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Augmenting Fertility in Anestrus and Subestrus Cows with Hormonal Interventions and Their Effect on Blood Biochemical Profile

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ABSTRACT

A study was carried out on forty-four problem breeder cows to evaluate the therapeutic efficacy of GnRH and $PGF_{2\alpha}$ for estrus induction response, treatment to estrus induction interval and conception rate and its effect on biochemical profile. Thirteen true anestrus cows were treated with GnRH (Inj. Receptal, 2.5 ml, IM, once), twenty three subestrus cows were treated with $PGF_{2\alpha}$ (Inj. Estrumate, 2 ml, IM, once) and eight animals kept as untreated anestrus control. The estrus induction response, treatment to estrus induction interval, overall conception rate and service per conception in GnRH treated, $PGF_{2\alpha}$ treated and positive control cows were 84.62%, 28.27±6.46 days, 72.73%, 3.13; 91.30%, 3.52±0.46 days, 71.43%, 2.53 and 50%, 48.25±10.8 days, 50%, 3.5 respectively. The plasma P_4 level was significantly lower in all three groups as compared to other two periodic values (d-0 & d-20-22 post AI) which were due to luteal demise. The mean serum total protein was significantly ($p<0.05$) lower in untreated anestrus (8.57±0.36 g/dl) as compared to GnRH (9.75±0.28 g/dl) and $PGF_{2\alpha}$ (9.62±0.31 g/dl) treated cows. However, total cholesterol was numerically lowest in control cows than those of treated contemporaries but did not differ significantly (168.22±17.22; 208.46±14.71; 163.84±10.64 mg/dl; in GnRH, $PGF_{2\alpha}$ and control group respectively). In conclusion, anestrus and subestrus cows can be well managed with GnRH and $PGF_{2\alpha}$ therapy. The normal hormonal and biochemical milieu is essential for normal functioning of reproductive system.

Key words: Anestrus, Biochemical profile, Cows, Hormone, Subestrus

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INTRODUCTION

Bovine anestrus syndrome is one of the most prevalent reproductive disorders of dairy cows (Butani *et al*., 2008; Ashoo *et al*., 2020) which results in significant economic loss due to prolonged calving interval, reduced calf crop, and shorter productive life (Kumar *et al*., 2020). To resume cyclicity threshold LH concentration is obligatory. Prolonged period of ovarian dysfunction and failure of ovulation is mainly due to suboptimal plasma LH concentration. The causes of anestrus are multifactorial in origin, and there is no single panacea to correct the clinical syndrome. In spite of nutritional management (Dhami *et al*., 2019), it is primarily addressed by hormonal therapy (Bhatnagar *et al*., 2020). Normal level of biochemical constituents are utmost important for normal reproductive function. Thus, the study was undertaken to assess the efficacy of hormonal treatment (GnRH and $PGF_{2\alpha}$) for management of clinical syndrome of anestrus and its effect on blood biochemical profile.

MATERIALS AND METHODS

The study was carried out in the milk shed areas of Anand district, Gujarat. Anestrus cows were screened by twice trans-rectal palpation ten days apart and the cows with smooth, small, round and hard ovaries were grouped as true anestrus (n=13), and cows with palpable CL were referred as subestrus (n=23) and 8 true anestrus cows were kept as positive control. All cows were dewormed with Albendazole 3000 mg and owners were supplied with 1 kg mineral mixture (Amul brand) for feeding to their cows @ 30g per day for one month. The anestrus cows were treated with single dose Buserelin acetate @ 10 µg, intramuscularly (GnRH, Inj. Receptal, 2.5 ml, MSD) whereas subestrus cows were subjected with Cloprostenol @ 500 µg, intramuscularly ($PGF_{2\alpha}$, Inj. Estrumate, 2 ml, MSD). The estrus induction response (EIR), treatment to estrus induction interval (TEI), cycle wise conception rate i.e. conception rate (CR) at induced estrus (first service conception rate; FSCR), two subsequent natural estrus, and overall conception rate (OCR) were recorded. Estrus detection was done by visual observation and per rectal examination. If found in estrus, cows were inseminated with good quality frozen thawed semen following AM-PM rule. The animals were observed for return to estrus up to three cycles and returning animals were bred again. Pregnancy was confirmed by transrectal palpation 60 days post-AI.

Blood samples were collected on the day of treatment (d-0), day of induced estrus/AI and day 21 post-AI by jugular vein puncture in heparinized vacutainers. In untreated control cows, three samples were taken at 21 days interval. The plasma was separated out by centrifugation at 1200g for 15 min at 4°C and stored at -20°C with a drop of merthiolate (0.1%) as preservative until analyzed. The plasma progesterone was estimated by employing standard RIA technique (Kubasic *et al*., 1984). The levels of protein, cholesterol, triglycerides, and minerals were estimated by using standard procedures and assay kits of Coral Clinical System, Goa on biochemistry analyzer. The generated data were analyzed statistically completely randomized design and Duncan's NMRT (Snedecor and Cochran, 1994).

RESULTS AND DISCUSSION

Estrus Induction response and Conception rate

Estrus induction response, treatment to estrus induction interval, and conception rate are depicted in Table 1. The present findings suggest that ovulatory estrus can be induced in a month or so, with use of GnRH in apparently healthy anestrus cows. Various EIR and CR with GnRH treatment in anestrus cows have been documented. Like current findings, Karmakar *et al*., (Karmakar *et al*., 2012) recorded similar EIR (85%) using GnRH and vitA in true anestrus crossbred cows. Conversely, varying EIR and CR were reported by many previous researchers (El-Shahat and Badr, 2011; Kamal *et al*., 2012; Gupta *et al*., 2012; Islam *et al*., 2013; Soni *et al*., 2018; Dutta *et al*., 2019; Kumar *et al*., 2020; Ratnaparkhi *et al*., 2020). Variation in EIR and CR might be due to variation in age, breed parity and nutritional status of animals. The present study clearly indicates that ovarian cyclicity with ovulatory estrus can be effectively induced in a month or so with GnRH in apparently healthy anestrus cows.

Like present observations in $PGF_{2\alpha}$ treated subestrus cows, comparable findings were recorded by Gupta *et al*. (2012)(90%, 2.37±1.33 days and 78%;EIR, TEI and CR respectively) and El-Shahat and Badr (2011)(87.5% EIR, 53.7±4.2 h TEI and 71.42 % CR).Conversely, El-Desouky and Hussein (2015) recorded varying response using standard dose versus over dose of $PGF_{2\alpha}$ (65%, 4.53±0.44 days, 1.61±0.24 and 84.60% with 500µg vs 85%, 2.47±0.10 days, 1.41±0.17 and 82.30% with 750 µg; EIR, TEI, SPC and CR respectively) in postpartum cows; Dailey *et al*. (1983) reported lower EIR (41.6%) whereas Ratnaparkhi *et al*.(2020) observed higher EIR and FSCR (100% & 40%) in $PGF_{2\alpha}$ treated dairy cows.

The present findings clearly indicate that subestrus condition in cattle can be successfully treated with $PGF_{2\alpha}$. Whether prostaglandins (PGs) have direct effects on follicular growth prior to luteinizing hormone (LH) surge to initiate ovulation in livestock is unknown (Weems *et al*., 2006), but $PGF_{2\alpha}$ increases pituitary responsiveness to GnRH to release LH in the postpartum cows (Randel *et al*., 1996), however, PGs produced by the ovulatory follicle are indispensable for ovulation (Murdoch *et al*., 1993). Furthermore, Pfeifer *et al*. (2018) opined that $PGF_{2\alpha}$ can induce ovulation of dominant follicle by an independent mechanism of luteolysis and can be successfully used as an ovulation inducer in timed artificial insemination protocols for beef (Pfeifer *et al*., 2014)] and dairy cows (Pfeifer *et al*., 2016). The EIR, TEI and CR of treated cows were far more better as compared to untreated contemporaries, which suggest positive impact of GnRH and $PGF_{2\alpha}$ in management of anestrus and subestrus cases respectively.

Effect of Hormone Therapy on Plasma Progesterone

The mean plasma progesterone concentration (P_4 values) was depicted in Table 2. Significantly higher (P<0.05) P_4 was recorded at d-0 in GnRH and untreated control group which might be due to failure to diagnose presence of corpus luteum during trans-rectal ovarian palpation at commencement of experiment. Similarly, Dawson (1975) and Hanzen *et al*. (2000) also stated that trans-rectal ovarian palpation is not a perfect method of assessment of ovarian structures. Numerically highest P_4 values were recorded at d-0 in subestrus cows than GnRH and control groups, as former cows screened on the basis of presence of CL on the ovary. At d-20-22 post AI, the P_4 level was the highest in $PGF_{2\alpha}$ group, it appears that exogenously administered $PGF_{2\alpha}$ strengthen the luteal activity of subsequently formed corpus luteum, as $PGF_{2\alpha}$ act at the level of hypothalamus and pituitary, enhance GnRH induced LH release (Randel *et al*., 1996). Moreover, the highest conception rate at induced estrus in $PGF_{2\alpha}$ treated subestrus cows supported the view that luteal insufficiency might be a cause of lower conception rate at induced estrus in GnRH treated anestrus cows. The mean P_4 value differed significantly at day of commencement of treatment (day 0), at induced estrus and day 20-22 post AI in conceived cows. Similar trends were also noticed in non-conceived cows.

Effect of Hormone Therapy on Plasma Biochemical Profile

The overall mean total serum protein in untreated anestrus cows was significantly lower than GnRH and $PGF_{2\alpha}$ treated cows at third sampling (Table 3). Lower total protein in untreated control cows was in accordance with many previous reports (Virmani *et al*., 2011; Kruparan, 2013;

Table 1: Estrus induction response, estrus induction interval and conception rate of anestrus and subestrus cows to GnRH or $PGF_{2\alpha}$ treatment

Group	Therapy	EIR	TEI (days)	Conception Rate (%) 1st AI	2nd AI	3rd AI	OCR	SPC
Anestrus (n=13)	GnRH (10 μg)	84.62% (11/13)	28.27 ±6.46	18.18% (2/11)	44.44% (4/9)	40.00% (2/5)	72.73% 8/11	3.13 25/8
Subestrus (n=23)	$PGF_{2\alpha}$ (500μg)	91.30% (21/23)	3.52 ±0.46	52.38% (11/21)	30.00% (3/10)	14.29% (1/7)	71.43% 15/21	2.53 (38/15)
Anestrus control (n=8)	None	50% (4/8)	48.25±10.80	25% (1/4)	33.33% (1/3)	None	50% (2/4)	3.5

EIR: Estrus induction response; TEI: Treatment to estrus induction interval; OCR: Overall conception rate; SPC: Service per conception

Table 2: Serum Progesterone profile at d-0, induced estrus and day 20-22 post AI in different categories of cows (Mean ± SE)

Therapy/status	Number of animals	Serum Progesterone (ng/ml) At d-0	At estrus/AI	At day-20-22 post AI	Overall or pooled
GnRH/true anestrus	13	1.94±0.40[b]	0.51±0.10[a]	4.62±2.01[c]	2.36±0.75
$PGF_{2\alpha}$/subestrus	23	3.99±0.58[b]	0.54±0.05[a]	6.09±1.42[c]	3.54±0.65
Control/untreated	8	1.58±0.31[b]	0.45±0.07[a]	2.78±1.27[c]	1.61±0.47
Overall	44	2.68±0.35[b]	0.56±0.05[a]	5.05±0.82[c]	2.77±0.36
Conceived	25	3.02±0.62[b]	0.52±0.08[a]	7.71±1.15[c]	3.75±0.66
Non-conceived	19	2.36±0.34[b]	0.61±0.05[a]	2.59±0.62[b]	1.85±0.27

Means bearing different superscripts within row differ significantly (p<0.05)

Table 3: Overall mean (± SE) blood biochemical and macromineral status in different treatment groups and conceiving and non-conceiving anestrus cows

Therapy/status	No. of animals	Total protein (g/dl)	Cholesterol (mg/dl)	Calcium (mg/dl)	Phosphorus (mg/dl)	Magnesium (mEq/L)
GnRH/true anestrus	13	9.75±0.28[b]	168.22±17.22	9.06±0.28	8.61±0.27	3.40±0.15
$PGF_{2\alpha}$/subestrus	23	9.62±0.31[b]	208.46±14.71	10.68±0.26	9.48±0.22	3.70±0.14
Control/untreated	8	8.57±0.36[a]	163.84±10.64	9.39±0.12	8.28±0.15	3.09±0.14
Overall	44	9.57±0.18[b]	187.89±8.56	9.98±0.18	8.72±0.15	3.64±0.09
Conceived	25	9.41±0.27	182.82±11.76	10.04±0.29	8.91±0.27	3.84±0.11
Non-conceived	19	9.73±0.24	192.59±12.47	9.92±0.22	8.55±0.17	3.46±0.15

Means bearing different superscripts within column differ significantly (p<0.05)

Ray *et al.*, 2016; Kumar *et al.*, 2018; Dhami *et al.*, 2019; Mangrole *et al.*, 2019; Mondal *et al.*, 2019). Furthermore, the protein level was lower in conceived cows as compared to non-conceived contemporaries and the findings are in agreement with Dhami *et al.* (2015) who reported lower total protein in conceived than non-conceived cows (9.30±0.19 vs 10.80±0.20mg/dl) in ovsynch treated cows. Hypoproteinemia causing deficiency of certain amino acids required for biosynthesis of gonadotrophins and other gonadal hormones, might cause reproductive endocrine anomaly leading to dysfunction ovary (Roberts, 1986). Furthermore, Pandey *et al.* (2015) opined that rise of plasma total protein might be associated with high level of estrogenic activity.

The overall mean total cholesterol level was numerically lowest in untreated anestrus cows but did not differ significantly among different groups (Table 3). The findings are comparable with many previous reports (Soni *et al.*, 2018; Weems *et al.*, 2006; Kumar *et al.*, 2018) who recorded lower cholesterol concentration in anestrus cows. Like present study, nonsignificant difference in total cholesterol level was recorded by Dhami *et al.*(2015) in conceived and non-conceived cows (136.72±1.48 vs 120.46±3.05 mg/dl). Current findings are supported by Henerick *et al.* (1971) who opined that the highest adrenal cholesterol values occur at estrus when females are under estrogen dominance eventually facing a decline later when progesterone phase sets in. This is again supported by present lower overall pooled value of cholesterol in conceived (progesterone dominance) than those of non-conceived cows.

Effect of Hormone Therapy on Plasma Minerals Profile

The serum calcium level did not vary significantly among groups (Table 3). Like present findings many previous reports mentioned non-significantly or significantly lower calcium level in anestrus cows as compared to values at induced estrus or cyclic cows (Virmani *et al.*, 2011; Agrawal *et al.*, 2015; Ray *et al.*, 2016; Kumar *et al.*, 2018; Satapathy *et al.*, 2018; Soni *et al.*, 2018; Dhami *et al.*, 2019; Mangrole *et al.*, 2019;). The available reports suggest that lower calcium level might be a cause of anestrus (Youngquist and Threlfall, 2007). Furthermore, in the present study the calcium level was higher in conceived cows but did not differ significantly as compared to non-conceived contemporaries. Conversely, Dhami *et al.* (2015) reported nonsignificantly lower calcium level (9.52±0.14 vs 8.85±0.11 mg/dl) in conceived than non-conceived ovsynch treated cows. Calcium primarily has an indirect effect on reproduction (Gerloff and Morrow, 1986); hypocalcemia predisposes the cows to parturient paresis and sensitizes the tubular genitalia for action of hormone. Moreover, Capen and Rosol (2003) opined that estrogen increases intestinal calcium absorption. Calcium when severely deficient affects reproductive function adversely (Youngquist and Threlfall, 1997). Moreover, calcium, calcium phosphorus ratio, and balance of calcium with vitamin D are also linked to altered reproductive performance (Spain *et al.*, 2012).

The mean phosphorus level did not differ significantly in different groups but the value was lowest in untreated anestrus cows (Table 3). Similar patterns were documented in many previous reports (Virmani *et al.*, 2011; Ray *et al.*, 2016; Kumar *et al.*, 2018; Soni *et al.*, 2018; Mangrole *et al.*, 2019). Furthermore, Dhami *et al.* (2015) observed nonsignificant difference in serum phosphorus level among conceived and non-conceived cows using ovsynch protocols in crossbred cows. Blood level below 4 mg/dl usually indicates phosphorus deficiency (Roberts, 1986; Noakes *et al.*, 2019), normal values being 4 to 8 mg/ dl. Phosphorus is the mineral most frequently associated with reproductive abnormalities in cattle (Gerloff and Morrow, 1986). Some scientists (Morrow, 1969; Morris, 1976) suggest that phosphorus deficiency causes infertility (anestrus, subestrus, irregular cycle, and low conception rates) while others

Table 4: Overall mean (± SE) blood plasma micromineral status (ppm) in different treatment groups and conceiving and non-conceiving anestrus cows

Therapy/status	Number of animals	Zinc	Iron	Copper	Cobalt	Manganese
GnRH/true anestrus	13	1.13±0.08	2.94±0.14	0.66±0.03	0.18±0.02	0.09±0.01
$PGF_{2\alpha}$/subestrus	23	0.72±0.04	3.19±0.14	0.63±0.03	0.13±0.01	0.08±0.01
Control/untreated	8	0.88±0.04	2.63±0.12	0.45±0.02	0.13±0.01	0.06±0.01
Overall	44	1.04±0.07	3.06±0.09	0.64±0.02	0.14±0.01	0.08±0.01
Conceived	25	1.05±0.12	3.02±0.10	0.65±0.03	0.13±0.01	0.06±0.01
Non-conceived	19	1.03±0.06	3.09±0.14	0.63±0.02	0.15±0.01	0.09±0.01

(Castairs *et al*., 1980) found no evidence of hypophosphataemia associated infertility. Thus, there is conflicting opinion of several scientists about hypophosphataemia as a cause of infertility (Noakes *et al*., 2019).

The magnesium level was lowest in untreated anestrus cows as compared to treated cows, though the difference was nonsignificant (Table 3). Furthermore, the value was numerically higher in conceived cows than non-conceived one. Magnesium (Mg) requirement is more at time of occurrence of high energy demand, as association of Mg is well known as a co-factor in all ATP requiring enzymatic processes in general metabolism.

The overall pooled values of trace minerals (Zinc, iron, copper, cobalt and manganese) neither differed significantly among anestrus, subestrus and control cows nor among conceived and non-conceived cows (Table 4). The iron, copper and manganese were lower in untreated anestrus cows than those of treated contemporaries. The serum trace minerals in our study are in close conformity with findings of Desai *et al*. (1979). Low iron level may be attributed to low tissue oxygenation of uterus which in turn results in impaired nutrition to the uterus (Reddy and Reddy, 1988). Furthermore, copper plays important role in formation of hemoglobin, which is oxygen carrying pigment of the blood. Radostits *et al*. (2007) opined that reproductive function of male and females are most sensitive to manganese deficiency. Present findings of lower iron and copper concentrations are consistent with observations of Vadnere and Singh (1989) who reported significantly lower iron and copper level in anestrus cows than those of cyclic cows.

CONCLUSIONS

In conclusion, anestrus and subestrus conditions in crossbred cows can be effectively managed with aforesaid therapeutic regimens. Assessment of blood biochemical profile is important for better understanding of clinical syndrome of anestrus.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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