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Seasonal variations in superovulatory responses of Holstein cows treated with pregnant mare serum gonadotrophin under semi-arid environment

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This study was designed to examine the effect of hot summer season on superovulatory responses of 20 Holstein cows in a semi-arid environment. Oestrus was synchronised using two injections of 15 mg prostaglandin $F_{2\alpha}$ (PG; Prosolvin, Intervet, Holland) given 11 days apart. In each season, ten cows were superovulated with 2000 iu (International Units) of intramuscular pregnant mare serum gonadotrophin (PMSG) on day 10 of their synchronised oestrous cycle. All cows were treated with PG on day 12 of oestrous cycle and naturally mated during oestrus. Embryos were collected non-surgically on day 6 post mating. On the day of embryo recovery, the number of corpora lutea (CL) were counted by rectal palpation. Daily blood samples were collected during the study period. The results showed that ovarian responses were adversely affected by the hot summer as indicated by a reduced number of CL, total ova/embryos and quality of embryos during summer compared to winter. Although summer heat did not significantly affect progesterone (P_4) and oestradiol (OE_2) concentrations, P_4 levels tended to be higher in winter compared to summer. It is concluded that the superovulatory responses in cows in a semi-arid climate were adversely affected by hot summer months compared to winter.

Keywords: summer season; superovulatory responses; Holstein cows

Introduction

Superovulation is a major component for successful embryo transfer techniques. A main drawback of the superovulation technique is the high variation in the superovulatory responses to PMSG injection in terms of number of ovulations. Part of the variation can be attributed to the environmental conditions, mainly seasonal variations in ambient temperature. These seasonal variations are considered to impose physiological stress which affects the superovulatory responses. Reports concerned with seasonal variations in superovulatory responses are inconclusive. Several workers reported that the superovulatory responses were higher in winter compared to summer

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(Hasler *et al.*, 1983; Almeida, 1987; Gordon *et al.*, 1987). Others failed to observe any seasonal variations in the superovulatory responses of cows (Critser *et al.*, 1980; Putney *et al.*, 1988). A common breed of dairy cattle, raised in Saudi Arabia, is the exotic temperate-evolved Holstein cows. The environmental temperature prevailing in the central regions of Saudi Arabia remains above the thermoneutral temperature of Holstein (21°C) for at least 8 months of the year. Therefore, the objectives of the present study were to investigate the effect of hot summer and moderate winter seasons on superovulatory responses (endocrine responses, the number of CL, total and viable embryos and quality of embryos) of superovulated Holstein cows raised in the semi-arid environment.

Materials and methods

Twenty Holstein cows with normal reproductive tracts were used in this experiment during June–July of 1991 (summer season) and January–February of 1992 (winter season) at the farm of the Animal Production Department in Riyadh, Saudi Arabia. Animals were housed together in an open shaded barn (about 6 m × 25 m). No cows were lactating at the time treatment was carried out. Cows were fed according to the standard system practiced in the farm on concentrates and roughage. The concentrates mixture (containing 13·4% digestible protein and 72% total digestible nutrients) was offered to animals according to their actual requirements and was given at 0600h and 1600h. The roughage was provided *ad libitum* and consisted of 100% green fodder (*Medicago sativa*). Water was available at all times in a drinking basin and salt licks were provided *ad libitum*.

During each season, ten cows were utilized (with an average body weight of 510 ± 18 kg in summer, and 485 ± 20 kg in winter). Oestrous cycles of all cows were synchronised using two intramuscular (im) injections of 15 mg prostaglandin $F_{2\alpha}$ (PG; Prosolvin, Intervet International B.V., Boxmeer, Holland). The first injection (PG1) was given at the initiation of the study and the second one (PG2) was given 11 days after PG1. Animals were then observed for oestrus over a period of 4 days. On day 10 (0 = day of first standing oestrus) of their synchronised oestrous cycle, cows were treated with 2000 iu of PMSG (Folligon) im (all PMSG was from Intervet International B.V., Boxmeer, Holland with the same batch number). All cows were treated with 15 mg PG (PG3) on day 12 of their cycles. Cow were then observed for oestrus and naturally mated by a bull of proven fertility twice, 12 and 30 h after detection of induced oestrus. On day 6 after oestrus, cows were flushed, and ova/embryos were collected nonsurgically (Mapletoft, 1986), counted and classified (Lindner & Wright, 1983); both ovaries were palpated per rectum to estimate the number of CL and unovulated follicles.

Daily blood samples were collected from the day before PMSG injection until the day of ova/embryos collection. Blood samples were immediately cooled and centrifuged, and plasma was stored at –20°C for later analysis. Concentrations of P_4 and E_2 were estimated by a direct solid phase ^{125}I RIA method (Coat-A-Count TKPG and TKE; Diagnostic Products Corporation, Los Angeles, CA, U.S.A.) according to the manufacturer's methods with slight modifications in assaying E_2 (Bevers & Dieleman, 1987). The main cross reactivities for P_4 were 2·4, 2·0, 1·7 and 1·3% for 11-deoxycortisol, 20α-dihydroprogesterone, 11-deoxycorticosterone and 5-pregnan-3,20-dione, respectively, and for E_2, 10·0, 4·4, 1·8 and 1·8% for oestrone, d-equilenin, oestrone-β-d-glucuronide and ethinyl oestradiol, respectively. The intra-assay CV was 4·7 and 5·3% and the inter-assay CV was 8·0 and 6·4% for P_4 and E_2, respectively.

During the experiment period, daily maximum and minimum ambient air temperatures (°C) were recorded in the open shaded barn from the day of PG2 injection to the day of ova/embryos collection. Mean minimum and maximum

Table 1. *Ovarian response parameters as affected by summer and winter (means ± standard errors)*

Parameters	Summer	Winter	Overall
No. Animals	10	10	20
Corpora lutea	4·8±0·2	6·6±0·2**	5·7±0·2
Total ova/embryos	3·4±0·2	4·5±0·2**	3·9±0·1
Embryo quality			
Excellent	0·4±0·06	1·1±0·06**	0·75±0·04
Good	0·6±0·07	1·5±0·07**	1·05±0·05
Fair	0·9±0·07	1·1±0·07**	1·0±0·05
Poor	0·7±0·06	0·3±0·06*	0·5±0·04
Unfertilized ova	0·8±0·08	0·5±0·08*	0·65±0·06

**Significantly different ($p < 0·01$) between two groups.
*Significantly different ($p < 0·05$) between two groups.

temperatures in summer were 28°C and 43°C and in winter were 8°C and 18°C. Data were subjected to statistical analyses at King Saud University Computer Centre using the General Linear Models procedure of the Statistical Analysis System (Goodnight *et al.*, 1986).

Results

Mean numbers of CL, total ova/embryos collected, unfertilized ova, and embryo quality (ovarian responses) are presented in Table 1. The overall mean number of CL counted by rectal palpation on the day of embryo flushing was 5·7 ± 0·2 per cow. The overall mean total ova/embryos was 3·95 ± 0·1 per cow. The rate of embryo recovery was 69·3%. This was calculated as percentage of ova/embryos relative to the number of CL. There were large variations in ovarian responses of an individual cow in both summer and winter seasons. Summer season adversely affected the ovarian response parameters compared with winter. The means of CL, total ova/embryos, and excellent, good and fair embryos were significantly ($p < 0·01$) lower in summer compared with winter (4·8, 3·4, 0·4, 0·6 and 0·9 *vs.* 6·6, 4·5, 1·1, 1·5 and 1·1 per cow, respectively).

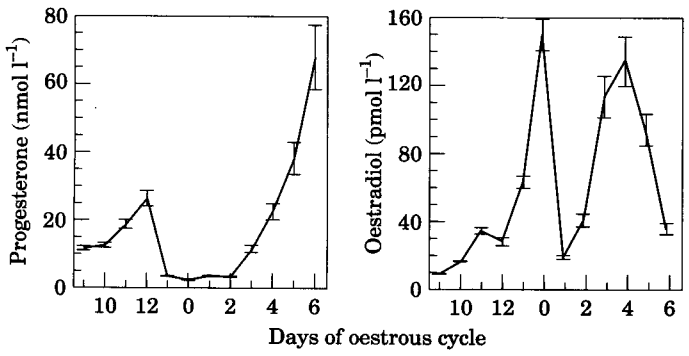

Figure 1. Overall mean of progesterone and oestradiol concentrations (± SEM) in PMSG treated cows ($N = 20$).

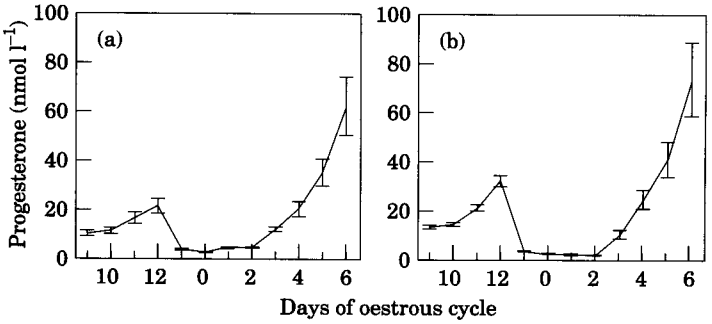

Figure 2. Mean progesterone concentration (± SEM) during (a) summer and (b) winter in PMSG treated cows ($N = 10$).

In addition, poor embryos and unfertilized ova were significantly ($p < 0{\cdot}05$) higher in summer compared with winter (0·7 and 0·8 *vs.* 0·3 and 0·5 per cow, respectively).

The overall mean of P_4 and OE_2 concentrations are shown in Fig. 1. P_4 levels started to increase from day 10 of oestrous cycle (day of PMSG in treated cows) until day of PG3 injection. In all treated cows, P_4 concentrations fell to $< 3{\cdot}2$ nmol l^{-1} after luteolysis (48–72 h post PG3 injection) and remained low until day 2 post oestrus. Thereafter, P_4 levels increased to a maximum level on day 6 post oestrus. As shown in Fig. 1, the overall mean of OE_2 started to increase 24 h post PMSG injection, and showed a peak level on the day of oestrus. One day post oestrus, OE_2 levels declined to the basal level and then increased to reach a maximum level on day 4 post oestrus. Thereafter, OE_2 declined until the end of the experiment.

Mean P_4 concentrations during summer and winter seasons are presented in Fig. 2. Levels of P_4 varied widely in both summer and winter seasons among individual superovulated cows, with no significant seasonal effects being observed. However, P_4 levels tended to be higher in winter than in summer during days 4, 5, and 6 post oestrus (25, 41 and 74 *vs.* 20, 35, and 62 nmol l^{-1}, respectively). There were significant ($p < 0{\cdot}01$) relationships between P_4 concentrations on day 10, 11 and 12 of oestrous cycle and subsequent numbers of CL and total number of ova/embryos ($r = 0{\cdot}84$ and 0·81, 0·89 and 0·84, 0·88 and 0·86, respectively). The levels of P_4 at day 5 post oestrus was significantly ($p < 0{\cdot}01$) correlated with the number of CL and total ova/embryos ($r = 0{\cdot}90$ and 0·96, respectively).

Mean OE_2 levels during summer and winter seasons are shown in Fig. 3. There were wide variations in the levels of OE_2 among individual superovulated cows in both summer and winter seasons with no significant seasonal effects. There were significant

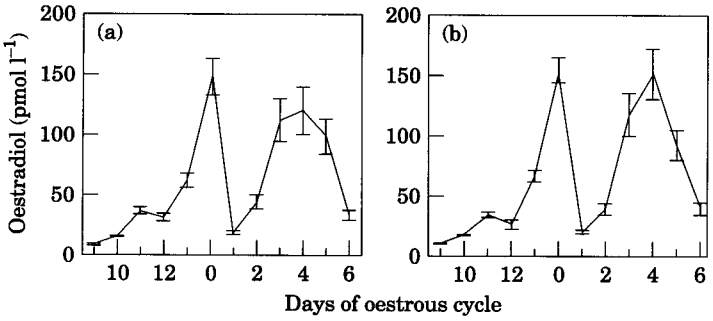

Figure 3. Mean oestradiol concentrations (± SEM) during (a) summer and (b) winter in PMSG treated cows ($N = 10$).

($p < 0{\cdot}01$) correlations between OE_2 concentration on day 3 post PMSG injection and the number of CL and total ova/embryos ($r = 0{\cdot}75$ and $0{\cdot}76$, respectively). Similar correlations were observed between OE_2 preovulatory peak and the number of CL and total number of ova/embryos ($r = 0{\cdot}74$ and $0{\cdot}64$, respectively). In addition, there was a significant ($p < 0{\cdot}05$) correlation between OE_2 level on day 5 post oestrus and poor quality embryos ($r = 0{\cdot}52$).

Discussion

The number of CL and total number of ova/embryos were in the normal range compared to other studies conducted in a hot climate using diary cattle (Almeida, 1987; Monty & Racowsky, 1987; Boland *et al.*, 1988). During summer season, ovarian responses were adversely affected as indicated by a reduced numbers of CL and total ova/embryos obtained during summer compared with winter. These adverse effects were consistent with several reports. Almeida (1987) observed that the number of palpated CL and recovered ova were significantly lower in summer as compared with other seasons in superovulated dairy cows. Similarly, the highest number of ova were recovered in winter and spring and lowest in summer and autumn (Hasler *et al.*, 1983). The increase in the number of CL and total ova/embryos during winter compared to summer may be attributed, in part, to the elevation in P_4 levels in winter during the day before PMSG and the day of PMSG injection. Goulding *et al.* (1994) compared superovulatory responses in heifers treated with porcine follicle stimulating hormone (pFSH) either at days 9–11 of the cycle or towards the end of a 7 day P_4 treatment (PRID) given at three different days of the cycle (days 1–3, 6–8 or 16–18). They found that the number of ovulations and embryos recovered were higher in heifers treated with P_4 on days 6–8 or 16–18 compared to those given P_4 on days 1–3 or given pFSH only. Furthermore, Scudamore *et al.* (1992, 1993) reported that 40 mg as opposed to 30 mg progestagen (FGA) or 30 mg FGA supplemented with 400 mg P_4 results in higher ovulation and ovum recovery rates in ewes superovulated with PMSG or pFSH. In addition, Yadav *et al.* (1986) observed a significant correlation between P_4 concentrations at the injection of gonadotrophin treatment and the ovulation rate in lactating cows. All these results confirm the important role which P_4, may play on superovulatory responses prior to gonadotrophin treatment. This can be attributed to the fact that P_4 is necessary for steroidogenesis and thereby responsiveness to gonadotrophin treatment.

The results show that poor quality embryos and unfertilized ova were significantly high during summer. Ryan *et al.* (1993) conducted a field study comparing early embryos mortality in dairy cows during hot and cool months in Saudi Arabia. They found that the embryo mortality rate increased during the hot season. Additionally, unfertilized ova were higher in hot-season cows compared to winter-season cows (Monty & Racowsky, 1987). Working on heifers housed in a temperature controlled chamber (20, 30, and 42°C), the proportion of embryos classified as good or excellent was significantly lower among heat-stressed heifers (Putney *et al.*, 1988). Putney *et al.* (1989) indicated that summer heat stress of superovulated Holstein heifers increased the incidence of retarded embryos as well as embryos graded fair to poor in quality. These adverse effects of heat stress on embryo quality and fertilization may be due to a direct action of heat on uterine environment and thereby on embryos (Ryan *et al.*, 1992) or to an indirect effect that is mediated by abnormal endocrine secretion in heat-stressed animals and the effect of heat stress on semen quality (Mieusset *et al.*, 1992; Salah *et al.*, 1992).

Although there was no statistical evidence on the effect of season on P_4 and OE_2 levels in PMSG treated cows, P_4 levels tended to be low in summer compared to winter. This decrease in P_4 level can be explained by the fact that heat stress decreases

the secretion of basal level of LH (Madan & Johnson, 1973) that is necessary for the maintenance of the CL (Auletta & Flint, 1988) which is the major source of P_4 circulating in plasma (Keyes & Wiltbank, 1988). After gonadotrophin treatment, P_4 concentration in the blood increased until the PG3 injection. Similar findings have been reported by other workers (Saumande, 1980; Yadav *et al.*, 1986; Kweon *et al.*, 1987). This increase in P_4 level reflected the stimulatory effect of PMSG on the CL (Stewart *et al.*, 1976; Hansel & Convey, 1983). P_4 concentrations from the day of PMSG to the day of PG3 were found to be correlated with the number of CL and total number of ova/embryos. Similar results were obtained using lactating cows (Yadav *et al.*, 1986). P_4 concentration dropped immediately after PG3 injection and remained low until day 2 post oestrus. After 48 h post oestrus, P_4 levels increased, which is in agreement with Saumande (1980) and Yadav *et al.* (1986). These increased levels of P_4 reflect the formation of CL from the ovulated follicles (Britt & Holt, 1988). The relationship that was observed between P_4 level on day 5 post oestrus and the number of CL and total ova/embryos is presumably due to the production of P_4 by numerous CL in the superovulated cows as reported by other workers (Donaldson, 1985; Goto *et al.*, 1987). Such a relationship has previously been demonstrated (Sreenan *et al.*, 1978; Goto *et al.*, 1988).

Data show that OE_2 concentrations on day 3 post PMSG injection and OE_2 preovulatory peak reflected the number of developing follicles, since there were correlations between OE_2 levels during those period and subsequent CL and total number of ova/embryos. These agree with the finding of other workers (Sreenan *et al.*, 1978; Britt & Holt, 1988; Alcivar *et al.*, 1992). After the preovulatory peak, OE_2 concentration decreased similar to that observed in the normally cyclic cow (Dieleman *et al.*, 1986). Then OE_2 had a second peak (post ovulatory peak) after oestrus which may be produced from large follicles in the ovaries which developed due to the presence of PMSG in the peripheral blood after ovulation, because the biological half-life of PMSG is very long, being about 5 days (Schams *et al.*, 1978). Similar results were obtained by other workers (Saumande, 1980; Bevers & Dieleman, 1987; Kaneko *et al.*, 1992). The correlation between OE_2 level on day 5 post oestrus and poor quality embryos reflects the adverse effect of postovulatory increase in OE_2 level on embryo quality (Kweon *et al.*, 1987).

In conclusion, ovarian responses in Holstein cows in a semi-arid environment were adversely affected during summer season. Further studies are needed on a large number of cows under semi-arid environment to confirm the role of summer heat stress on superovulatory responses of cows. In addition, it is important to study the role of exogenous P_4 with gonadotrophin treatment that can improve superovulatory responses during the hot season.

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