SHORT COMMUNICATION Agricultural Science Digest, Volume 40 Issue 4: 440-444 (December 2020)

Ovarian Insulin-Like Growth Factor-I

M. Bijna[1], K. Karthiayini[2]

10.18805/ag.D-5104

ABSTRACT

The insulin-like growth factor-I is a pleotropic growth factor that modulates cell replication. Insulin-like growth factor-I is produced by granulosa, theca and luteal cells and have autocrine and paracrine actions. It plays a crucial role in animal fertility acting as a monitoring signal. Researches have shown that synergistically with gonadotropins it stimulates the growth, survivability and steroidogenesis of follicular cells.

Key words: Growth, IGF-I, Ovary, Steroidogenesis.

Folliculogenesis in mammals is a tightly controlled process where only a small proportion of total follicles give rise to dominant follicles. Earlier it was thought that the folliculogenesis is entirely regulated by pituitary gonadotropins and ovarian steroids. Interestingly, recent reports on growth factors point to the fact that follicular microenvironment plays an important role in follicular development. Among the various growth factors involved in the regulation of follicular development and ovulation, insulin-like growth factor plays a pivotal role. The insulin-like growth factor (IGF) system is composed of two ligands (IGF-I and IGF-II), two trans-membrane receptors (IGFR-I and IGFR-II), six IGF binding proteins and varying number of IGFBP degrading proteases. They play an important role in sexual development and reproduction of both males and females. The IGF ligands regulate different aspects of gonadogenesis, sex determination, sex-specific development or reproductive performance (Neirijnck *et al.,* 2019). The bioavailability of IGF-I and IGF-II is regulated by a family of six high-affinity binding proteins (IGFBP1-6). The IGF binding proteins produced by granulosa cells (GCs), theca cells (TCs) and luteal cells increase the circulating half life of IGFs, transport IGF molecules to their receptors, potentiate or inhibit effects of IGFs (Spicer and Echternkamp, 1995; Kostecka and Blahovec, 2002). Insulin-like growth factor binding protein-3 (IGFBP3) gene is the gene responsible for growth and reproductive development in mammals and IGFBP3 when attached to IGF-I modifies the functions of IGF-I (Othman *et al*., 2014). However, the affinity of IGFBPs for IGF-I and IGF-II can be modulated by a serine metalloprotease that degrade IGFBPs. This protease was found to be pregnancy-associated plasma protein-A (PAPP-A) in rat, sheep, equine, buffalo and cattle (Nayan *et al.,* 2013). The rise in PAPP-A in future dominant follicle in response to rise in follicle stimulating hormone (FSH) led to a decrease in IGFBP-4 and 5 and an increase in free IGF-I. Moreover, the synergistic actions of IGF-I and FSH allowed future dominant follicle to upregulate estradiol production thereby preventing subordinate follicles from acquiring PAPP- A and becoming dominant. Thus change in follicular IGF system is an important determinant of follicle dominance in mammalian ovary (Fortune *et al.,* 2004). Nayan *et al.* (2013) and Matsui *et al.* (2004) suggested the stimulatory effect of FSH on PAPP-A mRNA expression in Indian water buffalo ovary and rat granulosa cells respectively. Insulin-like growth factor-I exerts its physiological effects by activating insulin like growth factor receptor-I (IGFR-I). The IGFR-I is a transmembrane tyrosine kinase receptor containing two extracellular alpha subunits which has major ligand binding determinants and two membrane spanning beta subunits lodging intrinsic tyrosine kinase. The IGF-I acting through IGFR-I may promote phosphatidylinositol-3-kinase and mitogen activated protein kinase mediated pathways to stimulate cell proliferation, differentiation, migration and protection from apoptosis (Clemmons, 2009; Kumar and Laxmi, 2015).

[1]Department of Veterinary Physiology, College of Veterinary and Animal Sciences, Kerala Veterinary and Animal Sciences University, Mannuthy-680 651, Kerala, India.
[2]Department of Veterinary Physiology, College of Veterinary and Animal Sciences, Kerala Veterinary and Animal Sciences University, Pookode-673 576, Kerala, India.

Corresponding Author: M. Bijna, Department of Veterinary Physiology, College of Veterinary and Animal Sciences, Kerala Veterinary and Animal Sciences University, Mannuthy-680 651, Kerala, India. Email: dr.bijnam@gmail.com

How to cite this article: Bijna M. and Karthiayini K. (2020). Ovarian Insulin-Like Growth Factor-I. Agricultural Science Digest. 40(4): 440-444.

Submitted: 10-12-2019 **Accepted:** 02-03-2020 **Published:** 22-08-2020

Insulin-like growth factor-I

Insulin-like growth factor-I is a pleotropic growth factor with endocrine, paracrine and autocrine actions. The insulin-like growth factors are polypeptides with 50 per cent structural homology to insulin which mediated the anabolic and linear growth stimulating effect of growth hormone (Laron, 2001). Insulin-like growth factor-I also plays an important role in reproduction as it is expressed in organs concerned with reproduction like hypothalamus, anterior pituitary, ovary, oviduct and uterus (Giudice, 1992; Mikawa *et al.,* 1995).

Expression of IGF-I mRNA was reported in the ovaries of mouse (Wandji, 1998), human (El-Roeiy), bovine (Rebouças, 2014), caprine (Martins, 2010) and bubaline (Dev *et al.,* 2010; Singh *et al.,* 2015). Wood and Strauss (2002) reviewed that in pigs, cows and rodents, IGF-I is the major IGF ligand produced by granulosa cells but in humans IGF-II is the major one produced by granulosa cells (GCs) and theca cells (TC). But its expression in ruminants remain controversial with reports suggesting both its presence in caprines (Martins, 2010) and absence in sheep (Hastie and Haresign, 2006). The IGFs are local mediators of gonadotropin action in the ovary (Giudice, 2001). The IGF-I stimulate the growth of ovarian cells (Lucy, 2000) and suppression of *in vitro* apoptosis of GCs (Yu *et al.,* 2003). The synergic actions of IGF-I and follicle stimulating hormone promote estradiol (Jones and clemmons, 1995) and progesterone secretion from GCs (Behl and Pandey, 1999). Insulin-like growth factor-I upregulates lutenizing hormone (LH) receptors in ovarian cells (Jones and Clemmons, 1995) and stimulate secretion of LH from anterior pituitary (Denley *et al*., 2005). Insulin like growth factor system also play an important role in angiogenesis required for the maintanence of corpus luteum along with other factor like vascular endothelial growth factor (Berisha and Schams, 2005). It also stimulate proliferation of cumulus oophorus, nuclear maturation and developmental competence of oocytes (Sirotkin, 2010).

Insulin-like growth factor-I in pre-antral follicles

Monget and Bondy (2000) cited the importance of IGF-I in early folliculogenesis. Zhao *et al.,* (2001) cultured rat secondary follicles in presence and absence of IGF-I (1–100 ng/mL) and noticed an increase in follicular cell proliferation and differentiation. In the same study it was also observed that ultrastructure of follicles cultured in presence of IGF-I is better sustained compared to that follicles cultured in medium without IGF-I. Moreover, theca cells had normal characteristics of steroid-secreting cells and oocytes contained cortical granules when the culture was supplemented with IGF-I. But in follicles cultured in the absence of IGF-I, theca cells were degenerated and in oocytes cortical granules were hardly present. They suggested that IGF I, in the presence of follicle stimulating hormone, enhances rat pre antral follicle development *in vitro* and supports the morphology of cultured pre antral follicles. Fortune *et al.* (2004) reported that in cows the early stages of follicular development were controlled at least in part by IGF. Armstrong *et al.* (2002) detected the mRNA of *IGFBP2* in GCs and oocytes of preantral follicles but IGF-I and IGF-II mRNA were not present and they reported that though IGF system was not involved in initiation of bovine primordial follicle growth, it was involved during subsequent events of follicular development. Thomas *et al.* (2007) observed increased follicle diameter and estradiol production when bovine preantral follicles were cultured for 6 days in medium containing IGF-I (10 ng/ml, 50 ng/ml, 100 ng/ml, 1000 ng/ml) and suggested that IGF system is required for bovine folliculogenesis during the early stages. *In vitro* studies in caprine preantral follicles by Martins *et al.* (2010) suggest that IGF-I maintained pre-antral follicle survivability, promoted primordial follicle activation and transition from primordial to primary and secondary follicles. Similarly, studies by Huanmin and Yong (2005) indicated that the IGF-I regulated the survival and growth of oocytes of caprine preantral follicles. These results were suggestive of the role of IGF-I in goat pre-antral follicle development. Contrary to these results, study by Brito *et al.* (2012) revealed that IGF-I and / or FSH did not influence the survival, antrum formation and follicular diameter in caprine pre-antral follicles. Gouveia *et al.* (2015) observed that ovine culture supplemented with100 ng/mL IGF-I for 7 days promoted survivability and activation of ovine preantral follicles suggesting the role of IGF-I in ovine folliculogenesis.

Insulin-like growth factor-I in antral follicles

Yu *et al.* (2003) studied the effect of FSH on follicular fluid IGF-I levels and on IGF-I expression in caprine ovary. Radioimmuno assay of follicular fluid and semi-quantitative RT–PCR analysis of ovarian cortex revealed the presence of both IGF-I and IGF-II in the caprine ovary. The concentrations of both IGF-I and IGF-II increased significantly in medium follicles (3-5mm) in FSH treated and untreated ovary, but in large follicles (>5mm) only IGF-I increased significantly, after FSH treatment. Since IGF-I was more sensitive to FSH treatment in caprine follicles they suggested that IGF-I is the major mediator of FSH in goat ovary. Moreover, Martins *et al.* (2010) observed the presence of IGF-I mRNA in the caprine granulosa cells of small (1-3 mm) and large (3-6 mm) antral follicles and reported that granulosa cells and theca cells from both follicles produce significantly more IGF-I than their respective cumulus oocyte complex. Magalhaes *et al.* (2013) demonstrated that *IGF-I* gene was involved in secondary and antral follicle development in goats using microarray analysis. Meiyu *et al.* (2011) reported that in cattle IGF is important in antral stage of follicular development as it was involved in stimulating somatic cell proliferation and biosynthesis of estrogen and progesterone. *In vivo* studies in beef cattle revealed an increase in concentration of IGF-I in the follicular fluid of large follicles (>8mm) compared to medium (5-8mm) follicles (Echternkamp, 1994). The mRNA expression sequence analysis of ovarian follicles from Holstein×Japanese Black F1demonstrated that IGF-I mRNA was not detected in granulosa cells but low levels were present in theca cells of bovines. Satrapa *et al.* (2013) reported that the mRNA expression of *IGF-I, IGF-II, IGFR-I, IGFR-II, IGFBP2* and *IGFBP4* was significantly high in oocytes from *Holstein Freisian* compared to *Nelore* cows of Andhra Pradesh and hypothesized that the greater bioavailability of IGF in *Nelore* oocytes may contribute to the superior oocyte competence of *Nelore* (*B. indicus*) when compared to *Holstein* (*B. taurus*) cattle. Hastie and Haresign (2006) demonstrated that IGF-I mRNA was localized to both granulosa and theca cell layer of ovaries of Scottish

Blackface ewes but its expression was generally low in all classes of follicles (small-<2mm, medium-2-4mm andlarge->4mm). In addition the mRNA expressions of *IGF-II, IGFR-I, IGFR-II* and *IGFBP2,3,4* and *6* decreased as follicles increased in diameter and progressed from healthy to atretic status. They concluded that IGF-II is the major IGF ligand in sheep. Similarly, Monte *et al.* (2019) reported that IGF-I protein was immunolocalized in oocytes and granulosa cells from secondary and antral follicles but IGF-I did not influence follicular viability and growth of secondary follicles in ovines. The study also suggested that the synergistic action of IGF-I with FSH is likely to be important for maintaining a critical level of luteinizing hormone receptor for oocyte maturation in the sheep ovary. Dubey *et al.* (2014) observed the absence of IGF-I mRNA in any of the preantral follicles (200-250 µm), antral follicles (1-3 mm), ovulatory follicles (5-8 mm) and immature and *in vitro* matured oocytes of buffalo using RT-PCR in *in vitro* studies.

Insulin-like growth factor-I in growth and steroido genesis of follicular cells

Insulin-like growth factor-I is involved in the control of ovarian follicular development and its role during folliculogenesis had been widely studied in mammals. Monniaux *et al.* (1997) reported that development of small antral follicles (2mm in diameter in sheep and 3-4 mm in cow) was not strictly dependent on gonadotrophin supply and factors like Epidermal growth factor & IGF enhanced GC proliferation. *In vitro* studies in rat, and porcine granulosa cells demonstrated that IGF-I enhanced FSH and stimulated steroidogenesis in both follicular cells and luteal cells (Giudice, 1992; Adashi *et al.,* 1985). This occurs due to the increased aromatase activity and lutenizing hormone receptor synthesis in GCs by synergistic actions of IGF-I and FSH as demonstrated by culture studies in rat granulosa cells (Adashi *et al.,* 1985). The culture of ovine granulosa cells obtained from small (1-3 mm in diameter) and large (>5mm in diameter) follicles when treated with graded doses of IGF-I (0.1-100 ml/mL) evidenced the proliferative effect of IGF-I on granulosa cells especially from small follicles. In granulosa cells from large follicles, these effects were lower and delayed. In contrast, IGF-I stimulated both basal and FSH induced progesterone secretion from large but not small follicles (Monget *et al.,* 1993). Similarly Behl and Pandey (1999) reported that supplementation of 10-500ng/mL of IGF-I to caprine granulosa cell culture from small (<3mm diameter), medium (3-6 mm) and large (>6mm) follicles promoted the secretion of oestradiol and progesterone from GCs in all the three categories of follicles. The mechanism responsible for both cell and tissue type variation in expression and function of IGF-I is not known. It is hypothesised that in follicles stimulated by IGF-I, higher levels of intracellular proteins like c-jun and c-myc may promote proliferation rather than the differentiation of granulosa cells especially in small follicles (Mazerbourg *et al.*, 2003) and that IGF-I stimulates the cell cycle events to promote the multiplication of granulosa cells (Spicer and Aad, 2007). The IGF system synergistically with gonadotropins increased the generation of cAMP, the gonadotropin receptor second messenger. The increased cAMP activated protein kinase-A, in turn, can directly influence the activity of components of steroidogenic machinery like aromatase and steriodogenic acute regulatory (StAR) protein (Wood and Strauss, 2002). Thus IGF-I stimulated proliferation or differentiation and differentiated function of follicular cells depending on stage of development of follicles.

CONCLUSION

The exact mechanisms underlying ovarian follicular growth are not yet fully elucidated. In most mammalian species, IGF-I do not appear to be required for primordial to primary follicles transition, but they promote secondary follicle growth and antrum formation. The IGF-I promote the survivability, multiplication and steroidogenic function of the follicular cells. However, the precise mechanisms by which IGF-I gene expression is regulated needs to be investigated.

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