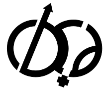

Luteinizing Hormone Receptor (LHR): basic concepts in cattle and other mammals. A review

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Abstract

Acquisition of the luteinizing hormone receptor (LHR) on granulosa cells of the dominant follicle is essential to physiological LH-mediated effects on the final stages of follicular growth, final maturation of the oocyte, ovulation and luteinization of the follicular wall. Therefore, LHR plays a key role in the final maturation of the dominant follicle from follicular dominance to ovulation. In this review, the basic molecular aspects of LHR (gene structure, alternative splicing and ligand mediated activation) and the physiological regulatory aspects (changes in LHR expression during antral follicle growth and a post-transcriptional model for downregulation of LHR transcripts) are addressed. Despite the accumulation of considerable amounts of information about LHR, a comprehensive and broad model for the role of LHR in bovine antral follicle growth is missing. Questions such as the function for alternative LHR transcripts, their transcriptional and translational regulation, and how the transcripts go to the cell surface and interact with ligands remain to be elucidated in bovine and other species.

Keywords: *Bos indicus*, *Bos taurus*, cattle, folliculogenesis, granulosa cells, LHR, luteinizing hormone receptor, ovulation.

Introduction

The development of ovarian follicles in the bovine species, from the appearance of the follicular antrum until the complete acquisition of ovulatory capacity, is essentially modulated by the action of gonadotropins (Mihm and Bleach, 2003), by ovarian steroid hormones (estradiol and progesterone), and by ovarian factors with endocrine and paracrine actions (Fortune, 1994; Gong *et al.*, 1996).

Some studies have indicated that the preantral follicular development is independent of acute gonadotropic stimulation, and paracrine control mechanisms play a primary role in this phase (Gong *et al.*, 1996). However, FSH receptors (FSHR) have been detected in primary follicles and FSH stimulates the growth of medium-sized preantral follicles *in vitro*, suggesting that FSH also acts to control the preantral stage (Gutierrez *et al.*, 2000; Webb *et al.*, 2003). Several intraovarian peptides (activin and inhibin), epidermal growth factor (EGF), fibroblast growth factor (FGF), stem cell factor (SCF or kit-ligand: KL), transforming growth factor family members (TGF-β), bone morphogenetic protein-15 (BMP-15 or GDF-9B), vascular endothelial growth factor (VEGF) and insulin-like growth factor 1 (IGF-1) have been identified as important regulators of follicular development in the preantral and antral stages (Monniaux *et al.*, 1997a, b; Berisha *et al.*, 2000; Mihm *et al.*, 2000; Juengel *et al.*, 2004).

Increased FSH plasma concentrations constitute the required stimulation for follicular recruitment and emergence of follicular waves (two or three per bovine estrous cycle; Adams *et al.*, 1992; Fortune, 1994). In the bovine species, only one follicle is selected and begins to exert dominance over the other follicles. Through the secretion of estradiol and inhibin, the growing follicle causes a reduction in circulating FSH levels, which are then insufficient to maintain the growth of the subordinate follicles (Ginther *et al.*, 1996). As LH interacts with LHR, it influences various activities such as steroidogenesis, follicular growth, oocyte maturation, ovulation, and corpus luteum formation, which are essential for reproductive function of the females (Hyttel *et al.*, 1997). Therefore, under physiologic conditions the appearance of LHR on granulosa cells is fundamental for folliculogenesis from the acquisition of follicular dominance until ovulation (Beg *et al.*, 2001; Ginther *et al.*, 2001; Sartori *et al.*, 2001; Barros *et al.*, 2010).

In this review, the following will be addressed: structural aspects and the activation of LHR, the occurrence of alternative splicing of LHR mRNA, the timing of LHR expression in the antral follicle cells, the participation of LHR in the acquisition and maintenance of follicular dominance, the modulation of alternative LHR transcripts, the biological activities of LHR isoforms, and the post-transcriptional model of LHR mRNA degradation through a downregulation mechanism.

Structure and activation of LHR

LHR is a protein that belongs to the guanine nucleotide coupled receptor superfamily (G proteins; Ascoli *et al.*, 2002). LHR contains an extracellular region (366 amino acids encoded by the first 10 exons

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of the gene), seven transmembrane helices, and a short intracellular region consisting of 72 amino acids (where the G protein is coupled to the carboxyl-terminal domain). The latter two regions are encoded by the long eleventh exon (Huhtaniemi, 2000). In the extracellular region, a sequence of amino acids near the first of the transmembrane helices appears to be essential for the activation of LHR by LH, but not the binding (Alvarez *et al.*, 1999). mRNA isoforms that do not contain exon 10 are translated into receptors with the ability to be activated by human chorionic gonadotropin (hCG), but not by LH (Gromoll *et al.*, 2003; Müller *et al.*, 2003). Galet and Ascoli (2005) observed distinct binding affinities depending on the origin of the ligand (bovine LH or human CG) and on origin of the receptor (human or mouse LHR). In this study, the differences in binding observed were related to the amino acid residues present in the extracellular domain of LHR, which differ among the studied species.

The trimeric G protein, coupled to the carboxyl-terminal in the intracellular region of LHR, is activated when the receptor undergoes a conformational change after binding of LH or hCG hormone (Lewin, 2004). The activation of LHR may trigger different pathways of cellular response (adenylate cyclase or phospholipase C) mediated by different G proteins in the activation of LHR. The Gi and/or Gq participate in the activation of phospholipase C while the Gs participates in the activation of adenylate cyclase (Herrlich *et al.*, 1996; Shemesh, 2001). The complexity of the activation process of the G protein coupled to LHR can be extended to protein kinase C. Although it has been suggested that LHR can activate protein kinases A and C (PKA and PKC), Salvador *et al.* (2002) demonstrated that in granulosa cells derived from pre-ovulatory follicles, the activation of LHR by hCG induced a cellular response (phosphorylation/activation of p42/44 MAPK) that was predominantly mediated by protein kinase A and independent of PKC.

In humans, LHR can have mutations that are either activating or inactivating. With activating mutations, even in the absence of circulating LH, chronic activation of the receptor occurs. This mutation is principally caused by localized changes in the third intracellular loop or in the sixth transmembrane segment of the LHR, resulting in constitutive activation of the receptor (Laue *et al.*, 1995; Zhang *et al.*, 1998). This mutation is the cause of the "familial precocious puberty in males" syndrome that occurs in humans. With inactivation mutations, even when LH is available there is no activation of LHR (Huhtaniemi, 2000) due to the absence of exon 10 in the LHR gene, which causes male hypogonadism. Treatment with hCG is effective in restoring the secondary sexual characteristics of patients with the syndrome, demonstrating the role of exon 10 in the discrimination between the actions of LH and hCG (Gromoll *et al.*, 2000).

LHR isoforms (alternative splicing)

The assembly of the primary transcript of an interrupted gene (pre-mRNA) by removing the genetic material that does not encode amino acids present in the mature protein (introns) and the alignment of the coding regions (exons) is required for the formation of mRNA. Gene transcription can generate mRNA containing all the exons of the gene (the complete or full-length form) or some forms of mRNA with total or partial deletions of one or more exons. Alternative splicing occurs when more than one sequence of mRNA is produced from the transcript of the same gene (transcripts or alternative isoforms; Lewin, 2004). According to Lareau *et al.* (2004), it is still not fully understood why some genes are more frequently transcribed with alternative splicing than other genes. Mammals probably use this process to amplify the synthesis of proteins, keeping the size of the genome at appropriate levels because a single gene could encode more than one functional protein.

Alternative splicing can participate in a self-control mechanism of gene expression (Lareau *et al.*, 2004) through the formation of microRNAs (miRNAs; Mattick, 2004; Chu and Rana, 2007). These miRNAs are part of intronic RNA or processed exonic RNA. These miRNAs can superimpose additional genetic instructions in a cell, modifying the production of protein at different levels. These introns encode shorter signals and may direct RNA molecules accurately to targets in other RNAs, DNA or proteins. Many of these miRNAs control processes such as stem cell maintenance, cell proliferation and apoptosis, thereby influencing the genetic programming of a cell in many ways (Mattick, 2004; Chu and Rana, 2007).

The study by Aatsinki *et al.* (1992), using ovaries from female rats, demonstrated the occurrence of four alternative full-length transcripts of the LHR gene; partial deletion of exon 9, deletion of exons 3 and 4, partial deletion of exon 11, and partial deletion of exon 11 and the total deletion of exon 5.

LHR isoforms have been described in sheep and cattle and not all appear to be functional. Investigation of the region between the ends of exons 9 and 11 revealed an alternative deletion of exon 10 and/or part of exon 11 (Abdennebi *et al.*, 2002; Robert *et al.*, 2003). Recently, the presence of four LHR transcripts was confirmed in follicular granulosa cells obtained from Nelore heifers (Ereno, 2008) and in follicular granulosa and theca cells of Nelore cows (Nogueira *et al.*, 2007a). These studies revealed the occurrence of an optional deletion of exon 3 (Nogueira *et al.*, 2007a; Fig. 1) in addition to the complete deletion of exon 10 and/or partial deletion of exon 11 of LHR (Nogueira *et al.*, 2007a; Ereno, 2008). In the literature, there is no information about the functional implication of the deletion of exon 3 in cattle and although Nogueira *et al.* (2007a) inferred a potential lower binding for LH

to that LHR protein, based on information from human and rat LHR exon 3, there is no hypothesized physiological role to that protein. In LHR transcripts in cattle, the deletion of exon 10 is implicated in the loss of affinity for LH, but not for hCG, whereas the partial deletion of exon 11 translates a truncated receptor without the transmembrane domain and without the G-protein coupled in the C terminal (Kawate *et al*., 2002).

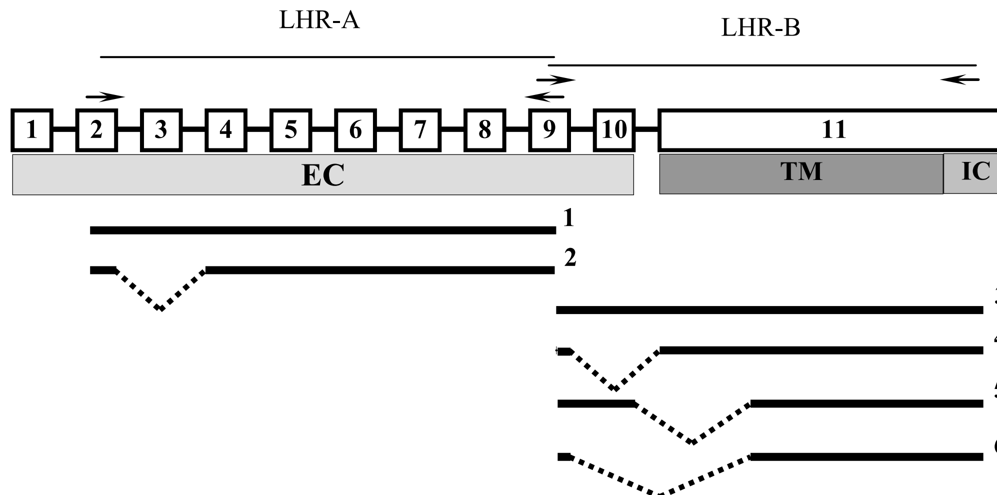

Figure 1. Position of PCR primers and alternative splicing of the bovine LH receptor gene in theca and granulosa cells. Exons are indicated by numbered boxes. The arrows indicate the priming sites used for amplification of fragment LHR-A and LHR-B. Specific protein domains are shown (EC, extracellular domain; TM, transmembrane region; IC, intracellular domain). The splice variants are represented by solid lines below the gene structure and are numbered on the right. Splice deletions are indicated by angled dotted lines (Adapted from Nogueira *et al*., 2007a).

Timing of LHR gene expression in the cells of the bovine antral follicle

It is postulated that ovarian follicle cells possess LHR mRNA in different stages of follicular development: from before the appearance of the *antrum* until the preovulatory phase in the theca cells (Bao and Garverick, 1998; Berisha *et al*., 2000; Braw-Tal and Roth, 2005) or only from follicular deviation through the preovulatory phase in the granulosa cells (Bao and Garverick, 1998; Beg *et al*., 2001; Ginther *et al*., 2001, 2003). Corroborating the results from Bao and Garverick (1998), Berisha *et al.* (2000) and Braw-Tal and Roth (2005), Nogueira *et al.* (2007a; Fig. 2) demonstrated that there was no significant difference in expression of LHR in the theca cells when compared with different classes of follicular diameter.

The expression of LHR in granulosa cells, studied by *in situ* hybridization, was shown to occur in follicles >8 mm in diameter, and the quantity of LHR mRNA increased with the follicle size and in steroidogenic follicles when compared with atretic follicles (Bao *et al.*, 1997). In a similar way, Evans *et al.* (2004), using quantitative real time PCR, verified increased LHR transcripts in granulosa cells of dominant follicles when compared to subordinate follicles. Bao and Garverick (1998) suggested that LHR expression increases with progressive follicular development, reaching a maximum when the dominant follicle is at its largest diameter.

Beg *et al.* (2001) detected LHR expression in granulosa cells of healthy follicles ≥8 mm in diameter 36 h after the onset of the follicular wave. LHR expression, measured by quantitative PCR, preceded by 0.5 mm the follicular diameter at the time of deviation in European breed heifers (±8 mm in diameter). Conversely, Fortune *et al.* (2001) did not detect expression of LHR by *in situ* hybridization in future dominant granulosa cells on days 1.5 and 2.5 of the first follicular wave. Recently, Nogueira *et al.* (2007a) and Ereno (2008), using semiquantitative RT-PCR, verified that the expression of LHR in follicular granulosa cells of follicles from Nelore cows (Nogueira et al., 2007a) or from Nelore heifers (Ereno, 2008) only occurs in follicles greater or equal to 7 mm in diameter although follicular diameter at morphological deviation in Nelore breed heifers was described as 6 mm (Sartorelli *et al.*, 2005) and 5.3 mm (Barros *et al.*, 2009). In the study by Ereno (2008), the minimum follicular diameter in which LHR expression was detected (≥7 mm) corresponded from 2.5 to 3 days after the determination of ovulation. Nogueira et al. (2007a) verified that only one sample (16.7%, 1/6) of follicles with a diameter of 7 mm and more than 87.5% of follicles with diameter ≥8 mm expressed LHR. Expression rates of LHR were similarly described by Ereno (2008), with 25% (2/8) in follicles with a diameter of 7 mm and 70% (7/10) in follicles with a diameter ≥8 mm.

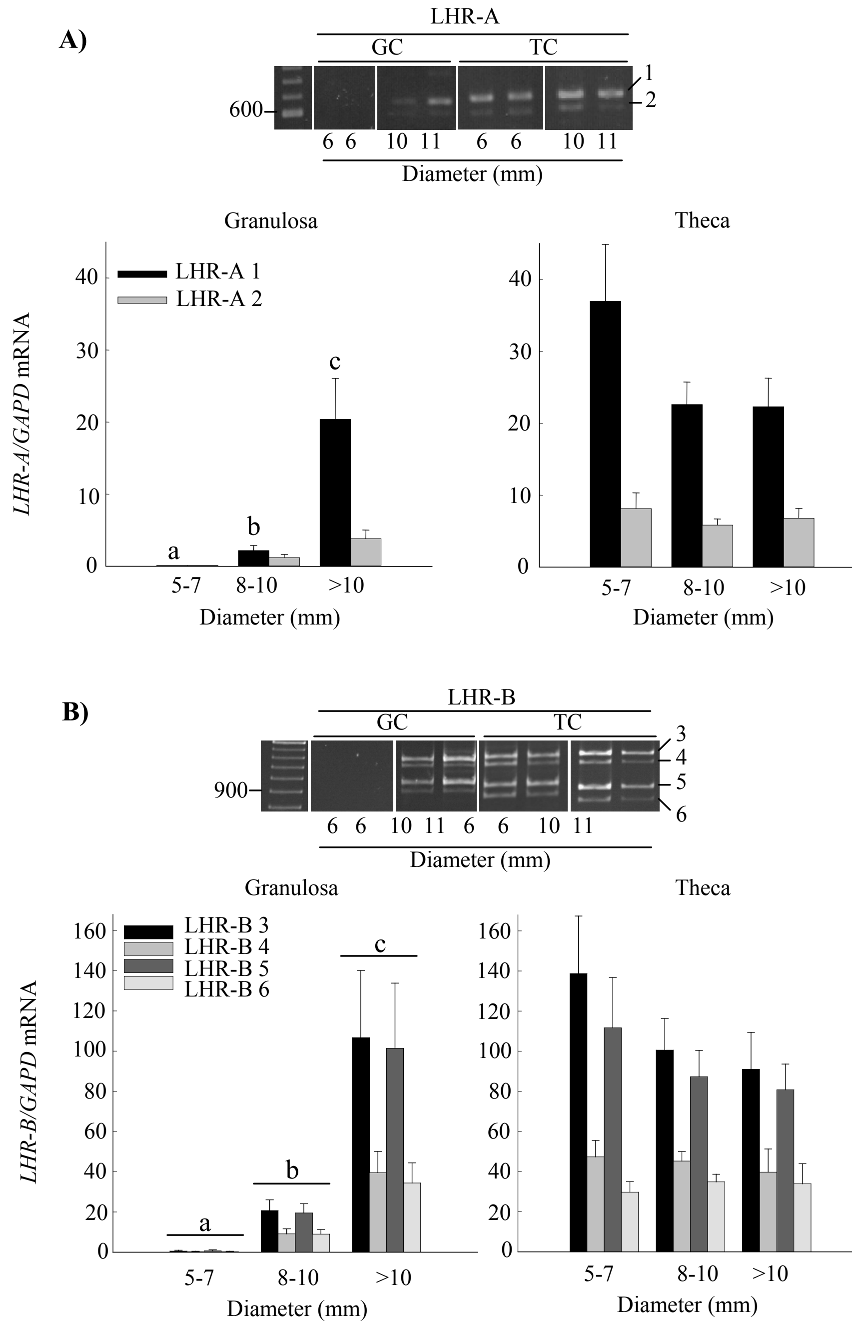

Figure 2. Developmental regulation of LHR expression in bovine antral follicles. Means (± SEM) relative abundance of LHR-A (A) and LHR-B (B) splice variants in granulosa and theca cells from non atretic follicles grouped according to follicle diameter (n = 10, 11, and 8 for the 5–7 mm, 8–10 mm, and >10 mm groups, respectively). Splice variants are numbered as depicted in Fig. 1. For each splice variant, means with different letters are significantly different ($P < 0.05$). The gels show LHR-A and LHR-B PCR products in granulosa (GC) and in theca cells (TC) of four representative follicles of the given diameters. Splice variants are numbered to right of the gel (Adapetd from Nogueira *et al*., 2007a).

Expression of LHR and the final maturation of the antral follicle

In the follicular wave of mono-ovulatory species, there is follicular selection either due to the advantage of a larger diameter in the initial emergence of the wave or from the later change in diameter between the largest follicles, which continue to grow (dominant) while others (subordinate) undergo atresia. In cattle, the IGF system, estradiol, and the LH receptors are involved in intrafollicular events that initiate this divergence (Ginther *et al*., 2001). Beg *et al.*

(2001) and Ginther *et al*. (2001) demonstrated that LHR expression (*i.e.*, mRNA) in bovine follicular granulosa cells (Holstein heifers) occurs on average 8 h before the follicular morphological deviation, which led the authors to suggest that the early expression of LHR is the cause for the deviation and consequently for the acquisition of follicular dominance.

Nonetheless, Fortune *et al*. (2001) suggested that acquisition of LHR by granulosa cells is not a key component of follicular selection, since it appears to occur after, rather than before, selection has occurred. Fortune *et al*. (2001), using *in situ* hybridization, and later Ereno (2008), using RT-PCR, did not detect LHR expression in the presumptive future dominant follicle immediately before the follicular morphological selection. The first difference observed by Rivera and Fortune (2003) between the future dominant follicle and the subordinates was an increased synthesis of the IGFBP-4 and 5 (IGF binding protein) protease (later called PAPP-A; Rivera *et al*., 2001) induced by FSH with subsequent decreases in the IGFBP-4 and 5 concentration by proteolytic degradation that occurred from recruitment until the preovulatory size (Mazerbourg *et al*., 2001; Rivera and Fortune, 2001; Rivera *et al*., 2001; Fortune *et al*., 2004). The low IGFBP-4 and 5 concentrations permit large quantities of IGF-1 to be biologically available to promote follicular growth, amplifying the effects of FSH and the estradiol synthesis (Fortune *et al*., 2001). Similarly, Mihm *et al.* (2000) and Austin *et al*. (2001) proposed that the primary intrafollicular change that distinguishes the follicle destined to become dominant from the other growing follicles is an increased capacity to produce estradiol and maintain low levels of IGFBPs. Therefore, in this biochemical model of acquiring dominance proposed by Fortune *et al*. (2004), the IGF system plays a critical role in the follicle becoming dominant.

The free IGF-1 concentration is greater in the follicular fluid of the largest follicle compared to the second largest in the same wave, even before the observed differences in the estradiol concentration or diameter (Beg *et al*., 2002). This finding suggests the existence of a system modulator of intrafollicular IGF availability that is differently regulated in dominant and subordinate follicles.

Despite the fact that LHR expression in granulosa cells is initiated in bovine follicles that are 8-8.4 mm in diameter (Ginther *et al*., 2001), the absence of pulsatile LH does not prevent the follicles from growing to a diameter of 9 mm (Ginther *et al*., 2003; Hampton *et al*., 2004). Therefore, these authors suggested that LHR expression may not be essential at this time for follicular growth.

Table 1. Summary of the differences between the published studies regarding the expression of the LHR gene in cattle and sheep species.

Study	Technique used	Amplicons produced	Follicular cells studied	Follicle at a known stage of the follicular wave?	∅ Minimum GC	Breed (species)
Xu *et al.*, 1995	ISH	NA	TC/GC	Yes	>9 mm	Cross-bred (ND)
Evans and Fortune, 1997	ISH	NA	TC/GC	Yes	>9.7 mm	Holstein (Bt)
Bao and Garverick, 1998	ISH	NA	TC/GC	Yes	≥7.8 mm	Holstein (Bt)
Soumano *et al.*, 1998	ISH	1	Follicle	Yes (FSH)	ND	Holstein cross (Bt)
Bacich *et al.*, 1999	QL	4	Follicle	Yes	ND	Merino (Oa)
Beg *et al.*, 2001	QT	1	GC	No	≥7 mm	Holstein (Bt)
Abdennebi *et al.*, 2002	SQ	3	TC/GC	Yes	≥3.5 mm	ND (Oa)
Robert *et al.*, 2003	QL	≥ 6	TC/GC	Yes (FSH)	<4 mm	Holstein (Bt)
Calder *et al.*, 2003	SQ	1	GC (COC)	No	NA	ND (ND)
Nogueira *et al.*, 2007a	SQ	≥ 4	TC/GC	No	≥7 mm	Nelore (Bi)
Ereno, 2008	SQ	4	GC	Yes	≥7 mm	Nelore PO (Bi)
Fernandes, 2008	SQ	4	GC	Yes (FSH)	≥8.5 mm	Nelore (Bi)
Simões, 2009	SQ	4	TC/GC	No	≥8.2 mm	Nelore (Bi)

Qualitative PCR (QL), semi-quantitative (SQ) or quantitative (QT), *in situ* hybridization (ISH); theca cells (TC) and granulosa cells (GC); ovarian stimulation with FSH (FSH); minimum diameter in which there was detected LHR transcripts in granulosa cells (∅ minimum GC); *Bos taurus* (Bt), *Bos indicus* (Bi) and *Ovis aries* (Oa). NA (not applicable) and ND (not described).

The diameter of follicular morphological deviation was determined to be approximately 6 mm in Nelore breed heifers, as shown by Sartorelli *et al*. (2005). Afterwards, Ereno (2008) and Barros *et al*. (2009) verified that the follicular deviation in Nelore breed heifers occurs when the follicular diameter is 5.3 mm and 4.9 mm for the dominant and subordinant follicles, respectively. These data are similar to those described by Castilho *et al*. (2007) and Gimenes *et al*. (2008) in Zebu females. The data presented by Sartorelli *et al*. (2005), Castilho *et al*. (2007), Ereno (2008) and Gimenes *et al*. (2008) combined with the data published by Nogueira *et al*. (2007a) demonstrate that LHR expression in Nelore occurs only after follicular deviation, which differs from the studies by Beg *et al*. (2001) and Ginther *et al.* (2003) for Holsteins (*Bos taurus*). Thus, it can be inferred that LHR expression in follicular granulosa cells ≥7 mm in diameter occurs later than the initiation of follicular deviation in Zebu females (*Bos indicus*).

Therefore, there are discrepancies among the published studies in regards to LHR expression depending on the species, breed, technique sensitivity, quantity of produced amplicons (meaning dependence on the location of primer annealing with LHR cDNA) and the minimum follicular diameter at which LHR expression was detected in the granulosa cells (Table 1).

LHR expression and acquisition of ovulatory capacity by the follicle

After the administration of 40 mg of LH, no Holstein breed cow with 7 mm follicles (0/9) or 8.5 mm follicles (0/9) ovulated compared with 80% (8/10) ovulation in cows with 10 mm follicles. Therefore, the ovulatory capacity in *Bos taurus* was verified in follicles of at least 10 mm in diameter (one day after follicular deviation, Sartori *et al*., 2001). These investigators inferred that the acquisition of ovulatory capacity may require increased LHR expression in granulosa cells of the dominant follicle, and this change could be important for the growth of the dominant follicle after selection. Gimenes *et al*. (2008) completed an experiment with Nelore, Gir and crossbred (Nelore x Gir) heifers in order to verify the diameter at which follicles acquired ovulatory capacity. In females treated with 25 mg of LH when the dominant follicle reached a diameter between 7.0 mm and 8.4 mm, 8.5 mm and 10.0 mm or >10.0 mm, the ovulatory rates were 33, 80 and 90%, respectively.

Recently, Simões (2009) verified the relationship between follicular diameter, ovulation rate and the expression of the isoforms of the LH receptor known as B3 (complete isoform), B4 (with total deletion of exon 10), B5 (with a partial deletion of exon 11) and B6 (combined deletions of the B4 and B5 isoforms) in Nelore cows. It was observed that with the increase in follicular diameter (follicles with 7.0 to 8.0, 8.1 to 9.0 and 9.1 to 10.0 mm) there was an increase in the ovulation rate (9, 36 and 90%, respectively; $P < 0.05$) and the expression of the sum of LHR isoforms in the granulosa cells (16.5, 21.0 and 37.6 LHR mRNA/GAPDH mRNA, respectively; $P < 0.05$). Additionally, there was a positive correlation between the increased expression of the LHR-B5 ($r = 0.54$; $P = 0.056$) and the LHR-B6 isoforms ($r = 0.63$; $P = 0.02$) with increased follicular diameter. However, in the theca cells, the expression profile was not altered ($P > 0.9$). The author concluded that in zebu females, the ovulatory capacity is related with increased follicular diameter and consequently the increased expression of the sum of the LHR isoforms in granulosa cells.

These data suggest that the ovulatory capacity in *Bos indicus* is acquired with smaller diameters when compared to what was observed in *Bos taurus.*

LHR expression under ovarian superstimulatory treatment

Soumano *et al*. (1998) evaluated the expression of the receptors for FSH (FSHR) and LH in follicles of Holstein heifers that had undergone superstimulation with eCG (n = 10, Folligon®, 2,500 UI on day 9 of the estrous cycle and PGF2α on day 12) or FSH (n = 10, FolltropinV®, 225 mg of FSH between day 9 and day 12 and PGF2α on day 12). These authors verified that the use of eCG in ovarian superstimulation of the heifers resulted in decreased follicular abundance of the LHR and FSHR transcripts when compared with the use of FSH; however, eCG did not affect the abundance of luteal LHR mRNA. The authors hypothesized that eCG could have induced a downregulation of the FSHR and LHR expression in the follicular cells because commercial preparations of eCG have greater LH bioactivity when compared to FolltropinV®.

Fernandes (2008) verified in Nelore cattle that the expression of the B3 and B5 isoforms in the granulosa cells obtained from larger follicles (10 mm diameter) is more intense than the B4 and B6 isoforms in both cycling heifers and in cows that had undergone ovarian superstimulation with Folltropin® (modified protocol P-36, based on Nogueira *et al*., 2007b). In granulosa cells obtained from follicles ≥8.5 mm in diameter, there were lower LHR transcripts in cows that were superstimulated with Folltropin® when compared with non treated heifers. The detection of B3 and B5 isoform expression was shown to be 100% in the samples from both groups, while the B4 and B6 isoforms differed among the cycling heifers (100% expression) and the superstimulated cows (30 and 25%, respectively). These results indicate that the superstimulation treatment with Folltropin®, associated with the time of follicle collection (*i.e.*, 36 h after removal of the progesterone source), decreases the expression of LHR in the granulosa cells. However, in this experiment, the follicles were obtained 36 h after

the final superstimulation treatment, a period in which the preovulatory surge of endogenous LH may have occurred. The occurrence of the endogenous LH surge may lead to a down regulation of LHR expression, which would explain the lower LHR expression in the granulosa cells of superstimulated cows when compared to the non treated cows. New experiments are currently ongoing to clarify this question.

Modulation of LHR and its transcripts

In sheep, the modulation of LHR expression is directly related to the physiologic condition of the ovarian activity during the season of the year, indicating that the study of alternative LHR transcripts may be partially related to the reproductive physiology of the ewes (Abdennebi *et al*., 2002). Abdennebi *et al*. (2002) reported prevalence in the expression of the full-length form of LHR in theca cells of the antral ovine follicle during the reproductive season. Nevertheless, during the seasonal anestrus, the alternative LHR transcript called 3 was demonstrated to be the most expressed transcript. Therefore, this modulation of LHR expression in theca cells may be partially associated with the regulation of the seasonal anestrus in sheep. However, it is interesting to note that the predominance of this alternative isoform (with the deletion of exon 10 and part of exon 11) does not impede the follicles from reaching preovulatory diameters, although without ovulatory capacity. Thus, it appears that in theca cells from sheep it is not necessary that a majority of the mRNA isoforms be the complete isoform for a dominant follicle to be selected although be necessary to the acquisition of ovulatory competence.

The modulation of LHR transcripts has not been observed during the sheep estrous cycle (Bacich *et al*., 1999). In this study, there was a predominance of the LHR transcript with partial exon 11 deletion in the follicles and in the corpus luteum. Additionally, the *in vivo* translation of this transcript was demonstrated by detection of the protein corresponding to the mRNA. Thus, Bacich *et al*. (1999) suggested that the abundance of this LHR alternative transcript, concomitant with its translation by the cells, may represent part of the mechanism by which LH regulates ovarian function. Similar to the results observed in sheep, Manikkam *et al*. (2001) verified that there is no difference in the LHR expression in the bovine theca and granulosa cells between the first and second follicular wave.

The expression of LHR in bovine granulosa cells is greater in follicles with estradiol concentrations in the follicular fluid >20 ng/ml compared to the follicles with lower estradiol concentrations (Berisha *et al*., 2000). Conversely, Nogueira *et al*. (2007a) verified that LHR expression in granulosa cells samples from Nelore cattle is not affected by the follicular concentrations of estradiol or progesterone in follicles of different diameters. It can be inferred that the differences between the utilized techniques, the animal breeds and the timing (of the follicular wave) of follicle collection may explain these discrepancies (as summarized in Table 1).

The inducer (FSH or estradiol) or inducers (FSH, estradiol and other factors) of the physiological pattern of LHR mRNA expression is a controversial point. The *in vitro* modulation of LHR expression in granulosa cells was demonstrated in cattle (Nogueira *et al*., 2007a). Treatment of the granulosa cells, that originated from ovaries obtained from slaughterhouse and derived from *Bos taurus* animals treated with FSH (1 or 10 ng/ml in the culture medium) induced an increase in abundance of the four principal LHR transcripts (Fig. 3). According to other authors (Shi and Segaloff, 1995; Hampton *et al*., 2004), the cause of induced LHR expression is FSH itself and not estradiol. Treatment with only exogenous estradiol does not induce an increase in LHR transcripts in rat granulosa cells, which is in contrast to the FSH treatment that increases the rate of LHR gene transcription (Shi and Segaloff, 1995). In granulosa cells derived from bovine follicles >10 mm, Hampton *et al*. (2004) did not observe differences in the abundance of LHR transcripts between the animals treated with FSH or FSH and LH (previously treated with a GnRH agonist). There was a difference between the intrafollicular concentrations of estradiol in the FSH group (259.0 ng/ml) and the FSH and LH group (790.7 ng/ml), which suggested that, at least for follicles with preovulatory diameters, estradiol is not an inducer of LHR gene expression. In bovine follicles with less than preovulatory diameters, estradiol may be a primary inducer of the basal expression of LHR transcripts (Nogueira *et al*., 2007a), although its function as a stabilizer of already transcribed LHR mRNA (Shi and Segaloff, 1995) has been suggested to explain the increased transcriptional abundance without a concomitant increase in transcription. Despite the fact that FSH induced LHR expression *in vitro* (Nogueira *et al*., 2007a), the relative abundance of LHR isoforms - in comparison to each other - was undoubtedly different from the physiologic pattern (*i.e.*, granulosa cells obtained *in vivo* and named as “>8 mm” in Fig. 3). We could conclude that FSH is an inducer of the *in vitro* LHR expression (mRNA), but other factors should be involved in the physiologic expression of LHR.

This modulation can also occur in the receptor-ligand complex mobility. Apparently, the ligand type (LH, hCG or deglycosylated hCG) or the receptor (functional or non-functional) induces structural differences among the formed hormone-receptor complexes (Roess *et al*., 2000a). Horvat *et al.* (1999) coupled a fluorescent protein (GFP) to the rat LHR and demonstrated that the non-occupied LHRs remained distributed at the plasma membrane in a disperse manner with lateral diffusion while the LHRs bound to LH or hCG were grouped with restricted lateral diffusion.

The desensitization to the agonist that is observed with LHR in cultivated granulosa cells or

follicular cells after the preovulatory LH surge appears to be mediated by auto-associations between two or more LHRs, the β-arrestin protein and regions of the cytoplasmic membrane known as rafts (Roess et al., 2000b; Hunzicker-Dunn et al., 2003; Roess and Smith, 2003). In these studies, it was demonstrated that the pattern of LHR grouping in the cytoplasmic membrane (size and lateral mobility) differs based on the ligand (LH or hCG) and the functional state (desensitized or functional).

Evans *et al*. (2004) verified that in granulosa cells the aromatase and abundance of LHR transcripts were positively and significantly correlated with dominant follicles when compared to its subordinates. The fact that the authors did not detect differences between the dominant and subordinate follicles in the expression of FSH receptor in granulosa cells, or LHR in theca cells, supports the hypothesis of dominant follicle growth through the utilization of circulating LH by activation of LHR on its granulosa cells (as proposed by Ginther *et al*., 2001). However, Hampton *et al*. (2004) did not observe a necessity of LH to promote growth of bovine dominant follicles although that study used a non-physiological model of FSH administration. That model of pharmacological FSH administration could account for the finding of dominant follicle growth without LH and that LH promotes the increasing of mRNA for 17-α hydroxylase (theca cells) but not for aromatase mRNA (granulosa cells).

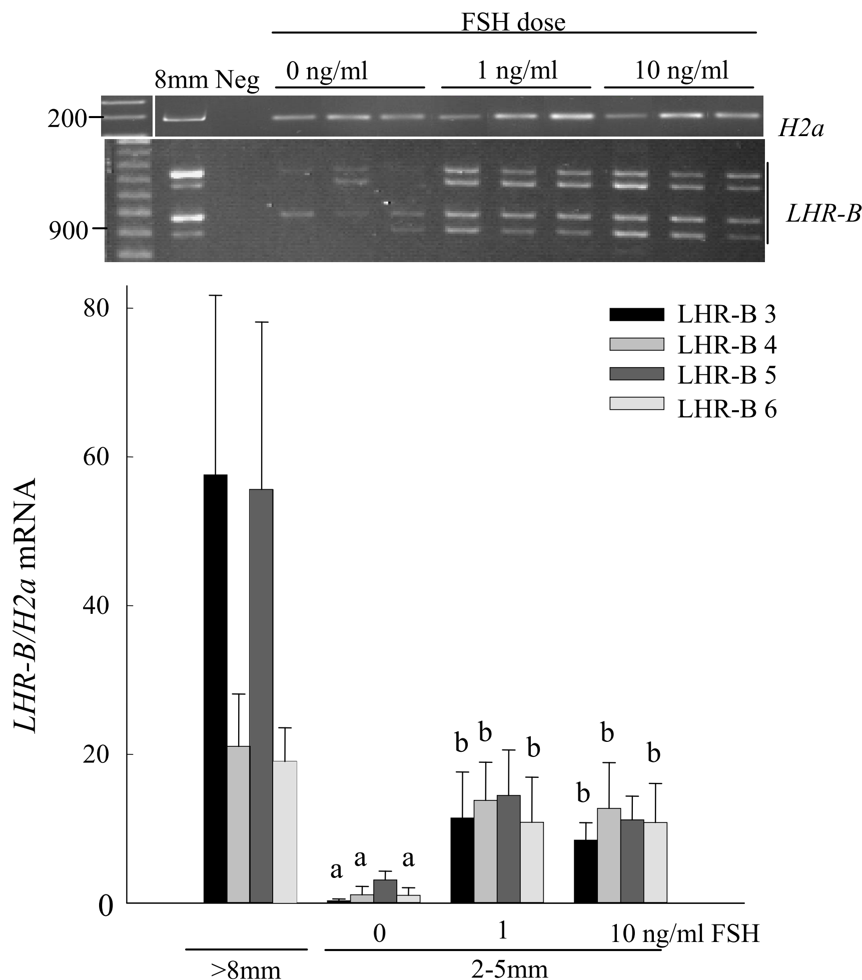

Figure 3. Regulation of LHR-B expression in granulosa cells by FSH in vitro. Granulosa cells from small (2-5 mm) follicles were cultured for 6 days in serum-free medium with the stated doses of FSH, and mean (± SEM) LHR-B mRNA levels are expressed relative to H2a (internal control). Data are derived from three independent experiments, and means with different letters are significantly different ($P < 0.05$). The data are plotted alongside PCR results from granulosa cells freshly recovered from large follicles (>8 mm, which represent a subpopulation of the 8-10 mm follicles presented in Fig. 2) for comparison. The gel shows H2a and LHR-B splice variants for the three replicates of cultured cells and positive (8 mm; fresh granulosa cells from 8-mm follicles) and negative (Neg; water) PCR controls (Adapted from Nogueira *et al*., 2007a).

Cumulus-oocyte complexes (COCs) aspirated from superstimulated Holstein cows were subjected to the procedure of *in vitro* embryo production to evaluate whether the presence of LHR in the granulosa cells (identified by RT-PCR) correlates with the potential of embryonic development in the oocyte. Despite the fact that expression of LHR does not serve as a marker to predict bovine oocyte competency in reaching the blastocyst phase, there was a greater proportion of oocytes that reached the blastocyst phase in the follicles that expressed LHR in the granulosa cells (Robert *et al*., 2003). Calder *et al*. (2003, 2005) demonstrated in cattle a positive correlation between the COC quality, the oocyte competency to support fertilization and early cleavage and the expression of LHR in the *cumulus oophorus*. The authors suggested that the LHR transcripts may be a subset of marker genes for oocyte maturation *in vitro*.

Biological activity of the LHR isoforms

In cattle, Kawate *et al*. (2002) demonstrated that there is transport and positioning of the LHR isoform with exon 10 deletion at the cellular surface. In humans, the deletion of exon 10 results in decreased affinity to LH in comparison to hCG (Gromoll *et al*., 2000; Müller *et al*., 2003; Fig. 4). In cattle, the alternative transcript with exon 11 deletion is translated as a truncated receptor by the introduction of a premature stop codon (Robert *et al*., 2003; Kawate, 2004). This truncated LHR is not transported to the cellular surface and is maintained in the cytoplasm even though it retains its capacity to bind (Fig. 4). In this condition, LH does not activate the G protein and this transcript would therefore be non-functional (Kawate *et al*., 2002; Kawate, 2004). However, the hypothesis of cooperation of dimers, trimers, or receptor oligomers in obtaining functional G protein coupled receptors (mediating the transport and positioning of the complete isoform or the control of the ligands bioavailability) could include a function for truncated isoforms (Roess *et al*., 2000a; Pierce *et al*., 2002; Robert *et al*., 2003; Nakamura *et al*., 2004).

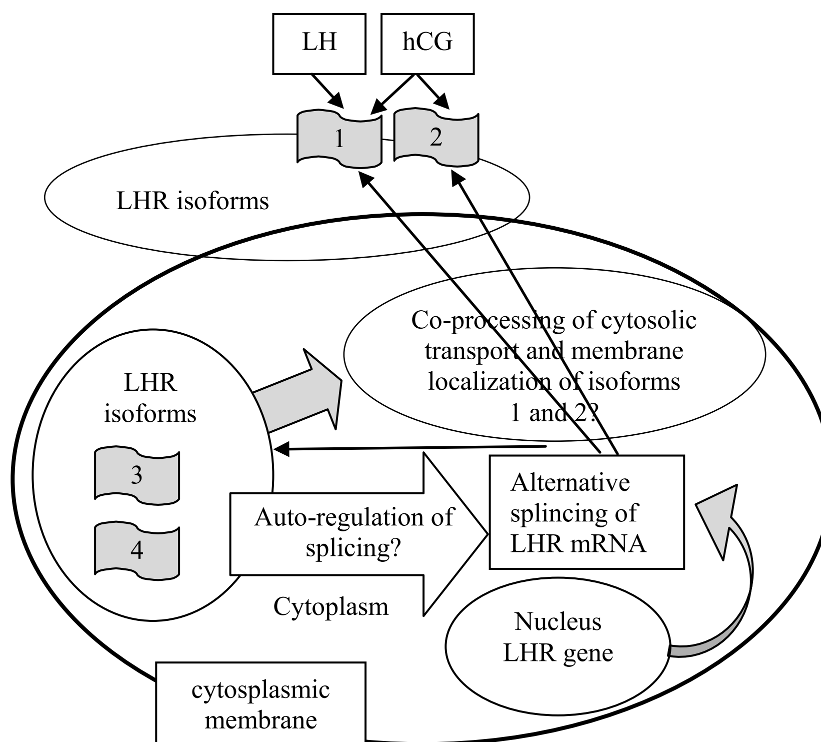

Figure 4. Schematic representation of possible LHR control mechanisms in an ovarian follicular cell. After LHR gene transcription to a pre-RNA, alternative splicing occurs to produce at least four mRNA isoforms. Two of these isoforms (1 and 2) can be translated into proteins capable of binding with a LH molecule (1) or hCG (1 and 2) and retain the ability to activate the G protein and the cellular response. Conversely, LHR mRNA isoforms 3 and 4 would be translated into truncated receptors (without the intracellular region) that are retained in an atypical location in the cytoplasm. In cattle it was inferred that these two isoforms (3 and 4) would be involved in transport to the cell surface of the so-called functional LHR isoforms (*i.e.*, 1 and 2) as well as being involved in the self-regulatory mechanism of splicing that could generate the four isoforms of LHR mRNA. (Adapted from Nogueira, 2005).

The biological variation in LHR expression includes a group of primates (*Platyrrhini*) in which exon 10 is constitutively lost during transcription and although there are no full length isoforms present, animals are reproductively normal. The evolution of this monkey produced a unique system in mammals in which a hormone structurally similar to hCG is produced in the pituitary gland instead of LH. This gonadotropin is responsible for the activation of the LH receptor in this subclass of animals (LHR type II, Gromoll *et al*., 2003).

Nogueira *et al*. (2005) utilized two ligands (LH and hCG) of high affinity and specificity in an attempt to promote the activation of LHR isoforms. When superstimulated donor cows were induced to ovulate with LH (control) or LH and hCG (treated), no significant difference was observed in the total production of structures or viable embryos as well as the rates of embryonic viability and conception after embryo transfer (Nogueira *et al.*, 2005).

Downregulation of LHR

The downregulation of the complete LHR mRNA isoform can be regulated by alternative splicing because the alternative transcripts of LHR promote reduced expression of the complete form (Roess *et al*., 2000a; Abdennebi *et al*., 2002; Licht *et al*., 2003).

Menon *et al*. (2004) reported that a protein which binds to LHR mRNA (LRBP) is synthesized in the cytoplasm of rat ovarian cells in response to the preovulatory surge of LH or the administration of therapeutic doses of hCG. In rats, the binding of LRBP to the coding region of mRNA inhibits the translation of LHR (Nair and Menon, 2005).

In rats, after the binding of LH or hCG to LHR, there is an increase in intracellular cAMP, which activates steroidogenesis and promotes the depletion of intracellular cholesterol. This is followed by increased transcription of genes associated with the synthesis and transport of cholesterol from the plasma to the interior of the cell (Menon *et al*., 2006) until the intracellular cholesterol is in excess and there is regulation of steroidogenesis. Mevalonate kinase (MVK), an enzyme that previously was described in rats as LRBP by Menon *et al.* (2004) and by Nair and Menon (2005), participates in cholesterol biosynthesis. MVK binds to LHR mRNA and accelerates its degradation by promoting its downregulation (Menon *et al.*, 2006, Wang *et al.*, 2007). The expression of MVK mRNA and protein is induced after treatment with hCG. According to this model, this downregulation is performed more by the rapid degradation of LHR mRNA than by inhibiting translation of the mRNA into LHR. In humans, the experimental results of Wang *et al.* (2007) indicate that there is direct participation of MVK in the regulation of LHR expression, and therefore the authors proposed a new hypothesis that involves the relationship between cholesterol metabolism and LHR expression in the ovary.

Final considerations

Although several aspects related to LHR have been addressed in this review, many others should be elucidated in the near future. The biological role of the various alternative LHR mRNA isoforms still needs to be understood both at the level of transcriptional and/or translational modulation as well as transport of the full-length receptor isoform. The hypotheses of whether the ovulatory mechanism is due to the increased number of LHR on granulosa cells (quantitative model), due to synergism between two or more intracellular signaling pathways (qualitative model) or due to a hybrid model should be tested in the future.

When new knowledge permits better comprehension of the biological role of LHR, it may suggest uses of LHR transcripts as efficient markers of follicular dominance, the establishment of ovulatory competence and follicular apoptosis. Similarly, modulation of these transcripts (by way of knock-out, knock-in or knock-down models) may optimize animal breeding and maximize the action of pharmacological inducers of ovulation and the final development of the follicle.

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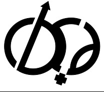

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