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Superovulatory response of Sistani cattle to three different doses of FSH during winter and summer

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Abstract

Objective of the present study was to investigate the effect of season and dose of FSH on superovulatory responses in Iranian *Bos indicus* beef cattle (Sistani). Cyclic cows, in summer ($n = 16$) and winter ($n = 16$), were assigned randomly to three dose-treatment groups of 120 ($n = 10$), 160 ($n = 12$) and 200 ($n = 10$) total mg of Folltropin-V with injections given twice daily for 4 days in decreasing doses. Estrous cycles were synchronized with two prostaglandin $F_{2\alpha}$ injections given 14 days apart. From day 5 after the ensuing cycle, daily ovarian ultrasonography was conducted to determine emergence of the second follicular wave at which time superovulation was initiated. Relative humidity, environmental and rectal temperatures were measured at 08:00, 14:00 and 20:00 h for the 3 days before and 2 days after the estrus of superovulation. Non-surgical embryo recovery was performed on day 7 after estrus. The effects of season, dose, time of estrous expression and all two-way interactions were evaluated on superovulatory responses: total numbers of CL, unovulated follicles (10 mm), ova/embryo, transferable and non-transferable embryos. Season (summer or winter), doses of Folltropin-V (120, 160 or 200 mg NIH) and time of estrous expression (08:00, 14:00 or 20:00 h) did not affect the number of transferable embryos (3.1 ± 0.58). When superovulatory estrus was detected at 08:00, a FSH dose effect was detected with the greatest numbers of CL (12.2 ± 0.87) and total ova/embryos (12.2 ± 1.46) occurring with 200 mg FSH (dose × time of estrous expression; $P < 0.01$).

Keywords: *Bos indicus*; Sistani cattle; Season; FSH; Estrus

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1. Introduction

Reproductive performance of dairy [1–3] and beef cattle [4,5] is reduced during hot seasons. The adverse effects of heat stress on follicular growth [6], corpus luteum function [7], expression of estrous behavior [8], superovulatory response [9,10], quality of embryos [3] and fertility [2] are documented in cattle. *Bos indicus* breeds are more adaptable to hot climates than *Bos taurus* breeds due to their superior thermo-regulatory ability [13–15] and thermotolerance at the cellular level [11,12]. Zebu cows have a better estrous expression and a higher conception rate during the summer months [16–18]. Sistani beef cattle are a native *Bos indicus* breed of Iran that is adapted to the harsh environment. There is no documentation of suitable doses of FSH for superovulation, and the effect of heat stress on superovulatory responses of Sistani cattle. The objective of this experiment was to evaluate the super-

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ovulatory response of Sistani cattle to three different doses of Folltropin-V during distinctly different summer and winter periods in Iran.

2. Materials and methods

2.1. Experimental location

The experiment was conducted at the research center for Agriculture and Natural Resources of the Sistan and Balouchestan province of Iran (latitude: $30'55''$ N; longitude: $61'41''$ E; altitude: 483 m). The region was considered to be an arid environment with an annual rainfall and temperature ranging from 1 to 10 mm and -2.8 to 47.1 °C, respectively.

2.2. Experimental animals

Cyclic Sistani cows were used in mid summer ($n = 16$; 368.9 ± 10.89 kg live weight; 62.3 ± 8.12 months of age; 185 ± 45.5 days open) and in mid winter ($n = 16$; 391 ± 34 kg live weight 68.3 ± 8.12 months of age; 246 ± 24.8 days open) seasons. All cows received a ration comprised of wheat straw (39%), Lucerne hay (28%), and concentrate (33%) with 10% crude protein and energy of 2.1 Mcal/kg dry matter, according to NRC recommendations [19] for beef cattle. Cyclicity was confirmed by presence of CL at either one of the examinations conducted 10 days apart with an ultrasound unit (Aloka 500, Japan) equipped with 5 MHz rectal probe.

2.3. Estrus synchronization

Cows, in each season, received two consecutive injections of a $PGF_2\alpha$ analogue (i.m.; 15 mg of Luprostiol, Prosolvin®, Intervet, Holland) given 14 days apart. Estrous detection was performed every 8 h for 3 days starting 24 h after the second $PGF_2\alpha$ analogue injection (D0 = day of standing estrus).

2.4. Experimental design

Beginning at 5 days after standing estrus, daily ovarian ultrasonography was performed to determine the emergence of the second follicular wave (detection of a cohort of $\geq$10 follicles, 4–5 mm in diameter, which did not exist the previous day) at which time superovulation was initiated. In order to determine the optimal dose of FSH, cows within each season were assigned randomly to three experimental groups of porcine FSH (120, 160 and 200 mg Folltropin-V®, Vetrepharm, Canada; a mg of Folltropin-V® is equivalent to a mg of NIH-FSH-P1 reference standard) after blocking for their body weight and age. Twice daily injections of porcine FSH were given in a decreasing dosage rate within: group 1 ($n = 10$) of 120 mg total given twice daily at 22.5, 17.5, 12.5 and 7.5 mg per injection on days 1, 2, 3 and 4, respectively; group 2 ($n = 12$) of 160 mg total given twice daily at 27.5, 22.5, 17.5, 12.5 mg per injection on days 1, 2, 3 and 4, respectively; group 3 ($n = 10$) of 200 mg total given twice daily at 32.5, 27.5, 22.5, 17.5 mg per injection on days 1, 2, 3 and 4, respectively. All cows received two i.m. injections of $PGF_2\alpha$ analogue at 48 and 60 h after initiating the first injection of FSH. Standing estrus was monitored continuously from 72 h after initiation of the superovulation protocol. The time of estrous expression was fixed at 08:00, 14:00 and 20:00 h for estruses expressed during the periods of 02:00–10:00 h, 10:00–18:00 h and 18:00–02:00 h, respectively. Cows were inseminated twice at 12 and 24 h after detection of standing estrus with frozen/thawed semen from a single ejaculate (i.e., characterized with >70% progressive forward motility of spermatozoa) of a fertile Sistani bull. Ovarian ultrasonography was performed on day 7 after standing estrus to determine number of CL and unovulated follicles (ovarian follicles $\geq$10 mm in diameter).

2.5. Embryo recovery

Embryo recovery was performed 7 days after standing estrus utilizing a standard non-surgical technique to flush the uterine horns [20]. Uterine flushing was conducted with Dullbecco's PBS (ZT156; IMV, France), a silicone two-way Foley catheter (25 in. and 20FR; AB technology, USA), and a long flushing tube set (66 in.; AB Technology, USA). Retrieved ova/embryos were transferred into a maintenance medium (ZA454; IMV, France) and assessed using a stereomicroscope (Olympus SZ40, Olympus, Japan). Embryos were evaluated and graded according to their stage of development (i.e., ova, 2–8 cell, 8–16 cell, early morula, compacted morula, early blastocyst, blastocyst, and expanded blastocyst) and quality (i.e., excellent, good, fair, poor and degenerated) [21]. Excellent and good quality embryos were considered as transferable and others were classified as non-transferable embryos.

2.6. Measurements

Daily rectal and environmental temperatures and relative humidity were measured at 08:00, 14:00 and

20:00 h from −3 to 2 days of the superovulatory estrus. Temperature humidity index (THI) was calculated (i.e., THI = environmental temperature ($^\circ F$) − [(0.55 − 0.55 × relative humidity)(environmental temperature ($^\circ F$) − 58)]) according to the method described previously [22].

2.7. Statistical analysis

Changes in environmental temperature and respective THI throughout the experimental periods were analyzed by least squares ANOVA using the general linear models procedure of SAS [23]. The mathematical model examined the main effects of season, day of experiment, time of measurement and higher order interactions. For rectal temperatures cow within season was considered random, and effects of season, day of experiment, time of measurement and higher order interactions were examined using the repeated measures analysis of the mixed models procedure of SAS [24]. The association between rectal and environmental temperature was investigated using the correlation procedure in SAS [23]. The effects of season, dose, time of estrous expression and all two way interactions of these effects on the superovulatory responses (number of CL, number of follicles and number of ova/embryos) were analyzed by least squares ANOVA using the general linear models procedure of SAS [23]. Data was expressed as least squares means ± standard error of mean.

3. Results

The mean day of follicular emergence and initiation of superovulation treatments was 8.9 ± 0.05 days of the estrous cycle or 4.9 days after initiation of daily ultrasound measurements that began on day 5 of the estrous cycle.

Environmental temperature from day −3 to 2 of the superovulatory estrus was greater in summer (38.3 ± 0.12; 25–44 °C) than in winter (20.04 ± 0.12; −2.8 to 32 °C; $P < 0.001$; Fig. 1). Environmental temperature did not differ among days within each season ($P > 0.05$; Fig. 1). Relative humidity was greater in the winter season (35.3 ± 0.5; 20–55%) compared to summer (15.73 ± 0.5; 9–37%; $P < 0.01$). Temperature humidity index differed ($P < 0.0001$) between winter (69.3 ± 0.62 THI) and summer (84.6 ± 0.60 THI) seasons. Rectal temperature in summer (38.8 ± 0.04; 37.29–41.8 °C) was elevated compared to winter (38.5 ± 0.03; 36.7–39.9 °C; $P < 0.01$). On the day after estrus, rectal temperature declined substantially in both seasons ($P < 0.01$; Fig. 1). There was an interaction between season and time of the day (08:00, 14:00 and 20:00 h) on rectal temperature ($P < 0.01$). Rectal temperature was similar among different times in summer (08:00 h: 38.8 ± 0.05 °C; 14:00 h: 38.8 ± 0.04 °C; 20:00 h: 38.8 ± 0.05 °C). However, it increased ($P < 0.01$) from 08:00 h (38.2 ± 0.05 °C) to 14:00 h (38.7 ± 0.04 °C) and then decreased ($P < 0.0001$) at 20:00 h (38.5 ± 0.04 °C) in winter. There was a correlation between environmental and rectal temperatures in winter ($r = 0.375$; $P < 0.01$) but not in summer.

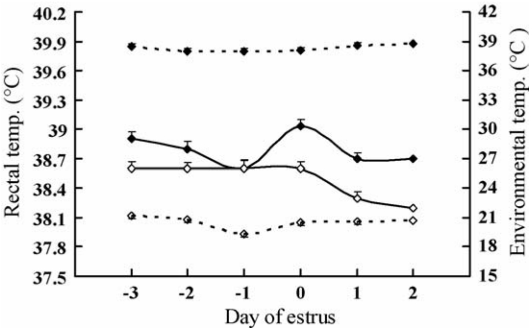

Fig. 1. Environmental (dotted line) and rectal (solid line) temperatures between days −3 and 2 after superovulatory estrus (day 0 = day of estrus) for summer (◆) and winter (◇) seasons.

Overall number of CL was 8.7 ± 0.62 and did not differ between summer (8.9 ± 0.54) and winter (9.2 ± 0.53; $P > 0.05$), or among different doses of FSH (120 mg: 8.2 ± 0.66; 160 mg: 9.6 ± 0.64; 200 mg: 9.4 ± 0.66; $P > 0.05$). There was an interaction between dose of FSH and time of estrous expression on number of CL measured at day 7 after the superovulatory estrus ($P < 0.05$; Table 1). There was an optimal time of day regarding occurrence of estrus and greatest number of CL as a consequence of superovulation with maximal responses at 20:00 h for the 120 mg FSH (12 ± 1.35 CL), 14:00 h for the 160 mg FSH (13 ± 1.35 CL) and 08:00 h for the 200 mg FSH (12.2 ± 0.87 CL; Table 1). The majority of animals in each dose group expressed estrus at 08:00 h. At 08:00 h, the 200 mg dose of FSH had the greatest CL response (200 > 120 mg FSH; $P < 0.02$), and a linear dose response to FSH was detected ($Y = -1.63 + 0.065x$; $P < 0.01$, $R^2 = 0.41$; x = mg of FSH). The large number of CL at 14:00 h for the 160 mg and at 20:00 h for the 120 mg doses of FSH was influenced largely by a single animal in each group (i.e., one of two cows; Table 1).

The number of unovulated follicles (overall mean: 4.1 ± 0.81) was not affected ($P > 0.05$) by season

Table 1
Effect of FSH dose for superovulation (120, 160 and 200 mg) and time of estrous expression (08:00 h, 14:00 h and 20:00 h) on number of CL (least squares means ± S.E.M.) in Sistani cattle

Dose (mg)	Time of estrous expression		
	08:00 h	14:00 h	20:00 h
120	5.9 ± 0.87 a, c (5)[a]	6.7 ± 1.17 a (3)	12 ± 1.35 b (2)
160	8.4 ± 0.73 a, c (7)	13.0 ± 1.35 b (2)	7.5 ± 1.17 a (3)
200	12.2 ± 0.87 a, d (5)	7.0 ± 1.17 b (3)	9 ± 1.35 b (2)

Dose × time of estrous expression, $P < 0.05$. Values within rows (a, b) or within columns (c, d) with different letters differ ($P < 0.05$).
[a] Number of cows in parenthesis.

Table 2
Effect of FSH (120, 160 and 200 mg) and time of estrous expression (08:00, 14:00 and 20:00 h) on total number of ova/embryo (least-squares means ± S.E.M.) recovered from Sistani cattle

Dose (mg)	Time of estrous expression		
	08:00 h	14:00 h	20:00 h
120	1.7 ± 1.6 a, c (5)[a]	10.5 ± 1.97 b (3)	2.0 ± 2.27 a (2)
160	5.2 ± 1.39 a, c (7)	5.5 ± 2.27 a (2)	6.0 ± 2.27 a (3)
200	12.2 ± 1.46 a, d (5)	6.5 ± 1.97 b (3)	6.0 ± 2.27 b (2)

Dose × time of estrous expression, $P < 0.05$. Values within rows (a, b) or within columns (c, d) with different letters differ ($P < 0.05$).
[a] Number of cows in parenthesis.

(summer: 3.5 ± 1.40; winter: 4.3 ± 1.37), FSH dose (120 mg: 2.5 ± 1.72; 160 mg: 3.7 ± 1.67; 200 mg: 5.4 ± 1.72) or the time of estrous expression (08:00 h: 4.6 ± 1.23; 14:00 h: 4.9 ± 1.84; 20:00 h: 2.1 ± 1.93).

Total number of ova/embryos (overall mean: 6.3 ± 0.84) did not differ between summer (6.5 ± 0.96) and winter (5.8 ± 0.89; $P > 0.05$), or among doses of FSH (120 mg: 4.7 ± 1.36; 160 mg: 5.6 ± 1.17; 200 mg: 8.2 ± 1.11; $P > 0.05$). However, there was an interaction between FSH doses and the time of estrous expression on number of ova/embryos ($P < 0.01$; Table 2). The dose of 200 mg FSH induced the greatest ova/embryo response at 08:00 h (12.2 ± 1.46); whereas, the response was greatest at 14:00 h for the 120 mg FSH dose (10.5 ± 1.97). At the intermediate FSH dose of 160 mg, the response was uniform at the different times of estrous expression. Since number of animals in the 14:00 and 20:00 h dose groups was minimal (i.e., 2 or 3; Table 2), it is prudent to examine the 08:00 h time for onset of estrous expression (i.e., time grouping with majority of animals). At 08:00 h a clear FSH dose effect was detected with greatest total number of ova/embryo in the 200 mg dose (200 mg > 120 mg FSH; $P < 0.001$; Table 2), and a linear dose response to FSH was detected ($Y = -14.212 + 0.126x$; $P < 0.001$, $R^2 = 0.59$; x = mg of FSH). The total number of ova/embryo paralleled the estimate for CL number.

The overall number of transferable embryos for the experiment was 3.1 ± 0.58. There was no effect of season (summer: 4.6 ± 0.95; winter: 2.4 ± 0.87; $P > 0.05$), dose (120 mg: 2.7 ± 1.11; 160 mg: 3.4 ± 1.15; 200 mg: 4.3 ± 1.09: $P > 0.05$) or the time of estrous expression (08:00 h: 2.4 ± 0.85; 14:00 h: 5.6 ± 1.17; 20:00 h: 2.3 ± 1.28; $P > 0.05$) on the number of transferable embryos. The number of non-transferable embryos (overall mean, 3.1 ± 0.64 including unfertilized ova [0.7 ± 0.27]) was not affected by season (summer: 1.9 ± 0.89 [0.3 ± 0.20]; winter: 3.5 ± 0.83 [1.1 ± 0.50]; $P > 0.05$), dose of FSH (120 mg: 2.0 ± 1.05 [1.6 ± 0.37]; 160 mg: 2.2 ± 1.08 [1.5 ± 1.36]; 200 mg: 3.9 ± 1.03 [1.2 ± 0.49]; $P > 0.05$) or time of estrus expression (08:00 h: 3.95 ± 0.8 [0.5 ± 0.21]; 14:00 h: 1.83 ± 1.11 [1.2 ± 0.9]; 20:00 h: 2.33 ± 1.22 [0.7 ± 0.56]; $P > 0.05$).

4. Discussion

The doses of Folltropin-V (120, 160 and 200 mg) did not differ in their superovulatory responses in Sistani cattle. It is accepted generally that the dose of FSH for superovulation of *Bos indicus* cattle is less than that of *Bos taurus* cattle. Lewis [25] recommended superovulatory doses of 360–400 mg Folltropin-V for *Bos taurus* cattle and that *Bos indicus* breeds require 25–30% less (i.e., about 250–280 mg) than the *Bos taurus* dose rates [25]. In our preliminary study, a superovulatory dose regime of 250 mg Folltropin caused an overstimulation and a high incidence of unovulatory follicles in Sistani cattle (unpublished observations). In two different studies with Nelore cows, optimal doses of 133 mg [26] and 200 mg [27] of Folltropin-V were suggested. In the Brahman breed, the doses of Folltropin-V for heifers and cows were 200 mg [28] and 240 [29] mg, respectively. In the present study, an interaction between time of the superovulatory induced estrus with dose of FSH influenced the superovulatory response (i.e., number of CL). The majority of cows expressed estrus in the 08:00 h period, and at that time the 200 mg dose gave the maximal CL and ova/embryo response without a disproportional increase in number of non-ovulatory follicles or unfertilized ova.

In this study, season of the year did not affect superovulatory responses (total number of CL, unovulated follicles, ova/embryo, transferable and non-transferable embryos). Although month of the year did not influence total number of ova/embryo, it did influence the number of transferable embryos in *Bos indicus* donor cows with the maximum number of transferable embryos occurring during the warmest months [18]. The numerical increase in transferable embryos in the present study during summer (summer: 4.6 ± 0.95 versus winter: 2.4 ± 0.87) was not significant ($P < 0.08$) even though there were 16 experimental cows in each seasonal period. Season has a major impact on the superovulatory response in *Bos taurus* [9,30,31] with total number of CL, ova/embryo and transferable embryos being lower in the mid summer than winter in Saudi Arabia [9,30]. A study in Mexico reported that the number of total ova/embryo was affected adversely by the wet season; whereas, no seasonal effect on ovulation rate was detected [31]. In spite of the significant climatic changes between seasons in Brazil, there was not any effect of season on superovulatory responses in Holstein cows [32].

In the present study, there was no association between rectal and environmental temperatures in summer; whereas, in the winter season a positive correlation was detected between rectal and environmental temperatures ($r = 0.375$; $P < 0.01$). This confirms a previous report in the southern hemisphere ($r = 0.41$; $P < 0.05$; 15). Rectal temperature in summer was elevated consistently throughout the day in the presence of elevated environmental temperatures. According to THI values and its association with heat stress for dairy cows [33], the cattle in the summer season of the present study were experiencing a medium heat stress. Nevertheless, mean rectal temperature (38.8 ± 0.04 °C) didn't rise up to the *Bos taurus* critical set point (rectal temperature of 39.6 °C) during summer [34,35]. *Bos indicus* breeds have a greater thermo-tolerability due to a better thermal regulatory response to high ambient temperature. This is due partially to a lower internal heat production [36] as a consequence of a lower basal metabolic rate [37], the ability to have a greater heat loss due to larger sweat glands [38], and diversion of blood flow from the body core to the skin [13]. The capability of *Bos indicus* cattle to adapt to environmental elevated temperature may explain in part their better estrous expression and a higher conception rate during the summer months [16–18], which is in agreement with our results in Sistani cattle (unpublished observations). In the present study, a greater number of ovulations (i.e., number of CL) were achieved when the females received 200, 160 and 120 mg of Folltropin-V and exhibited estrus at 08:00, 14:00 and 20:00 h, respectively. This interaction should be considered in a cautious manner because of lower number of cows expressing estrus at 14:00 and 20:00 h. Within the 08:00 h group, ovarian stimulation was greatest at the 200 mg dose without any indication of an overstimulation leading to higher incidences of anovulatory follicles and non-transferable embryos including unfertilitzed ova. Perhaps, the dose by time of estrus interaction is of biological relevance and associated with rectal temperature fluctuations throughout the day (08:00 h: 38.2 °C; 14:00 h: 38.7 °C; 20:00 h: 38.5 °C) in winter that did not occur during the summer season (38.8 °C). During the winter time, cows that exhibited estrus at 08:00 h experienced a greater level of rectal temperature fluctuation than those expressing estrus later in the day. Stressful conditions may have an adverse effect on the LH surge [39]. Indeed the cold season has adverse effects on the LH surge in *Bos indicus* cattle [17,40]. As the LH surge initiates a cascade of events leading to activation of the oocyte and ovulation of the preovulatory follicle [41], any disruption in the LH surge may alter follicular and oocyte responses. Rectal temperature fluctuations may be considered indicative of a stressor response. Administration of estrogen can attenuate the adverse effect of hypoglycemic stress on LH concentrations [42]. Perhaps the variation in CL number at various doses of FSH, as related to time of day that estrus was observed, may be explained partially by temperature fluctuations, the amount of estradiol secreted or dynamics of the preovulatory surge of LH. The cattle expressing estrus at 08:00 h would have experienced a greater temperature fluctuation (stress) compared to those in estrus at 20:00 h. This potential adverse effect may be reduced with a high dose regime of FSH that may stimulate greater total estradiol secretion by the FSH induced follicles. Indeed cattle expressing estrus at 08:00 h had a linear increase in CL numbers from 5.9 to 8.4 and to 12.2 at 120, 160 and 200 mg of FSH, respectively. Perhaps increased estradiol may reduce the detrimental effects of the subsequent temperature change or stress in the winter season. However, this cannot be substantiated in the present experiment since estradiol concentrations in plasma were not evaluated. Furthermore, such differences in sensitivity to FSH doses depending upon time of day superovulatory donors expressed estrus during different seasons needs to be examined with a greater number of cows in all categories of dose-time and season.

Total number of ova/embryos and transferable embryos are two important indices in evaluating superovulatory responses in cattle. In the present study with Sistani cattle of Iran, total number of ova/embryos and transferable embryos were 6.3 ± 0.84 and 3.1 ± 0.58, respectively. In the practical MOET scheme, the overall number of embryos recovered per donor flushed ranges from 4 to 7 [43–45]. In 2048 superovulated beef donors, a mean of 11.5 ova/embryos with 6.2 transferable embryos were characterized [46]. In another study with 987 Holstein dairy cows, the total yield of 10.1 ova/embryos and 4.5 transferable embryos were reported [47]. In a recent report, the number of transferable embryos was 4.6–4.8 [48]. The total number of ova/embryos and transferable embryos in Nelore cows were 10.9 ± 1.80 and 6.2 ± 1.37 [49].

In conclusion, season (summer or winter), different doses of Folltropin-V (120, 160 or 200 mg NIH) and the time of estrous expression (08:00, 14:00 or 20:00 h) did not affect the ultimate number of transferable embryos in Sistani cattle.

When the superovulatory estrus was detected at 08:00, linear FSH dose effects were detected with the greatest numbers of CL (12.2 ± 0.87) and total ova/embryos (12.2 ± 1.46) occurring with the 200 mg dose of FSH (dose × time of estrous expression; $P < 0.01$). This dose effect was not detected at 14:00 or 20:00 h. Whether differences in dose effects of FSH, as related to the time of estrous expression, has any potential application to enhance the output of a MOET program in cattle may warrant further investigation.

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