Insulin like growth factor 1 and regulation of ovarian function in mammals

Rahul Behl & Rajeev Kaul
Animal Genetics Division, National Bureau of Animal Genetic Resources, P. O. Box 129, Karnal 132001, India

Various growth factors have been proposed to play endocrine and / or paracrine role in mammalian ovarian follicular development. The insulin like growth factor 1 (IGF-1) is one such factor. More and more reports now support the existence of an intra-ovarian IGF system including receptors and binding proteins. The role of IGF-1 in ovary is to amplify gonadotropin hormone action in terms of increased steroidogenesis by ovarian granulosa cell and granulosa cell proliferation. The synthesis and proteolysis of insulin like growth factor binding proteins, under the control of follicle stimulating hormone, regulate the intra-follicular availability of IGF-1, which further determines the sensitivity of granulosa cells to gonadotropins. Besides gonadotropins, IGF-1 has been implicated in somatotropic hormone action in the ovarian function. Exact mechanism of IGF-1 action in the ovarian follicles needs to be worked out to elucidate whether or not IGF-1 is indispensable in addition to known endocrine factors like gonadotropin and ovarian steroid hormones. This will pave the way for better understanding of control(s) which ensure final development of dominant follicle(s) and atresia of other follicles of the cohort.

The ovarian follicular development is a long and complex process involving proliferation and differentiation of both somatic and germ cell elements. The follicular dynamics is largely controlled by conventional endocrine principles, such as pituitary gonadotropins and ovarian steroids. Importantly it has become increasingly apparent that several phenomena that are central to ovarian physiology are not fully accounted for by conventional endocrine principles. As an example, consideration must be given to the process of follicular selection whereby a predetermined number of follicle(s) is recruited, selected, allowed to assert dominance, and ultimately ovulate despite the fact that all follicles are afforded comparable gonadotropic support. These observations have given rise to the suggestion that the ovarian function may be under the control of yet another group of modulatory principles. The exquisitely timed and highly regionalised expression of these locally derived principles, known as autocrine and paracrine factors, may finally account for those aspects of the ovarian life cycle which defy conventional explanation. Different workers are investigating several classes of putative intra-ovarian regulators and significant attention has been paid to growth factors. The insulin like growth factor 1 (IGF-1) perhaps been the most thoroughly evaluated amongst the various growth factors being investigated for their role in ovarian follicular dynamics in the vertebrates including fish, poultry and mammals.

IGF-1 is a single chain basic polypeptide of 70 amino acids with molecular mass of 7649 Da. It is about 70% identical to IGF-2 and about 50% homologous to Κ and 2 chains of insulin, indicating the appropriateness of this nomenclature. Using molecular biology techniques it has been proved that IGFs are highly conserved proteins found in an array of vertebrate species. This review discusses the role of IGF-1 in mammalian ovarian physiology. The objective of this communication is to provide a general overview of the biology of IGF-1 in terms of ovary as a site of IGF-1 production, reception and action in the mammals.

IGF-1 production in the ovary

In liver and non-hepatic tissue two major classes of IGF-1 messenger ribose nucleic acid (mRNA) transcripts have been identified. Both transcripts code for the same mature peptide. The 'exon 2 transcripts' of IGF-1 (mRNA containing a 5'-untruncated region starting in exon 2) are believed to encode the 'endocrine form of the IGF-1' under the control of growth hormone (GH) in liver. The 'exon 1 transcripts' of IGF-1 (mRNA containing a 5'-untruncated region starting in exon1), which are found in all tissues, are regulated by the factors other than GH and may represent the autocrine/paracrine form of IGF-1.

The growing body of information now supports the view that the ovary is indeed bonafide site of IGF-1 gene expression. Murphy et al., subjected the total RNA from rat ovary to a liquid hybridisation/Rnase protection assay using a 32P-labelled riboprobe.
transcribed of a cDNA designed to highlight exon 1 and exon 2 transcripts of IGF-1 gene to establish the ovary as a site of IGF-1 production other than liver. The ovarian follicles and corpora lutea in pigs have been shown to express mRNA for IGF-1. The cellular localisation studies have clearly established the granulosa cells as the major ovarian cell type concerned with IGF-1 gene expression. In contrast theca-interstitial cells are virtually negative in this regard in rats. Oliver et al. using in situ hybridisation studies clearly localised IGF-1 to the membrane granulosa. The immuno-fluorescent localisation has also established the granulosa cells as the major producer of IGF-1. Note must also be made to the fact that healthy growing follicles proved IGF-1 replete whereas atretic, poorly growing follicles proved IGF-1 depleted.

Gong et al. reported that in vivo treatment of ewes with recombinant GH significantly increased the secretion of IGF-1 by ovarian follicles in vitro, suggesting that the IGF-1 gene expression be also modulated by GH dependence. Though, the GH has been shown to modulate IGF-1 mRNA expression in rat ovary, it is yet to be confirmed in other mammalian species that the increase in IGF-1 secretion by follicles is not just a reflection of elevated levels in follicular fluid resulting from increased peripheral concentration of IGF-1 induced by rGH treatment.

Ovary as a site of IGF-1 reception

The biological effect of IGF-1 is mediated through a cell membrane receptor. Two types of IGF cell membrane receptors have been identified. The type 1 receptor preferentially binds IGF-1 whereas type 2 receptor has more affinity for IGF-2. Type 1 IGF receptor is composed of two extra-cellular κ-subunits and two trans-membrane β-subunits. The κ-subunit contains the ligand-binding region of the receptor. The domain swapping experiments with the insulin receptor have implicated a cystein rich segment as being necessary for high affinity binding for IGF-1. The β-subunit is composed of a short extra-cellular domain, a membrane spanning segment, and a large intra-cytoplasmic region containing a tyrosine kinase domain and sites of tyrosine and serine phosphorylation. Ligand binding in the κ-subunit triggers activation of the intracellular tyrosine kinase, possibly by simulating a conformational change in the β-subunit. This leads to receptor phosphorylation by an intracellular trans-mechanism similar to that used by other receptor tyrosine kinases.

Studies have now clearly established the ovary as a site of IGF-1 reception. The conventional radio-ligand receptor assay has documented the presence of specific type 1 IGF receptor in granulosa cells. The pattern of IGF-1 receptor gene expression is highly conserved among the different mammalian species that have been studied, being heavily concentrated on the granulosa cells. Both in vitro and in vivo observations have clearly documented the ability of FSH to effect dose-dependent increment in granulosa cell IGF-1 binding. This increase in specific binding proved due to enhanced IGF-1 binding capacity rather than affinity as attested to by the apparent parallelism of the two scatchard plots. The ability of the FSH to induce ovarian type 1 IGF receptor was further confirmed at the molecular level when the cloned cDNA was used for the rat type 1 IGF1 receptor. Treatment of hypophysectomised immature rats with FSH resulted in a substantial increase in steady state levels of type 1 IGF receptor transcripts. New base levels of the type 1 receptors have been shown to decrease during atresia in the small antral follicles in sheep, but no change was observed in the follicular level of the IGF type 1 receptors during growth of antral follicle from 0.8 to 8.0mm in bovine granulosa cells. In the porcine ovary IGF-1 receptor mRNA level are highest in healthy growing and dominant follicle granulosa cells and reduced to undetectable levels in atretic follicles regardless of the follicular size, suggesting the loss of IGF effect due to receptor down regulation which may be involved in atresia in the pigs. Together these studies strongly indicate that the IGF-1 receptor system is strongly involved in the selection of follicles and atresia of other during ovarian follicular waves.

IGF binding proteins in ovary

The IGF binding proteins (IGFBPs) constitute a heterogeneous group of at least six distinct proteins capable of binding IGFs (but not insulin), with affinities in the range of $10^{-10}$ to $10^{-9}$ M. Although the exact role of IGFBP remains a matter of study, general consensus supports a role in the transport of IGFs from circulation to the peripheral tissues, to maintain reservoir of IGFs in the circulation, to potentiate or to inhibit IGFs action and to maintain IGF-independent biological effect. IGFBPs compete with IGF-1 receptors for binding IGFs. The IGF-1 bound to IGFBPs is biologically not available. In circulation IGFBP-3 is a major carrier protein for IGFs. The IGF and IGFBP-3 complex is unable to cross the capillary barrier and is retained in the circulation.
IGFBP-1 can cross the capillary barrier and is believed to regulate the acute changes of serum IGFs. It appears that the ovarian follicles are the site of IGFBP-2, 3, 4 and 5 and to some extent IGFBP 6 gene expression. In the follicular fluid also the IGFs are bound to IGFBPs that regulate the IGF bioavailability at the target cells. The level of IGFBPs is regulated by the control at the level of expression by gonadotropins or by proteolytic degradation of the protein. In bovine, ovine and porcine preovulatory follicles, IGF bio-availability depends on IGFBP-2 and IGFBP-3. The bio-availability of IGF is also regulated by the increased expression of IGFBP-2 in atretic follicles.

Role of IGF-1 / IGFBPs in ovary

IGF-1 has been shown to modulate a number of functions in the ovary either by itself or in concert with the pituitary gonadotropins. For the most part, however, IGF-1 acts at the level of granulosa cells to amplify gonadotropin hormone action in rat. Some workers have also shown the theca-interstitial cells as the IGF-1 action site. Indeed, the IGF-1 has been implicated in the regulation of ovarian function in several mammalian species. Monniaux et al. based on their experiment in ovine granulosa cells, proposed that the IGF-1, in synergy with FSH, clearly increases the percentage of cell expressing P450 side chain cleavage enzyme and cell proliferation. Similar observations were made in the pig, rabbit and rat granulosa cells. In the porcine granulosa cells the IGF-1 was also shown to stimulate estradiol and progesterone secretion in vitro. The IGF-1 has also been shown to increase estradiol and progesterone secretion alone and in the combination with FSH in murine, bovine, ovine and caprine ovaries. In summary, the IGF-1 has been shown to promote FSH supported granulosa cell proliferation and progesterone biosynthesis and estrogen production. Recently, the IGF-1 has been shown to enhance the FSH receptor expression in the granulosa cells.

It is important to emphasize that the terminal follicular growth is characterised by the increased sensitivity of granulosa cells to FSH and the increased steroidogenesis. The high responsiveness of the granulosa cell to FSH probably results from the intrafollicular system of amplification involving estradiol and IGF-1. As stated above, the concentration of bioavailable IGFs strongly increase in the large antral follicles during the terminal development of follicles. The future dominant follicles have been shown to contain increased IGF-1, highest estradiol and lowest IGFBPs especially the IGFBP-4. In contrast, the future atretic follicles have low IGF-1, high IGFBPs and low aromatase activity and estradiol synthesis. The granulosa cell death during follicular development and atresias occurs by apoptosis. FSH and IGF-1 has been shown to prevent apoptosis in bovine and porcine granulosa cells. It is suggested that FSH in the early and late follicular phase may contribute to increased concentration of bio-available IGFBPs in dominant follicle by regulating the synthesis and proteolysis of IGFBPs. In view of this and the stimulatory effect of IGF-1 on steroidogenesis and ability to enhance the FSH receptor expression, it is likely that the follicle(s) with the highest concentration of the estradiol and IGF-1 will be able to sustain the terminal maturation and will become the dominant follicle of the cohort.

Besides enhancing the gonadotropin action in ovarian follicles, the IGF-1 has been shown to affect follicular biology by mediating/synergising somatotropin hormone action in the ovarian follicles. The administration of the somatotropin in vivo leads to both an increase in the IGF-1 and an increase in the number of follicles. Since, the immunohistochemical studies in the bovine ovary failed to detect the GH receptor in ovarian follicular cells and GH did not enhance proliferation of granulosa cells in vitro, it was proposed that the GH may act in vivo by enhancing the peripheral IGF-1 concentrations as described in rats. But later, the GH has been shown to enhance the IGF-1 production by granulosa cells in vitro and recently, Izadyar et al. have localised the GH receptor in cumulus oophorus cells by RT-PCR amplification of GHR mRNA. It is proposed that the GH might act on the follicular development in vivo by enhancing IGF-1 production as well.

Conclusion

IGF-1 plays an important role in ovarian folliculogenesis by amplifying the gonadotropin action on follicular cells and enhancing steroidogenesis. The high responsiveness of the granulosa cells to gonadotropins during terminal follicular growth in mammals may be attributed to intrafollicular system of the amplification involving IGF-1 and the ovarian steroid hormones. The concentrations of the available IGFs strongly increase in the large antral follicles during terminal development, indirectly by inhibition of IGFBPs expression and increased IGFBP proteolysis and probably by the
selective expression of IGF-1 in developing healthy follicles. It is likely that IGF-1 may be the chief player in the selection of the follicle(s). The stimulatory effect of IGF-1 on follicular growth and steroidogenesis can be exploited for the improvement of existing regimens of reproduction in farm animals, but this needs a better understanding of the mechanisms by which IGF-1 controls the follicular dynamics.

References


