Relationship between Peripheral Plasma Inhibin and Progesterone Concentrations in Sahiwal Cattle (*Bos Indicus*) and Murrah Buffaloes (*Bubalus bubalis*)

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ABSTRACT : The present study investigated peripheral plasma immunoreactive inhibin (ir-inhibin) concentrations in relation to the stage of oestrous cycle and progesterone concentrations in cycling Sahiwal cattle (*Bos indicus*) and Murrah buffaloes (*Bubalus bubalis*). Blood samples were collected once daily for thirty-two consecutive days from cattle and buffaloes (5 each) during winter months of January and February. Mean (±S.E.M.) plasma ir-inhibin concentrations ranged from 0.40±0.01 to 0.59±0.03 ng/ml in cattle and from 0.29±0.03 to 0.52±0.05 ng/ml in buffaloes. In cattle, ir-inhibin concentrations increased from 0.47±0.07 ng/ml on day -4 (day 0=day of oestrus) to reach a maximum value of 0.59±0.03 ng/ml on day -2. Thereafter, ir-inhibin concentrations showed a decline to reach a low of 0.40±0.01 ng/ml on day 11 of the oestrous cycle. In buffaloes, ir-inhibin concentrations increased from 0.38±0.04 ng/ml on day -4 to reach a maximum concentration of 0.52±0.05 ng/ml on day -2. Ir-inhibin concentrations then declined to reach a low of 0.29±0.03 ng/ml on day 9 of the cycle. In both cattle and buffaloes, ir-inhibin concentrations which were lowest (0.43±0.02 and 0.34±0.02 ng/ml, respectively) during the mid-luteal phase of the oestrous cycle increased ($p<0.05$) to 0.52±0.03 and 0.44±0.04 ng/ml, respectively, during the late luteal phase, and then further to the highest value of 0.53±0.02 and 0.49±0.04 ng/ml, respectively, during the perioestrus phase, following which these declined to 0.50±0.02 and 0.39±0.03 ng/ml, respectively, during the early luteal phase. The variations in peripheral plasma ir-inhibin profile in both the species appear to be related to the changes in characteristics of follicular populations during the oestrous cycle. Peripheral plasma ir-inhibin concentrations were negatively correlated with progesterone concentrations in cattle ($r=-0.51$, $p<0.01$) and buffaloes ($r=-0.30$, $p<0.01$) indicating that the corpus luteum is not a source of peripheral ir-inhibin in these species. ***(Asian-Aust. J. Anim. Sci. 2003. Vol 16, No. 1 : 6-10)***

Key Words : Buffalo, Cattle, Inhibin, Progesterone

INTRODUCTION

Inhibin, a glycoprotein hormone produced by granulosa cells of ovarian follicles, suppresses hypophysial production and/or secretion of gonadotropins, preferentially Follicle Stimulating Hormone (FSH) through negative feedback at pituitary level (Burger, 1992). After induction of luteolysis, the follicular growth is stimulated by FSH which results in an increase in inhibin and oestradiol secretion by the dominant follicle leading to reduced peripheral FSH concentrations, which causes suppression of subordinate follicles during follicular phase of the oestrous cycle (Taya et al., 1991). Use of inhibin-based fecundity vaccines employing immunoneutralization of peripheral inhibin, which have been shown to increase ovulation rate and/or litter size in sheep and cattle (O'Shea et al., 1994) may hold high potential in improving fecundity in buffalo (Palta, 1998) and Zebu cattle. Progesterone as a marker for determining the functional status of corpus luteum as well as a diagnostic tool in identifying ovarian conditions such as oestrus confirmation, cyclicity monitoring have been well documented (Hoffman et al., 1976; Foote et al., 1979; Claus et al., 1983). We have earlier reported peripheral plasma inhibin concentrations in unstimulated (Palta et al., 1996a, 1997a) and superovulatory oestrous cycles (Palta et al., 1997b) in buffaloes. There is no information available on peripheral inhibin concentrations in indigenous Zebu cattle reared under tropical conditions. Also, information is lacking on the relationship between inhibin and progesterone in buffalo and Zebu cattle. The present investigation was, therefore, undertaken to i) measure peripheral plasma ir-inhibin concentrations during the oestrous cycle and ii) elucidate the interrelationship between ir-inhibin and progesterone in indigenous Zebu cattle (*Bos indicus*) and buffaloes (*Bubalus bubalis*), which exhibit silent oestrus even in the non-stressful seasons of the year (Kamboj and Prakash, 1993).

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MATERIALS AND METHODS

Experimental animals and blood sampling

Non-pregnant, non-lactating Sahiwal cattle and Murrah buffaloes (5 each; 5-6 year old and having body weight between 550-600 kg) were selected from the National Dairy Research Institute animal herd and were fed on the standard feeding and management conditions as practised in the general herd of the Institute. Blood samples were collected

from the jugular vein of the animals into heparinised tubes once daily for thirty-two consecutive days during the winter months of January and February. Blood samples were immediately transferred to an ice bucket and the plasma was harvested within 30 min of blood collection. Plasma samples were stored frozen at -20°C until analysis for inhibin and progesterone. Oestrus was detected by parading a vasectomized bull twice daily and/or visual signs, and later confirmed by plasma progesterone concentrations.

Hormone assays

Ir-inhibin concentrations were measured by a sensitive double antibody radioimmunoassay (RIA, Robertson et al., 1988) using highly purified 32kDa inhibin for iodination, bovine inhibin antiserum (rAs # 1989) and purified bovine 31 kDa inhibin (bINH-R-90/1) as reference preparation. The modified RIA procedure as standardised and validated in our laboratory (Palta et al., 1996a) was followed. It employed an ^{125}I-iodinated inhibin as tracer. The sensitivity of the inhibin assay was 16 pg/tube and the intra- and inter-assay coefficients of variation were <14% (n=5). The antisera used for the inhibin assay has been reported to have less than 0.05% cross-reactivity with free α- and beta-subunits of 31 kDa inhibin obtained by the reduction and alkylation of bovine inhibin, bovine activin A, bovine Mullerian inhibiting substance, human transforming growth factor-β as well as rat or human FSH, LH and TSH (Robertson et al., 1988). Progesterone concentrations in peripheral plasma were quantified by a simple, direct RIA described earlier (Kamboj and Prakash, 1993). The sensitivity of progesterone assay was 8 pg/tube. The intra- and inter-assay coefficients of variation were <17% (n=6). The progesterone antiserum (anti-progesterone-11-α-hemisuccinate-BSA) crossreacted with 4-pregnane-3,20-diene-11-α-hydroxyprogesterone and corticosterone to the extent of 100, 110 and 0.2%, respectively. The crossreactivity of the antiserum with cortisone, hydrocortisone was less than 0.01%, and with β-estradiol, estriol and testosterone was less than 0.001%.

Statistical analyses

For statistical analyses, the oestrous cycle was divided into 4 phases namely late luteal phase (day -4 to day -2, day 0 = day of oestrus), perioestrus phase (day -1 to day 1), early luteal phase (day 2 to day 5) and mid-luteal phase (day 6 to day 14). Differences between inhibin concentrations among different phases were compared by Analysis of Variance (ANOVA). The relationship between plasma ir-inhibin and progesterone concentrations in cattle and buffaloes was calculated by correlation analysis (Snedecor and Cochran, 1967).

RESULTS

Mean (±S.E.M.) plasma ir-inhibin concentrations ranged from 0.40±0.01 to 0.59±0.03 ng/ml in cattle and from 0.29±0.03 to 0.52±0.05 ng/ml in buffaloes during the oestrous cycle (Figures 1 and 2).

In cattle, the peripheral ir-inhibin concentrations increased from 0.47±0.07 ng/ml on day -4 to reach a maximum of 0.59±0.03 ng/ml on day –2. Thereafter, the mean ir-inhibin concentrations showed a decline to reach a low of 0.40±0.01 ng/ml on day 11 (Figure 1). Peripheral ir-inhibin concentrations which were lowest (0.43±0.02 ng/ml) during the mid-luteal phase of the oestrous cycle increased ($p<0.05$) to 0.52±0.03 ng/ml during the late luteal phase and then further to the highest value of 0.53±0.02 ng/ml during the perioestrus phase. Ir-inhibin concentrations then decreased to 0.50±0.02 ng/ml during

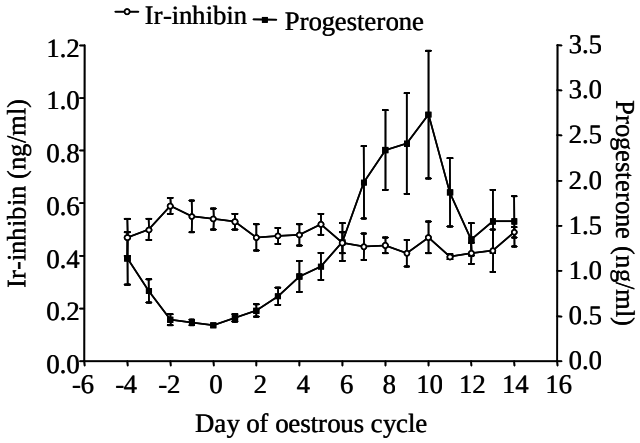

Figure 1. Peripheral plasma ir-inhibin and progesterone concentrations during different days of the oestrous cycle in cattle (day 0=day of oestrus).

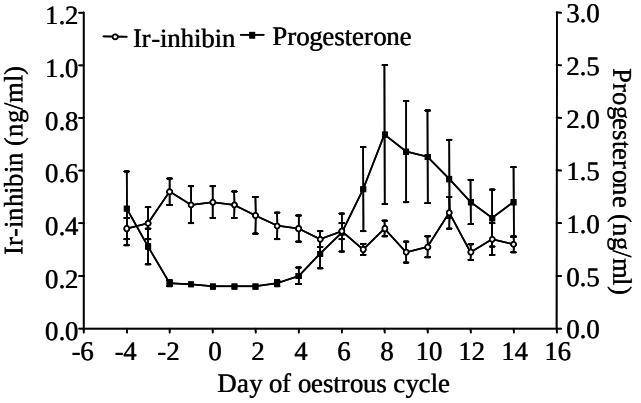

Figure 2. Peripheral plasma ir-inhibin and progesterone concentrations during different days of the oestrous cycle in buffaloes (day 0=day of oestrus).

the early luteal phase to reach the nadir at the mid-luteal phase (Figure 3).

A similar trend was observed in buffaloes in which peripheral ir-inhibin concentrations increased from 0.38±0.04 ng/ml on day -4 to reach a maximum concentration of 0.52±0.05 ng/ml on day -2. The mean ir-inhibin concentrations then showed a decline to reach a low of 0.29±0.03 ng/ml on day 9 (Figure 2). The peripheral ir-inhibin concentrations rose ($p<0.05$) from 0.34±0.02 ng/ml during the mid-luteal phase through 0.44±0.04 ng/ml during the late luteal phase to 0.49±0.04 ng/ml during the perioestrus phase of the oestrous cycle. Following this, ir-inhibin concentrations declined ($p<0.05$) to 0.39± 0.03 ng/ml during the early luteal phase to reach the nadir at the mid-luteal phase (Figure 3).

In cattle and buffaloes, the mean (±S.E.M.) plasma progesterone concentrations declined from 1.14±0.29 and 1.14±0.35 ng/ml, respectively, on day - 4 to 0.4 ng/ml on the day of oestrus and then rose to reach the peak values of 2.73±0.71 ng/ml on day 10 in cattle and 1.84±0.66 ng/ml on day 8 in buffaloes (Figures 1 and 2). A negative correlation was observed between peripheral ir-inhibin and progesterone concentrations in cattle ($r=-0.51$, $p<0.01$) and buffaloes ($r=-0.30$, $p<0.01$).

DISCUSSION

The present study demonstrates that peripheral plasma ir-inhibin concentrations exhibit a dynamic profile during the oestrous cycle and that the peripheral ir-inhibin and progesterone concentrations are negatively correlated in Sahiwal cattle and Murrah buffaloes.

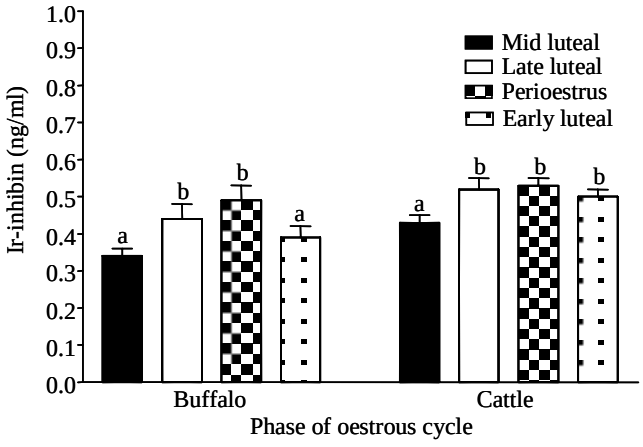

Figure 3. Peripheral plasma ir-inhibin concentrations during different phases of the oestrous cycle in cattle and buffaloes (mid-luteal phase: day 6 to day 14, day 0=day of oestrus; late luteal phase: day -4 to day -2; perioestrus phase: day -1 to day 1; early luteal phase: day 2 to day 5; Columns with different letters differ significantly a,b: $p<0.05$).

Use of diverse types of immunoassays by various authors for the estimation of inhibin has made the interpretation of inhibin data very difficult. The RIAs, which employed antibodies either against the α-subunit (Campbell et al., 1991; Martin et al., 1991) or against the mature 32 kDa α-β dimeric bovine inhibin (Robertson et al., 1988; present study) suffer from cross-reaction with free α-subunit and other monomeric precursors reported to be present in high concentrations in the bovine follicular fluid (Robertson et al., 1989). Although the two-site α-β dimer specific immunoradiometric assay (IRMA) is reported to be more reliable for measurement of dimeric 34 kDa bovine inhibin (Guilbault et al., 1993; Sunderland et al., 1996; Knight et al., 1996), neither RIA nor IRMA can accurately measure the concentrations of all molecular mass forms of inhibin (Ireland et al., 1994; Good et al., 1995; Sunderland et al., 1996). The antiserum used in the present study has been reported to cross-react with many molecular mass forms of inhibin (Robertson et al., 1989; Good et al., 1995). Therefore, the concentrations of inhibin reported in the present study have been referred to as immunoreactive inhibin concentrations.

In Sahiwal cattle as well as Murrah buffaloes, plasma ir-inhibin concentrations, which were lowest during the mid-luteal phase, increased through the late luteal phase to a maximum concentration during the perioestrus phase (Mondal et al., 2000a). These results are in partial agreement with earlier observations in sheep (Campbell et al., 1990; Findlay et al., 1990), cattle (Kaneko et al., 1992) and buffalo (Palta et al., 1997a) in terms of an increase in inhibin levels during the follicular phase of oestrous cycle and a decrease thereafter.

The antral follicles have been identified to be the primary source of circulating inhibin in sheep (Campbell et al., 1991), cattle (Guilbault et al., 1993) and buffalo (Palta et al., 1996b). In sheep, it has been shown that although large estrogenic follicles secrete the majority of inhibin, about 40% of inhibin is secreted by large non-estrogenic and small follicles (Campbell et al., 1991). In buffalo also, although the large (≥10 mm diameter) follicles contain concentrations of inhibin significantly higher than those in medium (6-9 mm diameter) and small (3-5 mm diameter) follicles, the medium and small follicles contain substantial amounts of inhibin (Palta et al., 1996b; 1998). As the population of the follicles of all size categories contributes to the circulating inhibin levels, the pattern of peripheral ir-inhibin concentrations observed in this study is probably a reflection of the overall follicular populations at different stages of the oestrous cycle. The growth and development of large, medium and small follicles has been shown to be bimodal in cattle (Pierson and Ginther, 1987) and buffalo (Manik et al., 1998a). The period between day 0 and day 8

(day 0=day of ovulation) which is marked by growth and development of the largest follicle is associated with an increase of short duration and a subsequent decline in the size of the second largest follicle and the number of medium and small follicles in cattle (Pierson and Ginther, 1987) and buffalo (Manik et al., 1998a) resulting in low follicular populations during the mid-luteal phase of the cycle. This could have resulted in the lowest concentrations of inhibin being observed during the mid-luteal phase of cycle. The onset of the next wave of follicular growth around day 10 is followed by an increase in the size of the dominant follicle and the total number of medium and small follicles in cattle (Pierson and Ginther 1987; Ginther et al., 1989) and buffalo (Manik et al., 1998b). This could have led to an increase in the peripheral ir-inhibin concentrations through the late luteal to the perioestrus phases of the oestrous cycle. An inverse relationship has been observed between peripheral plasma inhibin and FSH concentrations during the oestrous cycle in cattle (Taya et al., 1991) and buffaloes (Mondal et al., 2000b). The low concentrations of ir-inhibin during the mid-luteal phase, an increase through the late luteal and perioestrus phases and a decrease thereafter, as observed in the present study, may therefore be playing an important role in the regulation of FSH secretion in cattle and buffalo.

A negative correlation between peripheral plasma ir-inhibin and progesterone concentrations was due to occurrence of opposite patterns of peripheral concentrations of these two hormones. Following luteolysis, there was a sharp decline in progesterone concentrations through the late luteal to the perioestrus phase, with progesterone concentrations reaching basal levels around the day of oestrus. In contrast, ir-inhibin concentrations exhibited a progressive rise during this period. These results suggest that the corpora lutea are not the source of circulating ir-inhibin in both the species. In earlier studies in cattle, α and βA- inhibin mRNAs have been found to be present in antral follicles but not in corpora lutea in cyclic or pregnant cows (Rodgers et al., 1989; Torney et al., 1989). Kaneko et al. (1992) also reported that there was no positive correlation between circulating levels of inhibin and those of progesterone suggesting that bovine corpora lutea are not the source of circulating inhibin.

In conclusion, the results of this study indicate that peripheral plasma ir-inhibin concentrations exhibit a dynamic profile during the oestrous cycle and are negatively correlated with progesterone in Sahiwal cattle (*Bos indicus*) and Murrah buffaloes (*Bubalus bubalis*).

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