

Overview on Reproductive Endocrine Aspects in Buffalo

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Abstract: Buffalo is an important worldwide species in terms of milk and meat production as well as draft. The major constraints to full exploitation of the productive potential of buffalo are its inherent low reproductive efficiency due to delayed puberty, higher age at first calving, long post partum anoestrus period, long intercalving period, silent heat coupled with poor expression of oestrus, seasonality in breeding and low conception rate. Reproductive cycles in buffalo are regulated by endocrine-neuroendocrine interactions between hypothalamic, gonadotropic, gonadal and other hormones. To improve the buffalo reproductive efficiency, the investigation on endocrine aspects is useful to gain a better knowledge of buffalo reproduction. This review is focused on the information available on various basic hormones (Melatonin, Progesterone (P4), Estradiol 17B (E2), Follicle stimulating hormone (FSH), Luteinizing hormone (LH)) and on new hormones (Inhibin, Glycoproteins associated with pregnancy (PAGs), Leptin) related to reproductive function.

Keywords: Melatonin, Pituitary gonadotrophin, Gonadal steroid hormone, Inhibin, PAG, Leptin.

INTRODUCTION

Inherent reproductive problems (delayed puberty, higher age at first calving, long post partum anoestrus period, long intercalving period, silent heat coupled with poor expression of oestrus, seasonality in breeding and low conception rate) limit the productivity of buffalo, an important worldwide species in terms of milk and meat production as well as draft.

Since an understanding of the hormonal interaction is essential for relieving reproductive problems of endocrine origin, a considerable attention has been focused in the last two decades on utilizing reproductive endocrinology as a means to identify problems specific to this species and to devise means for improving reproductive performance.

The different phases of reproductive cycle are regulated by several sequential events and interactions between hypothalamic releasing hormones, hormones secreted from the pituitary and sex steroids secreted by the ovary. Lack of integration or synchronization or endocrine imbalances at any phase of the sequence may result in reproductive failure.

A sound knowledge of reproductive functioning in terms of interplay of hypothalamic, gonadotropic and gonadal hormones, with synergistic and antagonistic influences from other hormones and factors involved in the regulation of various reproductive stages can be

expected to lead to an improvement of the reproductive efficiency.

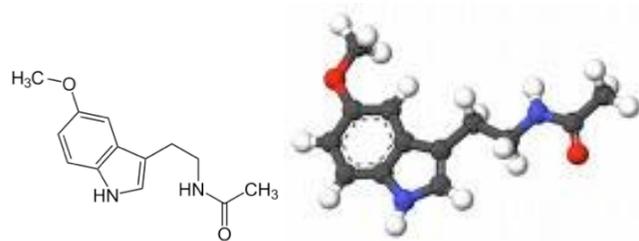
With regard to reproductive hormonal parameters, it is generally considered that the changes in the peripheral ovarian steroids and gonadotrophins profiles during the cyclic ovarian activity in buffalo cows are very similar to that in cattle [1,2]. On the other hand, in the past decade, lower levels of several sexual hormones and some differences in the oestrus behaviour and in other reproductive aspects in buffalo species in comparison to that of bovine, have been reported [3].

The ovarian activity in buffalo, as in other mammals, begins with puberty. Puberty is a complex physiological phenomenon whose origins are the neuro-endocrine mechanisms that determine: first ovulation in the female, the ability coeundi et generandi in the male, the changes in primary and secondary sexual characters in both. It is the hypothalamic neuro-hormone GnRH (gonadotrophin releasing hormone) to stimulate the secretion of gonadotropic hormones in the adenohypophysis: FSH (follicle stimulating hormone) and LH (luteinizing hormone) that promote the maturation of ovarian follicles until high and pulsatile production of LH determine the dehiscence of the mature follicle and the release of the first egg, as it indicates the advent of puberty.

Melatonin

Melatonin is a hormone of the brain, produced and stored in the pineal gland during the day and secreted during the dark, starting after sunset and ending at

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sunrise. Its secretion is the endocrine signal of the light-dark rhythm. The best known role of melatonin is the regulation of circadian rhythm as well as the annual cycle in many species, from the more primitive to the man. In the endocrinology of ruminants its role has been studied to induce ovarian cyclicity in seasonal animals such as goats and sheep, especially at higher latitudes [4-6].

Although buffaloes are polyoestrus, their reproductive efficiency shows wide variation throughout the year; in fact buffalo cows exhibit a distinct seasonal change in displaying oestrus, conception rate and calving rate [7]. This reproductive seasonality does not seem to depend on diet, food availability or metabolic status, while climate and particularly photoperiod, depending on melatonin secretion, play a pivotal role [8-11].

Parmeggiani *et al.* [8,12] have measured blood levels of melatonin in Mediterranean Italian buffaloes raised in farms where reproductive activity was characterized by strong seasonality and in other farms where the calving distribution tended to be uniform throughout the year (Figure 1). The more seasonal buffalo showed a melatonin profile reflecting the

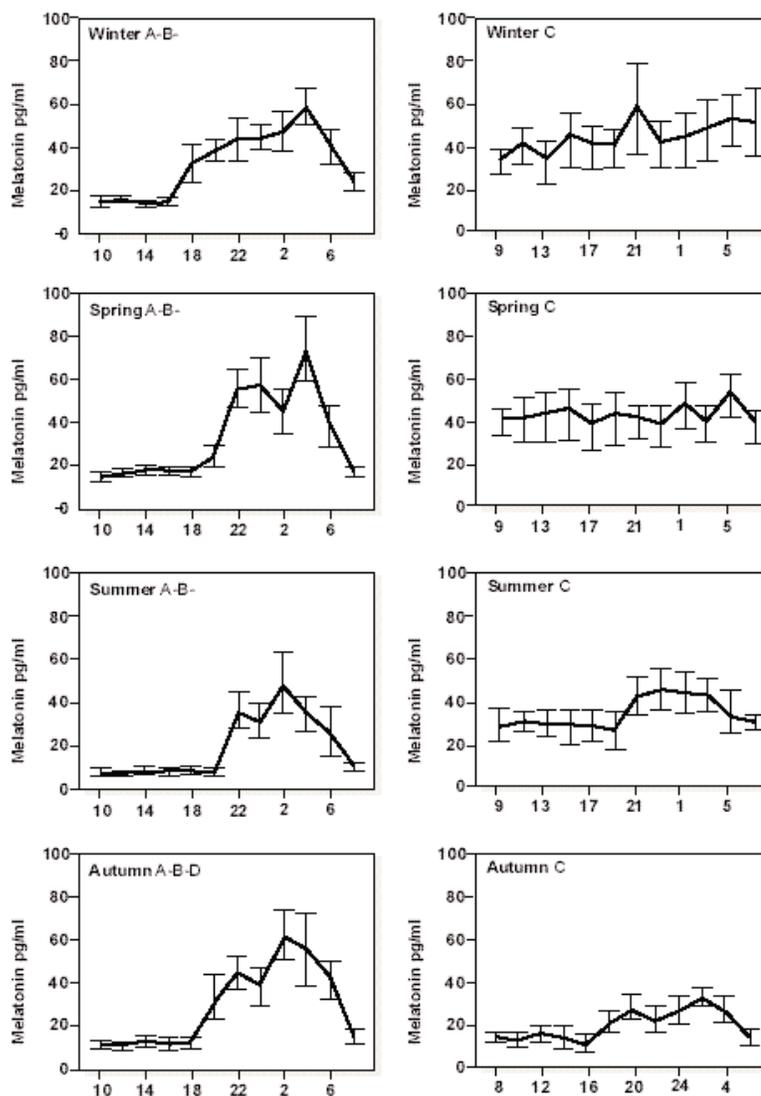
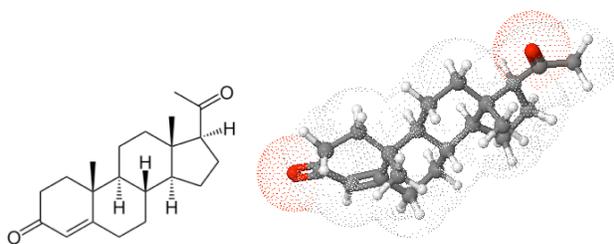


Figure 1: Circadian trend of melatonin in buffalo cows in different seasons. On the left, farms A-B-D characterized by a higher trend of seasonal reproduction activity; on the right, farm C characterized by a lower trend of seasonal reproduction activity [12].

changes of photoperiod, with a hormone concentration below 20 pg / ml during the hours of light and systematic peaks after sunset (with an average of 60 pg / ml). In contrast, less seasonal buffalo showed high concentrations of melatonin frequently during daylight hours (30-40 pg / ml), with a lack of a clear increase in melatonin during the night. The author's view is that the different trends of melatonin are the result of a selection made in the last farms, targeted to the elimination of seasonal buffaloes [13]. Borghese *et al.* [9], also report, in a study carried out on buffalo heifers and cows in Italy, that the melatonin trend shows remarkable differences between seasons. In June at the summer solstice, the lowest values and less persistence of melatonin peak were found because of the shortest night, while the highest values were noted at the equinoxes, particularly in September, the month corresponding to the start of hypothalamus-pituitary-ovarian axis activity, in the Northern hemisphere. The heifers showed significantly higher values during the day than in cows and in September also during the night, probably because they were close to the onset of puberty. These data suggest a relationship between photosensitivity and the seasonal reproductive trend in this species, as reported also by [14].

GONADAL STEROID HORMONES

Progesterone (P4)



Progestagens are a group of hormones with similar physiological activities, the most important of which is progesterone (P4), which plays a key role in the regulation of the oestrous cycle. Several studies have been reported on peripheral P4 concentrations during oestrous cycle in buffalo [15-20] showing that peripheral plasma P4 profile in buffalo is very similar to that in cattle (Figure 2).

P4 levels rise and fall in coincidence with the growth and regression of corpus luteum (CL) since CL is the source of P4 in cycling buffalo [21].

Peripheral P4 concentrations are lower on the day of oestrus (0.1 ng/ml), rise to peak concentrations of

1.6-3.6 ng/ml on days 13 to 15 of the cycle [1,21,22] or even on day 17 [23] before declining to basal levels at the onset of next oestrus; it goes down 2 or 3 days before the peak of luteinizing hormone (LH), begins to rise 2 to 4 days after the LH peak while the highest values are characteristic of the luteal phase (dioestrus) (5-12 ng/ml and 4-6 ng/ml in Mediterranean and Murrah buffaloes respectively).

P4 levels continue to increase in animals that conceive but drop 3 days before the next oestrus in those that fail to conceive [24]. The onset of the decline in P4 concentrations is variable, depending upon the time of regression of CL.

Peripheral P4 concentrations may change during the seasons [25]: lower P4 levels at oestrus as well at midluteal phase in hotter (0.14±0.05 and 2.05±1.16 ng/ml, respectively) than in cooler months (0.49±0.06 and 3.11±0.20 ng/ml, respectively) are believed to be responsible for the poor expression of oestrus and low conception rate during summer season by some Authors [48]. Others have, however, observed P4 concentrations to be significantly higher during summer compared to those in winter season [17,26]. A significant increase in peripheral plasma P4 concentrations during prolonged heat exposure could be from a stress-induced rise in P4 from adrenal cortex [27-29]; P4 concentrations have also been found to vary with the nutritional status [30]: suboptimal nutrition coupled with stress due to high environmental temperatures may be responsible for long anoestrus in buffaloes.

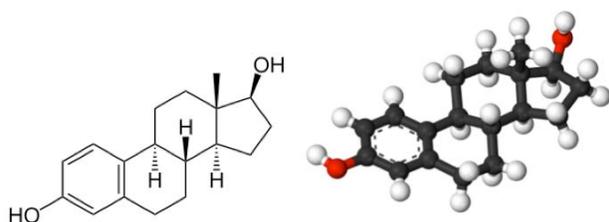
The pattern of P4 concentrations in milk has been found to be similar to that in plasma, although the concentrations in milk are much higher than those in plasma [16]. The average concentration of P4 in milk, which was 0.5 ng/ml at oestrus increased to 18 ng/ml on day 15 and declined thereafter to 4.4 ng/ml 3 days preceding the next oestrus in non pregnant buffaloes [24].

P4 concentrations in ovarian follicular fluid are much higher than those in peripheral circulation [31, 32]. Palta *et al.* [33] reported that P4 concentrations (pmol/ml) were not related to follicular diameter and were not different among small (330.99±27.32), medium (384.84±26.20) and large follicles (253.25±32.23). P4 concentrations of ovarian follicular fluid have also been reported to be lowest in monsoon (July to Oct) and highest in summer (March to June) [34].

Hattab *et al.* [35] showed that the levels of P4 metabolites in faeces are a valid means to monitor the functionality of the corpus luteum as they reflect the blood levels of P4 with a high correlation coefficient ($R = 0.77$). In addition, it was reported that the P4 hastens the transportation of oocyte in the oviduct; it has a dominant role in the maintenance of pregnancy, especially during the first phase, and acts in synergy with estrogens by stimulating the growth and development of alveolar tissue of mammary gland.

The blood level of P4 is considered a good indicator of the beginning of puberty and heifers are considered to achieve puberty when plasma P4 levels exceeded 1 ng/ml for two consecutive samples with a low value interval. Many environmental effects influence the age of puberty. In general, all the factors slowing the growth, thus preventing the expression of full genetic potential, delay puberty. A buffalo heifer with a good level of nutrition reaches puberty at 19 months [36-38].

Estradiol 17B (E2)



Estrogens are hormones produced by the ovary and transported by carrier proteins, the most important of which is estradiol 17 β (E2). They act on the central nervous system causing the oestrus behaviour of the females. Peripheral plasma E2 profile in buffalo is not very different from that reported in cattle, with peak concentrations observed before and during the preovulatory surge of gonadotropins after which the levels come down to base values in the next few days, with minor fluctuations throughout the oestrous cycle. In buffalo the general pattern of secretion of E2 indicates a peak that takes place the day before the LH peak [39,40] or frequently very close to this with blood levels between 9 and 13 pg/ml in Murrah buffaloes and Mediterranean, respectively (Figure 2) [1]. After the LH surge the E2 levels come down to basal values in the next few days, involving a block of oestrus after a time interval relatively constant (approximately 12 hours). The basal levels of E2 during the luteal phase of the cycle, are between 3 and 8 pg/ml, but lower values can be observed in the early luteal phase (1.0-1.5 pg / ml) [39,40]. During the luteal phase, a minor peak of 10

pg/ml and a more sustained peak of 20 pg/ml were observed on days 4-5 and day 10 after oestrus. These minor peaks might have resulted from waves of follicular growth since buffaloes have been reported to undergo two or three waves of follicular growth during the oestrous cycle, with the second wave occurring during days 10-11 of the cycle [41,42]. The prooestrus rise of oestradiol may be associated with triggering of LH release by positive feedback on hypothalamo-hypophyseal axis [43].

In Swamp buffaloes, E2 profile during the oestrous cycle is more or less similar to that in the riverine buffaloes, but the overall concentrations have been reported to be lower in Swamp buffaloes [34].

Whole milk E2 concentrations have been reported to be higher and positively correlated with those in plasma during oestrous cycle in buffaloes [43,44]. The higher levels of E2 in milk than in plasma raised the question whether mammary gland is active in the uptake of E2 or whether the synthesis of hormone takes place partly in the mammary tissue also.

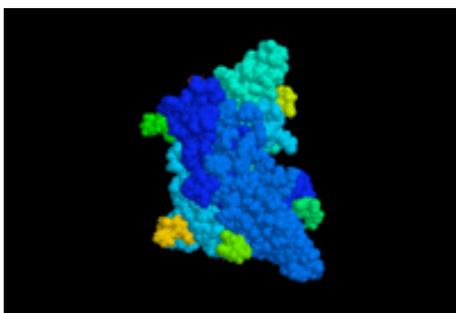
The ovarian follicular fluid E2 concentrations are much higher than those in peripheral circulation [32,45]. E2 concentrations have been reported to be positively related to follicular diameter and to be significantly higher in large (118.46 ± 30.25 pmol/ml) compared to those in medium follicles (50.32 ± 8.29 pmol/ml) which, in turn had higher E2 concentration than the small follicles (19.70 ± 5.57 pmol/ml, [33]. Parmar and Mehta [46], however, reported that the medium sized follicles contained significantly higher E2 compared to small and large follicles.

Plasma E2 concentrations are also affected by weather [30]. When oestradiol concentrations were compared among hot-dry (April to June), hot-humid (July to September), warm (October to December) and cold (January to March) seasons, E2 concentrations were found to be lower in summer compared to cooler months [47]. Lower peak values of E2 around oestrus coupled with decreased P4 concentrations was attributed to be the major reason responsible for a higher incidence of silent oestrus during summer by [48].

PITUITARY GONADOTROPHIN HORMONES

Follicle Stimulating Hormone (FSH)

Follicle stimulating hormone (FSH) promotes follicular growth and estrogen production by granulosa



cells in ovarian follicles. This hormone has been studied by several Authors in buffalo. Seren *et al.* [1], found that the FSH peak (1.6-6.0 ng/ml) coincides with the LH peak and lasts from 6 to 9 hours, with a remarkable lack of additional peaks during the luteal phase of the cycle, when hormone levels fluctuate between 0.2 and 1.5 ng/ml, before rising again to peak levels at the next oestrus (Figure 2). Moreover [39] found that the FSH peak coincides with that of LH, with an average around 25 ng/ml, but reported that the highest peak of this hormone occurs at day 10 after oestrus and the last increase is present at 4 and 15 days after oestrus. After the occurrence of simultaneous pre-ovulatory surges of FSH and LH, LH levels decline sharply to the basal levels whereas FSH concentrations show a gradual decline [49].

Palta and Madan [50,51], reported FSH peak values on average of 70-80 ng / ml and a duration of 5.8 ± 0.07 hours (during pregnancy) and 5.5 ± 0.02 hours (during the post-partum) after stimulation with GnRH.

It has recently been established, in buffalo, a temporary peak of FSH in the blood begins at each follicular wave [39,41,52] and decreased secretion of LH is associated with the loss of dominance and the end of the follicular wave that does not lead to ovulation. Palta and Madan [50,51] have found alterations in the release of pituitary gonadotrophins in pregnant buffaloes and during postpartum. The basal level of FSH showed significant differences during pregnancy; in fact, it was higher at 60 days compared to 240 days of pregnancy. During the postpartum baseline levels of LH and FSH increased significantly from day 2 to day 20, but only the LH levels were higher on day 35 compared to 20 days. The authors explain this phenomenon with a progressive reduction in pituitary sensitivity to GnRH during pregnancy and this could be due to the chronic negative feedback exerted by gonadal steroids, along with a probable reduction in pituitary GnRH receptors.

Peripheral FSH concentrations are also influenced by weather [30]. FSH concentrations have been reported to be significantly higher at oestrus and during the luteal phase in peak breeding seasons (November to December) in comparison to corresponding phases of medium (July to October) and low breeding season (March to June) in Surti buffaloes [53]. The FSH/LH ratio was higher during the peak breeding season compared to those in the medium and low breeding periods. In another study, the peak FSH concentrations on the day of oestrus were found to be similar during hotter and cooler months [54].

Peripheral plasma FSH concentrations increased from 1.67 ± 0.28 ng/ml and 1.23 ± 0.14 ng/ml on day -4 to reach a peak concentration of 4.13 ± 0.53 ng/ml and 3.42 ± 0.41 ng/ml on day 0 (day of oestrus) and decreased thereafter to 0.91 ± 0.08 ng/ml on day 6 and 0.63 ± 0.29 ng/ml on day 4 in buffaloes that exhibited overt oestrus and silent oestrus, respectively [34].

Luteinizing Hormone (LH)

The LH structure is similar to that of the other glycoprotein hormones, follicle-stimulating hormone (FSH), thyroid-stimulating hormone (TSH), and human chorionic gonadotropin (hCG). The LH is important in studies of ovarian activity since its pre-ovulatory peak is responsible for the follicular wall rupture and ovulation. Evident preovulatory LH surge have been detected in buffalo either with RIA or ELISA assay [55]. As also in case of cattle [56], peripheral LH remain at basal levels throughout the oestrous cycle till the day of oestrus when a pre-ovulatory LH surge occurs. Whereas the baseline values is around 0.72-3.0 ng/ml during a major part of the oestrus cycle, peak values of 20-40 ng/ml have been observed on the day of oestrus [49,57-61].

The interval from E2 peak to LH peak has been reported to be 14-15 h and the length of the LH peak in the Mediterranean Italian buffalo has been estimated to be 6-12 hours [1,55,62]. The interval between the LH surge and the onset of oestrus has been reported to be 8 h by [63]. Kanai and Shimizu [64] reported that LH surge occurred in association with behavioural oestrus in Swamp buffaloes and lasted for 7-12 h. Peak LH concentrations of 61-126 mg/ml were observed 4-15 h after the onset of oestrus whereas the interval between LH peak and the end of oestrus was much less variable. A direct positive feedback effect of E2 on LH secretion has not been demonstrated, however, occurrence of an E2 peak before the pre-ovulatory LH

surge and a positive correlation between E2 and LH during the 24 h period before the LH surge [64] indicate a role of E2 in mediating LH release. A pulsatile secretory pattern of LH has been demonstrated in buffalo (Figure 2) [1,65].

The LH time (in relation to oestrous symptoms and ovulation) is more important than the peak itself. Seren *et al.* [1] found that the average interval LH peak-ovulation was 35.5 hours in buffaloes with a single ovulation and 60 hours in those with double ovulation. Moili *et al.* [66], in a study on buffalo oestrous behaviour, found a mean interval peak LH-ovulation of 25.2 ± 13.1 hours for those animals which became pregnant and $46.1 \pm 18, 8$ hours for those which did not become pregnant. The same Authors found that the mean interval peak LH-end of courtship (interest shown by the bull towards a buffalo cow) was 2.4 ± 10.4 hours and 14.7 ± 15.2 hours in the two groups, respectively.

Studies on the evaluation of LH peak have been also carried out following oestrus synchronization

treatment [7,62,67]. In buffaloes synchronized in spring with a P4 intravaginal device (PRID) the mean interval from PRID removal to LH peak was 54.7 ± 12.3 hours ranging from 40 to 76 hours and the one from LH peak to ovulation was 31.0 ± 8.9 hours, similar to that found by [1] in non treated buffaloes. Evaluating the time of LH peak in oestrus synchronized buffaloes in two different seasons, it was found that the interval from PRID removal to LH peak was 46.87 ± 21.53 hours in November and 61.00 ± 12.05 hours in March. The ovulation occurred within 72 hours from PRID removal in November and within 96 hours in March [67].

Comparing PRID with Ovsynch treatment, no differences were reported in the mean values of LH peak and LH peak-ovulation interval, although a higher variability in the LH peak and ovulation time in the PRID treatment leads to a lesser degree of synchronization respect to the Ovsynch one [7].

Following different superovulation treatments no significant differences were found in the mean values

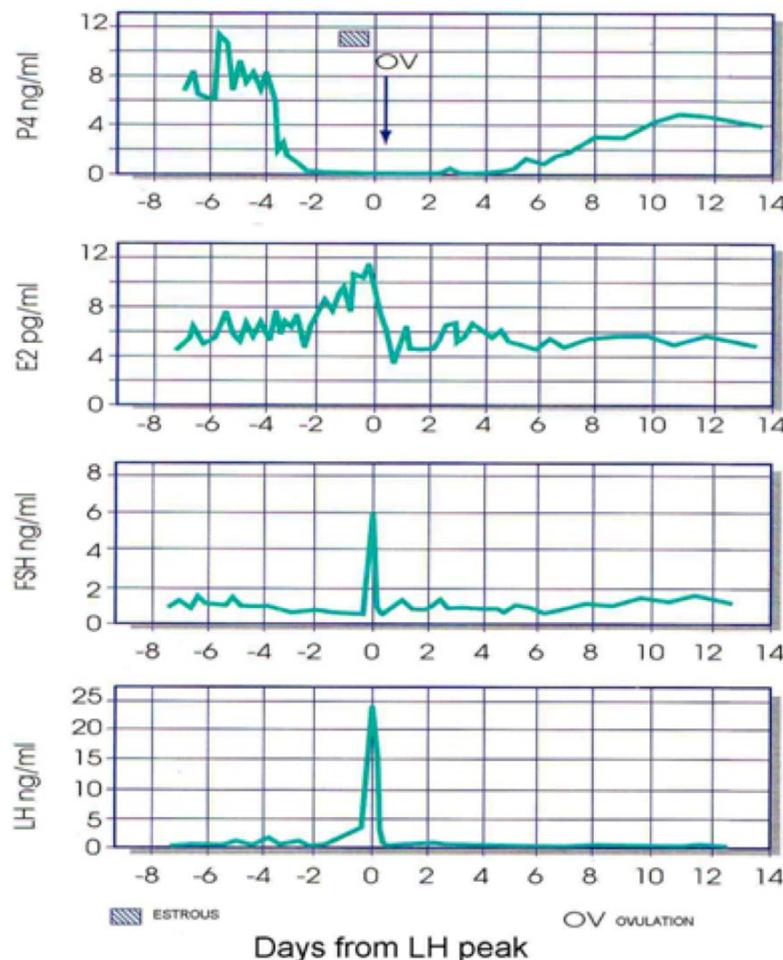


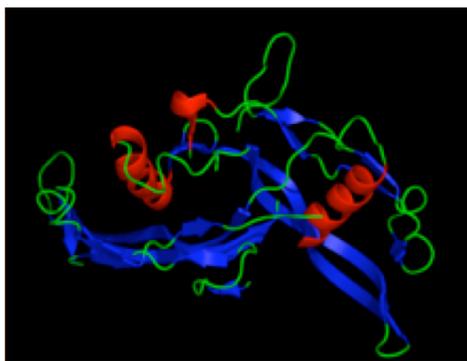
Figure 2: Perioestrus endocrine changes in the buffalo cow [1].

of LH peak (6.33 ± 3.64 ng/ml) nor in the ovulation rate (mean number of CL = 2.67 ± 1.97), while the timing of LH peak and the P4 concentration increase after ovulation, were affected by the different superovulatory treatments. [68].

As also for many other hormones, peripheral LH levels are affected by season in buffalo. Peak LH levels have been found to be higher on the day of oestrus in cooler compared to those in the hotter months [47] which may be due to inability of the hypothalamo-hypophyseal axis to produce a sustained increase in LH release in response to E2. The frequency and amplitude of pulses during the follicular phase have been reported to be significantly higher during winter than during summer season (frequency: 3.6 pulses/8 h vs. 2.8 pulses/8 h, amplitude: 3.7 ng/ml vs. 2.5 ng/ml, respectively) [65]. Lower basal LH level and lack of pre-ovulatory surge have been reported to be associated with ovarian inactivity and anoestrus condition during summer [69]. In contrast, [49] observed similar mean peak LH concentrations at the time of oestrus in hot and cool months. As oestrus behaviour is controlled either by accumulation of E2 in specific areas of hypothalamus [70,71] or by balanced plasma concentrations of E2 and P4 [72], the decrease in peak value of LH around oestrus together with decrease in P4 concentrations [48] may also be responsible for higher incidence of silent oestrus during summer.

NEW HORMONES RELATED TO REPRODUCTIVE FUNCTION IN BUFFALO

Inhibin



The inter-relationship between the pituitary and the gonad has long appeared too complex to be regulated only through the mediation of the two pituitary gonadotrophins, follicle-stimulating hormone (FSH) and luteinizing hormone (LH). Within the last decade, discoveries of additional regulatory mechanisms involved in the neuroendocrine control of the testis and

ovary have provided new insight into the control of this sophisticated biological phenomenon. The possible contribution of inhibins to the hypothalamo-pituitary-gonadal axis, and their role as paracrine regulators in the testis and ovary has received increasing attention over the years following their isolation and sequencing.

Inhibins were first isolated in 1985 and consist of an α -subunit (18 kDa) linked by a disulfide bridge to one of two highly homologous β -subunits (14 kDa), designated β_a and β_b , to form either inhibin A or inhibin B. The name 'inhibin' is derived from its ability to inhibit the release of FSH from the pituitary, and was initially isolated from ovarian follicular fluid as a classical endocrine hormone [75]. However, since its isolation, inhibin, along with its antagonist, activin, has been shown to be a member of the transforming growth factor- β (TGF β) superfamily, to be widely expressed, and to have local regulatory roles in a variety of tissues (in addition to the ovary), including the brain, the adrenal, bone marrow, the foetus and the placenta [76].

Inhibin A and B are differentially secreted across the ovarian cycle. Inhibin A is thought to be a marker of the maturity of the dominant follicle [73] whereas Inhibin B is the predominant form in the smaller antral follicles and may be an indicator of ovarian reserve [74] (Figure 3).

Over the past years, many works have focused on measurement of inhibin concentration and/or activity in biological samples in order to understand its role in physiology and disease: there are studies about possible use of inhibins in reproductive medicine and recent data indicate the role of Inhibin A in obstetrics.

Inhibins are so today used as diagnostic markers in human reproduction [77]: inhibin A is a marker of dominant follicle and corpus luteum activity and decreases in polycystic ovary syndrome (PCOS); increases in gestational diseases such as pre-eclampsia and fetal Down's syndrome and this increase in inhibin A improves early diagnosis of both conditions; it also provides useful information about the likelihood of pregnancy loss.

With regard to the gonadotrophic control of inhibin secretion, early studies were compromised by the use of unspecific assays of inhibin which detected precursors and free subunits in addition to dimeric forms [78,79]. Radioimmunoassay was the first assay developed, providing a wealth of insight into reproductive physiology [80]. More recently, with the

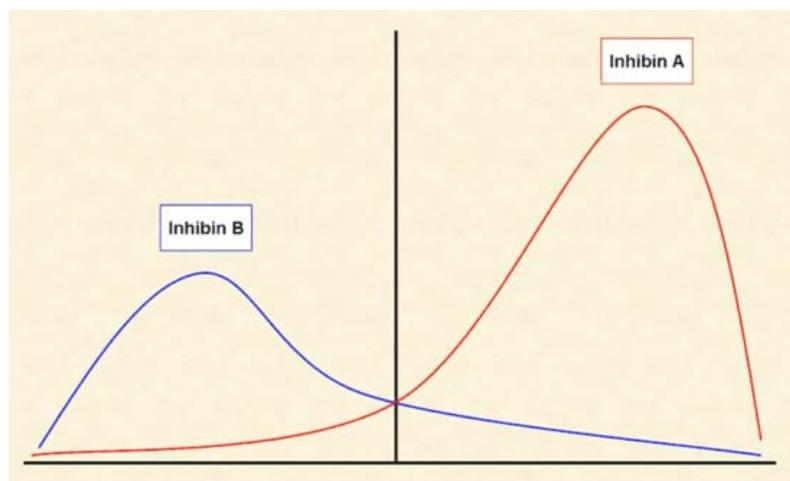


Figure 3: Inhibin A and B level change throughout the cycle: (Inhibin B is produced in small developing follicles, which is re-placed by the Inhibin A as the follicles approach the preovulatory stage).

advent of the specific assays for the various species of inhibins (Inhibin-A, inhibin-B, inhibin pro-alphaC) has an investigation of the control of inhibin secretion been possible, providing precise and replicable results for use in clinical and physiological research.

To date the changes in serum inhibin have been reported: in prepubertal and pubertal bovine bulls [81]; in male monkeys to verify seasonal plasma inhibin variations [82]; in rams to study the inhibin levels during seasonal cycles [83]; in mares at the time of ovulation and during oestrous cycle [84,85]; in cattle undergoing treatment for superovulation [86], before and after ultrasound guided follicular aspiration [87], during normal oestrous cycle and after treatment with steroid-free bovine follicular fluid (bFF) [88]; in heifers from birth to puberty [89].

In buffalo the research on inhibin level was undertaken to investigate the changes in peripheral inhibin levels: in superovulated buffalo cows [90-92] and in relation to climatic variations and stage of oestrous cycle [93]. Singh *et al.*, [39] in a study on plasma inhibin levels in relation to steroids and gonadotrophins during oestrous cycle, reported that plasma inhibin levels ranged between 391.25 and 631.97 pg/ml during various phases of the oestrous cycle and were found to be higher than that reported in cows. The mean plasma inhibin concentration on the day of oestrus was 562.5 +/- 18.9 pg/ml. Levels of FSH in the plasma showed three mid-cycle elevations which corresponded to comparatively lower inhibin and elevated E2 levels during the same period. From this observation it was deduced that both inhibin and E2 have a feed-back regulatory effect on FSH secretion in buffalo.

Terzano *et al.* [94] evaluated the relationship between inhibin A in plasma (analyzed in duplicate by a sandwich immunohistochemistry) and follicular development in prepubertal Mediterranean Italian buffaloes subjected to two different ovarian stimulation protocols. The data suggested that the medium and large follicles are the most important site of production of this hormone and the concentration of inhibin A during treatment with FSH might be used as a marker in the control of ovarian hyperstimulation.

Terzano [92] focused the research on developmental approaches to utilize Inhibin-A in ways to benefit buffalo production: evidence is so emerging that monitoring the stimulation phase with inhibin-A estimations as well as ultrasound scans give a better indication of follicular recruitment and development than the classic follicle stimulating hormone (FSH)-dependent marker E2 [91]; monitoring the time of ovulation with inhibins estimations as well as ultrasound scans might be a useful method for detecting the time of ovulation; it might be a good marker for follicular development and pregnancy rate in synchronized and artificially inseminated buffalo cows. The same author reported that monitoring the prepubertal phase with inhibin-A estimations as well as ultrasound scans might give an earlier indication of onset of puberty than the classic P4 hormone.

Glycoproteins Associated with Pregnancy (PAGs)

The PAGs are a large family of glycoproteins synthesized by the binucleate cells of trophoblast and released in maternal blood from the time of the implant until the calving. During the last week of pregnancy were isolated from the placenta of various species of

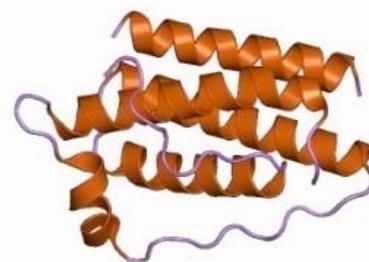


ruminants [95]. Recently it became evident that there are more than 100 genes PAG and that many of these are expressed [96]. Among the best known of these is the specific protein B (PSPB), first found in bovine placenta [97], and now used for the diagnosis of pregnancy in many animals. This is, like other PAGs, a real marker of fetal-placental welfare and could be used as a technique for early diagnosis in animals with a high risk of abortion [98-100]. The first study of the presence of PAGs in pregnant buffaloes was made by [101] and showed that the blood concentration is clearly related to pregnancy. Malfatti *et al.* [102], using a double antibody RIA [103] and antibodies to bovine PSPB, they found that the hormone in this species becomes detectable in 33% of the buffaloes between the 20th and the 25th day after conception. At the 30th days it is detectable in all the animals (1.6 ± 1.1 ng / ml) and the 35th day, the 91% of the animals has a blood level of PAGs more than 1.0 ng / ml. The hormone concentration reaches values of 6.6 ± 3.6 ng / ml at the 50th day and at the end of pregnancy the values are similar (6.28 ± 1.87 ng / ml).

The PAGs decrease after delivery: 5 days after calving the value is 45% less. The subsequent decline was much slower: the average hormone concentration was reduced by half in 10 days, 50 days after birth, plasma concentrations of PAGs are no longer recordable (less than 0.3 ng / ml). Barbato *et al.* [95], have described the first isolation and partial characterization of PAGs from the buffalo placenta which allows the study of their blood concentration and its trend during pregnancy becoming a pregnancy test for the buffalo species.

Leptin

Leptin serves as a metabolic signal that acts on the hypothalamic-pituitary-ovarian axis to enhance GnRH and LH secretion and ovarian function. It has been



reported in several farm animals, that leptin stimulates steroidogenesis and modulated follicular development [104-107]. Leptin effects on gonadotropin-releasing hormone (GnRH) and LH secretion are mediated by neuropeptide Y (NPY) and kisspeptin, thus, leptin appears to be an important link between metabolic status, the neuroendocrine axis and subsequent fertility in farm animals [108].

Leptin, discovered 15 years ago in rodents, is a 16 kDa protein produced by the obesity (OB) gene and it is believed to be involved in regulation and deposition of fat. In ruminants, as in other species, leptin is secreted predominantly by adipose tissue. Plasma leptin level increases linearly with increased body fat mass; it reduces feed intake in rodents, chicken, pig, sheep and other livestock species. Leptin appears to have a role as: growth factor in a range of cell types; mediator of energy expenditure; permissive factor for puberty; signal of metabolic status; modulation between the foetus and the maternal metabolism. Leptin also interacts with other hormonal mediators and regulators of energy status and metabolism such as insulin, glucagon, the insulin-like growth factors (IGF-1), growth hormone (GH) and glucocorticoids.

The physiological properties support leptin as a strong candidate gene for evaluation of genetic polymorphisms. If a polymorphic genetic marker for the leptin gene is identified, it may be used for selection of animals with desired traits [109,110]. Studies made in this field indicate that this hormone can influence buffalo milk's quality as in bovine, ovine and caprine [111]. Several Authors, studying the bovine *leptin* gene, have also found association between polymorphisms and traits like energy balance, feed intake and fertility [112], fat carcass content, milk and protein yield [113]; carcass and meat quality [114]. Terzano *et al.* (2011, unpublished data) carried out a study aimed to produce the sequence of the 5' flanking (promoter) region and entire coding regions of this gene in buffalo, to look for Single Nucleotide Polymorphism (SNP) useful for association studies with body traits and hormonal

values at the onset of puberty in buffalo. Seventeen SNP's were segregating in the sample population, significantly associated with width chest and inhibinA assayed forty days before puberty; with width pelvis and leptin assayed two months before puberty. This study has provided preliminary evidence that polymorphisms in the *leptin* gene might be useful as genetic markers for association studies in buffalo selection.

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