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Inhibin: A Role for Fecundity Augmentation in Farm Animals

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ABSTRACT

Inhibin is a non-steroidal glycoprotein hormone of gonadal origin with major action as negative feedback control of the production of Follicle Stimulating Hormone (FSH) by anterior pituitary gland which in turn modulates male and female reproductive functions. Its physiological role has led to the development of inhibin based immunogens for fertility enhancement in farm animals. It is envisaged that a reduction of endogenous inhibin secretion would increase FSH concentrations and thus offers a potential for increasing the number of ovulatory follicles in the ovary. Immunization against inhibin has been reported to be a useful method for inducing multiple ovulations in farm animals. Inhibins play important roles in the regulation of fertility based on their dual inhibitory action on the process of folliculogenesis in the ovary and FSH secretion by the pituitary. Inhibins are also recognized as paracrine ovarian and testicular regulators and have multiple paracrine effects in the utero-placental unit, representing a promising marker for male and female infertility, gynecological and gestational diseases.

Key words: Inhibin, fertility, reproduction, immunization, animals

INTRODUCTION

Livestock plays an important role in Indian economy and productivity in farm species is controlled by many factors. Reproduction is essential for the continuation of the farm species and for most of the life form itself. In biological terms, living and reproducing are essentially one and the same. Reproduction is a complex process, affected by environmental factors and involving a series of physiological events that are properly timed and are supported by endocrine system, through production of several hormones. Environmental factors significantly influence the gestation duration (Meliani *et al.*, 2011). Rizwan *et al.* (2005) in a short term study found significant differences for LH, FSH, progesterone and estradiol due to occupational exposure to pesticides. The cyclic reproduction pattern is controlled by a finely orchestrated balance and interplay between the hormones of the hypothalamus (GnRH), anterior pituitary (LH, FSH) and gonads (estrogen, testosterone and inhibin), which is classically referred to as the hypothalamo-hypophyseal-gonadal axis. Many hormones work in conjunction with each other to regulate reproductive system in farm animals. Inhibin is a non-steroidal glycoprotein hormone of gonadal origin which selectively inhibits the production of follicle stimulating hormone (FSH) by anterior pituitary gland. Inhibin molecule is a heterodimer composed of two dissimilar subunits of 134 and 116 amino acid residues which are designated as alpha and beta, respectively. Dimerization of these subunits leads to two forms of inhibin: inhibin-A and inhibin-B in which a common alpha

subunit is either linked to beta-A or beta-B ($\alpha\beta_A$ = Inhibin-A, $\alpha\beta_B$ = Inhibin-B) produced primarily by gonads, which are processed into a diverse array of different molecular mass $\alpha\beta$ dimers (inhibins) and free α and β subunits. Like other members of the transforming growth factor- β gene family, they undergo processing from larger precursor molecules as well as assembly into functional dimers. The concept of inhibin emerged from the studies showing that castration cells appear in the pituitary following damage to seminiferous tubules. McCullagh (1932) first described inhibin in the 1930s and proposed the term to denote the activity of an aqueous extract of the testis that has the capacity to suppress castration cell formation in the anterior pituitary gland. A major breakthrough came, when Frank De Jong and Richard Sharpe in the MRC Reproductive Biology Unit in Edinburgh, UK discovered that fluid from cow ovarian follicles contained high amounts of a substance that suppressed FSH secretion (De Jong and Sharpe, 1976). This provided the source for the eventual purification of inhibin by groups in Australia, Japan and the USA (Miyamoto *et al.*, 1985).

The function of inhibin in the control of FSH secretion suggests that it is an important component of many hormones that control the mammalian reproductive cycle. Accordingly, roles suggested for inhibin in farm animals include maintenance of an appropriate FSH/LH ratio triggering of the secondary surge of FSH in late proestrus and control of folliculogenesis (Hillier, 1991). Apart from their essential role in the selective control of FSH secretion, inhibins are currently recognized as paracrine ovarian and testicular regulators and have multiple paracrine effects in the utero-placental unit, representing a promising marker for male and female infertility, gynecological and gestational diseases. Therefore, in addition to its endocrine role, inhibin may also act as a paracrine/autocrine regulator of gonadal functions. Price *et al.* (1987) reported that the dimers comprised of inhibin subunits possess diverse functions and may act as growth/differentiation factor as well as hormone.

Optimization of reproduction in females requires control of seasonality and litter size. In species like sheep, goat and cattle, ovulation rate is the major factor limiting the number of young, while uterine capacity is limiting in sows and mares. Hence, techniques such as hormone treatments and GnRH injection (Kalaba and Abdel-Kahlek, 2011; Gharbi *et al.*, 2012), whereby ovulation rate can be increased in amounts compatible with uterine capacity and are valuable tools to maximize offspring numbers from naturally cycling animals. Various methods employing hCG, GnRH, PMSG, interferon and supplementing Omega-3 fatty acids have been tried using different preparations in dairy animals for improving pregnancy rates by reducing embryonic mortality (Bajaj and Sharma, 2011). Research has been directed towards developing inhibin immunization treatment for commercial use in both sheep and cattle (Voglmayr *et al.*, 1992). Researchers were able to demonstrate that ewes could respond to an inhibin vaccine with a sustained (at least 3 years) antibody response and a recurrent increase in litter size. Different types of inhibin based immunogens were used by several workers viz. follicular fluid (steroid free), native/purified inhibin, synthetic inhibin- α peptides and recombinant inhibin α -subunits (Findlay *et al.*, 1993). Bovine follicular fluid in sheep and ovine FF in sheep (Mann *et al.*, 1993; Tannetta *et al.*, 1998) and in cattle have been used as an immunogen to achieve higher FSH levels and higher ovulation rate. Synthetic peptides based on amino acid sequence of immunogenic region of inhibin are proved to be potent immunogens. Synthetic peptides based on bovine inhibin (bI α (1-29) Tyr30) were used to achieve higher ovulation rates in sheep (O'Shea *et al.*, 1994) and in cattle (Glencross *et al.*, 1994). Porcine inhibin- α (1-26) in goat (Medan *et al.*, 2003a) and in cattle (Medan *et al.*, 2005) has been used with positive results. Recently synthetic peptides have been used to increase sperm production in rams (Voge and Wheaton, 2007). A three to five fold increase in ovulation rate was

observed in ewes immunized against recombinant bovine and human inhibin alpha subunit fusion proteins (Findlay *et al.*, 1993). Active immunization by recombinant alpha subunit has been demonstrated to increase the ovulation rate up to 2-4 folds in sheep (Forage *et al.*, 1987). Isolation of inhibin from natural sources can only produce limited quantities of bioactive protein. To purify large-scale quantities of recombinant inhibin, recombinant DNA technology provides an eminent tool with production of large amount of recombinant protein in suitable host cell.

INHIBIN FAMILY AND STRUCTURE

Inhibin family comprised of three non-steroidal glycoprotein hormones: Inhibin, activin and follistatin. Inhibins and activins belong to the transforming growth factor- β (TGF- β) superfamily, which mediates embryonic growth and development (Kingsley, 1994). The TGF- β superfamily contains molecules that encompass diverse functions during embryogenesis and adult tissue homeostasis. TGF-beta ligands are initially synthesized as precursor proteins that undergo proteolytic cleavage. The mature segments form dimers via disulfide links, which serve as the active molecule. The subunits (alpha, beta A and beta B) can be processed in several ways to produce different isoforms. Each subunit is produced from a separate gene and is produced as a large precursor protein (Pangas and Woodruff, 2002). Each subunit has multiple cleavage sites, such that subunits of different size are routinely found in follicular fluid (Sugino *et al.*, 1992). The free alpha subunit is often found in higher molar levels than the dimer in many biological fluids (Knight *et al.*, 1989). Various combinations of alpha-beta subunits appear possible, giving rise to a number of different dimeric inhibin forms. Commonly found forms in bovine follicular fluid are approx. 29, 34, 48, 58, 68, 77, 122 and >160 kDa (Ireland *et al.*, 1994). Ganguly *et al.* (2010) reported two major forms of inhibin in buffalo follicular fluid with a little proteolytic cleavage/processing of the larger precursor. Within the TGF- β superfamily, the agonist-antagonist relationship between activin and inhibin is unique and critical to integrated reproductive function. Activin acts at the pituitary to stimulate follicle-stimulating hormone (FSH) and is antagonized by endocrine acting gonadally-derived inhibin (Mather *et al.*, 1992).

Activin only has inhibin-beta subunits: Activin-A has two beta-A subunits; activin-B has two beta-B subunits; Activin-AB has beta-A and beta-B subunits. These beta subunits are expressed in the same cells as inhibin beta subunits. Activin stimulates FSH and LH production and/or secretion from the pituitary gland (DePaolo *et al.*, 1991).

Follistatin (FS) is not a member of the TGF- β superfamily. It exists in two monomeric forms of 288 and 315 kDa. FS was discovered in gonadal extracts as an inhibitor of FSH secretion from pituitary gonadotrophs. Follistatin suppresses FSH by irreversible binding and neutralization of activins (Vale *et al.*, 1988) (Fig. 1).

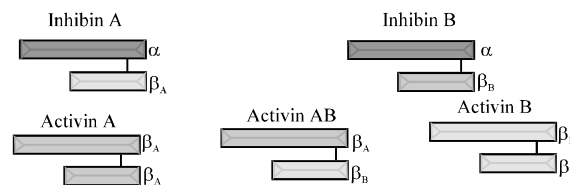


Fig. 1: Various molecular forms of inhibins and activins

PHYSIOLOGICAL/BIOLOGICAL ROLE

In mammals, inhibin is predominately expressed in the testis [sertoli cells] and ovary [granulosa and theca cells] (Robertson *et al.*, 1991; Krummen *et al.*, 1994). Ovarian follicular fluid (FF) is a rich source of inhibin. The physiological role of inhibin in reproduction i.e., regulation of folliculogenesis in females and spermatogenesis in males, can be classified into three types- endocrine, paracrine and autocrine (Findlay, 1993; Knight and Glistler, 2001). Inhibin has been shown to exert a negative feedback effect on follicle-stimulating hormone (FSH) at the pituitary gland (Schwartz and Channing, 1977) and to affect gonadal function (Findlay *et al.*, 1993). Apart from their essential role in the selective control of FSH secretion, inhibins are also recognized as paracrine ovarian and testicular regulators and have multiple paracrine effects in the utero-placental unit, representing a promising marker for male and female infertility, gynecological and gestational diseases. *In vitro* studies have indicated two separate mechanisms of action of inhibin on FSH secretion: at low concentrations, inhibin suppressed FSH release and synthesis while at higher concentrations, the pituitary gonadotroph content of both FSH and LH is reduced due to degradation of intracellular stores of these hormones (Burger, 1992). Various studies indicate that inhibin regulates long term mean levels by producing an overall damping effect.

Physiological role in females: The role of inhibin as an important component in the feedback regulation of FSH secretion from anterior pituitary and thus in folliculogenesis is well established by various researchers.

Functional relationships among FSH, follicles and inhibin and regulation of ovarian follicular development: FSH is the key hormone inducing the recruitment and growth of ovarian follicles. An association between a FSH surge and recruitment of follicles has been demonstrated by various workers in the course of development of reproductive biology (Kaneko *et al.*, 1995; Medan *et al.*, 2005, 2003a). During the estrous cycle of cows (Kaneko *et al.*, 1995, 2002) and goats (Medan *et al.*, 2003a; Medan *et al.*, 2005), periodic fluctuations of FSH concentrations are responsible for the regular emergence of follicular waves (Fig. 2). The rate of reduction in FSH concentrations is directly related to the number of growing cohort follicles. Thus, secretion of inhibins from the growing cohort follicles into the systemic circulation is probably responsible for the decline of FSH, indirectly causing their own atresia (Medan *et al.*, 2007). FSH also stimulates the production of inhibin and follistatin.

Various experiments confirm the involvement of inhibin in the regulation of FSH secretion during the growth phase of the dominant follicle in the early luteal phase in females. Inhibin neutralization during the early luteal phase, produces hypersecretion of FSH with an associated stimulation of follicular development, indicating that inhibin is an important factor for the negative regulation of FSH secretion during the early luteal phase when secretion of estradiol and progesterone are normally high (Hillier, 1991). By mid follicular phase, in the follicle responding most rapidly and extensively to FSH, granulosa cell aromatase activity, inhibin production and LH receptor expression, rise to critical levels. Since aromatase activity and inhibin production are coupled to the LH receptor, this follicle is selected and becomes destined to ovulate. During the second half of follicular phase, inhibin production in the preovulatory follicle continues to increase, paralleling the aromatase activity and estrogen secretion (Hillier, 1991). After induction of luteolysis, FSH stimulates follicular growth resulting in an increase in inhibin and estradiol-17 β

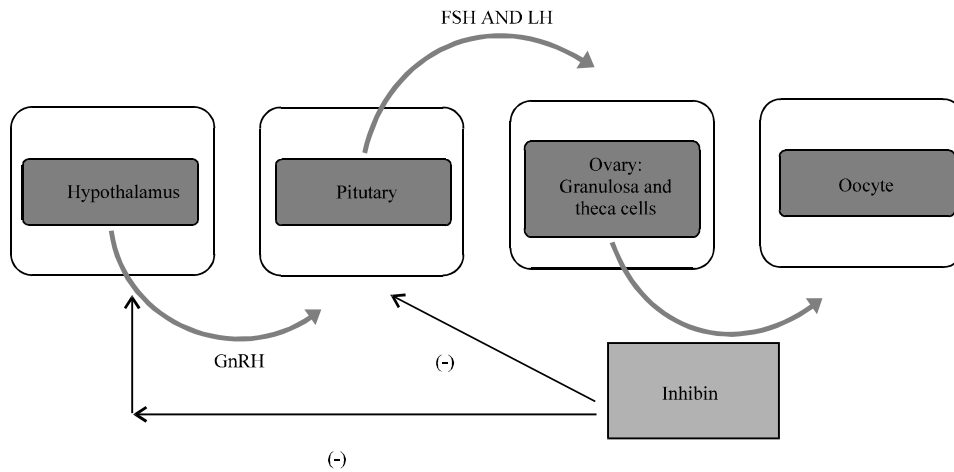


Fig. 2: Hypothalamo-hypophyseal axis-role of hormones

secretion by the dominant follicle, leading to reduced peripheral FSH levels. This results in suppression of subordinate follicles from the same wave of recruited follicles.

Secretion of inhibins during the estrous cycle: Inhibins are produced by follicles as they develop from small antral to preovulatory stages (Medan *et al.*, 2007). Follicle turnover continues throughout estrous cycle in a wave-like pattern in domestic animals, such as cattle (Welt *et al.*, 1997; Kaneko *et al.*, 1995), mares (Buratini *et al.*, 1997), sheep (Bartlewski *et al.*, 1999) and goats (Medan *et al.*, 2003b; Medan *et al.*, 2005). The emergence of each follicular wave is preceded by an elevation in FSH secretion. Circulating FSH levels decline after the selection of the dominant follicle(s) through a negative feedback effect of the inhibins produced by the developing follicles. In the golden hamster, Ohshima *et al.* (1999) found that inhibin A increased from the early morning of day 1 (day 1 = Day of ovulation) and reached plateau levels on day 2, being followed by an abrupt increase at the time of LH surge on day 4. However, inhibin B increased on day 1 and declined on day 4. These differential patterns of inhibin A and B secretion in the hamster were also observed after induced atresia and subsequent follicular development (Ohshima *et al.*, 2002). These findings suggest that the dominant form of dimeric inhibins may shift from inhibin B to inhibin A with follicular development. Also, the distinct patterns of inhibin A and inhibin B secretion suggest the unique regulation of inhibin A and inhibin B by gonadotropins and the stage of follicular development. These differences in regulation are apparent when comparing stimulation of secretion *in vivo* and *in vitro*. *In vivo*, FSH readily stimulates both inhibin A and inhibin B secretion in the early follicular phase, when small antral follicles are present (Welt *et al.*, 1997; Welt and Schneyer, 2001). In contrast, while both FSH and cyclic adenosine monophosphate (cAMP) stimulate inhibin A secretion from the granulosa cells of small antral follicles *in vitro*, neither stimulates inhibin B. Furthermore, both FSH and LH stimulate inhibin A from the preovulatory follicle but neither stimulates inhibin B *in vivo* (Welt *et al.*, 1999, 2001). Consistent with the hypothesis that FSH stimulates inhibin A but not inhibin B, inhibin A increases in follicular fluid with increasing follicle maturity, whereas inhibin B does not (Schneyer *et al.*, 2000).

In cattle (Kaneko *et al.*, 2002) and goats (Medan *et al.*, 2003b; Medan *et al.*, 2005) an inverse relationship between FSH and inhibin A was demonstrated, indicating the key role of inhibin A produced by dominant follicle(s) in terminating the transient peaks of FSH secretion. There was

a highly significant inverse correlation between inhibin A and FSH and a highly significant positive correlation between inhibin A and estradiol-17 β as the mean concentrations of estradiol-17 β increased during the follicular phase and reached a peak 2 days before ovulation. These findings clearly indicate that follicles increase the secretion of inhibin A as well as estradiol-17 β during final maturation in small ruminants. Moreover, there is a unique phenomenon in the secretion of inhibin, the so-called ovulatory inhibin surge, during the estrous cycle in farm animals such as mares, goats etc. Circulating inhibins (ir-inhibin, inhibin pro- α C and inhibin A) but not estradiol-17 β sharply increases on the day of ovulation in mares (Nambo *et al.*, 2002). These findings suggest that circulating inhibins may be useful for determining the time of ovulation in the farm animals.

Inhibins and activins during pregnancy: Circulating levels of inhibin and activin during pregnancy have been reported in human and non-human primates. Also, both inhibin β_A and inhibin β_B subunit mRNAs and associated proteins have been identified in placentas, deciduas and fetal membranes (Kondo *et al.*, 2003). Placentas and fetal membranes in humans (Florio *et al.*, 2001) and non-human primates (Kondo *et al.*, 2003) are major sites of the production and secretion of inhibin and activin in maternal serum and amniotic fluid. The levels of inhibin A and activin A significantly decreased after the removal of the feto-placental unit (Muttukrishna *et al.*, 1997). There are a few reports on the secretion of inhibins and activins from the placenta in species other than primates. In the rat and hamster, ovarian follicles but not placentas are major source of circulating inhibin, whereas the placenta secretes a large amount of activin A in the hamster (Ohshima *et al.*, 2002). On the other hand, equine female ovaries and testes secrete large amounts of inhibin pro- α C and inhibin A but not activin during late pregnancy (Ohshima *et al.*, 2002; Kondo *et al.*, 2003).

Physiological role in males: Physiological inhibin production by the adult testis requires a normal population of sertoli cells, FSH stimulation and spermatogenesis to be present. The two later factors are not absolutely necessary for a basal inhibin B release, which is seen in some forms of hypogonadism, impaired spermatogenesis (Foresta *et al.*, 1999). Not only is the contribution of leydig cells to the circulating inhibin negligible (Anderson *et al.*, 1998), but the LH effect on inhibin release also appears to be inhibitory rather than stimulatory (Ramaswamy and Plant, 2001). In humans, among infertile males with elevated FSH levels, the FSH concentration is inversely correlated with inhibin B but not with pro- α C inhibin, suggesting that the physiologically important hormone that exerts potential negative feedback upon FSH secretion is inhibin B (Illingworth *et al.*, 1996). In male primates (Illingworth *et al.*, 1996) and rodents (Woodruff *et al.*, 1996; Jin *et al.*, 2001), inhibin B is known or believed to be the primary dimeric form in the blood circulation. Inhibin concentration in rams is increased in late fetal life and in the first few months after birth (Sanford *et al.*, 2000) when sertoli cells are completing proliferation. A comparatively small increase in inhibin occurs in mature rams during seasonal testicular recrudescence (Sanford *et al.*, 2000). Inhibin is involved in suppression of FSH secretion in late fetal life, during puberty and in adulthood (Tilbrook *et al.*, 1999). Furthermore, dimeric inhibin and forms of the α -subunit of inhibin may act as paracrine/ autocrine regulators in the testis (Chong *et al.*, 2000). In rams, FSH administration increases inhibin production by fetal testis and the output of inhibin into the testicular lymphatic and vascular systems in adults (Voglmayr *et al.*, 1992). Corresponding increases in FSH, immunoactive inhibin B (Sanford *et al.*, 2000) and inhibin A (Lincoln *et al.*, 2001) during testicular redevelopment and greater numbers of FSH receptors, in the testis in the

breeding season compared with the non-breeding season, provide additional evidence for FSH regulation of inhibin secretion in rams. However, the mode of FSH action (altered secretion versus number of receptors) and the regulation of gene expression for FSH receptors as testes pass through the different stages of the seasonal cycle are poorly understood. Inhibin secretion by sertoli cells may also be regulated by paracrine mechanisms acting within the testis. Sertoli cells in rodent species and human males have androgen receptors and normal cell function requires both testosterone and FSH (Baird and Smith, 1993). Thus, testosterone from neighboring leydig cells is a candidate for inhibin regulation. An effect of testosterone may also be mediated indirectly by other androgen target cells such as peritubular myoid cells. Other studies concerned mainly with sertoli cells in culture denote conflicting indications as to whether testosterone stimulates or inhibits inhibin secretion. Sertoli cells and peritubular cells in ram testis contain androgen receptors and seasonal variations in blood inhibin and testosterone concentrations are positively correlated (Sanford *et al.*, 1993). Razie *et al.* (2011) studied the inter-relationship between the level of inhibin B and ultra-structure of sertoli cells in contra-lateral testis after unilateral blunt testis trauma in rats and suggested that UTT affected the contra-lateral testis and levels of inhibin B in serum reflected the spermatogenetics and other functions of sertoli cells.

IMPORTANCE OF INHIBIN IN REPRODUCTION MANIPULATION

The combination of equine chorionic gonadotropin and human chorionic gonadotropin has been a common method to induce superovulation in farm animals (Rahman *et al.*, 2008; Moeini *et al.*, 2009) except equines. The pattern of folliculogenesis, eCG and progesterone profile was studied in cyclic, pregnant and irregular cyclic equine mares (Pal and Gupta, 2005; Meenakshi *et al.*, 2006, 2008; Bansal *et al.*, 2006, 2009). It has been demonstrated by various workers that uterine capacity is the major limiting factor for increasing the number of offspring in equines. The assisted reproductive technologies in Farm animals have also contributed significantly in increasing the productivity (Sejian *et al.*, 2010). Inhibin acts directly on pituitary to inhibit FSH synthesis and secretion. A reduction of endogenous inhibin secretion would increase FSH concentrations and thus offers a potential for increasing the number of ovulatory follicles in the ovary. Thus inhibin mediated pathway also found to be potential alternate methodology for superovulation (Findlay *et al.*, 1993; Palta, 1998; Medan *et al.*, 2007).

Practical uses of inhibin as fecundity augmenting agent: Inhibins act as chemical signals to the pituitary gland on the number of growing follicles in the ovary. Inhibins reduce the secretion of FSH to a level, which maintains the species-specific number of ovulation in both single and litter bearing species. By inhibiting FSH release without altering LH release, inhibins may be partly responsible for the differential release of FSH and LH from the pituitary (Hafez and Hafez, 2000). Inhibin in the form of steroid-free follicular fluid preparation or purified bovine inhibin results in the specific suppression of plasma concentrations of FSH in the ewes. This suppression of FSH is associated with a failure of growth of large preovulatory follicles greater than 2.5 mm diameter. On cessation of FF treatment, a rebound release of FSH results in an increase in ovulation rate in the subsequent cycle. These effects are mediated by the direct inhibitory effect of inhibin on the secretion of FSH from pituitary. However, the follicular fluid contains not only inhibin but also many other proteins that could be immunoreactive and the results were variable and inconsistent. Bovine follicular fluid in sheep (Cumminst *et al.*, 1986) and ovine FF in sheep (McNeilly, 1984; McNeilly *et al.*, 1991; Tannetta *et al.*, 1998) and in cattle (Price *et al.*, 1987; Bindon *et al.*, 1988) have been used as an immunogen to achieve higher FSH levels and higher ovulation rate. In early

days, native inhibin, purified from follicular fluid, has also been used; bovine inhibin in ewes (Henderson *et al.*, 1984), sheep inhibin in cow (Price *et al.*, 1987) and ovine inhibin in cow (O'Shea *et al.*, 1994) resulted in higher FSH levels and ovulation rate. Purification of inhibin from follicular fluid involved a costly and cumbersome process; moreover, purity or immunoreactivity may be less in these cases as compared to synthetic peptides and recombinant α -inhibin proteins.

Immunization of ewes against recombinant bovine inhibin α -subunit (expressed in prokaryotic system) resulted in increased basal levels of FSH in the luteal phase and GnRH-stimulated concentrations of FSH in the follicular phase of the oestrous cycle (Findlay *et al.*, 1989), though LH levels were unchanged and neither gonadotrophin was affected by immunization during anoestrous. Unconjugated α - β I as an immunogen elicited a strong immune response, reflected by the presence of antibodies in the ewes capable of recognizing native inhibin. It implies that the epitopes present in the non-glycosylated α -subunit expressed by the prokaryotes are also present in the native molecule, presumably on the glycosylated α -subunit. Increase in ovulation rate of three fold were achieved in sheep immunized with a recombinant fusion protein of α - β I-(165-300 amino acid) with an N-terminal extension of 20 amino acid of β -galactosidase (Forage *et al.*, 1987), whilst use of a recombinant human α -inhibin to immunize Rambouillet ewes led to ovulation rates 4-6 times greater than in controls (Mizumachi *et al.*, 1990). The latter study also reported a concomitant enhancement of both pre- and postovulatory levels of FSH, but not LH, the postovulatory surge also persisting for longer periods of time in immunized animals. In guinea pigs, immunized with recombinant ovine alpha inhibin, Shi *et al.* (2000a) observed increase in ovulation rate in a dose dependent manner and suggested that immunization results in the production of circulating antibodies that are able to bind to endogenous native guinea pig inhibin. Use of recombinant bINH- α to immunize gilts (Brown *et al.*, 1990) led to rise in mean ovulation rates from 12 to over 16, the number of ovulations being highly correlated with levels of inhibin antibodies present in the serum. The cloning and heterologous expression of Indian Sahiwal cattle (*Bos indicus*) bINH- α (bovine alpha inhibin) encoding gene was successfully done in *E. coli* and the purified recombinant bINH- α was characterized (Bhardwaj *et al.*, 2007; Bhardwaj, 2008). Recombinant bINH- α (25 $\mu\text{g mL}^{-1}$) immunized guinea pigs had a significant increase in litter size compared to control group. These results also indicated a role for recombinant bovine alpha inhibin as a fecundity vaccine to enhance the ovulation rate and litter size in animals (Bhardwaj *et al.*, 2006, 2012).

A chimeric recombinant ovalbumin/inhibin (ovalin) vaccine was prepared by Geary and Reeves, 1996. Rabbits were immunized subcutaneously against crude ovalin (300 μg of antigen) and significant antibody titers were observed against ovalbumin and inhibin. Similarly, a novel chimeric antigen (Sewani *et al.*, 1998) consisting of the non-toxic B subunit (EtxB) of an *E. coli* enterotoxin and the first 14 N-terminal amino acid residues of the C-terminal portion of the α -subunit of bovine inhibin (bINH¹⁻¹⁴) was produced in prokaryotic host. Rabbits immunized subcutaneously with EtxB::bINH¹⁻¹⁴ developed significant titers of antibodies that recognized an inhibin peptide fragment containing bINH¹⁻¹⁴, native inhibins and EtxB during separate enzyme-linked immunosorbent assay (ELISA). Passive immunization of mice with the rabbit anti-EtxB::bINH¹⁻¹⁴ serum increased concentrations of follicle-stimulating hormone (FSH) in serum twofold compared with controls, whereas serum concentrations of luteinizing hormone (LH) were unaltered.

The effect of selective immunosuppression of endogenous inhibin in goats on FSH, LH, progesterone and estradiol-17 β profiles during the breeding and non-breeding seasons by immunization against the recombinant human inhibin α -subunit (hINH- α) was studied by Hennies *et al.* (2001). They found that, mean basal concentrations of FSH were not affected by

immunosuppression of endogenous inhibin, nor was there a difference in the amplitude of the pre-ovulatory FSH surge. By contrast, concentrations of circulating estradiol were significantly elevated after immunization. Extending immunization into the anoestrous season by a booster injection of hINH- α , implicating oestrus induction with a progestogen and eCG, produced no discernible differences in FSH and LH profiles in comparison with non-immunized control goats. Their findings suggest that, paracrine factors may play a more significant role in controlling follicular activity than a feedback mechanism acting via the pituitary. In one study, Findlay *et al.* (1993) reported that active immunization against a fusion protein consisting of amino acid 1-166 of the alpha subunit (α N fragment) of bovine inhibin led to impairment of fertility in sheep in terms of lambs born. This could not be attributed to changes in circulating inhibin, progesterone or gonadotrophin levels and the number of corpora lutea were greater than control animals. However, corpora lutea had the appearance of luteinized unruptured follicles and egg recovery was significantly lower, thereby indicating ovulation failure in these animals and implying a facilitatory role for α N fragment in the ovulatory process (Terqui *et al.*, 1995).

Immunization of ewes against an synthetic N-terminal fragment (1-29) of bovine inhibin α -subunit conjugated to tuberculin purified protein derivative resulted in a twofold rise in ovulation rate during the breeding season, 25% increase in FSH levels and 37% increase in lambs born. Although, conception and pregnancy rates and length of gestation were unaffected, lambs born to immunized ewes had lower birth weights and a higher proportion was stillborn. Thus, overall there was no significant increase in the number of viable lambs produced (Wrathall *et al.*, 1990). Kaneko *et al.* (2002) showed that a dominant follicle during luteal phase secreted inhibin and the peripheral concentration of inhibin increased according to the growth of dominant follicles in cows. The number of ovulations is reduced, when a superovulatory treatment is initiated in the presence of a functional dominant follicle and removing the dominant follicle improves the superovulatory response. Inhibin suppresses the development of FSH sensitive follicles or the response to superovulatory treatment by inhibiting the secretion of endogenous FSH. Active Immunization of goats (Medan *et al.*, 2003b) and cattle (Takedomi *et al.*, 2005) against inhibin (porcine α N-1-26 conjugated to rabbit serum albumin) resulted in significant increase in ovulation rate. These results have attributed to the stimulatory effect of inhibin immunoneutralisation on the ovary, either endocrinologically, by removing the inhibitory effect of inhibin on FSH secretion or locally, through a paracrine effect and finally enhancing ovarian follicular development and increasing the number of growing follicles.

On the other hand, passive immunization against inhibin also increased the ovulation rate through elevated FSH secretion in mice (Medan *et al.*, 2004a), hamsters (Kishi *et al.*, 1997), rats (Rivier and Vale, 1989), guinea pigs (Shi *et al.*, 2000b), ewes (Mann *et al.*, 1993), goats (Medan *et al.*, 2003b, 2004b), cows (Akagi *et al.*, 1997; Takedomi *et al.*, 1997) and mares (Nambo *et al.*, 1998). In cattle, i.v., injections of 25, 37.5 and 50 mL of inhibin antiserum (against inhibin produced in a castrated male goat) were given and multiple ovulations (2-4) were recorded in all animals after injection of 50 mL inhibin antiserum, however all cows in 25 mL group experienced only one ovulation and injection of 37.5 mL resulted in a variable number of ovulations (1-5) (Akagi *et al.*, 1997). In rats, i.p., injection of inhibin antiserum (obtained from a castrated goat immunized against Tyr30-porcine inhibin- α (1-30)-NH₂ conjugated to rabbit serum albumin) at doses- 50, 100, 200, 400 μ L was administered and higher ovulation rate was achieved in dose dependent manner (Wang *et al.*, 2001). The rate of blastocyst development for animals treated with

50-200 μ L inhibin antisera was significantly higher than control animals (Wang *et al.*, 2001). The oocytes induced to superovulate using immunization against endogenous inhibin have normal developmental competence (Medan *et al.*, 2007).

In another study, two experiments were conducted to induce superovulation in goats using passive and active immunization against inhibin (Sasaki *et al.*, 2006). There was a significant increase in plasma FSH concentration compared with the controls. The numbers of follicles in passively and actively immunized goats were significantly greater than those in the controls. In addition, the ovulation rate was greater in the immunized animals compared with the controls. Therefore, either passive or active immunization against inhibin could be used to induce superovulation.

In males, immunization against inhibin immunogens increases FSH and testis functions (Terqui *et al.*, 1995). In rams, immunization against inhibin increased testis diameter and daily sperm output in one study (Al-Obaidi *et al.*, 1987), whereas body weight, scrotal circumference and plasma FSH were unaffected in another (Wheaton and Godfrey, 2003). However, in the latter study, the age at puberty was delayed and Luteinizing Hormone (LH) and testosterone were reduced, in the inhibin-immunized lambs. In rams, immunomodulation of inhibin led to delayed, amplified and extended season related increases in serum gonadotropins (Voglmayr *et al.*, 1990). Al-Obaidi *et al.* (1987) observed significant rise in both testis diameter and daily sperm production in rams after inhibin immunization. Whereas, in bulls, Kaneko *et al.* (2002) found levels of plasma immunoreactive inhibin and FSH to be relatively low for the first few weeks of life and then to rise steadily thereafter, treatment at a young age with inhibin antiserum (passive immunization) produced elevations in plasma FSH without affecting. Schanbacher (1991) showed enhancements in serum FSH and testicular sperm density, although testes and epididymides weight, daily sperm production and blood levels of LH and testosterone were not altered. Similarly, Martin *et al.* (1991) found active immunization against inhibin to increase serum levels of FSH but ineffective in altering testicular growth (measured as scrotal circumference). However, unlike Schanbacher (1991), Martin *et al.* (1991) found inhibin immunoneutralization increased circulating concentrations of testosterone and daily sperm production along with a decrease in blood levels of LH. In another study, Holstein bulls (Bame *et al.*, 1999) were actively immunized against bovine inhibin α^{1-26} gly-tyr (bINH) conjugated to human alpha globulin and enhancement of sperm output after immunization against inhibin was observed. Satterlee *et al.* (2006) found that, injection of quail and breeder hens with a recombinant protein antigen (MBP-cINA521)-a fusion of the bacterial maltose-binding protein (MBP) and a fragment of the α -subunit of chicken inhibin (cINA521) accelerate puberty and enhances lay. The gonadal hormone inhibin regulates daily sperm production (DSP) indirectly through negative feedback control of FSH secretion and may also affect DSP via direct actions within the testis (Voge and Wheaton, 2007).

Hence, immunization against inhibin in the males and females increases FSH secretion with no significant changes in LH. However, in addition to the classical endocrine feedback role of inhibin, inhibin molecules also subserve local actions at the intragonadal. Such local actions may also be perturbed following inhibin immunization. Moreover, consideration must be given to the natural fecundity of the species or breeds to be immunized, since although technically feasible, there may be little to be gained by artificially raising litter size beyond the optimum. Maternal behaviour and milk production, for example, have to be adequate to raise extra litters. As with other approaches to increase fecundity (genetic selection, gonadotrophin treatment etc.), the increased ovulation rates resulting from inhibin immunization may also have a detrimental effect on perinatal survival owing to decreased birth weight.

Other clinical uses of inhibin

Inhibin measurements in ovarian and gestational disorders: One of the most important developments in the field of reproduction is the use of inhibin as an ovarian tumor marker. Inhibin α -subunit and dimeric inhibin A and B have been detected in the serum of females with granulosa cell tumors and epithelial ovarian cancers (Burger *et al.*, 2001). In farm animals, studies were conducted in mares only for the detection of tumors with inhibins. Granulosa theca cell tumors (GTCT) in the mare secrete high levels of ir-inhibin (Yoshida *et al.*, 2000; Bailey *et al.*, 2002); therefore measuring inhibins may be useful in diagnosing and confirming GTCT. Davis *et al.* (2005) reported that two cases of GTCT in mares displayed positive immunoreactivity for inhibin α -subunit, but were negative for inhibin β_A and β_B subunits. This indicates that the appearance of the inhibin α -subunit can be used as an immunohistochemical marker in the diagnosis of GTCT in the small ruminants.

Several reports have measured inhibins and activins in pregnancy disorders (studies yet limited to humans). Inhibin A and activin A levels were elevated in women carrying a child with Down syndrome (Dalglish *et al.*, 2001) and the levels of α , β_A mRNAs also increased in the placentas of Down syndrome pregnancies (Lambert-Messerlian *et al.*, 1998). Pre-eclampsia is a pregnancy specific disease that is a major cause of maternal and fetal morbidity. Inhibin A levels in the maternal serum of women who developed pre-eclampsia were higher than that of controls (Aquilina *et al.*, 1999). Studies investigating the source responsible for the rise in serum activin A and inhibin A in pre-eclampsia have reported an increased expression of inhibin α and inhibin/activin β subunit genes (Florio *et al.*, 2002; Casagrandi *et al.*, 2003) and proteins (Manuelpillai *et al.*, 2001; Bersinger *et al.*, 2002) in the placenta. Serum inhibin and activin levels were found to be high in patients with the hydatidiform mole (Florio *et al.*, 2002), suggesting that inhibins and activins can be used as reliable tumor markers. In addition, measurement of inhibins is useful in hypothalamic amenorrhoea (Welt *et al.*, 1997), polycystic ovarian syndrome (Lockwood *et al.*, 1998) and premature ovarian failure (Petraglia *et al.*, 1991). Inhibin B has been found to be a reliable marker in ovarian aging and the menopause transition (Sowers *et al.*, 2008).

CONCLUSION AND FUTURE PERSPECTIVES

Inhibins are multifunctional molecules involved in the control of pituitary FSH secretion. They are glycoprotein hormones produced in the gonads and are capable of regulating FSH secretion by the pituitary gland. Inhibins show a reciprocal relationship with FSH during estrous cycle. Immunization against inhibin enhances FSH secretion and follicular growth and finally increases the ovulation rate. The different studies on inhibin for increasing ovulation rate and fecundity in animals opens the possibility of eliminating the use of exogenous gonadotropins for fertility enhancement. The significance of inhibin as a marker in other pregnancy related and ovarian functions paves the way for further study and research.

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