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# Combined effect of heat and nutritional stress on superovulation of Malpura ewes in a semi-arid region

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ABSTRACT

Sheep reared in hot semi-arid environments are generally exposed to heat and nutritional stress in some seasons of the year, which affects both production and reproduction. To assess the effect of high ambient temperature and feed scarcity on superovulation, 16 adult Malpura ewes were randomly divided into two groups of 8 animals each. G1 (control) was kept under a shed and offered a maintenance diet, and G2 (combined stress) was subjected to both nutritional (30% less of maintenance diet) and heat (38–44 °C for 6 h/day) stress. Ewes were superovulated without estrus synchronization by a combination of single injection of 200 IU eCG and 8 injections of FSH (Folltropin-V) at 12-h intervals in tapering doses of 5 mg/kg body weight, starting from the day 7 of natural estrus. eCG was given with the first injection and PGF2α (10 mg) was given with the second last FSH injection. G2 increased respiration rate and rectal temperature ($P < 0.01$), and blood urea level ($P < 0.05$), whereas it decreased average daily gain, plasma T4 concentration ($P < 0.01$) and body weight ($P < 0.05$). Plasma estradiol level was lower ($P < 0.05$) in G2 ewes as compared to control (G1) ewes. However, the number of ewes showed a superovulatory response (88 vs 66% ewes ≥ 3 corpus luteum), ovulation rate (8.75 vs 5.88) and embryo production (5.5 vs 3.9) decreased, and the number of large follicles (anovulation) increased (1.0 vs 2.14) in G2 ewes. G2 had a comparable effect on the superovulatory response compared to control ewes although physiological changes occurred as an adaptive mechanism to stress. Therefore, the well-adapted cyclic sheep of the semi-arid region may be used for superovulation despite the stressful condition of heat exposure and nutritional insufficiency.

## 1. Introduction

Prolong hot summer and limited availability of forage is the characteristics climatic features of the arid and semi-arid region (De et al., 2017a). Despite such climatic extreme variability, sheep husbandry is the sustainable livelihood resource for the peasants residing in hot arid and semi-arid region (Naqvi et al., 2013). However, the animals reared in hot arid and semi-arid environments are mostly subjected to more than one stress at a time which severely affects production and reproduction of animals (Sejian et al., 2010a, 2010b). The majority of sheep of arid and semi-arid regions are simultaneously subjected to high ambient temperature and scarcity of feed (Naqvi et al., 2004). However, most of the studies on stress effect on animal production and reproduction have been carried out considering a single stress effect at a time (Indu et al., 2015; De et al., 2017b, 2018).

Heat stress is the major limiting factor in the arid and semi-arid region for productivity as they disrupt the physiology and reproductive performance of animals (De et al., 2017a, 207b, 2017c). Although the well-adapted animals present physiological response mechanism to counterbalance the heat stress effect which is helpful for survival but is detrimental to the productive performance (Sejian et al., 2010a, 2010b). Similarly, the nutritional scarcity and availability of low-quality feed also affect the reproductive efficiency of sheep (Kumar et al., 2015). Furthermore, if the animals are exposed to heat and nutritional stress at the same time, the summed effect of both stressors might prove detrimental to the animals (Silva et al., 2016).

For maintaining the genetic diversity of locally endangered sheep genotype (Mayorgaa et al., 2011) as well as increasing the contribution of superior females to breeding program (Chang et al., 2006), superovulation and embryo transfer is an essential assisted reproductive technology. Superovulation and harvesting embryo from donor females is an efficient means of obtaining embryos at a given stage of

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development. However, due to some limitations, it is difficult to use the embryo transfer technology for commercial purpose (Forcadaa et al., 2011). The known influencing factors affecting superovulation and quality of embryo are breeds (Terawaki et al., 2002), nutrition (O'Callaghan et al., 2000; Armstrong et al., 2001), season (Hansen et al., 2001; Chagas et al., 2003), gonadotrophin preparations (Lopes da Costa et al., 2001; Gonzalez-Bulnes et al., 2002) and many more (Chang et al., 2006). Heat stress adversely affects the quality of embryos (Naqvi et al., 2004). Similarly, nutritional limitations also affect superovulation (O'Callaghan et al., 2000). However, in the hot semi-arid region, both heat and nutritional stress occur simultaneously and may severely affect the superovulation. But most of the studies on this area have been carried out considering a single stress factor. To the best of our knowledge, no literature is available considering the combined stress effect on superovulation for evaluating the efficiency of this technique in the practical condition of the hot semi-arid region on well-adapted native sheep for their conservation as well as propagating superior female genotypes. Therefore, the present study was carried out to assess the effect of the combined heat and nutritional stress on superovulation of Malpura ewes.

## 2. Materials and methods

### 2.1. Study site

The experiment was conducted at the experimental animal farm of ICAR-Central Sheep and Wool Research Institute, Avikanagar located in the semi-arid region of India, (75°-28′ E longitude, 26°-26′ N latitude, at an altitude of 320 m above mean sea level). The climate is typically hot with annual minimum and maximum temperature of 4 ° and 46 °C, respectively. The annual rainfall ranges from 200 to 500 mm with an erratic distribution throughout the year. The microclimate during the experimental period is described in Table 1. The experiment was carried out over four weeks with 15 days for animal adaptation to diets and daily management. All the experimental procedures were approved by the institutional animal ethics committee (NFBSFARA/AS2002/2010-11).

### 2.2. Animals

Malpura sheep which is a well-adapted native breed of the semi-arid tropical region reared for its mutton, wool, and skin were used (Gowane et al., 2014). Sixteen 2–4 years old non-pregnant cyclic Malpura ewes were selected for the study and average body weight of the ewes was 38.9 ± 1.3 kg. Their cyclicity was confirmed through heat detection by aproned ram for two consecutive estrous cycles. Ewes were housed in mud-floored well-ventilated asbestos roofed shed and maintained under proper hygienic conditions throughout the experimental period. The prophylactic action was taken against sheep pox, peste des petits ruminants (PPR), enterotoxaemia, endoparasitic and ectoparasitic infestations as per the schedule of the Institute Animal Health Division.

**Table 1**
Ambient temperature and humidity values are means ± SEM.

| | Time (h) | Temp (°C) | RH (%) | THI |
|---|---|---|---|---|
| Climatic chamber | 1000 | 37.2 ± 0.2 | 37.5 ± 0.9 | 33.4 ± 0.1 |
| | 1100 | 38.9 ± 0.2 | 36.1 ± 0.7 | 34.9 ± 0.1 |
| | 1200 | 41.4 ± 0.1 | 33.8 ± 0.9 | 36.3 ± 0.1 |
| | 1300 | 42.7 ± 0.1 | 33.0 ± 0.7 | 37.0 ± 0.1 |
| | 1400 | 43.7 ± 0.1 | 33.5 ± 0.9 | 37.9 ± 0.1 |
| | 1500 | 42.8 ± 0.2 | 38.4 ± 1.8 | 36.7 ± 0.2 |
| Shed | 0700 | 26.8 ± 0.2 | 88.4 ± 0.7 | 26.4 ± 0.2 |
| | 1400 | 32.0 ± 0.5 | 78.8 ± 1.2 | 30.8 ± 0.4 |

RH Relative humidity, THI temperature–humidity index.
Temperature humidity index calculated as THI = db°C-{(0.31-0.31 RH)(db°C − 14.4)}; Marai et al. (2007).

### 2.3. Experimental procedure

The study was conducted for a period of 28 days covering two estrous cycles under controlled conditions. The 16 ewes were allocated randomly into two groups of eight animals each viz., G1 (n = 8; Control) and G2 (n = 8; combined stress). All ewes assigned to G2 were habituated to the novel conditions of the climatic chamber management for behavioral desensitization for 15 days at ambient temperature (without artificial manipulation) in the chamber, whereas G1 ewes were kept in asbestos roofed shed. During this 15 day period, ewes were accustomed daily to handling and blood sampling procedures to reduce their stressful impact (Guesdon et al., 2012). All ewes were stall-fed individually with a diet consisting of 70% *Cenchrus ciliaris* hay (buffelgrass) and 30% concentrate (barley, 650 g/kg, groundnut cake, 320 g/kg, minerals 30 g/kg including 10 g/kg NaCl, with crude protein = 180 g/kg and total digestible nutrients = 650 g/kg). G1 ewes were provided with their daily maintenance requirement, calculated as per ICAR (2013) recommendation while G2 ewes were offered 30% less than their maintenance requirement as it is presumed that at least 30% nutritional shortage occur during the dry period in this region. The chemical composition of feed is described in Table 2. All ewes were superovulated. The G1 ewes were kept under the shed, where the average maximum temperature and the average minimum temperature was 32.8 ± 0.4 °C and 25.2 ± 0.02 °C, respective and relative humidity was 86.4 ± 0.1 and 76.1 ± 1.4% at 0700 h and 1400 h, respectively during the experimental period. The ewes of G1 were maintained in an asbestos-roofed shed. Four sides of the shed were covered by wire net fencing, and the floor was made up of dirt filled with a sand bed. Whereas G2 ewes were kept inside the climatic chamber in an individual tubular iron partitioned pen from morning (0800 h) to afternoon (1730 h). After that, G2 ewes were withdrawn from the climatic chamber and kept (1730–0800 h) in the asbestos-roofed shed the same as the control group. All the experimental ewes were individually stall-fed only during daytime from morning (0800 h) to afternoon (1700 h) and restrained individually with a 1.5 m long iron chain. G2 ewes were kept in a climatic chamber for 6 h a day between 1000 h and 1600 h to expose them to varying temperatures, i.e. 38 °C at 1000–1100 h; 40 °C at 1100–1200 h; 42 °C at 12:00–1300 h; 43 °C at 1300–1400 h; 44 °C at 1400–1500 h and 42 °C at 1500–1600 h. This model of temperature control inside the climatic chamber is simulated with near-natural heat stress model for sheep under this hot semi-arid region (Indu et al., 2015). The climatic chamber was made up of stainless steel. The dimension of the psychrometric chamber was 3.7 m × 2.4 m × 2.1 m in length, width, and height, respectively, and the total floor area was 8.9 $m^2$. The temperature inside the psychrometric chamber can be thermostatically controlled within the range of 5–60 ± 2 °C and monitored. The climatic chamber possesses a temperature and humidity regulator which maintains the desired temperature and humidity as per the requirement of the experiment. Blood samples were collected and physiological response, i.e. respiration rate (RR), pulse rate (PR) and rectal temperature (RT) were recorded at

**Table 2**
Chemical composition (%) of concentrate and buffelgrass hay.

| Nutrient | Buffelgrass | Concentrate |
|---|---|---|
| Dry matter | 96.78 | 89.71 |
| Organic matter | 92.45 | 96.89 |
| Total Ash | 7.55 | 3.11 |
| Acid-insoluble ash | 3.01 | 1.03 |
| Crude protein | 6.01 | 12.09 |
| Neutral detergent fibre | 70.35 | 54.19 |
| Acid detergent fibre | 40.76 | 5.61 |
| Hemicellulose | 34.79 | 48.58 |
| Cellulose | 29.60 | 12.93 |
| Lignin | 4.91 | 2.38 |

The values are in percentage on dry matter basis.

weekly intervals. The physiological response was recorded twice at 0700 h and 1400 h in a day. The study was conducted after obtaining approval from the institutional ethical committee for subjecting the animal to both heat and nutritional stresses.

### 2.4. Superovulation of ewes

Ewes in both groups were superovulated individually during the natural estrous cycle without estrus synchronization. Superovulation was done by a combination of single injection of 200 IU eCG (Folligon, Intervet-Netherlands) and multiple injections of FSH (Folltropin-V; Bioniche Animal Health Canada Inc, Belleville, Ontario, Canada). Each animal received FSH according to its body weight 5 mg/kg starting from day 7 of natural estrus. The total dose of FSH was injected intramuscularly in a tapering manner i.e. 40%, 30%, 20% and 10% of the total dose, over a 4 day period at 12-h intervals. The eCG was given with the first injection of FSH i.e. commencement of the superovulation treatment (Naqvi et al., 1998). The PGF2α (10 mg; Lutalyse, Pfizer, Puurs- Belgium) was administered at the time of the second last injection of FSH.

### 2.5. Estrus detection and mating

Estrus detection was started from the end of the superovulation treatment (4th day of the FSH injection) with an aproned ram. Ewes detected in estrus were subjected to mating twice a day (morning and evening) with Malpura rams of proven fertility. Individual rams of proven fertility were assigned to each ewe.

### 2.6. Embryo collection

Ewes were subjected to laparoscopy for ovarian activity evaluation on days 4–8 after mating to determine whether it was worth attempting surgical embryo collection. Embryo recovery was performed according to a procedure previously described (Naqvi et al., 2004). In brief, ewes were fasted for at least 24 h prior to the laparoscopy. The abdominal area anterior to the udder was shaved and sprayed with 70% alcohol. Ewes were sedated with xylazine hydrochloride (Xylazine, Indian Immunologicals, India) and locally anesthetized by infiltration of lignocaine hydrochloride (Xylocaine, SG Pharm, India). Ewes with 3 or more corpora lutea (CL) were deemed as having a superovulatory response and embryos were recovered from these animals through midventral laparotomy. The fallopian tubes were flushed with 20 ml Dulbecco's phosphate-buffered saline (DPBS), pH 7.5 (0.3), supplemented with 2% bovine serum albumin (Sigma) at room temperature, introduced near the tubal junction. The tubal washings were collected in sterile Petri dishes and examined for ova or embryos under a Stereozoom microscope (Nikon) equipped with a warm stage platform (37 °C) at ×50 magnification. The numbers of recent ovulations (corpora lutea) and persistent large follicles were recorded and the ewes' ovarian responses were estimated by adding the numbers of corpora lutea and large follicles.

### 2.7. Blood sample collection and analysis

Five ml of blood were collected once a week at 1100 h from each ewe in heparinized tubes from the jugular vein. Blood samples were centrifuged at 1500 g for 15 min and plasma was separated. Plasma glucose, total protein, albumin and cholesterol were determined using reagent kits supplied by Span Diagnostics Ltd., India through standard spectrophotometric method. Plasma cortisol (analytical sensitivity 10 nM; the intra-assay and inter-assay coefficient of variations 5.8% and 9.2%, respectively), T3 (analytical sensitivity, 0.1 nmol/L; intra- and inter-assay coefficients of variation, 3.3% and 8.6%, respectively), T4 (analytical sensitivity, 13 nmol/L; intra- and inter-assay coefficients of variation, 5.1% and 8.6%, respectively), estradiol (analytical sensitivity, 6 pg/ml; intra-assay and inter-assay coefficient of variations were 5.8% and 9.0% respectively) and progesterone (analytical sensitivity, 0.05 ng/ml; intraassay and inter-assay coefficient of variations, 12.1% and 11.2% respectively) were estimated by radioimmunoassay (RIA) using the Packard Cobra II gamma counter employing RIA kits supplied by Immunotech, Marseille Cedex, France. All hormones were determined by RIA using a gamma counter (PC-RIA MAS; Stretec, Germany).

### 2.8. Statistical analysis

Data were analyzed using a student T-test (SPSS 14.0). The effect of treatment on the FI, WI, body weight, ADG, physiological responses (RR, PR, and RT), blood biochemical parameters, endocrine parameters were determined. For percentages of ewes in estrus, superovulatory response, percent recovery of embryo were analyzed using the chi-square test; whereas; ovulation rate; ovulation rate in super ovulatory responded ewes and embryo recovery rate per ewe were analyzed using the Maan-Whitney test. The superovulatory response and embryo production parameters were analyzed using the student T-test. Statistical significance levels were set at $P < 0.05$ for each parameter.

## 3. Result

### 3.1. Body weight, feed intake and water intake

The combined effect of temperature and feeding stress on body weight, feed intake, and water intake are described in Table 3. Body weight at the end of the experiment, body weight gain and average daily gain (ADG) was significantly ($P < 0.05$) lower in G2 ewes as compared to G1 ewes. Feed intake was significantly ($P < 0.01$) lower in G2 as compared to G1. The water intake was significantly ($P < 0.01$) higher in G2 as compared to G1.

### 3.2. Physiological response

The effect of the combined stress on physiological response is depicted in Table 4. RR did not differ ($P = 0.07$) in the morning; however, it was significantly ($P = 0.01$) higher in G2 as compared to G1 in the afternoon. The PR did not vary between the groups during the morning

**Table 3**
Effect of combined heat and nutritional stress on feed intake and water intake of superovulated Malpura sheep.

| | $\bar{x}$ ± SE | Control | Combined stress | P value |
|---|---|---|---|---|
| VFI (DMI g/$W^{0.75}$/day) | 52.3 ± 0.89 | 59.3 ± 1.26 | 45.2 ± 1.26 | 0.001 |
| WI (L/kg DMI) | 3.9 ± 0.11 | 3.0 ± 0.16 | 4.9 ± 0.16 | 0.001 |
| Initial weight (kg) | 31.4 ± 0.60 | 31.6 ± 0.85 | 31.1 ± 0.85 | |
| End weight (kg) | 31.1 ± 0.60 | 32.4 ± 0.84 | 29.8 ± 0.84 | 0.046 |
| Gain (kg) | − 0.287 ± 0.25 | 0.788 ± 0.354 | − 1.362 ± 0.354 | 0.001 |
| ADG (g) | − 20.5 ± 17.89 | 56.3 ± 25.30 | − 97.3 ± 25.30 | 0.001 |

$\bar{x}$ indicates the overall mean ± SEM.
VFI, voluntary feed intake; WI, water intake.

**Table 4**
Effect of combined heat and nutritional stress on physiological response of superovulated Malpura sheep.

| | $\bar{x}$ ± SE | Control | Combined stress | P value |
|---|---|---|---|---|
| RRM (breaths/min) | 32.4 ± 1.13 | 34.5 ± 1.60 | 30.3 ± 1.60 | 0.07 |
| RRA (breaths/min) | 69.1 ± 3.20 | 60.5 ± 4.53 | 77.7 ± 4.53 | 0.01 |
| PRM (beats/min) | 57.3 ± 1.18 | 57.7 ± 1.66 | 57.0 ± 1.66 | 0.78 |
| PRA (beats/min) | 64.6 ± 1.54 | 65.2 ± 2.18 | 64.0 ± 2.18 | 0.71 |
| RTM (°C) | 38.5 ± 0.04 | 38.5 ± 0.06 | 38.5 ± 0.06 | 0.95 |
| RTA (°C) | 39.1 ± 0.05 | 39.9 ± 0.06 | 39.3 ± 0.06 | 0.01 |

$\bar{x}$ indicates the overall mean ± SEM.
RRM, respiration rate at morning; RRA, respiration rate at afternoon; PRM, pulse rate at morning; RTM, rectal temperature at morning; RTA, rectal temperature at afternoon.

and afternoon. RT at afternoon was higher ($P < 0.01$) in G2 as compared to G1.

### 3.3. Blood biochemical and endocrine profile

The effect of the combined stress on blood biochemical and endocrine profile is described in Tables 5 and 6. Combined stress had no significant ($P > 0.05$) effect on plasma total protein, albumin, and cholesterol level. Plasma urea was significantly ($P > 0.05$) higher in G2 as compared to G1. Plasma T3 and insulin level did not differ ($P > 0.05$) between groups. However, plasma T4 level was lower ($P < 0.05$) in G2 as compared to G1. Plasma cortisol level did not differ ($P > 0.05$) among groups. Plasma estradiol level was lower ($P < 0.05$) in G2 ewes as compared to control ewes; whereas, the reverse trend was found in plasma progesterone level; although the difference was not statistically significant.

### 3.4. Ovarian response and super-ovulation

The effect of combined stress on ovarian response and super-ovulation is described in Table 7. All ewes (100%) exhibited estrus after the superovulatory treatment in both groups. The number of ewes showing superovulatory response, ovarian response, ovulation rate and embryo production were the same in G1 and G2 although it was numerically lower in G2 as compared to G1. However, the number of large follicles (anovulation) increased in the G2 group.

## 4. Discussion

The present study establishes the modifications of the biological function of sheep to struggle with the effect of both the nutrition and heat. Results also indicated that the superovulation without progesterone device also produces a good number of the embryos and ovarian response. The present study further showed the effect of combined stress on superovulatory response in native well adapted sheep of a semi-arid region.

In the present study feed intake decreased significantly in the combined stress group. The reduced feed intake is obvious as the

**Table 5**
Effect of combined stress on blood biochemical of superovulated Malpura sheep.

| | $\bar{x}$ ± SE | Control | Combined stress | P value |
|---|---|---|---|---|
| Glucose (mg/dl) | 48.52 ± 1.88 | 50.55 ± 2.65 | 46.50 ± 2.65 | 0.284 |
| Total protein (g/dl) | 7.72 ± 0.18 | 7.78 ± 0.25 | 7.67 ± 0.25 | 0.757 |
| Albumin (g/dl) | 2.70 ± 0.06 | 2.72 ± 0.09 | 2.68 ± 0.09 | 0.739 |
| Cholesterol (mg/dl) | 37.88 ± 4.95 | 39.45 ± 7.00 | 36.31 ± 7.00 | 0.752 |
| Urea (mg/dl) | 41.29 ± 1.07 | 38.78 ± 1.52 | 43.80 ± 1.52 | 0.023 |

$\bar{x}$ indicates the overall mean ± SEM.

**Table 6**
Effect of combined heat and nutritional stress on endocrine profile of superovulated Malpura sheep.

| | $\bar{x}$ ± SE | Control | Combined stress | P value |
|---|---|---|---|---|
| T3 (nmol/l) | 1.97 ± 0.08 | 2.08 ± 0.11 | 1.82 ± 0.11 | 0.186 |
| T4 (nmol/l) | 59.16 ± 2.70 | 70.27 ± 3.81 | 48.05 ± 3.81 | 0.001 |
| Cortisol (nmol/l) | 18.97 ± 1.22 | 19.20 ± 1.72 | 18.74 ± 1.72 | 0.849 |
| Estradiol (pg/ml) | 24.15 ± 2.96 | 30.39 ± 4.03 | 17.91 ± 4.33 | 0.042 |
| Progesterone (ng/ml) | 8.11 ± 0.93 | 6.55 ± 1.32 | 9.68 ± 1.32 | 0.098 |
| Insulin (nmol/l) | 10.5 ± 0.94 | 11.98 ± 1.33 | 9.20 ± 1.33 | 0.144 |

$\bar{x}$ indicates the overall mean.
T3, triidothyronoine; T4, tetraiodothyronine.

combined stress group ewes were offered with less feed as compared to control. The increase of water intake in stressed animals might have occurred to compensate the deficit in body water, which was caused by the increase of evaporation through the respiration and skin surface to counter the heat stress as they were exposed to a higher temperature (Sejian et al., 2010a, 2010b).

The average body weight and ADG decreased significantly in the G2 as compared to the control group. The high ambient temperature influences the ADG which might be attributed to the decrease in anabolic activity and increase tissue catabolism (Indu et al., 2015). The marked reduction in body weight indicated the severity of combined stress in the present experiment.

The physiological functions like RR, PR, and RT are good indicators of environmental stress (Daramola et al., 2009). In the present study, RR at afternoon increased significantly in the G2 group as compared to control group which might be an adaptive mechanism of ewes for thermolysis and maintenance of homeothermy to avoid an increase in body temperature because of thermal exposure (McManus et al., 2009). Increase in PR during thermal exposure is a common physiological adaptive change to increase blood flow from the core to the surface for increasing heat loss by sensible and insensible means (De et al., 2017c). However, in the present study, the PR did not increase in G2, which might be due to a decrease in metabolic rate as a result of restricted feeding (Sejian et al., 2010a, 2010b). RT increased significantly in G2 as compared to G1. The body temperature increased during the afternoon as a nicthermal variation (De et al., 2017c) in sheep, which is reflected as an increase in RT in G1 in the afternoon. However, the higher RT in G2 might be attributed to the thermal exposure. Although they maintained the RT within a narrow range, that indicates their adaptive capacity to combine stress.

In the present study plasma glucose, total protein, albumin, and cholesterol were fairly similar in G2 and G1; although the values were numerically lower in G2. The non-significant changes in the blood biochemical parameters indicated the adaptive capability of Malpura sheep to these combined stress and reflect their ability to cope with the stress for a short time period without any serious physiological changes. The increased plasma urea level in G2 as compared to G1 might be attributed to the consequence of skeletal muscle breakdown (Indu et al., 2015), which is indicated by a significant reduction in body weight in G2 ewes.

Plasma T4 and T3 level decreased in stressed animals. The reduction of these hormones might be due to reduced thyroid gland activity by high environmental temperature (Gowane et al., 2014). Furthermore, feed restriction could play a major role in reducing plasma thyroid hormone (Todini et al., 2007). In the present study, the lower plasma estradiol concentration in G2 as compared to G1 might be attributed to the diminishing follicular development in G2 which is supported by lower mean ovulation rate. In previous studies, the reduction of blood estradiol levels due to thermal stress (Sejian et al., 2011) and nutritional insufficiency (Kiyma et al., 2004) were observed.

The occurrence of behavioral estrous in all ewes of both groups

**Table 7**
Effect of combined heat and nutritional stress on ovarian response and embryo production in superovulated Malpura sheep.

| Observation | Control | Combined Stress |
|---|---|---|
| Number of ewes | 8 | 8 |
| Number (%) of ewes in estrus | 8 (100%) | 8 (100%) |
| Interval to onset of estrus (d) (mean ± SE) | 7.67 ± 0.387 | 7.97 ± 0.387 |
| Duration of estrus (h) (mean ± SE) | 25.7 ± 3.3 | 27.4 ± 3.3 |
| Duration of superovulated estrus (h) in stress (mean ± SE) | 25.5 ± 7.2 | 27.8 ± 7.2 |
| Mean ovulation rate (CL/ewe) | 8.8 ± 1.7 | 5.9 ± 1.7 |
| No. of LF | 1.0 ± 0.8 | 2.1 ± 1.4 |
| Mean ovarian response (CL + LF) | 9.6 ± 1.6 | 7.8 ± 1.7 |
| Superovulatory response (ewes ≥ 3 CL) | 7 (87.5%) | 5 (62.5%) |
| Ovulation rate in super ovulatory responsive ewes | 9.8 (68/7) | 9.0 (45/5) |
| Embryo recovery | | |
| Morula recovered | 17 | 12 |
| Blastocyst recovered | 26 | 19 |
| Unfertilized egg | 0 | 0 |
| Total | 43 | 31 |
| Embryo recovery (%) | 62.9% (44/70) | 66.0% (31/47) |
| Recovery rate per ewe | 6.2 (43/7) | 6.2 (31/5) |

LF, large follicle, SE, Standard error of the mean.

clearly indicated that Malpura sheep are well adapted to this region. However, the slight delay in the onset of estrus and duration of estrus after superovulatory treatment could be due to a decrease in blood estrogen concentration (Naqvi et al., 2004). This is further supported by the lower number of ewes showing superovulatory response when subjected to the combined heat and nutritional stress compared to the control group. This indicates that most of the Malpura ewes had the capacity to present normal estrus cycles under stress but they fail to perform superovulatory response as good as in control, might be due to extra demand of the body during superovulation, which is not possible to meet during combined stress by some of the ewes. The lower value of ovulation rate (CL/ewe) and ovarian response (CL + LF) in the present study in ewes subjected to the combined heat and nutritional stress might be attributed to the deficiency of energy in the diet which presumably reduced the number of responsive follicles (Albuquerque et al., 2012). The embryo recovery rate is generally related to the operator and flushing conditions rather than treatment (Mayorgaa et al., 2011). In the present study, the operator and flushing conditions remained the same, therefore, the embryo recovery percentage also remained the same.

## 5. Conclusions

It was concluded that despite a marked decrease in feed intake, body weight and hormonal profile, cyclic Malpura ewes subjected to the combined effect of heat and nutritional stress were equally responsive to superovulation than control ewes, which reaffirms the high adaptability of these ewes to harsh environments of India.

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## Conflict of interest

None.

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