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Review

# Regulation of the reproductive cycle and early pregnancy by relaxin family peptides

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ABSTRACT

The relaxin family of peptide hormones are structurally closely related to one another sharing a heterodimeric A–B structure, like that of insulin. They may also be active as unprocessed B–C–A pro-forms. Relaxin has been shown to pay a key role within the ovary, being involved in follicle growth, and ovulation. Relaxin is produced in large amounts also by the corpus luteum where it acts as an endocrine hormone positively affecting implantation, placentation and vascularization during the all-important first trimester phase of pregnancy establishment. Relaxin exerts its functions via the receptor RXFP1. Insulin-like peptide 3 (INSL3) in contrast acts through the related receptor RXFP2, and plays an essential role in the production of androgens within growing antral follicles. INSL3 is also produced in large amounts by the male fetus shortly after sex determination, where it controls the first transabdominal phase of testicular descent. However, this fetal INSL3 is also able to influence placental and maternal physiology, indicating associations with later preeclampsia and/or fetal growth restriction. Other members of this relaxin-like family of peptides, such as INSL4, INSL5 and INSL6 are less well studied, though all suggest modulatory roles in ovarian and/or placental function.



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## 1. Introduction

The relaxin family of peptide hormones, whilst structurally related to insulin and the IGFs, appears to have evolved as a separate branch of informational molecules already very early in evolution. Whilst there appear to be no members of the relaxin-like subfamily in insects and worms, several members have been characterized in vertebrates, and particularly in mammals. In deuterostomes, recent discoveries from starfish now suggest that an ancestral relaxin-3-like molecule was already present in this phylum and most importantly was playing a key role in the maturation and release of oocytes (Mita et al., 2009). Significantly, this

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molecule, called GSS (gonadal stimulating substance), is made within the radial nerves and appears to be performing a role much like the pituitary gonadotropins in other species.

Besides their cladistic similarity, the relaxin-like group of hormones – at least in mammals – is characterized by having receptors which belong to the G-protein coupled receptor (GPCR) family, unlike the insulin branch which all make use of receptor tyrosine kinases. The vertebrate ancestor of the relaxin-like family of peptides, called relaxin-3, is predominantly also a neurohormone, like GSS, and together with the related INSL5 (insulin-like peptide 5), both recognize GPCRs of class A, with small N-terminal extracellular domains (called RXFP3 and RXFP4) (Bathgate et al., 2006). In fish and amphibians, relaxin-3-like molecules are also involved in reproductive processes being highly expressed also in the gonads (Wilson et al., 2009). At some time prior to and concomitant with the emergence of mammals, with their very sophisticated system of viviparity which frees the reproductive process from the arbitrariness of the external environment, the relaxin family of peptide hormones underwent a further radiation. Thus in mammals, particularly in humans, we find altogether seven members of the relaxin hormone family: relaxin-3 and INSL5, predominantly associated with the brain and gut respectively; H1-relaxin, H2-relaxin, INSL3, INSL4 and INSL6, are all associated with reproductive functions specifically linked to viviparity. This has led to the coining of the term 'neohormone' for this group of peptide hormones (Anand-Ivell et al., 2013), which serve specifically mammalian physiologies, though others, such as hCG or interferon-tau, are also members. Significantly, H1-relaxin, H2-relaxin and INSL3 make use of different GPCRs (RXFP1 and RXFP2) from those used by relaxin-3 and INSL5 (INSL4 and INSL6 have as yet no known receptors). These GPCRs are affiliated to the class A, rhodopsin-like GPCRs and are distantly related to the receptors for the glycoproteohormones, LH, FSH and TSH, within subclass C of the LGR (leucine-rich repeat-containing GPCR) family (van Hiel et al., 2012). Thus, from an evolutionary and signaling viewpoint, the relaxin family of peptide hormones shares several features with the hormones of the HPG axis, though unlike these have evolved to accommodate additional functions related to viviparity and internal fertilization.

The present review explores the specific roles of relaxin family peptides in female physiology with emphasis on ovarian function, embryo formation and implantation, and early pregnancy up to the end of the first trimester. The role of these peptide hormones in later pregnancy, in lactation and in male reproductive function have been recently covered in other reviews (e.g. Bathgate et al., 2006; Parry and Vodstrcil, 2007; Ivell et al., 2011) and will not be further discussed here.

## 2. Relaxin

### 2.1. Relaxin and ovarian function

The term relaxin is used here to refer to the peptide called H2-relaxin in humans, relaxin-1 in rodents, and its homologs, and is thus distinct from the ancestral neurohormone relaxin-3 and its homologs, or from the recently evolved H1-relaxin found in humans and chimpanzees. Relaxin is the major relaxin-like peptide produced within the ovary of most mammalian species, and the hormone which was first extracted and shown to have relaxing-like properties on the term pubic symphysis in guinea-pigs and other rodents, hence its name. As this function suggests, relaxin is a major product of the corpus luteum of pregnancy, and has been identified in this structure in almost all mammals (except bovids) (Sherwood, 1994).

The corpus luteum develops in every estrous cycle from the mural cells of the ovulating follicle(s), and times its development accordingly from the day of ovulation within the normal cycle. In ruminants and probably also rodents the corpus luteum comprises cells of both the follicular granulosa as well as theca interna layers, following the dissolution of the follicle basement membrane and the LH surge-induced epithelial–mesenchymal-transition (EMT) that these cells then undergo. In humans and other primates, the corpus luteum appears to derive mostly from granulosa cells, with little contribution from the theca cell layer. This is important, because at least in humans and possibly in other species, relaxin in the corpus luteum appears to be made exclusively by the granulosa-derived luteal cells, beginning a few days after ovulation and the EMT. Consequently, in cultured human granulosa-lutein cells collected by aspiration at ovum pick-up following appropriate hormonal stimulation as part of an IVF program, relaxin production and secretion into culture medium occurs only after 6–10 days of culture and in vitro cell differentiation (Stewart and Vandevoort, 1997). In vivo this luteal expression of relaxin would normally be interrupted at luteolysis, and the commencement of a new cycle. If pregnancy occurs, then the corpus luteum is retained and relaxin continues to be produced and secreted by the corpus luteum throughout pregnancy, or as long as the corpus luteum functionally persists. It is luteal relaxin which appears to be the major contributor to circulating relaxin in female mammals, at least during the luteal phase of the cycle and in pregnancy. This is supported by the complete absence of circulating relaxin in ovum donor pregnancies in women with non-functioning or absent ovaries (Johnson et al., 1991).

Why this is important is that relaxin expression can also be detected in theca interna cells of antral follicles in both humans and pigs before the LH surge (Blankenship et al., 1994; Ohleth and Bagnell, 1999), as well as in both cell types after culture with luteinizing levels of the gonadotropin (Ohleth and Bagnell, 1999). Whilst this relaxin probably does not contribute to the circulation it likely is the major contributor to the relaxin detected in follicular fluid (Wathes et al., 1986). Thus within the ovary there appear to be two different sources of relaxin – the theca interna cells of follicles and the corpus luteum.

As mentioned above, relaxin acts primarily via the GPCR called RXFP1 (Bathgate et al., 2006). It may also activate the alternative receptor RXFP2 (which is specific for INSL3), but only in some species such as the human, and then only at highly supraphysiological concentrations. In transfected cells, and in some naturally receptor-expressing primary cells, such as human endometrial stromal cells (Bartsch et al., 2001, 2004; Ivell et al., 2007) or human myometrial cells (Heng et al., 2008), relaxin interacts with RXFP1 to activate $G_s$-mediated adenylyl cyclase causing an elevation of intracellular cAMP. It may also in some circumstances activate PI3-kinase in a $G_{i/o}$-dependent manner involving PKC-zeta (Nguyen and Dessauer, 2005). Within the ovary, RXFP1 relaxin receptors have been identified at the transcript or protein level on granulosa and cumulus cells of pig antral follicles (Feugang et al., 2011a), and possibly also on oocytes themselves (Feugang et al., 2011b). Moreover, treatment with relaxin of porcine cumulus–oocyte-complexes in vitro, whilst appearing to have little effect on oocyte maturation, did appear to positively influence the resulting embryos (Feugang et al., 2011a; Kim et al., 2010). RXFP1 is also expressed within the corpus luteum of monkeys and cats (Braun et al., 2012; Maseelall et al., 2009), though the precise cellular localization in these tissues has not been ascertained. One report also suggests the presence of RXFP1 in human granulosa cells of primordial, primary and secondary follicles (Shirota et al., 2005a), with relaxin treatment of ovarian cortical fragments leading to development of those follicles. Moreover the same authors show that relaxin treatment of cortical fragments can also cause