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Review

The role of relaxin in mare reproductive physiology: A comparative review with other species

Claudia Klein*

Faculty of Veterinary Medicine, University of Calgary, Calgary, Alberta, Canada

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A B S T R A C T

Relaxin is a peptide hormone best known for its action during the latter half of pregnancy, in particular for its softening effect on pelvic ligaments that aids in preparation of the birth canal for the impending delivery of the fetus. The source of relaxin during early pregnancy varies across species, with the CL being the main source in a number of species. The main source of relaxin during late equine pregnancy is the placenta. In mares with impaired placental function, circulating relaxin levels decline before abortion. During early pregnancy, relaxin promotes endometrial angiogenesis through upregulating endometrial expression of vascular endothelial growth factor. The horse is unique in that the equine conceptus expresses relaxin messenger RNA as early as 8 days after ovulation, with levels increasing as conceptus development proceeds. Although secretion of functional relaxin has not been verified, it is likely, given that the embryo also expresses transcripts coding for enzymes processing the prohormone to yield the mature hormone. Furin, an enzyme which belongs to the subtilisin-like proprotein convertase family known to process preprorelaxin, appears to be the foremost convertase expressed by equine conceptuses. Conceptus-derived relaxin could drive endometrial angiogenesis and also act in an autocrine fashion to promote the embryo's own development. Relaxin is also expressed by ovarian structures during the nonpregnant estrous cycle. In the mare, follicular expression of relaxin is comparable among follicles of varying size and has been localized to granulosa and theca cells. In women and pigs, relaxin appears to promote follicular development. In the rat, multiple lines of evidence indicate that relaxin is involved in the ovulatory process. In the mare, relaxin might play a similar role in the ovulatory process, as in equine ovarian stromal cells relaxin promotes the secretion of gelatinases and tissue inhibitors of metalloproteinases; local proteolysis of the follicular wall is integral to the ovulatory process. However, functional studies addressing the role of relaxin in the ovulatory process are missing in the mare.

1. Introduction

Relaxin is best known for its action during the latter half of pregnancy, in particular for its softening effect on pelvic ligaments that aid in preparation of the birth canal for the impending delivery of the fetus. It was this effect that Frederick Hisaw discovered when he injected the serum of pregnant guinea pigs and rabbits into virgin guinea pigs and observed the relaxation of the pubic symphysis [1]. The unknown hormone was subsequently named "relaxin" [2]. Relaxin belongs to the insulin superfamily, which consists of a number peptides marked by a structural similarity to insulin, i.e., being composed of two peptide chains connected through disulfide bridges. The number of relaxin peptides encoded in the genome differs among species; in humans and higher primates, three relaxin genes are present, *RLN1*, *RLN2*, and *RLN3*, whereas in other mammals, including the horse, only two genes are present, *RLN1* and *RLN3*. *RLN2* of

* Corresponding author. Tel. +1 (403) 210-7233; fax: +1(403) 210-3929.
E-mail address: cklei@ucalgary.ca

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higher primates is the functional ortholog of *RLN1* in other species. Cattle are unique in that they lack a *RLN1* gene [3]. Relaxin 1 (relaxin 2 in primates) is expressed by reproductive tissues and can be detected in the systemic circulation, whereas relaxin 3 is predominantly expressed in brain tissue and is unlikely to be a circulating hormone. The function of the *RNL1*, the gene not orthologous to relaxins in other mammals, in humans, and higher primates is unknown. The remaining members of the insulin superfamily are the insulin-like (INSL) peptides, INSL3, INSL4, INSL5, and INSL6, of which only INSL3 has a well described function in reproduction [4]. Sequence homology of relaxin is quite low among domestic animals, yet its function is conserved, as the receptor-binding region of the B-chain is highly conserved among species [5]. Relaxin peptides are synthesized as preprohormones, amino acid chains consisting of a signal peptide (which directs the protein to the endoplasmic reticulum) followed by a B-, C- (connecting), and A-domain in that order. The chain circles to allow bonding between the B and A domains; the mature B-A heterodimer results after cleavage first of the signal peptide, and then of the C-domain, through the action of a proprotein convertase [4].

Four relaxin receptors, RXFP1-4, have been identified, that, unlike the insulin receptor, are G-protein–coupled receptors and not tyrosine kinase receptors. RFXP1 and RFXP2 used to be known as LGR7 and LGR8; RXFP3 and RFXP4 used to be called GPCR135 and GPCR142, respectively. *RXFP1* and *RXFP2* genes contain introns and alternative splicing occurs, whereas *RXFP3* and *RXFP4* are intronless genes. RFXP1 is the receptor-binding relaxin (RLN2 in higher primates and RLN1 in other mammals), RXFP2 is the main receptor-binding INSL3, and RXFP3 and RXFP4 bind RLN3 and INSL5, respectively, [4]. To date, endometrial expression of RXFP1 has been addressed in human, with two studies describing an increase in RXFP1 expression during the secretory phase of the menstrual cycle [6,7], and one study reporting no difference in RXFP1 abundance across the menstrual cycle [8]. Ectopic endometrial tissue found in women suffering from endometriosis expresses a significantly lower amount of RXFP1 than does eutopic endometrium [9]. We have confirmed the expression of RXFP1 in equine endometrium at the messenger RNA (mRNA) and protein level, with no variation in expression levels across the estrous cycle or depending on pregnancy status (unpublished observations).

2. Relaxin's role during late pregnancy

The placenta is the major source of relaxin during the second half of gestation in most mammals studied to date including but not limited to rabbit [10], dog [11], dromedary camel [12], cat [13], and horse [14]. The pig presents a notable exception, with the corpora lutea being the source of relaxin during pregnancy [15]. In pregnant mares, circulating relaxin levels peak coinciding with the second stage of parturition; after passage of the fetal membranes, relaxin declines to undetectable levels within 36 hours, suggesting that the placenta is the sole source of relaxin during late pregnancy [14]. In early equine embryos, relaxin transcript localizes to the noninvasive trophoblast of the allantochorion, whereas the invasive chorionic girdle does not express relaxin transcript [16]. Breed differences exist with regards to levels of circulating relaxin during pregnancy. Standardbred mares have higher concentrations than Thoroughbred mares, and Pony mares have lower circulating levels of relaxin than both Standardbred and Thoroughbred mares [17]. Relaxin concentrations decline in mares with impaired placental function, both in the case of spontaneously occurring pregnancy loss [17] and in the case of experimentally induced placentitis with subsequent pregnancy loss [18]. Relaxin has therefore been explored as a potential marker of treatment success in mares undergoing treatment for placentitis; it was however determined that circulating relaxin levels are not a reliable indicator to assess therapeutic efficacy [18].

In general, relaxin is most widely known for its softening effect on pelvic ligaments that aid in preparation of the birth canal for delivery of the fetus. It has been postulated that relaxin is not an absolute requirement for normal vaginal delivery to occur, as aluteal women, who are hyporelaxinemic (the CL is the major source of circulating relaxin), go through normal vaginal delivery without any overt clinical problem [19]. However, the placenta produces relaxin that does not enter the systemic circulation, and local effects of placental relaxin cannot be excluded [19]. Relaxin knockout mice suffer from inadequate development of the pubic symphysis, mammary gland, and nipples, as well as atrophic vaginal and cervical luminal epithelium during pregnancy, mainly attributed to increased collagen content and, in case of the pubic symphysis, also reduced water content [20,21]. Neutralization of circulating relaxin in pregnant rats results in a phenotype similar to relaxin knockout mice, i.e., reduced vaginal growth [22], reduced mammary gland development, and prolonged labor (likely due to a smaller and less extensible cervix than untreated animals) [23]. RXFP1 negative mice show normal fertility and litter size; however, an increase in dystocia rate occurs, and an increased number of dead pups can be observed. As in relaxin knockout mice, mammary gland development, in particular nipple development is impaired [24]. Conditional knockout of RXFP1 in smooth muscle results in reduced pubic symphysis length in pregnant mice and overall more collagen content of the reproductive tract [25]. The pig presents an interesting model to study the role of relaxin during pregnancy; the corpora lutea are the sole source of relaxin, and therefore, bilateral ovariectomy followed by progesterone supplementation results in relaxin-deficient pregnancies [26]. Using this model, Min et al. [26] reported that relaxin promotes the growth of vagina and uterus during pregnancy, and Zaleski et al. [27] observed greatly reduced mammary gland development in relaxin-deficient gilts, which however was overcome once piglets started nursing.

Relaxin's effect on uterine contractility is well described. In pigs and rats, relaxin clearly inhibits contractility of myometrial strips mounted in a physiologic organ bath [28–31]. In the pregnant rat, the inhibitory effect of relaxin on uterine contractility depends on the stage of pregnancy: during the first half of pregnancy, relaxin inhibits contractility, whereas during the second half of pregnancy, and at term, no such inhibitory effect of relaxin can be observed [29,31]. In human myometrium, no such clear