 1981. Hormone ontogeny in the ovine fetus. VII. Circulating luteinizing hormone and follicle-stimulating hormone in mid- and late gestation. *Endocrinology*, 108:874-880.

Smyth C, Wilkinson M. 1994. A critical period for glutamate receptor-mediated induction of precocious puberty in female rats. *J Neuroendocrinol*, 6:275-284.

Suttie JM, Foster DL, Veenvliet BA, Manley TR, Corson ID. 1991. Influence of food-intake but independence of body-weight on puberty in female sheep. *J Reprod Fertil*, 92:33-39.

Sørensen K, Mouritsen A, Aksglaede L, Hagen CP, Mogensen SS, Juul A. 2012. Recent secular trends in pubertal timing: implications for evaluation and diagnosis of precocious puberty. *Horm Res Paediatr*, 77:137-145.

Tata B, Huijbregts L, Jacquier S, Csaba Z, Genin E, Meyer V, Leka S, Dupont J, Charles P, Chevenne D, Carel JC, Léger J, de Roux N. 2014. Haploinsufficiency of Dmxl2, encoding a synaptic protein, causes infertility associated with a loss of GnRH neurons in mouse. *PLoS Biol*, 12:e1001952.

Teles MG, Bianco SDC, Brito VN, Trarbach EB, Kuohung W, Xu S, Seminara SB, Mendonca BB, Kaiser UB, Latronico AC. 2008. A GPR54-activating mutation in a patient with central precocious puberty. *N Engl J Med*, 358:709-715.

Terasawa E. 2005. Role of GABA in the mechanism of the onset of puberty in non-human primates. *Int Rev Neurobiol*, 71:113-129.

Thompson IR, Kaiser UB. 2014. GnRH pulse frequency-dependent differential regulation of LH and FSH gene expression. *Mol Cell Endocrinol*, 385:28-35.

Tilbrook AJ, Turner AI, Clarke IJ. 2000. Effects of stress on reproduction in non-rodent mammals: the role of glucocorticoids and sex differences. *Reproduction*, 5:105-113.

Tillet Y, Caldani M, Batailler M. 1989. Anatomical relationships of monoaminergic and neuropeptide y-containing fibers with luteinizing-hormone-releasing hormone systems in the preoptic area of the sheep brain - immunohistochemical studies. *J Chem Neuroanat*, 2:319-326.

T**opaloglu AK, Reimann F, Guclu M, Yalin AS, Kotan LD, Porter KM, Serin A, Mungan NO, Cook JR, Ozbek MN, Imamoglu S, Akalin NS, Yuksel B, O'Rahilly S, Semple RK**. 2009. TAC3 and TACR3 mutations in familial hypogonadotropic hypogonadism reveal a key role for Neurokinin B in the central control of reproduction. *Nat Genet*, 41, 354-358.

Topaloglu AK. 2010. Neurokinin B signaling in puberty: human and animal studies. *Mol Cell Endocrinol*, 324:64-69.

Uenoyama Y, Tanaka A, Takase K, Yamada S, Pheng V, Inoue N, Maeda K-I, Tsukamura H. 2015. Central estrogen action sites involved in prepubertal restraint of pulsatile luteinizing hormone release in female rats. *J Reprod Dev*. 61:351-359.

Urbanski HF, Ojeda SR. 1990. A role for n-methyl-d-aspartate (nmda) receptors in the control of lh secretion and initiation of female puberty. *Endocrinology*, 126:1774-1776.

Vargas CA, Elzo MA, Chase CC, Chenoweth PJ, Olson TA. 1998. Estimation of genetic parameters for scrotal circumference, age at puberty in heifers, and hip height in Brahman cattle. *J Anim Sci*, 76:2536-2541.

Veneroni O, Cocilovo L, Müller EE, Cocchi D. 1990. Delay of puberty and impairment of growth in female rats given a non competitive antagonist of NMDA receptors. *Life Sci*, 47:1253-1260.

Viguie C, Jansen HT, Glass JD, Watanabe M, Billings HJ, Coolen L, Lehman MN, Karsch FJ. 2001. Potential for polysialylated form of neural cell adhesion molecule-mediated neuroplasticity within the gonadotropin-releasing hormone neurosecretory system of the ewe. *Endocrinology*, 142:1317-1324.

Walton JC, Weil ZM, Nelson RJ. 2011. Influence of photoperiod on hormones, behavior, and immune function. *Front Neuroendocrinol*, 32:303-319.

Wańkowska M, Polkowska J. 2009. Gonadotrophin-releasing hormone and GnRH-associated peptide neurobiology from the rearing period until puberty in the female sheep. *J Chem Neuroanat*, 38:9-19.

Watanabe G, Terasawa E. 1989. In vivo release of luteinizing hormone releasing hormone increases with puberty in the female rhesus monkey. *Endocrinology*, 125:92-99.

Winter JSD, Faiman C. 2009. Serum gonadotropin concentrations in agonadal children and adults. J *Clin Endocrinol Metab*, 35:561-564.

Wray S, Grant P, Gainer H. 1989. Evidence that cells expressing luteinizing hormone-releasing hormone mRNA in the mouse are derived from progenitor cells in the olfactory placode. *Proc Natl Acad Sci USA*, 86:8132-8136.

Wu FC, Butler GE, Kelnar CJ, Huhtaniemi I, Veldhuis JD. 1996. Ontogeny of pulsatile gonadotropin releasing hormone secretion from midchildhood, through puberty, to adulthood in the human male: a study using deconvolution analysis and an ultrasensitive immunofluorometric assay. *J Clin Endocrinol Metab*, 81:1798-1805.

Xu N, Kim H-G, Bhagavath B, Cho S-G, Lee JH, Ha K, Meliciani I, Wenzel W, Podolsky RH,. Chorich LP, Stackhouse KA, Grove AMH, Odom LN, Ozata M, Bick DP, Sherins RJ, Kim S-H, Cameron RS, Layman LC. 2011. Nasal embryonic LHRH factor (NELF) mutations in patients with normosmic hypogonadotropic hypogonadism and Kallmann syndrome. *Fertil Steril*, 95:1613-1620.e1-7.

Young J, Metay C, Bouligand J, Tou B, Francou B, Maione L. Tosca L, Sarfati J, Brioude F, Esteva B, Briand-Suleau A, Brisset S, Goossens M, Tachdjian G, Guiochon-Mantel A. 2012. SEMA3A deletion in a family with Kallmann syndrome validates the role of semaphorin 3A in human puberty and olfactory system development. *Hum Reprod*, 27:1460-1465.

Yu W, Dahl G, Werner R. 1994. The connexin43 gene is responsive to oestrogen. *Proc Biol Sci*, 255:125-132.

Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. 1994. Positional cloning of the mouse obese gene and its human homologue. *Nature*, 372:425-432.

Zhu H, Shah S, Shyh-Chang N, Shinoda G, Einhorn WS, Viswanathan SR, Takeuchi A, Grasemann C, Rinn JL, Lopez MF, Hirschhorn JN, Palmert MR, Daley GQ. 2010. Lin28a transgenic mice manifest size and puberty phenotypes identified in human genetic association studies. *Nat Genet*, 42:626-630.