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Oxytocin : The Cuddle Hormone- A RAVIEW

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ABSTRACT

The oxytocin hormone has a well established role in let-down of milk and parturition process in dairy animals. However, its harmful effect on health of lactating ruminants has not been established till date though short term effects are known in some species. During the last one decade various actions of oxytocin has been reported but research in lactating animals are still needed to prove the harmful effects. The present review discuss about the synthesis, storage and its wider action in male and females.

Key Words: Oxytocin, Let-down of milk, Role in male and females, Release and inhibition

INTRODUCTION

Oxytocin and vasopressin were isolated and synthesized by Vincent du Vigneaud in 1953, for which he received the Nobel Prize in Chemistry in 1955. The name oxytocin is derived originally from the Greek for "swift birth".Oxytocin is naturally released by hypothalamus section of the brain during labor to stimulate uterine contractions. In addition, normal suckling by offspring/ calf also releases oxytocin which stimulates contractions in secretory tissue of mammary gland leading to milk let-down. Oxytocin is produced in both males and females and receptors for it are found all over the brain and reproductive system of both sexes. Human preparations of oxytocin are available in various trade forms like Pitocin, Syntocinon and *various other generic preparations*. But oxytocin sometimes is also known as the "cuddle hormone" because of its wider influence on maternal behavior, pair bonding, socio-sexual problems, eating disorders, pedophilia, and child abuse and neglect. Information on animal models, including non-human primates reveals that oxytocin at the level of brain, play an important role in the expression of maternal, sexual, social, stress, learning, memory and feeding behaviors. Oxytocin (OT) receptors have also been identified in tissues including kidney, heart, thymus, pancreas, and adipocytes.

Structure

All neuro-hypophysial hormones are nona-peptides with a disulfide bridge between Cys residues 1 and 6. This results in a peptide consisting of a six-amino acid cyclic part and a COOH-terminal α-amidated three-residue tail. Based on the amino acid at position 8, these peptides are classified under vasopressin and oxytocin (OT) families. The vasopressin family contains 2 basic amino acid (Lys, Arg), and the OT family contains a neutral amino acid at this position (Table1). Iso-leucine in position 3 is essential for stimulating OT receptors and Arg or Lys in position 8 for acting on vasopressin receptors as shown in Fig1. The difference in polarity of these amino acid residues enable the vasopressin and OT peptides to interact with the respective receptors (Barberis *et al*, 1998).

Oxytocin has amino acids orderly sequence of cysteine - tyrosine - isoleucine - glutamine - asparagine - cysteine - proline - leucine - glycine (CYIQNCPLG). The cysteine residues form a sulfur bridge. Oxytocin has a molecular mass of 1007 daltons and one international unit (IU) of oxytocin is equivalent to about 2 micrograms of pure peptide. The structure of oxytocin is very similar to that of vasopressin (cysteine - tyrosine - phenylalanine - glutamine - asparagine - cysteine - proline - arginine

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- glycine), also a nonapeptide with a sulfur bridge as shown in fig 2. Oxytocin and vasopressin are the only known hormones released from the posterior pituitary gland to act at a distance. However, oxytocin neurons make other peptides, including corticotropin-releasing hormone (CRH) and dynorphin in humans. The magnocellular neurons that make oxytocin and vasopressin are similar in many respects.

Formal chemical name (IUPAC) : (S)-N-((S)-1-(2-amino-2-oxoethylamino)-4-methyl-1-oxopentan-2-yl)-1- ((4R,7S,10S,13S,16S,19R)-19-amino-7-(2-amino-2-oxoethyl)-10-(3-amino-3-oxopropyl)-13-sec-butyl-16-(4-hydroxybenzyl)-6,9,12,15,18 -pentaoxo-1,2-dithia-5,8,11,14,17-pentaazacycloicosane-4-ca.

Oxytocin Synthesis, Storage and Release

Oxytocin is synthesized in magnocellular neurosecretory cells of supraoptic and paraventricular nucleus of hypothalamus and is released into blood from the posterior lobe of pituitary gland. Oxytocin is also made by some neurons in the paraventricular nucleus that project to other parts of brain and to the spinal cord. After the synthesis, oxytocin is packaged in large dense-core vesicles and bound to neurophysin-I, a large carrier protein fragment. Secretion of oxytocin from the nerve endings is regulated by electrical activity of oxytocin cells in the hypothalamus. These neuronal cells generate action potentials in response to tactile or milking stimulus that propagate down axons to the nerve endings having oxytocin-containing vesicles

TABLE 1: Oxytocin and related peptides in different species

	1	2	3	4	5	6	7	8	9	
Oxytocin	Cys	Tyr	Ile	Gln	Asn	Cys	Pro	Leu	Gly (NH_2)	Placentals, some marsupials, ratfish (Hydrolagus colliei)
Mesotocin	*	*	*	*	*	*	*	Ile	*	Marsupials, non mammalian tetrapods, lungfishes
Oxytocin	Cys	Tyr	Ile	Gln	Asn	Cys	Pro	Leu	Gly (NH_2)	Placentals, some marsupials, ratfish (Hydrolagus colliei)
Isotocin	*	*	*	Ser	*	*	*	Ile	*	Osteichthyes
Glumitocin	*	*	*	Ser	*	*	*	Gln	*	Skates (Chondrichthyes)
Valitocin	*	*	*	*	*	*	*	Val	*	Sharks (Chondrichthyes)
Aspargtocin	*	*	*	Asn	*	*	*	*	*	Sharks (Chondrichthyes)
Asvatocin	*	*	*	Asn	*	*	*	Val	*	Sharks (Chondrichthyes)
Phasvatocin	*	*	Phe	Asn	*	*	*	Val	*	Sharks (Chondrichthyes)
Cephalotocin	*	*	Phe	Arg	*	*	*	Ile	*	Octopus vulgaris (Molluscs)
Annetocin	*	Phe	Val	Arg	*	*	*	Thr	*	Eisenia foetida (Annelids)
Vasotocin	*	*	*	*	*	*	*	Arg	*	Nonmammalian vertebrates, cyclostomes
Vasopressin	*	*	Phe	*	*	*	*	Arg	*	Mammals
Lysipressin	*	*	Phe	*	*	*	*	Lys	*	Pig, some marsupials
Phenypressin	*	Phe	Phe	*	*	*	*	Arg	*	Macropodids (Marsupials)
Locupressin	*	Leu	*	Thr	*	*	*	Arg	*	Locusta migratoria (Insects)
Arg-conopressin	*	Ile	*	Arg	*	*	*	Arg	*	Conus geographicus (Molluscs)
Lys-conopressin	*	Phe	*	Arg	*	*	*	Lys	*	Lymnaea stagnalis (Molluscs)

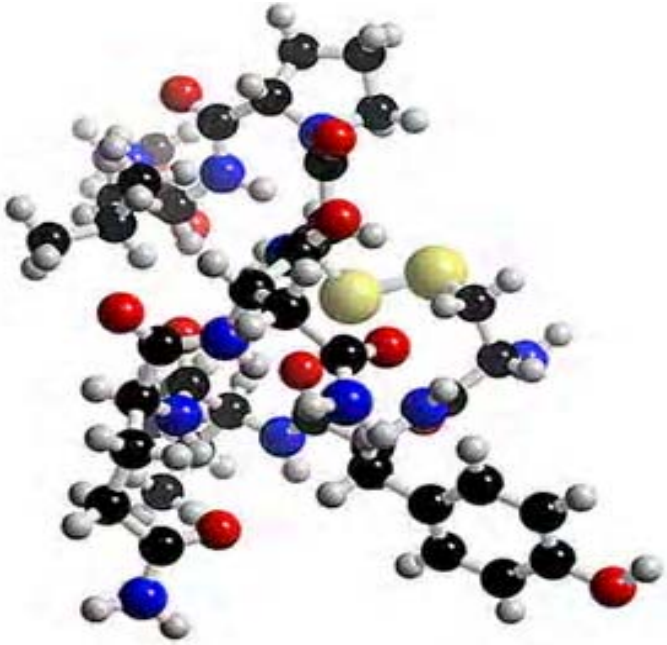

Figure 1: 3- D Structure of oxytocin molecule

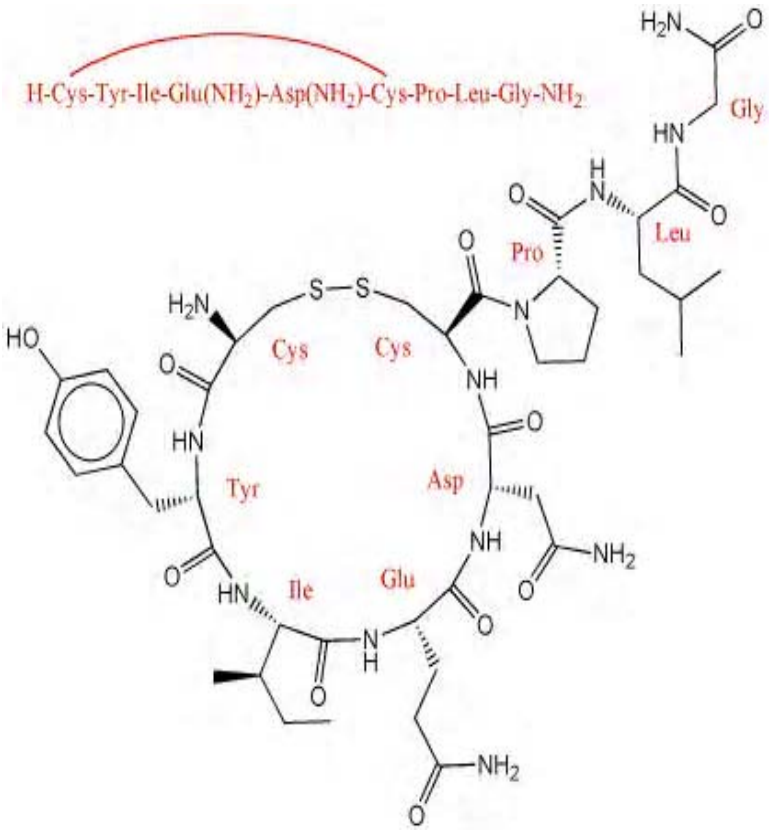

Figure 2: Chemical structure of oxytocin molecule

in pituitary. From these nerve endings, oxytocin is released by exocytosis process. Pineal gland secretion has an inhibitory impact on both oxytocin synthesis and release and oxytocin synthesis rate increase markedly eight weeks after pineal removal in rat (Bojanowska *et al*, 1999).

Physiological role of oxytocin

≈ The primary use of oxytocin is to induce the labor in animals suffering from weak or no contractions at parturition. It enhances uterine contractions by increasing the permeability of sodium ions across muscle cells and helps to move the foetus into the birth canal. Oxytocin causes contractions during the second and third stages of labor. Studies have found increases in plasma oxytocin levels at orgasm – in both men and women but whether such oxytocin release occurs in livestock requires investigation. Due to its similarity to vasopressin, it can slightly reduce the excretion of urine.

≈ Oxytocin is used medically to expel any residual placental material that might be left/ retained in the uterus to prevent metritis incidence in animals.

≈ After the parturition, oxytocin helps to stimulate milk release by a process called *let-down of milk* from the alveoli of mammary gland. Oxytocin acts at peripheral (hormonal) and central (brain) level through specific and high affinity oxytocin receptors. The oxytocin receptor is a G-protein-coupled receptor which requires Mg2+ and cholesterol. It belongs to the rhodopsin-type (class I) group of G-protein-coupled receptors.

Let-down of milk

The massage of teat or mechanical stimulation of tactile receptors generates sensory impulses that are transmitted from the teats to spinal cord and then to the secretory oxytocinergic neurons in the hypothalamus. These neurons display a synchronized high-frequency bursting activity, consisting of a brief (3-4 seconds) high-frequency discharge of action potentials recurring after every 5-15 min. Each burst leads to massive release of OT into the bloodstream by which OT is carried to the mammary gland. In response to elevated OT concentrations, stimulation of the OT receptors causes myoepithelial cell, the smooth muscle cells that surround the alveoli like a basket, to contract. The small milk ducts are also covered longitudinally by myoepithelial cells. Thus, when alveoli contract, ducts are shortened, diameter of ductal lumen increase, and alveolar milk is forcefully drained into the larger free-draining ducts and then into the cisternal space (Bruckmaier and Blum, 1996, Bruckmaier *et al.*, 1994. Schams *et al.*, 1984). Alveolar milk ejection causes a rapid increase of pressure within cistern up specific to an individual capacity (Bruckmaier and Blum, 1996; Bruckmaier *et al.*, 1994) and enlargement of cisternal cavity size (Bruckmaier and Blum, 1992). However, not all alveolar milk can be ejected if no milk is removed from the udder. Milk ejection occurs only up to a maximal intra-mammary pressure which occurs as soon as OT concentrations increase and a threshold concentration is maintained (Bruckmaier *et al.*, 1994). Partial milk ejection occurs due to insufficient OT release or when OT is transiently released (Bruckmaier and Blum, 1996; Bruckmaier *et al.*, 1996). Oxytocin is also used to measure the inherited retained milk in the udder called "Residual milk" after normal milking. Oxytocin stimulates phosphorylation of myosin light chain in rat mammary myoepithelial cells within 30 seconds of binding. Influx of extra-cellular calcium ions regulated the duration of response. The *milk ejection or milk let-down reflex* continues to function until weaning and is inhibited by factors like stress, fear, beating of animals at milking, loud sounds and unfavorable environmental condition (Bruckmaier *et al.*, 1996). The OT in conjunction with continued milk removal is required for postpartum alveolar proliferation and normal mammary gland function. It has been found that alveolar density and mammary epithelial cell differentiation at parturition were similar in wild-type and OT-deficient dams, but despite suckling and presence of systemic lactogenic hormones, mammary tissue in OT-deficient dams involutes partially (Wagner *et al.*, 1997). Thus continuously elevated OT concentrations such as those during infusion or during normal milking are necessary for complete milk removal in dairy cows (Bruckmaier *et al.*, 1994). Before milking, less than 20% of the milk yielded by dairy cows is stored within the cistern (cisternal milk) and is immediately available for removal (Michelle and Singh, 2008). However, most of the milk (alveolar milk) is available by hand or

machine milking only after milk-ejection reflex. Pre-milking teat stimulation causes alveolar milk ejection before the start of milking. Thus, bimodal milk flow curves (i.e., interruption of milk flow after removal of the cisternal milk) could give an idea about the incidence of both the milk fractions in dairy animals. Administration of single injection of oxytocin (10IU i.v.) or its long acting analogue carbetocin (280ìg i.m.) at day 7 after ovulation did not affect progesterone secretion, pregnancy rate and embryonic growth in mares (Handler et al., 2003).In mares, no differences in oxytocin release have been shown between suckling and milking, as milking are carried out with mares that are usually nursing (Doreau and Boulot, 1988). Cherepanova and Belokobylenko (1974) noted that 20% of milked mares withheld their milk when their foal was at a distance farm them.

Role of milking stimulus and oxytocin concentration

Timings of oxytocin release may influence milk ejection reflex in dairy animals. Oxytocin levels are higher in cows after manual pre-milking stimulation than in cows receiving no stimulation. Concentrations of oxytocin in blood are elevated within minutes of milking stimuli in cows as determined by RIA methods (Gorewit, 1979, Knaggs, 1963; Lawson and Graf, 1968; Merrill and Gorewit, 1981; Momongan, 1969; Sagi *et al.*, 1980, 1980a, b). The oxytocin concentration in blood plasma decline rapidly after attainment of peak concentration and by 5 minutes after teat cup application, the levels are below 2.5 U/ml. By 5-minutes post milking oxytocin drop to less than 0.01 U/ml. The oxytocin concentration peak also decreases with advancement of lactation. The half-lives of oxytocin is very small and estimated between 0.8 -1.4 and 0.85 to 1.30 minutes with and with out milking stimulus. The average total circulating oxytocin has been reported to be 22.1 /lg, whereas average amount of hormone stored in the pituitary gland is estimated as 814/lg. On an average, there is about 38 times as much stored posterior pituitary oxytocin as circulating oxytocin in the lactating cow during resting conditions. Thus, concentration of circulating oxytocin represents only about 2.7% of the total. The cow can release approximately one-third of pituitary stores at a given milking. Maximal concentrations during milking decrease across lactation. Peak concentrations of endogenous oxytocin in sera of cows ranged from 11 to 65/aU/ml (Gorewit, 1979). A mature dairy cow having approximately 40 liters of blood release a bolus of about 0.4 to 2.6 IU of endogenous oxytocin to establish these oxytocin concentrations. Thus intravenous injection of as little as 0.02 IU oxytocin could elicit milk ejection in cows (Donker, 1958). Non-lactating cows also release oxytocin upon udder stimulation and conditioning. Virgin heifers do not respond in the same way to udder stimulation as lactating cows suggesting that sensitivity of the milk ejection reflex depends upon the physiological state of animal. Cows probably release about one-third of their stores of oxytocin at milking, and it appears that little hormone is necessary to elicit its effect in the udder.

Basal concentrations of oxytocin decrease from early to mid lactation increase from mid to late lactation and from late lactation to involution. In late lactation, the stimulatory response of mammary gland to oxytocin decreases due to reduced responsiveness of neuroendocrine reflex, capacity of hypothalamus to synthesize oxytocin, increased rate of hormone clearance from the body, or a combination of all factors.

Peripheral and central inhibition of oxytocin release

The interruption of milk ejection process can disturb milk removal by peripheral inhibition of oxytocin effects on the mammary gland or by central inhibition of oxytocin release by central nervous system. Peripheral inhibition is induced by elevated concentrations of catecholamines (epinephrine and nor epinephrine) through stimulation of á-adrenergic receptors in the mammary gland and changed ductal resistance. Inhibition of oxytocin release by central nervous system has been observed in primiparous cows and buffaloes immediately after parturition, during peak estrus, and during disturbed milking operations and unfamiliar surroundings. Under such conditions, â-endorphin and cortisol levels are elevated in blood. Such animals will not letdown the milk in spite of apparent engorgement of udder and can be corrected by clinical use of oxytocin for 2-3 days. However, role of endogenous opioid peptides in inhibition of oxytocin release in bovines remains

unclear (Bruckmaier and Blum, 1998). During machine-milking, the physiological requirements of cows and specially buffaloes need to be considered carefully and any unfamiliar incident and stressors must be minimized.

Role in buffaloes

Buffalo because of the highly sensitive nature and temperamental behavior at milking needs utmost care at milking. Some buffaloes take long time to let-down the milk and are called as *hard milkers*. Inadequate milking stimulus increases the stripping yield and decreases fat significantly which indicates incomplete milking. The buffaloes are easily disturbed even by small changes in milking routines and release of oxytocin is impaired (Bruckmaier and Blum, 1998; Thomas *et al.*, 2005). Under such conditions, alveolar milk is not fully drawn and the amount of residual milk increases in udder which decreases milk synthesis and secretion. Such incidences may occur in freshly calved buffaloes, which need to be cured for impaired lactation by short term clinical treatment with exogenous oxytocin so that elite buffaloes do not dry up and loss to the farmers does not occur. This fact has been further proved by a positive correlation between the total time oxytocin concentration remain elevated over threshold levels and the machine yield (r=0·86, P<0·05).

Effect on milk production and composition

Long term administration of oxytocin before and after milking significantly increase milk production by 3% caused by increased gland output of milk and not by removal of residual milk (Linda *et al.*, 1993), Oxytocin treatment did not influence milk plasmin activity, fat, protein, SCC, and lactose. It has been found that oxytocin treated cows produced 849 kg more milk during the lactation than the control group, with a significant difference occurring after peak milk yield, due to maintenance of greater persistency during lactation without affecting fat, protein percentages and health of cows. Further, short-term oxytocin treatment increases bovine milk yield by enhancing by more milk-ejection reflex and milk removal rather than a direct stimulatory action on mammary metabolism. Oxtytocin treatment (1 I.U.) decreases milk Na and Cl while K, lactose, fat and protein increases within 24 hr. The passage of sucrose, Na and Cl from blood to milk also increases due to changed permeability of mammary epithelium and the pathways for ion movements (Linzell *et al.*, 1975). The higher dose of oxytocin (10 IU) treatment affects composition of milk by increase in milk fat from av. 5.2 to 6.4%, while lower dose of oxytocin (1 IU) injected intramuscularly to let-down milk and the composition of milk is not affected with a dose <5 IU (Bencini,1995; Prasad and Singh, 2001; Dang *et al.*, 2002).

Fate of oxytocin

Oxytocin hormone does not remain for a longer time in the blood and is destroyed by the enzymes in the gastrointestinal tract. It has a small half-life which also eliminates it from the blood at a faster rate. Oxytocin once administered is destroyed by enzyme oxytocinase present in liver and kidney and is also utilized by the mammary gland receptors for let-down of milk.

Precautions and side effects

Oxytocin is generally safe and effective in physiological doses against prescribed by a veterinarian, but may cause side effects in animals which are hypersensitive or allergic to this hormone. Oxytocin should not be used if the fetus is in an abnormal position or is too large to pass through the birth canal, but its use is safe when cervix is dilated. Further, oxytocin should not be used if an animal has low blood calcium. More important, in several species, oxytocin can stimulate sodium excretion from the kidneys (natriuresis). Indiscriminate and higher dose of oxytocin for let-down of milk can cause uterine rupture, fetal injury, death of the fetus or pain due to excessive uterine cramping. It has been found that a doses of <5.0 IU administered intramuscularly did not adversely affect the health, water and feed intake, milk production and composition of dairy animals (Ludri and Singh, 1987). However, it affects mineral content and increases SCC of milk in buffaloes, but increased SCC are within normal physiological range (Prasad and Singh, 2001; Singh and Aggarwal, 2001). The indiscriminate use of oxytocin in buffaloes is one of main constrains in clean milk production programme under field conditions. The farmers need to be educated about the ill effects of oxytocin so that buffaloes could be prevented from infertility owing to over doses of oxytocin pituitary extract containing vasopressin and other active peptides.

Actions of oxytocin within brain

Oxytocin secreted from the pituitary gland cannot re-enter the brain because of the blood brain barrier. The behavioral effects of oxytocin reflect release of it from centrally projecting oxytocin neurons, which are different from those that lead to the pituitary gland. Oxytocin receptors are expressed by neurons in many parts of brain and spinal cord including amygdala, ventromedial hypothalamus, septum and brainstem. Oxytocin has a role in social behaviors and bonding in many species.

Maternal behavior- Sheep and rat females given oxytocin antagonists after giving birth do not exhibit typical maternal behavior. By contrast, virgin female sheep show maternal behavior towards foreign lambs upon infusion of oxytocin in cerebrospinal fluid. Experimental evidence indicates that OT injection into the lateral ventricles of multiparous ovariectomized rats induces maternal behavior (Pedersen and Prange, 1979; Kendrick *et al.*, 1997; Williams and Griffith, 1992). However, OT is effective only to initiate the maternal behavior, but not for the performance of maternal behavior per se. When the females become maternal or enter into estrus, an OT antagonist had no effect (Witt and Insel., 1991). Circulating oxytocin as well as neurogenic oxytocin stimulates maternal interaction and attachment between mother and young. Oxytocin participates in the metabolic, digestive and anabolic prerequisites for milk production by stimulating glucagon release, mobilization of glucose and increased vagal nerve activity. It also induces antistress like pattern by lowering cortisol levels and blood pressure in monogastric animals as well as in ruminants (Uvnas-Moberg, 2003).

≈ Increasing trust and reducing fear. Nasally administered oxytocin displayed "the highest level of trust" twice as often as the control group. However, there is no conclusive evidence for access of oxytocin to the brain through intranasal administration.

≈ According to some studies in animals, oxytocin inhibits the development of tolerance to various addictive drugs (opiates, cocaine, and alcohol) and reduces withdrawal symptoms.

≈ Preparing fetal neurons for delivery. Maternal oxytocin passes through the placenta and induces a switch in the action of neurotransmitter (GABA) from excitatory to inhibitory on fetal cortical neurons. This silences the fetal brain for the period of delivery and reduces its vulnerability to hypoxic damage.

≈ Certain learning and memory functions are impaired by centrally administered oxytocin.

≈ Experiments with transgenic mice suggest that OT acts as a luteotrophic hormone opposing the luteolytic action of $PGF2_{\alpha}$. Thus, to initiate labor, it might be essential to generate sufficient PGF2 to overcome the luteotrophic action of OT in late gestation.

≈ OT also plays an important role in control of the estrous cycle length, follicle luteinization in the ovary, and ovarian steroidogenesis. In the male, OT is a potent stimulator of spontaneous erections and ejaculation in rats. In buffaloes, administration of 2.5 and 5 IU (i.m.) for 30days has no effect on ovarian status, conception rate, pregnancy and total milk (Annual Report, 2006.

≈ The central actions of OT range from the modulation of the neuroendocrine reflexes to the establishment of complex social and bonding behaviors related to the reproduction and care of the offspring. The later has got more importance in case of buffaloes. The buffaloes fail to give complete milk and dry off early in event of calf death after the parturition due to strong bonding. Such buffalo needs immediate attention and the damage to udder can be prevented by exogenous oxytocin (1 I.U.).

≈ OT exerts potent anti-stress effects that may facilitate pair bonds. The regulation by gonadal and adrenal steroids is one of the most remarkable features of the OT system and is being least understood.

Role of oxytocin in males

Oxytocin is traditionally thought of as a 'female' neurohypophysis hormone due to its role in parturition and milk ejection. However, OT also has endocrine and paracrine roles in male reproduction. A burst of OT is released from the neurohypophysis into the systemic circulation at ejaculation to facilitate sperm release and transport in reproductive tract. Conclusive evidence indicates synthesis of OT within the mammalian testis, epididymis and prostate gland (Thackare *et al.*,

2006). OT receptors are present in reproductive tract for a local action. It has a paracrine role in stimulating contractility of the seminiferous tubules, epididymis and the prostate gland and modulate androgen levels in these tissues via stimulation of conversion of testosterone to dihydro-testostone (DHT) by 5-reductase.

CONCLUSION

In view of the above facts there is no doubt that oxytocin administration affects the endocrine milieu of animals and if administered for longer period, adversely affects animal health and productivity. Though till date, no information is available on oxytocin secretion in milk but unscientific report always find a place in news papers and medias which needs scientific validity to prove the same. The author has not found any literature on these aspects. The effect of oxytocin on health of animals may vary in different breeds under different feeding regimes and management. Therefore, a systematic study needs to be undertaken on effect of oxytocin on milk production, composition, health and reproduction for consecutive lactation to generate the facts and figures. In addition, analysis of carcass of animals injected with oxytocin may reveal residual effects if any. Till the above facts are proved oxytocin hormone, because of its cuddliness will always be controversial. In addition, physiological regulation of OT system will remain puzzling as long as the molecular mechanisms of genomic and nongenomic actions is not been clarified.

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