

# Gnrh and its Applications in Bovine Reproduction

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## Abstract

The identification and sequencing of gonadotropin releasing hormone (GnRH) are comparatively recent events. The roles of gonadotropin in reproductive endocrinology had been recognized before 1950. The early 1960's the neuroendocrine roles of the hypothalamus in releasing and synthesizing LH were defined. Three types of GnRH are present- GnRH - I, GnRH - II, and GnRH - III. The regulation of both synthesis and the secretion of GnRH are affected by neurotransmitter system in the brain. GnRH secretory cells of hypothalamus are regulated by feedback effects of gonadal steroids (estrogen, progesterone) via intermediary neurons that possess the relevant steroids receptors. Two major intracellular pathways exist to control of the synthesis and secretion of LH (leutinising hormone) and FSH (follicle stimulating hormone) by GnRH. The first involves Ca<sup>++</sup> and participates in early GnRH stimulated LH release and in GnRH receptor regulation, whereas the second involves protein kinase and modulates gonadotropin synthesis and sustained secretion and activates receptors. GnRH analogues and GnRH agonists are also available commercially. Applications of GnRH are discussed in detail.

**Key words-** Gonadotropin releasing hormone, leutinising hormone, follicle stimulating hormone

## Introduction

Gonadotropin Releasing Hormone (GnRH) is a hypothalamic-releasing hormone chemically a straight chain decapeptide synthesized in the cell bodies of neurosecretory neurons located in the mediobasal hypothalamus and transported by their axons to circumscribed areas of the median eminence. The actions of GnRH lead to the physiological synthesis and release of gonadotropins, LH (leutinising hormone) and FSH (follicle stimulating hormone) and in turn control the process of gamatogenesis and steroidogenesis<sup>1</sup>. Plasma half-life of GnRH is short, 2-4 minutes<sup>2</sup>. GnRH has been found in central nervous

system of all species of vertebrates examined. Both of natural and synthetic decapeptides release LH and FSH and induce ovulation in all mammalian species so far examined. Passive immunization of a variety of mammalian species with an antiserum to GnRH reduces blood levels of both FSH and LH and blocks ovulation. Collectively, these observations indicate that the decapeptide represents the moiety responsible for the release of LH and FSH<sup>3</sup>.

The identification and sequencing of gonadotropin releasing hormone (GnRH) are comparatively recent events. The roles of gonadotropin in reproductive endocrinology had been recognized before 1950. The early 1960's that the neuroendocrine roles of the hypothalamus in releasing and synthesizing LH were defined<sup>4</sup>. Gonadotropin releasing hormone (GnRH), the central initiator of reproductive hormonal cascade was first isolated from mammalian hypothalamus as the decapeptide<sup>5,6</sup>. GnRH is processed in specialized neurons of the hypothalamus from a precursor polypeptide by enzymic processing and packaged in storage granules that are transported down axons to the external zone of median eminence<sup>7</sup>. Each GnRH pulse stimulates a pulse of LH but FSH pulses are less clear. The frequency of pulses is highest at ovulatory LH and lowest during the luteal phase of the ovarian cycle.

## Types of GnRH

Three types of GnRH are present

- (1) GnRH - I / mammalian GnRH
- (2) GnRH - II / chicken GnRH / midbrain GnRH
- (3) GnRH-III / telencephalic GnRH.

## GnRH-I

It was first isolated during the early 70s by Dr. A. Schally Dr. R. Gulliemann and Co-workers. There are about 14 structurally variant forms of hypothalamic GnRH that are known 16 regulate reproduction across a wide variety of organisms. These organisms range from yeast to mammals. All these forms are of classical 10 amino acid peptide.



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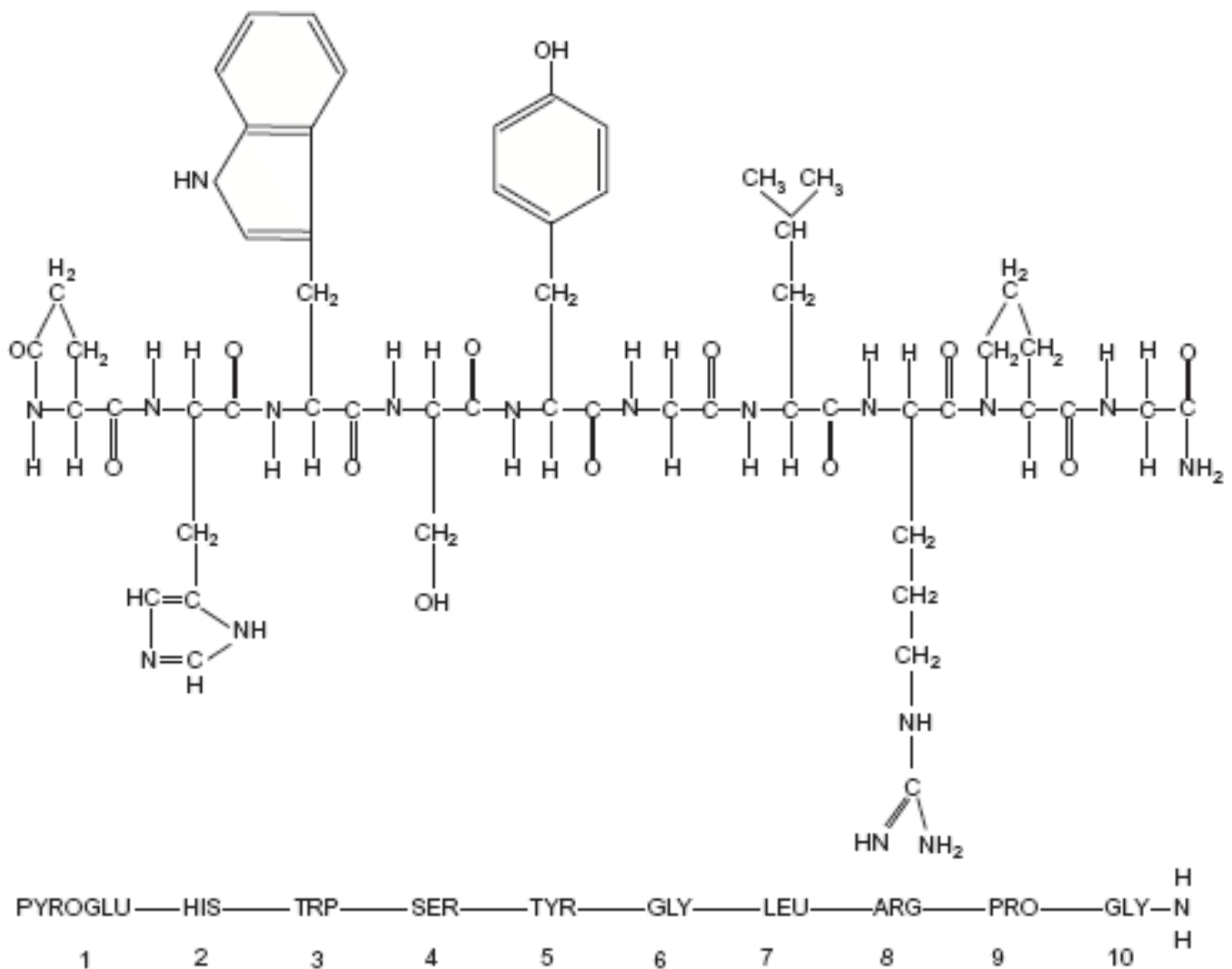
### GnRH-II

A midbrain GnRH on GnRH-II was first identified in chicken brain. It is structurally concerned in special ranging from teleost fish to humans.

### GnRH-III/ Telencephalic GnRH

These preferentially exist in the terminal part of the olfactory neuronal cell in the brains<sup>8</sup>. It is clear that each form has its unique locations within the brain, which suggests a difference in developmental origin and or adult function. The most importantly, the GnRH-II peptide is the one that appears to be predominantly expressed in other issue such as the reproductive system thereby attracting the special interest of reproductive biologist. However the exact function of these non-hypothalamic GnRH molecules is yet to be defined.

### Chemical structure of GnRH<sup>3</sup>



### Synthesis and secretion of GnRH

The regulation of both synthesis and the secretion of GnRH are affected by neurotransmitter system in the brain. Overriding effects on synthesis and secretion of GnRH can be observed during aging and under nutrition and stressful situations; these involve various neuronal system, which may have serial or parallel effects on GnRH cells. Gonadotropin releasing hormone (GnRH) is the brain factor that provides primary arrive for the reproductive axis without GnRH, the gonad tropes and the gonads do not function. Regarding the control of secretion, information is most abundant for studies done in sheep, since there is a robust means for accessing the hypophyseal portal blood vessels in this species<sup>9</sup>. This allows minute-to-minute analysis of patterns of secretion in detail and the changes occurs during the

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estrous cycle and in response to gonadal steroids which has been reviewed in detail<sup>10</sup>. The hypothalamic control of anterior pituitary is carried out by small peptide hormones that releases in the median eminence and transported to anterior pituitary via portal system of capillary. GnRH secretory cells of hypothalamus are regulated by feedback effects of gonadal steroids (estrogen, progesterone) via intermediary neurons that posses the relevant steroids receptors. The serum progesterone level may have not any direct effect or a little effect on secretion of GnRH which is not clearly understand. The major control of GnRH secretion occurs by steroids via estrogen responsive system within brain<sup>11</sup>. The secretion of GnRH during the luteal phase of the cycle by low frequency pulse at the end of the luteal phase of estrus cycle with fall in plasma progesterone the frequency of GnRH and LH pulse increased. As a follicle phase the plasma estrogen level rise the GnRH phase frequency increases the preovulatory LH surge which is associated with and unamambiguous rise in secretion of GnRH. These effects occur due to high level of estrogen which are involved in the positive feedback mechanism. Progesterone acts in two ways on secretion of GnRH during the luteal phase. Continuously elevated plasma progesterone level prevents the preovulatory LH surge, which ultimately block the GnRH surge. The second way by progesterone controlled GnRH secretion during the luteal phase of cycle. It prime the brain to the action of estrogen which may affect the GnRH/LH surge.

#### Mechanism of action of GnRH

Anterior pituitary acts as the main target of the gonadotrophin releasing hormone. The control of the synthesis and secretion of LH and FSH by GnRH in laboratory mammals and livestock is reviewed<sup>12</sup>. Two major intracellular pathways exist. The first involves  $Ca^{++}$  and participates in early GnRH stimulated LH release and in GnRH receptor regulation, whereas the second involves protein kinase and modulates gonadotropin synthesis and sustained secretion and activates receptors. The ability of pituitary to response to GnRH is restored by day 20 post-partum. This gonadotropin release in turn stimulates the release of gonadal steroids. A single injection of GnRH results in an enhanced and simultaneous release of both LH

and FSH in a log-dose response manner. Clearance of GnRH injected i/m is parallel to the LH and FSH (maximum concentrations in 15-30 min and return to base levels in about 6 hrs). The magnitude of gonadotropin release in response to GnRH depends upon both gonadal and adrenal steroids. Benefit of this property of GnRH has been utilized to treat cow and buffaloes having true anoestrous condition post-partum<sup>13</sup>. Under normal physiological condition GnRH is secreted from the hypothalamus in discrete pulsatile bursts<sup>14</sup>. These pulses usually occur every 2 to 6 hours. GnRH or GnRH agonist bind to anterior pituitary and activate Gq 11, which in turn stimulates phospholipase activity and increases the intracellular concentration of  $Ca^{++}$ , thereby increasing both the synthesis and secretion of LH and FSH. If GnRH is administered exogenously in a cyclically pulsed schedule, it can induce the pituitary to secrete increased amount of LH and FSH.

#### GnRH analogues: Agonists and Antagonists

The United States Food and Drug Administration (FDA) had produced recommendations for the testing and evaluation of GnRH analogues. Busereline had received most attention of all analogues synthesized<sup>15</sup>. Alterations in the chemical structure of the native GnRH molecule have led to the synthesis of potent GnRH agonists. Substitutions usually involve replacement of glycine molecules at positions 6 and 10 with a D-amino acid at position 6 and/or an N-ethylamide group at position 10. The design of GnRH agonists has been directed toward stabilization of the molecule against enzymatic attack, increasing binding to plasma proteins and membranes, and increasing the affinity of the agonist for GnRH receptor1. At least the following GnRH analogues and GnRH agonists are available commercially: gonadorelin (native-like GnRH; gonadorelin diacetate tetrahydrate or gonadorelin hydrochloride), buserelin (D-serine at position 6 and ethylamide at position 10), fertirelin acetate (ethylamide at position 10), and deslorelin (D-tryptophane at position 6 and ethylamide at position 10). There are mainly two analogues, (i) GnRH agonist and (ii) GnRH antagonist. Structure and potencies of GnRH and its analogues are discussed in Table 1.

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Table 1 Structure and potencies of GnRH and its analogues

Name	RP	Amino acid sequence										Dose form
		1	2	3	4	5	6	7	8	9	10	
GnRH	1	Pyro-Glu	His	Trp	Ser	Tyr	Gly	Leu	Arg	Pro	Gly-NH2	IV
Leuprolide	15	Pyro-Glu	His	Trp	Ser	Tyr	D-Leu	Leu	Arg	Pro	N-Et-NH2	SC, depot IM
Buserelin	20	Pyro-Glu	His	Trp	Ser	Tyr	D-ser (tBu)	Leu	Arg	Pro	N-Et-NH2	SC, IN
Nafarelin	150	Pyro-Glu	His	Trp	Ser	Tyr	D-Nal	Leu	Arg	Pro	N-Et-NH2	SC, IN
Deslorelin	150	Pyro-Glu	His	Trp	Ser	Tyr	D-Trip	Leu	Arg	Pro	N-Et-NH2	SC, depot IM
Histrelin	150	Pyro-Glu	His	Trp	Ser	Tyr	D-His (ImBzl)	Leu	Arg	Pro	N-Et-NH2	SC
Goserelin	100	Pyro-Glu	His	Trp	Ser	Tyr	D-ser (tBu)	Leu	Arg	Pro	AzGly-NH2	Depot SC

### 1. GnRH agonist

These are synthetically modelled after the natural GnRH decapeptide with specific amino acids, substitutions in position 6 and 10. They include (i) Leuprolide, (ii) Buserelin, (iii) Nafarelin, (iv) Deslorelin, (v) Histrelin and (vi) Goserelin.

### 2. GnRH antagonist

They includes Cetrorelix and Granirelix.

**Buserelin :-** It had received most attention among all analogues synthesized<sup>15</sup>. In the treatment of follicular cyst in cattle its response to treatment should be checked after 10-14 days. If no CL is detectable or if cysts have formed treatment should be repeated. An improvement in conception rate is expected if the time of ovulation can be established more exactly. This is also achieved by giving receptal injection at the time of insemination or witnesses the proceeding 6 hrs.

**Deslorelin:-** A GnRH super agonist appears to be particularly stable in solution with an estimate life greater than 20 years when stored 6°C only minor degradation was noticed after 5 years when stored at higher temperature (25, 40, to 50°C)<sup>16</sup>. Thermal stability is also a future of GnRH.

### Route of Administration

Early studies of GnRH against primarily used parental routes of administration i.e. s/c, l/m. GnRH agonists are susceptible to gastrointestinal peptidase degradation, making oral administration unsuitable with only 0.1 % bioavailability<sup>17</sup>. Route of administration also depends on structure, potency, dose and duration of slow release GnRH agonist formulation.

### Clinical application of GnRH and GnRH analogues

The pharmacological basis for the therapeutic use of GnRH derives from its physiological effect of stimulating of LH and FSH, which constitute the preovulatory surges, are preceded by a similar surge of GnRH release in the species (It has been known for 30 years that all aqueous solution of the decapeptide results in LH and FSH release within 30 min of injection). The goal of animal science research is to provide quality food to feed the growing need of human population. As the production directly depends on reproduction, optimum reproductive efficiency is a pre-requisite for animal production. The optimum

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reproductive efficiency and productivity can be achieved by (1) genetic selection animals for high reproductive efficiency and production traits (2) ideal management conditions (3) proper feeding with due considerations for the physiological needs of the animals and production. However, when these measures fall short in achieving the goals a corrective hormone therapy is necessary<sup>18</sup>.

#### Applications of GnRH

- A) Treatment at the time of AI (Artificial Insemination) in normal and repeat breeder cows to improve fertility.
- B) Treatment of cystic ovarian disease.
- C) Treatment of anoestrous.
- D) Estrous synchronization programme.
- E) Post-insemination treatments to improve fertility.
- F) Postpartum reproductive management to improve fertility later at the breeding period.
- G) Treatment of embryo transfer recipients to enhance embryo survival.

#### Prevention of embryo mortality

Approximately 25% of bovine embryos are lost in the first two weeks of life<sup>19</sup>. The maintenance of progesterone secretion by a luteolysis is probably a major cause of embryo loss. Use of GnRH as a 'holding' injection on the day of insemination improves the chances of successful pregnancy. GnRH has been shown to stimulate progesterone secretion in not pregnant cows when injected mild luteal phase<sup>20</sup>. It is now well established that cows typically exhibit during each cycle<sup>21</sup> depending on follicular status at the time, treatment with GnRH during the luteal phase likely advance either atresia, luteinisation or ovulation followed by luteinisation. Apart from stimulating progesterone secretion these changes also result in a decrease in oestradiol -  $17\beta$  production<sup>22</sup>. In turn this could result in all inhibition of up-regulation of oxytocin receptors and consequent inhibition of  $PGF_2$  secretion<sup>23</sup>. Therefore a reduction in oestradiol secretion at this time might be expected to inhibit the luteolytic mechanism allowing some pregnancies to continue. GnRH attenuates or weakens the luteolytic signal allowing embryos more time to develop their own luteotrophic capability<sup>22</sup>.

#### Synchronization protocols

An experiment was carried out<sup>24</sup> to find out the estrous synchronization use in lactating dairy cows using  $PGF_{2\alpha}$ , GnRH and estradiol cypionate (ECP). In lactating dairy cows received two injections of  $PGF_{2\alpha}$  on days 0 and/or two injection of  $PGF_{2\alpha}$  (days 0 and 11) and 100  $\mu$ g of GnRH on day 3. A higher percentage of GnRH treated cows ovulated after 1st  $PGF_{2\alpha}$  injection (90%). The GnRH treated cows have a larger dominant follicle present at the time of second injection of  $PGF_{2\alpha}$ . The 60 % of cows ovulated after 2nd injection of  $PGF_{2\alpha}$ .

#### Induction of ovulation in post partum period

The resumption of ovulatory cycle is clearly depends on the critical path to reestablishment of pregnancy and the dominant scientific opinion has been that it is desirable for ovarian cycles to begin as early as possible after parturition<sup>25</sup>. The environmental and endocrine interaction results in delayed resumption of ovarian cycle post-partum<sup>26</sup>. A single injection of GnRH results in an enhanced and simultaneous release of both LH and FSH in a log-dose response manner. Clearance of GnRH injected I/m is parallel to the LH and FSH (maximum concentration in 15-30 min and return to base level in about 6 hrs.). The ability of pituitary to respond to GnRH is restored by day 20 - post-partum. This gonadotrophin release in turn stimulates the release of gonadal steroids. The magnitude of gonadotrophin release in response to GnRH depends upon both gonadal and adrenal steroids. Benefit of this property of GnRH has been taken advantageously to treat animals having true estrus condition 15 weeks or more post-partum.

#### Effect on post- partum early resumption of ovarian activity

Early resumption of ovarian activity and there by narrowing down the service period or length of days open should be the milestone of livestock breeders in order to achieve better economy and make the daily business most popular or profitable. In spite of being this practical fact, delayed resumption of ovarian activity in post-partum period may overt loopholes to attain this target. Suckling has inhibitory effect on female pituitary activity and is regarded to prolong the service period and delay the conception rate in dairy cows and buffaloes. Ovulation can be



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induced and normal estrous cycles initiated when daily cows were treated on day 13 post-partum with GnRH and same scientist reported service period for GnRH treated cows to be  $93.9 \pm 35.5$  days as against  $118.4 \pm 33.8$  days for control group. Treatments resulted into significant reduction in the intervals from parturition to first detected estrus and first ovulation. Low doses of GnRH delivered in a pulsatile fashion equivalent to that found in all the portal vessels (pg/ml) restore fertility in hypogonadal male and female and also effective in treatment of delayed puberty. However, high doses of natural GnRH or agonist analogues desensitize the gonadotropes with resultant decrease in LH and FSH and a decline in ovarian and testicular function. This desensitization phenomenon is extensively applied in clinical medicine for treatment of a wide range of diseases.

#### Ovsynch protocol

GnRH peptide antagonist also inhibits the reproductive system through competition with endogenous GnRH but the doses required of agonist are higher than the desensitizing against doses and antagonist is currently less extensively employed.

#### Conclusion

The efficacy of GnRH treatment have been reviewed in relation to prevention of embryo mortality, control of follicular development, synchronization program using  $\text{PGF}_{2\alpha}$  as luteolysis, induction of ovulation in post partum period. There are sufficient evidences in cattle that GnRH is effective in increasing pregnancy rate when given either at the time of AI (first or repeat) or between days 11 to 14. Use of Ovsynch protocol appears to be effective in increasing the synchrony of ovulation in controlled breeding programmes. However, the main synchronizing effect seems to reside in the second GnRH injection. GnRH is the treatment of choice in bovine cystic ovarian disease through stimulating ovulation of a new follicle and luteinization of the cyst. The therapeutic applications of GnRH analogues have promise as new generation male and female contraceptive in conjunction with steroid hormone replacement.

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