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Preeti Verma
Department of Physiology & Biochemistry, College of Veterinary Science & Animal Husbandry, NDVSU, Jabalpur, Madhya Pradesh, India

Anand Kumar Jain
Department of Veterinary Physiology and Biochemistry, CoVSc.&AH Jabalpur, Madhya Pradesh, India

Aditya Mishra
Department of Physiology & Biochemistry, College of Veterinary Science & Animal Husbandry, NDVSU, Jabalpur, Madhya Pradesh, India

Deepika D Jesse
Department of Physiology & Biochemistry, College of Veterinary Science & Animal Husbandry, NDVSU, Jabalpur, Madhya Pradesh, India

Sanju Mandal
Department of Physiology & Biochemistry, College of Veterinary Science & Animal Husbandry, NDVSU, Jabalpur, Madhya Pradesh, India

Anil Gattani
Department of Physiology & Biochemistry, College of Veterinary Science & Animal Husbandry, NDVSU, Jabalpur, Madhya Pradesh, India

Pragati Patel
Department of Physiology & Biochemistry, College of Veterinary Science & Animal Husbandry, NDVSU, Jabalpur, Madhya Pradesh, India

Purnima Singh
Department of Physiology & Biochemistry, College of Veterinary Science & Animal Husbandry, NDVSU, Jabalpur, Madhya Pradesh, India

Dr. Maneesh Jatav
Department of Veterinary Pathology CoVSc.&AH Jabalpur, Madhya Pradesh, India

Corresponding Author:
Anand Kumar Jain
Department of Veterinary Physiology and Biochemistry, CoVSc.&AH Jabalpur, Madhya Pradesh, India

Role of inhibin hormone: An update

Preeti Verma, Anand Kumar Jain, Aditya Mishra, Deepika D Jesse, Sanju Mandal, Anil Gattani, Pragati Patel, Purnima Singh and Dr. Maneesh Jatav

Abstract
Inhibin hormone is synthesize and secreted by granulosa and theca cells of ovary having glycoprotein in nature. Inhibin is effectively important for the control of reproductive functions in mammalians. It also produced during pregnancy in females from different foetal structure whereas it is secreted from sertoli cells of testis in males. In cattle and goats FSH and inhibin A were found to be inversely correlated, highlighting the crucial role of dominant follicle-produced inhibin in bringing the FSH transitory peaks to an end. FSH and Inhibin have an inverse correlation whereas; inhibin and estradiol-17 are positively correlated in the plasma of the animals. Plasma levels of Inhibin B are now known to be a non-invasive diagnostic tool to measure the amount of spermatogenesis. Clinical applications of inhibin for induction of fertility, inhibin inhibits FSH concentration via directly acts on pituitary gonadotrophs and thus offer offers potential for increased fertility. Guo *et al*. (2020) reported that immunisation against inhibin, increases the frequency of ovulations that can increase either in-vivo or in-vitro fertility in dairy cows. Ma *et al*. (2021) performed meta-analysis and quality evaluation techniques for evaluation of fertility in cattle immunised with inhibin. Superovulation technology in cattle is more effective and labor-saving because to inhibin immunity. The ability to maximise the number of ovarian developing follicles (Superovulation response) depends on the methods employed to induce superovulation in cattle.

Keywords: Inhibin, superovulation, spermatogenesis, FSH and granulosa cells

Introduction
In India livestock plays an important role in production and economy development. For continue production and maintain the biology of livings, reproduction is essential tool and complex process depends on the various factors such as environmental factors and physical status of animals that are properly timed and supported by hormones. Hormones secreted from endocrine system work other hormones of the body system to regulate reproduction cycle in domestic animals (Bhardwaj *et al.*, 2012) [5]. Inhibin is important hormone for the control of reproductive functions in animals. It was first introduced by D. Roy McCullagh in 1932 via isolation as peptides from gonads and considered as the main regulator of reproduction (Makanji *et al.*, 2014) [26]. Inhibin also produced during pregnancy in females from different foetal structure. The Molecular weight of inhibin is 32 kDa molecule composed of two active heterodimers subunits forms in circulation with 134 amino acid and 116 amino acids residue designated as inhibin A which is made up by α (alpha) with βA subunits and inhibin B, which is made by an α with βB subunit. By modulating FSH biosynthesis, inhibin plays a crucial role in the negative feedback regulation of pituitary gonadotropin hormones secretion. This is done by either reducing steady-state FSH m-RNA in pituitary gonadotropins or decreasing the stability of FSH m-RNA.

Synthesis and secretion of inhibin hormone
The synthesis of inhibin hormones initiates as long chain peptides, which are called as preprohormones on ribosomes. Following that "pre" component, the rough endoplasmic reticulum (RER) is instantly attached. The "pre" component of the molecule can be quickly eliminated because to the peptidase enzymes found inside the RER wall, and the prohormone can then exit the RER in vesicles. The golgi apparatus is where these vesicles subsequently travel, where they combine with golgi membranes to generate secretary granules. Exocytosis then results in the release of this active hormone in response to a particular signal. Adenosine triphosphate (ATP) and calcium (Ca^{2+}) are needed for exocytosis. The intracellular release of calcium from mitochondria or the endoplasmic reticulum, as well as the inflow of extracellular calcium, both lead to an increase in cytoplasmic calcium (Fig 1).

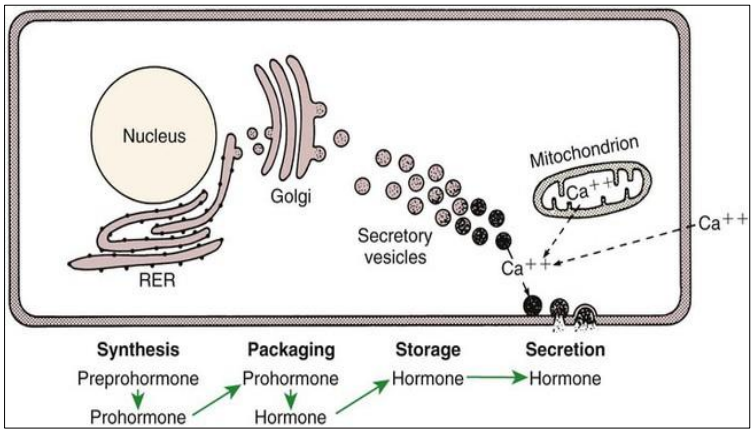

Fig 1: Inhibin hormone synthesis and secretion. (Source: Text book of Veterinary Physiology by Cunningham JG. 1992. WB Saunders.)

Inhibin receptors

Inhibin is antagonist to activin and most of its effects are associated with its antagonism. There are certain cells that bind with higher affinity to inhibin than activin because of the existence of specific inhibin-binding molecules. One of these molecules was discovered to be betaglycan recently, these acts as an inhibin co-receptor which basically is a membrane-anchored proteoglycan.

Mechanism of action

It is a water-soluble or hydrophilic hormone, as they cannot diffuse through cell membrane; they interact with the cell surface receptor to activate a signalling cascade mediated by a protein known as a second messenger cyclic adenosine monophosphate (cAMP). This receptor is linked to a G – protein (intracellular component) activates them to encounter hormone. The adenyl cyclase enzyme is then activated, which converts adenosine triphosphate (ATP) to cAMP. The activity of a protein kinase enzyme present in the cytosol is stimulated by cAMP. Activated protein kinases create a phosphorylation cascade, which results in the execution of the function of the protein hormone inhibin. (Fig 2).

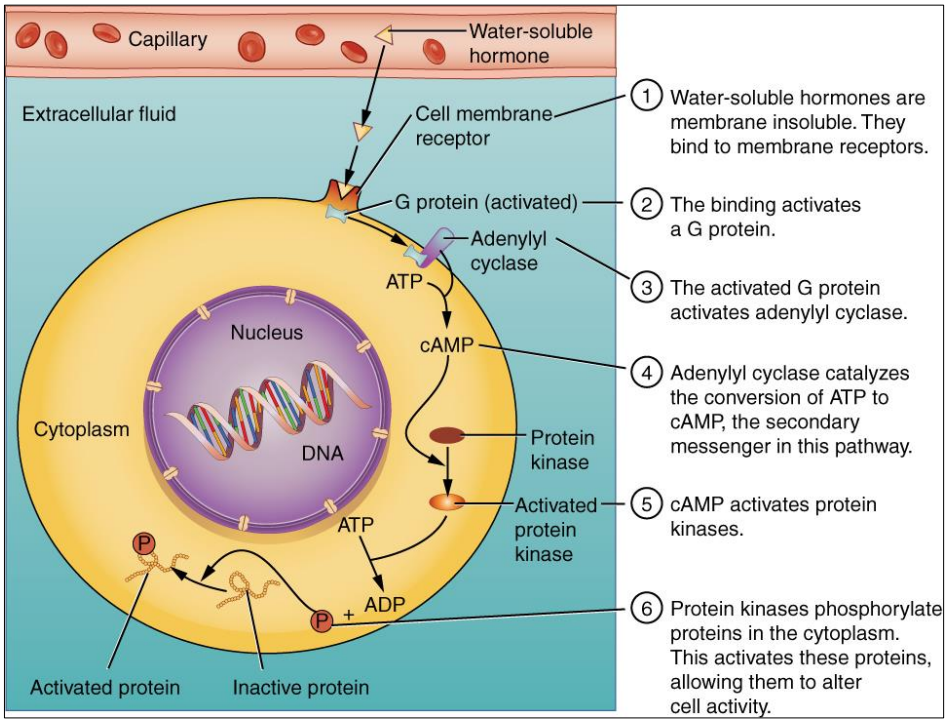

Fig 2: Mechanism of action of inhibin hormone (Source: Wikipedia)

Regulation of inhibin secretion

Inhibin impairs gonadal activity by causing less secretion of FSH via negative feedback mechanism. It recognised as the paracrine ovarian, testicular and uterus-placenta complex, inhibin is a suitable marker for male or female infertility, any gynaecological disorders and gestational diseases. At low concentrations, inhibin inhibited the production and release of FSH. Inhibin is able to bind type II activin and BMP receptors with their subunit, counteracting the effects of activins by blocking the recruitment of type I receptor.

Paracrine action

Inhibins also show the paracrine action by giving signals to cells that are close together. For quick action of inhibins occur via paracrine mode that last only a short duration of time.

Role of inhibin

In Female Reproductive System

Estrous cycle

When follicle in mares, sheep and goats change from the small antral to preovulatory phases, they release inhibitins

(Medan *et al*., 2007) [28]. A rise in FSH production precedes the appearance of each follicular wave. Following the selection of the dominant follicle, circulating FSH levels decline due to a negative feedback effect of the inhibins released by the growing follicles. It was discovered that inhibin A increased in the golden hamster starting in the morning of day one (ovulation day), peaked on day two and then suddenly surged on day four during the LH surge. Inhibin B on the other hand, increased on day 1 and decreased on day 4. In the hamster, induced atresia and subsequent follicular development resulted in the same variable patterns of inhibin A and B release (Ohshima *et al*., 2002) [31]. Furthermore, the different secretion phages of inhibin A and inhibin B indicate that they are controlled independently by gonadotropins and the stage of follicular growth.

An inverse relation between FSH and inhibin A in cattle and goats was found, highlighting the crucial role of dominant follicle-produced inhibin A in ending the fleeting peaks of FSH secretion. Animals such as mares, goats and others exhibit an unusual phenomenon in inhibin production throughout the estrous cycle known as the "ovulatory inhibin surge." Circulating inhibins concentration may be useful estimation in reproductive physiology to determine the time of ovulation in farm animals. In mares, on the day of ovulation inhibins in the blood circulation markedly increases.

Folliculogenesis

FSH is the primary hormone responsible for ovarian follicle recruitment and growth. A connection has been indicated between an increase in FSH and follicle recruitment. FSH stimulates aromatase enzyme activity in granulosa cells. It also promotes the synthesis of inhibin and follistatin. Periodic variations in FSH concentrations during the estrous cycle of bovines and caprines produce continuation of follicular waves. Continuous suction of cohort follicles following their appearance prevents FSH reduction (Tohei *et al*., 2001) [39]. Following their appearance, cohort follicles are continuously aspirated to prevent the FSH drop. So, growing cohort follicles due to inhibin secretion is neccasory for the declining of FSH that leads to their own atresia.

In pregnancy

Non-human primates have been shown to have circulating amounts of inhibin during pregnancy (Kojima *et al*., 2002) [21]. Placenta, decidua, and foetal membranes have been found to contain the inhibin A and B subunit, mRNAs, and related proteins. According to Florio *et al*. (2002) [14], the placenta and foetal membranes play a significant role in the generation and production of inhibin.

In male reproductive system

Plasma concentrations of inhibin B are low before puberty and gradually increase as sertoli cell function is activated with the start of spermatogenesis, with inhibin B being the only biologically active dimeric inhibin produced by the testes in males after an initial increase in level shortly after birth (Crofton *et al*., 2002) [9]. According to research by Myers *et al*. (2009) [30], measuring plasma inhibin B can reliably predict the degree of spermatogenesis. The measurement of inhibin B concentration in the plasma appears to play a crucial part in offering a non-invasive way to assess the amount of spermatogenesis.

Inhibin is induced by FSH and regulates FSH synthesis by servo mechanism (negative) at pituitary. FSH, which acts to upregulate subunit A and B synthesis, is hypothesized to induce sertoli cells inhibin B synthesis. Inhibin, in turn, acts as a negative feedback signal, regulating FSH secretion. (Figure-03).

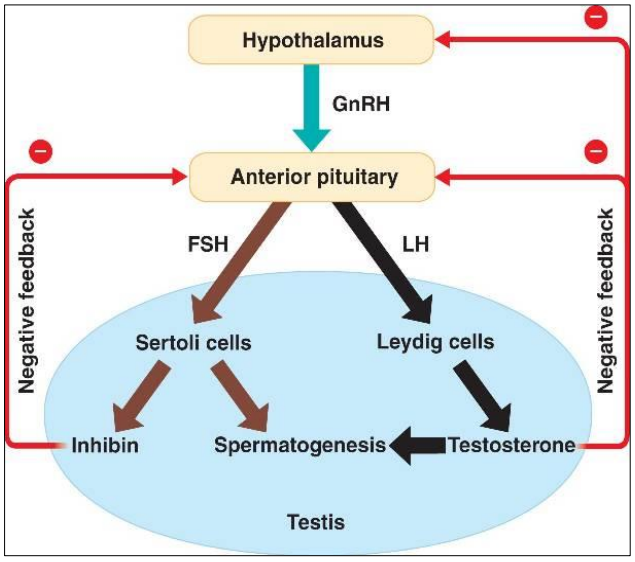

Fig 3: Role of inhibin in male reproductive system (Source: Medscape reference)

Plasma Level of Inhibin in Bovine

During the induced follicular phase the concentration of inhibin A is fifty picogram per millilitre (in plasma) before luteolysis. At some stage in the preovulatory E_2/LH/FSH surge, it reaches to 125 picogram per millilitre. With the surge in secondary FSH after ovulation, inhibin A drops significantly to 55 pg/mL. Inhibin A levels increased to around 90 pg/mL throughout the formation of the new dominant follicle (Bleach *et al*., 2001) [6].

Clinical Applications of Inhibin

Induce Fertility

Inhibin has the potential to boost fertility since it directly inhibits the production of FSH by pituitary gonadotrophs. Multiple ovulation and embryo transfer methods are now employed more and more in cow breeding. Nowadays, the majority of superovulation (SOV) therapy regimens rely on the administration of pituitary extracts containing follicle-stimulating hormones (FSH), often twice daily at a decreasing

dose for 4 days (Sanderson *et al*., 2020) [34]. However, the production of embryos is not very effective (Hasler *et al*., 2014) [18]. The growing follicle overproduces inhibins during superovulation.

Yan *et al*. (2015) [41] reported decrease oocyte quality and embryo development by altering follicle development due to higher concentration of inhibins. Numerous investigations have shown that active immunisation against inhibins and their components, as well as removal of the source, neutralize the true activity of endogenous inhibins. This encourages the formation of additional or new follicles by increasing pituitary production or plasma concentrations of FSH (Ginther *et al*., 2015) [16]. Inhibin recombinant DNA vaccines have become the most common method for immunisation against inhibins in recent years, and many studies have shown that they can improve reproductive function in other animals such as rat, mice, hen and sheep (Hui *et al*., 2014, Dan *et al*., 2016, Mao *et al*., 2016, Forage *et al*., 1995) [20, 11, 27, 15]. Guo *et al*., 2020 [17] studied immunization against inhibins can increase the number of ovulations and improve fertility in dairy cows *in vivo* and *in vitro*. Ma *et al*. (2021) [25] used meta-analysis and quality evaluation methodologies for the first time to assess the fertility of cattle immunised with inhibin. All results showed that immunisation against inhibin recombinant DNA had a substantial effect on improving fertility when compared to the control treatment. As a result of being immunized against inhibin, cow superovulation technology becomes more labor-saving and efficient.

Superovulation

It has been a regular practise to induce superovulation by combining human chorionic gonadotropin with equine chorionic gonadotropin. As a result, it is conceivable to induce superovulation by eliminating inhibin's inhibitory effect on the hypothalamus-pituitary axis' release of gonadotropin. Active immunisation against inhibin improved ovarian activity and ovulation rate in cattle (Medan *et al*., 2004) [29], ewes (Anderson *et al*., 1998) [1], goats and guinea pigs (Shi *et al*., 2000) [37] by increasing FSH secretion. Other investigations in ewes (Tannetta *et al*., 1998) [38] and goats (Hennies *et al*., 2001) [19] found that active immunisation against inhibin increased ovulation rate without changing FSH levels. These results suggest that immunisation against inhibin either promotes ovarian activity via increasing FSH secretion or has a direct stimulatory effect on follicular growth that is independent of FSH secretion.

In Ovarian and Gestational Disorders

The widespread use of inhibin as an ovarian tumour marker is one of the most significant advancements in the science of reproduction. Females with granulosa cell tumours and epithelial ovarian malignancies have been found to have blood levels of inhibin -subunit and dimeric inhibin A and B (Burger *et al*., 2001) [7]. In research involving farm animals, inhibins were only used to identify tumours in mares. In mare, Ir-inhibin is a protein that is highly secreted by granulosa theca cell tumours (GTCT) (Yoshida *et al*., 2000; Bailey *et al*., 2002) [42, 3]. As a result, quantifying inhibins may be helpful in identifying and confirming GTCT. Higher inhibin A levels in female bearing a child suffering from down syndrome (Dalgliesh *et al*., 2001) [10]. Pre-eclampsia, a condition that only affects pregnant women is a major factor in maternal and foetal morbidity. Inhibin A concentrations in maternal serum were higher in pre-eclamptic mothers than in controls (Aquilina *et al*., 1999) [2]. Studies looking into the cause of the elevation in serum inhibin A levels in pre-eclampsia have discovered an increased expression of inhibin and inhibin subunit genes as well as proteins in placenta (Casagrandi *et al*., 2003; Bersinger *et al*., 2002) [8, 4]. Florio *et al*., (2002) [14] reported levels of high serum inhibin in patients with the hydatidiform mole suggesting that inhibins can be used as reliable tumor markers. Welt *et al*., (1997) [40] reported estimation of inhibins is useful in premature ovarian failure, POCS and hypothalamic amenorrhoea (Petraglia *et al*., 1991) [32]. In ovarian aging and the menopause transition inhibin B has been found to be a reliable marker.

Tumor Diagnosis

Inhibin used as a marker for ovarian cancer clinically which serves the significant advances in the reproductive disorders. Females with epithelial ovarian cancer and granulosa cell tumours have dimeric inhibin A and B, as well as the inhibin A subunit, in their serum. In mares, GTCT release substantial amounts of inhibin, evaluation of which may be helpful in identifying and confirming GTCT.

Estimation of Inhibin

The inhibin bioassays use disseminated cultured rat or sheep anterior pituitary cells are the most popular. Particularly the sheep assay system is sensitive enough to be used for determining the amounts of circulating inhibin. Additionally, the identification of follistatin (FSH-suppressing drugs) has been made possible thanks to bioassays. Characterization of inhibin and related compounds produced through recombinant technologies now heavily rely on bioassays based on the reduction of circulating FSH levels in animal species such as sheep and rats.

Radioimmunoassay (RIA)

Inhibin can also be estimated by Radioimmunoassay. Schneyer *et al*., 1990 [36] developed radioimmunoassays and “the Monash assay” provides a lot of knowledge into reproductive physiology. Monash assay is unable to distinguish between dimeric bioactive inhibin forms and other forms of free subunit A that circulate in 20-fold excess. However, it use a polyclonal antibody produced against 31 kDa bovine inhibin with antibody epitopes on the inhibin A subunit. Several researchers use mono- and poly-clonal antibodies with the goal of developing two-site immunoassays to precisely test the bioactive inhibin (Poncelet and Franchimont, 1994) [33].

The first inhibin RIAs used heterologous systems with very pure 31 kDa bovine inhibin as the tracer while 58 and 31 kDa natural bovine inhibin as antigens. The currently utilised heterologous assay (with antiserum 1989) has been used to evaluate inhibin in a variety of animals using species-specific standards. In addition to assay techniques that use native bovine or porcine inhibin as antigen, the synthesis and application of synthetic peptide immunogens, notably those derived from the first 26 amino acids of the inhibin LYC subunit, have been documented. These techniques have been used to explore the physiology of inhibin in animals in particular. All of these assays may exhibit various degrees of cross reactivity with inhibin a-subunit related peptides and proteins (Burger *et al*., 2001) [7].

Elisa

Inhibin is a weak immunogen and because of the excellent structural conservation between the species, antibodies thus

produced have a low affinity. Using a panel of monoclonal antibodies to synthetic peptide immunogens, ultrasensitive ELISA for inhibin A, inhibin B and the inhibin pro-alpha C were developed recently. The hydrogen peroxide sample pre-treatment process employed in the inhibin A and inhibin B assays that oxidises the residues of methionine in the B subunits, considerably improving the assay's sensitivity. To increase specificity, inhibin B assay samples are heated with a sodium dodecyl sulphate solution. This permanently breaks down activin-follistatin complexes, removing the impact of heterophil antibodies. Assessment of serum concentration of other members of inhibin family necessitates very high specificity and sensitivity as low as 5.0 pg/mL can be detected. The Groome ELISA, which is currently accessible, provides precise and reproducible data for use in reproductive research.

Conclusion

Inhibins are multifunctional molecules that influence pituitary follicle stimulating hormone secretion. They are gonad-produced glycoprotein hormones that regulate the pituitary gland's release of FSH. During the estrous cycle, inhibitins and FSH have a mutually beneficial relationship. Immunisation against inhibin promotes follicular growth and FSH release, resulting in an increase in ovulation frequency. Exogenous gonadotropins may no longer be required to stimulate fertility as a result of studies on inhibin's ability to increase ovulation rate and fecundity in animals. Inhibin as a marker in various ovarian and pregnancy - associated functions opens the door to further research and study.

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