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Alterations in Camels' Vaginal Temperature, Oxidative Stress, Antioxidants, and Steroid Hormones in Response to Exogenous Progesterone Insert During Cold Ambient Temperature

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Abstract

TO DETERMINE changes in the vaginal temperature, oxidants-antioxidants, and ovarian hormones to new, blank, and used CIDR in Dromedary camels, Ten healthy, non-pregnant dromedary camels of age (6-12) years were divided equally into two groups. The first group was inserted previously used-CIDR (uCIDR)-data logger for eleven days. The second group was inserted a new-CIDR (CIDR)-data logger for 8 days. All ciders were removed for 5 days (control-rest interval) and then re-inserted after washing and cleaning for another 11 days (R-uCIDR, R-CIDR). Blood samples were collected and sera were used to measure estradiol (E2), progesterone (P4), total antioxidants capacity (TAC), malondialdehyde (MDA), nitric oxide (NO), glutathione reduced (GSH), and catalase (CAT). Results showed increased ($P<0.0001$) vaginal temperature after re-inserting CIDR. The control rest intervals had the lowest ($P<0.0001$) P4 and GSH while E2 and MDA reached their highest levels. CIDR and R-CIDR insertions indicated higher ($P<0.0001$) vaginal temperature, P4, E2, with lower MDA and NO compared to uCIDR. The use of the univariate general linear model (Intercept + 2 treatment +11 Days CIDR + 24 Hour + 5 animals) revealed that vaginal temperature is influenced ($P<0.0001$) by treatment, Days, hour, Treatment ×Day, Treatment ×hour. Vaginal temperature correlated ($P<0.0001$) with ambient temperature of uCIDR ($r=0.37$); R-uCIDR and CIDR ($r=0.28$); and R-CIDR ($r=0.40$). In conclusion ambient temperature, day during CIDR, hour of the day, and type of CIDR affects vaginal temperature. Ovarian hormones, oxidants, and antioxidants vary according to the type of CIDR, days of insertion and after removal.

Keywords: Body temperature; Ovarian hormones; Oxidants-antioxidants; Dromedary camels.

Introduction

In dromedary camels, progesterone-releasing intra-vaginal devices (PRID) [1] and devices of controlled intra-vaginal drug release (CIDR) [2,3], are commonly used for the management of breeding. CIDR was used in dairy cattle [4,5], beef cattle [6], buffaloes [7], llamas [8], and dromedary camels [9] for synchronizing folliculogenesis. For improving the endometrial environment to maintain the conceptus, short-interval application of progesterone-releasing devices (5 to 7 days) is preferred [10]. CIDR was re-used to decrease the costs since it is designed to release progesterone if kept in the vagina for 14 days [1,11,12]. Repeated insertion of the same CIDR was used in heifers during embryo transfer programs [13]. In beef heifers and lactating beef cows, new and once previously used CIDR achieved higher pregnancy rates after fixed-time artificial inseminations compared to twice used CIDR [6]. For the management of breeding in buffaloes [14], and sheep [15] re-used CIDR was practised.

In cattle, body temperature is considered as a marker of heat stress (HS) using the vaginal temperature to express any elevation in the body temperature in response to HS [16]. Body temperature is related to several physiological functions such as pregnancy, parturition, estrus, and ovulation [17-19]. The vaginal temperature could by assessed by using a temperature probe [20], a temperature logger enchased in a plastic anchor [21], or fixed in the CIDR [22]. Vaginal temperature is used as an indicator to evaluate the thermoregulatory

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response in Nellore heifers [23] and dairy cows [24] which increased during estrus phase and estrus day [25,26,27,28]. Ovulation time could be predicted by monitoring the fluctuations in the reticulo-rumen temperature in cows [19] and the follicle diameter and shape in mares [29]. The increase in body temperature of pregnant animals was attributed to increased progesterone [30] and luteinizing hormone surge in cyclic animals. In cyclic dairy cows, body temperature was assessed rectally and vaginally in response to administration of exogenous progesterone [31].

In another experiment, the vaginal temperature in cows that received CIDR-containing progesterone did not differ from those implanted with CIDR-free progesterone after excluding 4.1% of temperature values lower than 37.5°C [31]. In dairy [24], and beef cows [26], vaginal temperature increased 0.3 to 0.8°C during estrus for 7 to 12 h [27,32] and could be used to predict the suitable time for artificial insemination [25]. Camels as unique animals possess a thermal gradient in their skin that enables them to balance between the ambient temperature and their body temperature and their thermal gradient became minimum at high ambient temperature and maximum at lower ones [33].

The use of CIDR containing 0.33 g of progesterone in non-pregnant and non-lactating llamas [8], increased plasma progesterone concentration to the maximum values on Day 1 after treatment. The association of vaginal temperature with circulating endogenous or exogenous progesterone was studied in cattle [28,31] for the detection of estrus but the association of CIDR-free progesterone with follicular changes was reported in dromedary camels [22]. Both progesterone and estradiol fluctuate during the growth, maturation and ovulation of the dominant ovarian follicle [34,35]. In Egypt, ovarian activities in camels commence between December and May every year [36] and usually, breeding is preferred during the cold season to expect calves in the next winter. So, the current research aimed to investigate the daily and hourly changes in the vaginal temperature determined during inserting CIDR used for the first time for 8 days and 11 days and the association of the type of CIDR with ambient environmental temperature, vaginal temperature, peripheral progesterone, estradiol, oxidants and antioxidants in dromedary camels during the coldest month of the breeding season.

Material and Methods

Animals and Management

All Institutional and National Guidelines for the care and use of animals were followed and approved by the Faculty of Veterinary Medicine Animal Care in Cairo University and Use Committees and Desert Research Center Animal Care and Use Committee with an approval number (Vet CU01122022583). The National Research Center Animal Care and Use Committee Approval certificate (NRC-19-143). This experiment included 10 multiparous, non-lactating female camels (Camelus dromedarius) of 6- 8 years old, and average body weight of 400-500 kg belonging to Desert Research Institute (Agriculture Research Center) and kept in Marsa-Matruh (Research Station were used for this experiment. Latitude 31° 00' N; Longitude 29° 47' E. The average environmental temperature during the study was 15.52±3.21. Camels were fed as one group a maintenance ration composed of a concentrated mixture at the rate of 3-5 kg/head/day in addition to Egyptian clover hay (Trifolium Alexanrinum). Fresh water was presented once daily. This experiment was conducted during January in the middle of the breeding season. Camels were equally divided into two groups (5 animals each). Camels of the control group were inserted with previously used CIDR for 11 days (uCIDR) after thorough cleaning and disinfection with distilled water and alcohol, removed for 6 days (Control), and re-inserted for another 11 days (R- uCIDR) except one animal that its CIDR was kept inside until the end of the experiment. The second treated camels were inserted a new controlled internal drug release insert (CIDR) containing 1.38g progesterone (EAZIBREED, inter Age, Hamilton, New Zealand) for 8 days (CIDR), removed for 6 days (Control), then cleaned an also disinfected with distilled water and alcohol then reinserted for another 11 days (R-CIDR).

Rest control interval were inserted neither CIDR nor uCIDR for 5 days (n=9) according to Swelum and Alowaimer [9] where ovulatory phase contain large ovulating follicles was from two to four days after CIDR removal.

Measurements of environmental and vaginal temperature

The ambient temperature of the farm was recorded using hourly (24 reading/day) recording data logger (Ondo-tori Jr.; Climatic, Tokyo, Japan). The environmental data logger was placed in the yard, 1.5 meters above the ground to avoid direct sunlight. Another data logger (Thermoclone type SL; KN Laboratories, Osaka, Japan) was fixed to each CIDR to record the vaginal temperature every hour. CIDR was inserted vaginally in each animal after cleaning and washing the perineum region with water and a povidone iodine-based detergent solution. In addition, 10 ml tetracycline was introduced during the application of CIDR in the vagina of each animal to minimize the contamination and inflammatory response. At the end of the experiment, each data

logger was removed, all temperature data (ambient or vaginal) were collected by the software (Rh Manager; KN Laboratories) and the average temperature was calculated by T&D Recorder; Climatic).

Blood sampling, hormonal and oxidant-antioxidants analysis

Starting from the day of CIDR insertion, blood samples were collected via jugular venipuncture each other day from inserting, during, and after removing the CIDRs. Blood samples were left at room temperature to clot. After centrifugation, sera were harvested and kept at -20°C until measuring estradiol, progesterone, nitric oxide (NO), lipid peroxide product (Malondialdehyde, MDA), catalase (CAT), glutathione reduced (GSH), and total antioxidant capacity (TAC, Biodiagnostics, Egypt). Quantitative progesterone and estradiol were measured using an ELISA commercial EIA kit (Legal Manufacturer, DRG instruments, GmbH, Germany) [5]. The sensitivity of the progesterone assay was 0.05 ng/ml and the intra- and inter-assay precisions were 5.9% and 10.1%. The sensitivity of estradiol assay was 2.0 pg/mL and test precisions were 6.81 % and 7.25%.

Statistical analysis

Data are presented as mean ± standard error of the mean (SEM). Simple one-way ANOVA was performed to study the effect of days during CIDR implantation on mean daily and hourly vaginal temperature, oxidants-antioxidants and ovarian hormones using IBM-SPSS 20.0 IBM Corporation [37]. Duncan's Multiple Range test was used to differentiate between significant means. Pearson correlation coefficient was also performed to correlate the vaginal temperature with ovarian hormones, ambient temperature, and oxidants-antioxidants. Univariate analysis of variance of General Linear model was used to study the effect of treatments, days during CIDR, hours during the day on the vaginal temperature, the model: $Xi\ j\ k\ l = \mu + \alpha i + \beta j + \gamma k + \theta\ ijk\ l + \varepsilon i\ j\ k\ l$ was used, where, μ is the grand mean, α is the effect of treatment, i is the level of treatments, β is the effect of the day during CIDR insertion, j is the index of the days during CIDR, γ is the effect of the hour within treatment, k is the index of the hours, θ is the interaction of treatments, day, and hour, and ε is the error variability. For studying the effect of Days during CIDR and hour during the day on the vaginal temperature, the model: $Xijk = \mu + \alpha i + \beta j + \gamma ijk + \varepsilon ijkl$ was used, where, μ is the grand mean, α is the effect of the day during CIDR, i is the level of days, β is the effect of hour of the day during CIDR insertion, j is the index of the hours during CIDR, γ is the effect of the hour within treatment, k is the interaction of days and hours, ε is the error variability. For studying the effect of treatment, day, and animal on the ovarian hormones and oxidants-antioxidants the model: $Xijkl = \mu + \alpha i + \beta j + \gamma k + \theta\ ijkl + \varepsilon ijkl$ was used; where μ is the grand mean, α is the effect of treatment, i is the level of treatments, β is the effect of the day during CIDR insertion, j is the index of the day during CIDR, γ is the effect of the animals within treatment, k is the index of the animals, θ is the interaction of treatments, day, animal, and ε is the error variability.

Results

The vaginal temperature of camels inserted with CIDR for 8 days is higher than the vaginal temperature of camels implanted with uCIDR for 11 days (Fig. 1). Camels inserted with R-CIDR for 11 days indicated higher vaginal temperature compared to those inserted with R-uCIDR (Fig. 1). The high ambient temperature ($P<0.0001$) during insertion of uCIDR (15.68±0.16), and CIDR (15.64±0.18) for the first time were associated with lower ($P<0.0001$) vaginal temperature (37.29±0.01; 37.43±0.01). The mean ambient temperature along the insertion of R-uCIDR (15.10±0.12) and R-CIDR (15.09±0.12) for 11 days was associated with higher ($P<0.0001$) vaginal temperature (37.37±0.01; 37.52±0.01, respectively; Table1).

The increase of the ambient temperature from 16.5 to 20.4, 22.6, >24°C increased the vaginal temperature ≥ 37°C in camels implanted uCIDR with a total of seven peaks (Fig. 2a). In the same treatment, ambient temperatures of 11.5, 15.3, and 23.1°C were associated with vaginal temperature <37°C. During R-uCIDR application, ambient temperatures ($P<0.0001$) from 14.2 to 15°C, and >17.2°C were associated with vaginal temperature >37.52°C with frequent decreases between this temperature to reach 37.22°C with a total 12 peaks (Fig. 2b). During CIDR insertion, ambient temperature from 10.3 to 24.3 showed several peaks in the vaginal temperature >37.45°C with the largest number (N=28) of peaks (Fig. 2c). The R-CIDR increased the vaginal temperature ≥37°C for 22 times when the ambient temperature ranged from 10.7 to 21.7°C (Fig. 2d). Vaginal temperature decreases as the ambient temperature decease and increase with the increase of the ambient temperature when uCIDR (Fig. 2a), R-uCIDR (Fig. 2b), and R-CIDR (Fig. 2d) were inserted but the vaginal temperatures were high regardless the ambient temperature increased or decreased when CIDR was implanted (Fig. 2c). The correlation between the ambient temperature and the vaginal temperature during uCIDR ($r= 0.369$; $P=0.0001$), R-uCIDR ($r=0.275$; $P=0.0001$), CIDR ($r=0.282$; $P=0.0001$), and R-CIDR ($r=0.398$; $P=0.0001$) were fair but significant. Days ($P<0.0001$; Fig. 1) and hours ($P<0.0001$; Fig. 3) indicated

significant effects on the vaginal temperature after all types of CIDR application (Table 2). Individual variations between animals significantly affected the vaginal temperature of R-uCIDR (P<0.0001), CIDR (P<0.0001), R-CIDR (P<0.0001), and uCIDR (P<0.032). Treatment × Days (P<0.0001) and treatment × hour (P<0.002) influenced the vaginal temperature (Table 3). The vaginal temperature reached the highest values in all types of CIDR from 4:00 to 5:00 PM and declined to reach the lowest values at midnight and 1:00 a.m. (Fig. 3). The vaginal temperature had low and significant correlations with the hour of the day during uCIDR (r=0.13; P=0.002), R-uCIDR (r=0.139; P=0.0001), CIDR (r=0.222; P=0.0001), and R-CIDR (r=0.236; P=0.0001).

Camels inserted with uCIDR (Fig. 1) had the lowest (P<0.0001) vaginal temperature on Days 2 (37.16±0.02), 3 (37.18±0.02), 4 (37.19±0.02) and Day 1 (37.19±0.03) and the highest ones on Day 6 (37.49±0.03). Inserting R-uCIDR (Fig. 1) showed the lowest (P<0.0001) vaginal temperature on Day 6 (37.28±0.03) and Day 3 (37.1829±0.03), and the highest ones on Day 9 (37.45±0.02) and Day 10 (37.49±0.02). The application of CIDR for 8 days has declined (P<0.0001) the vaginal temperature on the first two days (37.21±0.064; 37.23±0.033) that reached high values on Day 7 (37.571±0.023), Day 5 (37.574±0.025), and Day 6 (37.649±0.024). The application of R-CIDR for 11 days decreased (P<0.0001) the vaginal temperature on Day 3 (37.322±0.028), Day 2 (37.392±0.025), Day 11 (37.400±0.036), and Day 4 (37.428±0.042) with nearly stabilized values during the other days with no significant differences between them.

During uCIDR insertion and throughout the 24 hours of the day, the vaginal temperature (Fig. 3) was minimum (P<0.0001) from midnight (37.145±0.041) and 3.00 am (37.152±0.038). The highest value was observed at 4.00 p.m. (37.447±0.03) and 5.0 p.m. (37.491±0.04). When R-uCIDR was implanted (Fig. 3), the vaginal temperature was minimum (P<0.0001) and kept low values from midnight (37.26±0.044) till 5 a.m. (37.23±0.043). The highest value was observed from 11.00 a.m. (37.517±0.027) till 5.00 p.m. (37.536±0.032). The Vaginal temperature declined (P<0.0001) to the lowest values after CIDR application at 4:00 a.m. (37.286±0.065), 8:00 a.m. (37.286±0.056), and 9:00 a.m. (37.23±0.65) and reached the highest values at 5:00 p.m. (37.705±0.036) and 6:00 p.m. (37.638±0.044). The insertion of R-CIDR showed the lowest (P<0.0001) vaginal temperature at 9:00 a.m. (37.353±0.046), 8:00 a.m. (37.367±0.042), and 4:00 a.m. (37.393±0.042) and the highest vaginal temperature became high from 2:00 p.m. (37.66±0.044) to reach the highest value at 6:00 p.m. (37.79±0.028).

The vaginal temperature increased >37.5°C for more than six successive hours on Days 6, 8 and 11 after inserting uCIDR (Fig. 4A); Days 4 and 10 after R-uCIDR (Fig. 4B); Days 5 and 6 of CIDR (Fig. 4C); and Days 4, 5, and 7-10 of R-CIDR (Fig. 4D).

In comparison to progesterone (P4) during the control interval (4.06 ±0.13), camels inserted with uCIDR (Table 1) have high significant concentrations (P<0.0001; 4.54 ±0.15ng/ml) which became higher after inserting R-uCIDR (6.61 ±0.16 ng/ml) but those inserted with CIDR (7.66 ±0.07 ng/ml) and R-CIDR (7.96 ±0.08 ng/ml) indicated the highest P4. Estradiol (E2) achieved the lowest concentration in camels implanted with uCIDR (60.69±2.47pg/ml) and R-uCIDR for another 11 days (76.38±3.69pg/ml) whereas; CIDR and R-CIDR increased its concentrations.

MDA values in camels during the control interval (13.33±0.17 nmol/mL) and camels implanted with uCIDR (13.24±0.22 nmol/mL) is higher (P<0.0001) than the other treatments (Table 1). Nitric oxide (NO; P<0.0001) varied significantly between treatments. NO levels were high after inserting R-uCIDR for 11 days (Table 1). Though TAC of uCIDR (0.696±0.019 mM/L) is higher (P<0.0001) than the control interval (0.648±0.01mM/L), R-uCIDR (0.606±0.009 mM/L) and R-CIDR (0.609±0.003 mM/L) but all of them are lower than CIDR (0.827±0.024 mM/L; Table 1). The levels of glutathione reduced (GSH, P<0.0001) during the uCIDR insertion (9.42±0.42 mg/dl) are higher than the control (7.85±0.08 mg/dl) but lower than the other three treatments (Table 1). Compared to uCIDR the value of 303±7.2 U/L indicating the activity of catalase (CAT, Table 1) increased in camel's inserted R-uCIDR for another 11 days (433±6.5 U/L) whereas that R-CIDR (373±6.7 U/L) indicated lower CAT activity than CIDR (394±2.1 U/L) and control (400±7.5 U/L).

After uCIDR implantation, the highest (P<0.0001) P4 concentration (Fig. 5A) was observed on Day 1 (9.34 ±0.17 ng/ml) which declined linearly to reach the lowest concentrations on Days 7 (2.78 ±0.19 ng/ml) and 9 (2.99 ±0.08 ng/ml) then re-increased again on Day 11 (5.61 ±0.00 ng/ml). After R-uCIDR was inserted, the highest (P<0.0001) P4 concentration (Fig. 5A) was observed on Day 2 (11.86 ±0.25 ng/ml) which declined linearly from Day 7 (5.64 ±0.19 ng/ml) to reach the lowest concentrations on Days 9 (4.53 ±0.08 ng/ml) and 1 (3.02 ±0.09 ng/ml). CIDR increased (P<0.0001) P4 on Day 1 (9.03 ±0.17 ng/ml) after its application and reached the lowest concentration on Day 5 (7.21 ±0.09 ng/ml). R-CIDR maintained high P4

(P<0.0001) until Day 4 (9.04 ±0.27ng/ml) then declined to reach the lowest concentration on Day 10 (6.07 ±0.21ng/ml). P4 reached the highest (P<0.0001) concentrations on Day 5 after uCIDR and CIDR removal during the rest-control interval. The effect of vaginal temperature on circulating progesterone concentration of camels inserted uCIDR tended to be significant (P≤0.074; Fig. 5B). The mean of P4 concentrations (5.47±3.03 ng/ml) was recorded with the vaginal temperature of 37.3□C. When the uCIDR was inserted again after 5 days of rest, P4 concentrations less than 6.0 to 9.91 ng/ml were recorded at a vaginal temperature of 37.3 to 37.8□C. An increase (P<0.0001) in the vaginal temperature to 37.8□C was observed when P4 concentrations reached 4.74±3.40 ng/ml (Fig. 5B). After CIDR insertion, vaginal temperatures from 37 to 38.2 □C were associated with increased P4 concentrations from 6.25 to 10.14 ng/ml (P<0.001; Fig. 5B). The vaginal temperature of 36.8 to 38 was observed at higher P4 concentrations from 7.22 to 9.81ng/ml after R-CIDR insertion (P<0.0001) following 5 days' rest.

The camels implanted with uCIDR had the lowest (P<0.0001) estradiol (E2) concentrations (Fig. 6). E2 rose (P<0.0001) from Day 1 (74.39 ±9.66 pg/ml) to reach the highest value on Day 3 (78.19 ±5.54pg/ml), and started declining from Day 5 to reach the lowest concentration on Day 7 (43.09 ±3.93pg/ml). After implanting R-uCIDR, E2 reached the highest (P<0.0001) concentration on Day 2 (282.48 ±9.42pg/ml) and Day 10 (139.48 ±3.87pg/ml). Lower E2 concentrations were observed on Day 5 (0.07 ±0.00pg/ml), Day 3 (6.26 ±0.23pg/ml), Day 9 (13.00 ±0.93pg/ml), and Day 7 (13.90 ±2.03pg/ml). CIDR and R-CIDR applications did not influence E2. The significant increase of E2 on Day 4 was followed by a significant decrease on Day 10 after inserting R-CIDR. E2 achieved the highest (P<0.0001) concentrations on Day 1 and Day 3 after removing all CIDR compared to Day 4 and Day 2.

When uCIDR treatment was inserted, TAC (Fig. 7) declined (P<0.0001) from Day 1 (1.11 ±0.13 mM/L) to reach nearly similar low values on Days 5,7,3,9, and 11 (0.57 ±0.01mM/L). By implanting the R-uCIDR, TAC declined (P<0.0001) to low values on Day 3 (0.46 ±0.03mM/L) and Day 8 (0.39 ± 0.01mM/L) but reached high values on Days 2 (0.62 ±0.01mM/L), 11 (0.68 ±0.01mM/L) and 9 (0.71 ±0.07mM/L). TAC increased (P<0.0001) after CIDR insertion to reach the highest value on Day 3 (1.02 ±0.04mM/L) then declined to reach the lowest value on Day 7 (0.67 ±0.003mM/L). After inserting R-CIDR, TAC increased (P<0.0001) except on Day 10. TAC has declined from Day 1 (P<0.0001) reached the lowest level on Day 3 then started increasing till Day 5.

The use of uCIDR declined MDA (Fig. 8; P<0.0001) from Day 1 (14.05 ±0.78 nmol/mL) till Day 5 (11.87 ±0.21nmol/mL) then after a transient increase on Day 7 (15.43 ±0.92nmol/mL) re-decreased again on Day 9 (11.92 ±0.28nmol/mL). The use of R-uCIDR declined MDA (P<0.0001) from Day 1 (14.02 ±0.50nmol/mL) till achieved low levels on Day 3 (10.59 ±0.21nmol/mL), Day 6 (10.56 ±0.70nmol/mL), and Day 10 (10.39 ±0.48nmol/mL) that increased reaching the highest level on Day 9 (14.87 ±0.20nmol/mL). After the CIDR application, the lowest (P<0.0001) MDA levels could be observed on Day 1 (11.17 ±0.02nmol/mL) with significantly high values on Day 3 and Day 7. Re-insertion of CIDR increased (P<0.0001) MDA on Day 6. The removal of CIDR increased (P<0.006) MDA till reached maximum levels on Day 3 (Fig. 8).

During the uCIDR (Fig. 9), levels of NO increased (P<0.0001) from Day 1 (35.05 ±0.34µmol/L) till Day 3 (51.31 ±1.76 µmol/L) then declined till Day 7 (29.10 ±0.89µmol/L), then it showed a transient increase on Day 9 (34.42 ±0.53µmol/L) then re-decreased again on Day 11 (21.27 ±0.21µmol/L). NO of the R-uCIDR declined (P<0.0001) from Day 1 (37.79 ±2.45µmol/L) till Day 3 (26.49 ±0.09µmol/L) and after reaching a high value on Day 4 (48.95 ±1.49µmol/L), it continued increasing till reaching the maximum value on Day 6 (56.91 ±1.98µmol/L) and achieved the lowest one on Day 11 (23.54 ±0.09µmol/L). After CIDR application, NO increased from Day 1 (31.53 ±0.80) to achieve the highest levels (P<0.0001) on Day 3 (45.49 ±1.45µmol/L). The application of R-CIDR increased (P<0.0001) NO levels on Days 10 and 6 compared to Days 4 and 2. NO declined sharply (P<0.0001) to reach the lowest levels on Day 3 after removing all CIDR then continued increasing till Day 5 (Fig. 9).

The insertion of uCIDR declined the values of GSH till reached the lowest level on Day 9 (6.13 ±0.06 mg/dl; Fig. 10) then continued increasing till Day 11. Contrary, GSH of R-uCIDR decreased (P<0.0001) from Day 1 (8.48 ±0.03 mg/dl) to reach the lowest level on Day 4 (6.89 ±0.55mg/dl) followed by the highest level on Day 5 (13.93 ±0.98 mg/dl) then declined with showing high values on Day 7 (10.67 ±0.08 mg/dl) and 9 (12.13 ±0.18 mg/dl). The transient decrease (P<0.0001) of GSH on Day 5 after CIDR insertion (7.19 ±0.18mg/dl) was followed by the highest peak on Day 7 (15.75 ±1.29mg/dl). After R-CIDR insertion, GSH attained (P<0.0001) the highest levels on Day 8 compared to Day 10. GSH declined (P<0.0001) from the Day after removing all CIDR till achieved the lowest levels on Day 3 then ascended again till reached the highest value on Day 5 (Fig. 10).

CAT levels of uCIDR treatment declined from Day 1 (332.79 ±12.02U/L) to achieve the lowest (P<0.0001) value on Day 7 (102.24 ±3.48U/L; Fig. 11) then increased till reached the highest value on Day 11 (456.58 ±7.18U/L). CAT of R-uCIDR showed the lowest levels (P<0.0001) on Day 4 (139.83 ±13.78U/L) and Day 9 (406.16 ±4.29U/L) reaching the highest value on Day 2 (647.73 ±18.93U/L). After the CIDR application, CAT declined (P<0.001) from Day 1 (414.69 ±6.26U/L) to reach the lowest value on Day 7 (384.69 ±3.88). CAT concentrations increased (P<0.0001) markedly on Day 10 after R-CIDR reached the lowest levels on Day 8. Removing all CIDRs increased (P<0.0001) CAT on Days 3 and 5 compared to Day 4 (Fig. 11).

In all camels, the vaginal temperature has a weak positive correlation with the hours of the day (r=0.18; P<0.0001), ambient temperature (r=0.29; P<0.0001), P4 (r=0.22; P<0.0001), E2 (r=0.16; P<0.0001), and NO (r=-0.15; P<0.0001). In camels inserted uCIDR, the vaginal temperature showed positive and weak correlations with MDA (r=0.24; P<0.0001). Inverse and weak correlations are observed between vaginal temperature with P4 (r=-0.13; P<0.031), E2 (r= - 0.18; P<0.003), and GSH (r= - 0.18; P<0.003) that became moderate with NO (r=- 0.32; P<0.0001). In camels R-uCIDR, the vaginal temperature got weak positive correlations with E2 (r= 0.21; P<0.0001), and CAT (r=0.11; P<0.035), but weak negative correlations with P4 (r= - 0.34; P<0.0001), NO (r= - 0.12; P<0.017), and GSH (r= - 0.23; P<0.0001), and a negative moderate one with MDA (r= - 0.35; P<0.0001). In camels inserted CIDR, correlations of the vaginal temperature are positive and moderate and positive with MDA (r=0.37; P<0.0001) but are negative and weak with P4 (r= -0.24; P<0.0001) and NO (r= -0.18; P<0.004). In camels inserted R-CIDR, the vaginal temperature had negative and fair correlations with P4 (r= - 0.15; P<0.005) and CAT (r= - 0.18; P<0.001).

Discussion

In camels of our study, the vaginal temperature correlated with the ambient temperature whether used CIDR for the first time or after repeated insertion. In agreement with our results, vaginal temperature varied in response to changes in the ambient temperature [31]. Similar to camels treated with uCIDR, R-uCIDR and R-CIDR, the vaginal temperature increased with the increase of the vaginal temperature in cattle inserted a long anchor fitted with Data Logger [20]. The higher vaginal temperature throughout the low to high ambient temperature after CIDR insertion could be related to the release of high concentrations of progesterone. In agreement with our camels, progesterone in the new CIDR increased the vaginal temperature in dairy cows on Day 3 compared to blank-CIDR and the re-insertion of uCIDR showed lower vaginal temperature compared to R-CIDR [31]. In contrast to the significant difference in the vaginal temperature of all treated camels except CIDR throughout the hour of the day, the time of day did not affect vaginal temperature but influenced the rectal temperature in dairy cows [31]. In our camels, the vaginal temperature increased on Day 6 after inserting uCIDR and CIDR, but in dairy cows, this increase was on Day 3 than on Day 1 [31].

In domestic cattle, body temperature increased 1.3°C every 21 days on the day of estrus with slight seasonal variations [38]. Similar to cows where the start of estrus could be determined by recording an increase of 0.3°C in the vaginal temperature than it has been at the same time the day before or the day after estrus [39] that continued for more than three hours [28]. The start of estrous could be determined in camels of the current study by recording an increase in the vaginal temperature for more than six hours. Regardless of the season, the vaginal temperature was used for discriminating between estrus and non-estrus animals [28]. The increase in the vaginal temperature for six hours persisted during estrus in our camels is similar to that was recorded in dairy cattle [24,27,40], and beef cows [26]. In Bororo zebu cows [41] and dairy cows [24], body temperature decreased on days 7 and 1 before estrus, and one day post-estrus. The different days of increased vaginal temperature for 6 successive hours could be referred to as the significant animal effect indicating individual variations and the mean vaginal temperature increase when at least one animal approaches estrous. In the current study, vaginal temperature increased at 6:00-7:00 p.m. and became the minimum from midnight to 5:00 am. In cows [17,38] and dromedaries [22], body temperature increased at dusk compared to dawn and body temperature cycle length was similar to estrus cycle length that showed Rhythmicity. Similar to the increase of vaginal temperature from noon till 6:00 p.m. and decreased from midnight till 6:00 am, skin and body surface temperatures increased in camels afternoon and decreased after midnight and showed Rhythmicity [33].

In cattle treated with prostaglandin, estrous was associated with high vaginal temperature and lower progesterone concentrations that decreased around ovulation [42]. Similar to dairy cows [31] and dromedary camels [43]. The results of our study showed that Days influenced the progesterone concentrations in our treated and non-treated camels. In camels, the decreased progesterone concentrations to lower levels (2.2 ng/ml) on day 3 [8] could be noticed in our camels on Days 7-9 for camels

implanted blank-CIDR for 11 days. In agreement with Japanese Black cows, the decrease in the vaginal temperature when treated with prostaglandin but the un-change in the vaginal temperature after treatment with prostaglandin-exogenous progesterone combination (CIDR) [28] reflected the relation between progesterone and vaginal temperature which is evident when CIDR impregnated with progesterone increased the vaginal temperature throughout the low ambient temperature. In contrast, no association was observed between progesterone and body temperature in cattle treated with prostaglandin [42]. The decrease of P4 observed after removing CIDRs on Days 2 and 4 was also noticed on Day 4 in camels synchronized with CIDR for 12 days [43]. The negative and weak correlation between the vaginal temperature and progesterone was also reported in cows [31]. The decrease of progesterone to low levels during blank-CIDR suggests that CIDR or PRIDs induced ovulation [44] and its decrease during the rest-control interval refers to spontaneous ovulation [45]. The high P4 for 8 days after CIDR insertion in camels of this study was referred to as the presence of luteinized preovulatory follicle at the time of CIDR insertion to dromedaries which produced progesterone concentrations similar to corpus luteum [9].

The negative correlation between estradiol and vaginal body temperature in camels treated with uCIDR and the positive one after re-inserting uCIDR though significant but still weak is similar to the transient increase in estradiol concentrations during the mature ovarian follicles and ovulation after removal of CIDR in camels [43] and during estrus in dairy cows [27]. The decrease of estradiol after inserting CIDR on Day 3 and uCIDR on Day 7 agrees with our previous results when CIDR was implanted for 7 days during the breeding season [2]. The increased AC on Day 3 after CIDR insertion and then its linear decrease until Day 7 associated with a decline in P4 and E2 could refer to the decrease of superoxide dismutase activity on day 7 after a sharp increase on Day 3 [2] which indicated the ability of dromedary camel to modulate their redox defense mechanisms to adapt to their environment [45], and the protective effects of estradiol on lipid peroxidation [46]. The decrease of TAC on Day 3 after inserting uCIDR and rest control interval, on Day 3 and Day 8 after inserting R-uCIDR and on Day 5 after R-CIDR correlated with low estradiol and the absence of its protective effects on lipid peroxidation. The increase of MDA on Day 7 after uCIDR insertion and Day 3 of the rest-control interval and CIDR on Day 6 after inserting R-uCIDR and CIDR was also noticed during the breeding season in dromedary camels synchronized with CIDR [2]. The transient decrease of the vaginal temperature on Days 3 and 6 of inserting R -uCIDR and R-CIDR and Day 7 after CIDR insertion and Day 4 after CIDR insertion may refer to the induced ovulation via increasing the oxidative stress. In contrast to the reach of MDA to maximum levels on Day 7 and the vaginal temperature on Day 6 in dromedaries implanted with uCIDR or CIDR, NO and catalase reached their minimum with minimum estradiol and progesterone on Day 7. All these events agree with non-lactating Japanese Black cows subjected to high ambient temperatures during summer which increased their body temperature, oxidative stress, and declined antioxidants [47].

Conclusion

It could be concluded that the measuring of vaginal temperature can predict estrus in camel and might help predict ovulation using other tools rather than a data logger. Exogenous progesterone, environmental temperature, and the time of the treatment Day influenced vaginal temperature. The animal showed a distinct effect on vaginal temperature, ovarian hormones, and oxidants-antioxidant status. The increase in vaginal temperature a few successive hours is associated with oxidative stress and lowered ovarian hormones and might indicate the approach of induced ovulation. The increase of vaginal temperature above 37°C for 3 to 6 hours might indicate induced ovulation whereas the decline of progesterone after CIDR removal indicates spontaneous ovulation. Vaginal temperature reached the highest values from the afternoon till 6:00 p.m. and reach minimum values from midnight till 9:00 am. CIDR exogenous progesterone increase the vaginal temperature during low ambient temperature.

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Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical of approval

This study follows the ethics guidelines of the Faculty of Veterinary Medicine, Cairo University, Egypt (approval number:Vet CU01122022583).

TABLE 1. Mean± SEM of vaginal temperature, ambient, P4, E2, TAC, MDA, NO, GSH, and CAT in treated and control dromedary camels

Treatment	Temperature		P4	E2	TAC	MDA	NO	GSH	CAT
	Vaginal	Ambient	ng/ml	pg/ml	mM/L	nmol/ml	µmol/L	mg/dl	U/L
CIDR	37.43^{c} ±0.01	15.64^{b} ±.18	7.66^{d} ±0.07	95.88^{c} ±2.54	.827^{d} ±0.024	12.59^{b} ±0.08	38.87^{b} ±0.77	10.40^{c} ±0.45	394^{c} ±2.1
R-CIDR	37.52^{d} ±0.01	15.09^{a} ±0.12	7.96^{d} ±0.08	88.86^{c} ±1.53	.609^{ab} ±0.003	12.06^{a} ±0.07	35.16^{a} ±0.35	10.17^{c} ±0.15	373^{b} ±6.7
uCIDR	37.29^{a} ±0.01	15.68^{b} ±0.16	4.54^{b} ±0.15	60.69^{a} ±2.47	.696^{c} ±0.019	13.24^{c} ±0.22	36.97^{ab} ±0.75	9.42^{b} ±0.14	303^{a} ±7.2
R-uCIDR	37.37^{b} ±0.01	15.10^{a} ±0.12	6.61^{c} ±0.16	76.38^{b} ±3.69	.606^{a} ±0.009	12.97^{bc} ±0.16	42.42^{c} ±0.66	10.34^{c} ±0.16	433^{d} ±6.5
Control		15.52^{b} ±0.14	4.06^{a} ±0.13	121.52^{d} ±2.43	.648^{b} ±0.01	13.33^{c} ±0.17	38.52^{b} ±0.98	7.85^{a} ±0.08	400^{c} ±7.5
P-value	0.0001	0.002	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

*Mean with different superscript letters (a,b,c,d) in the same column are significantly different at (P<0.05).

TABLE 2. The effect of Day within treatment and Animals on vaginal temperature, ambient, P4, E2, TAC, MDA, NO, GSH, and CAT in treated and control dromedary camels

Treatment	Effect	Temperature		P4	E2	TAC	MDA	NO	GSH	CAT
		Vaginal	Ambient	ng/ml	pg/ml	mM/L	nmol/ml	µmol/L	mg/dl	U/L
CIDR	Day	0.0001	0.087	0.0001	NS	0.0001	0.0001	0.0001	0.0001	0.001
	Animal	0.0001	NS	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
R-CIDR	Day	0.0001	0.0001	0.0001	NS	0.0001	0.0001	0.0001	0.0001	0.0001
	Animal	0.0001	NS	0.063	0.0001	0.0001	0.0001	0.0001	0.0001	.018
uCIDR	Day	0.0001	0.023	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
	Animal	0.032	NS	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
R-uCIDR	Day	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
	Animal	0.0001	NS	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Control	Day	0.0001	0.0001	0.0001	0.0001	0.0001	0.006	0.0001	0.0001	0.0001
	Animal	0.001	NS	0.0001	0.0001	0.027	0.0001	0.0001	0.009	0.0001
All treatments	Animal	0.0001	NS	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

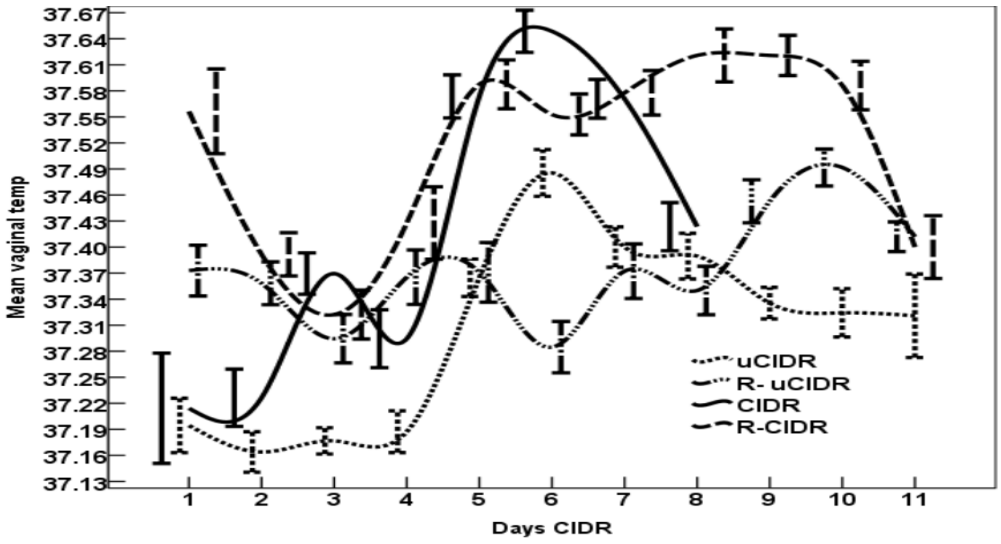

Fig. 1. Mean change in the vaginal temperature °C in camels treated with blank-CIDR and CIDR with error bars

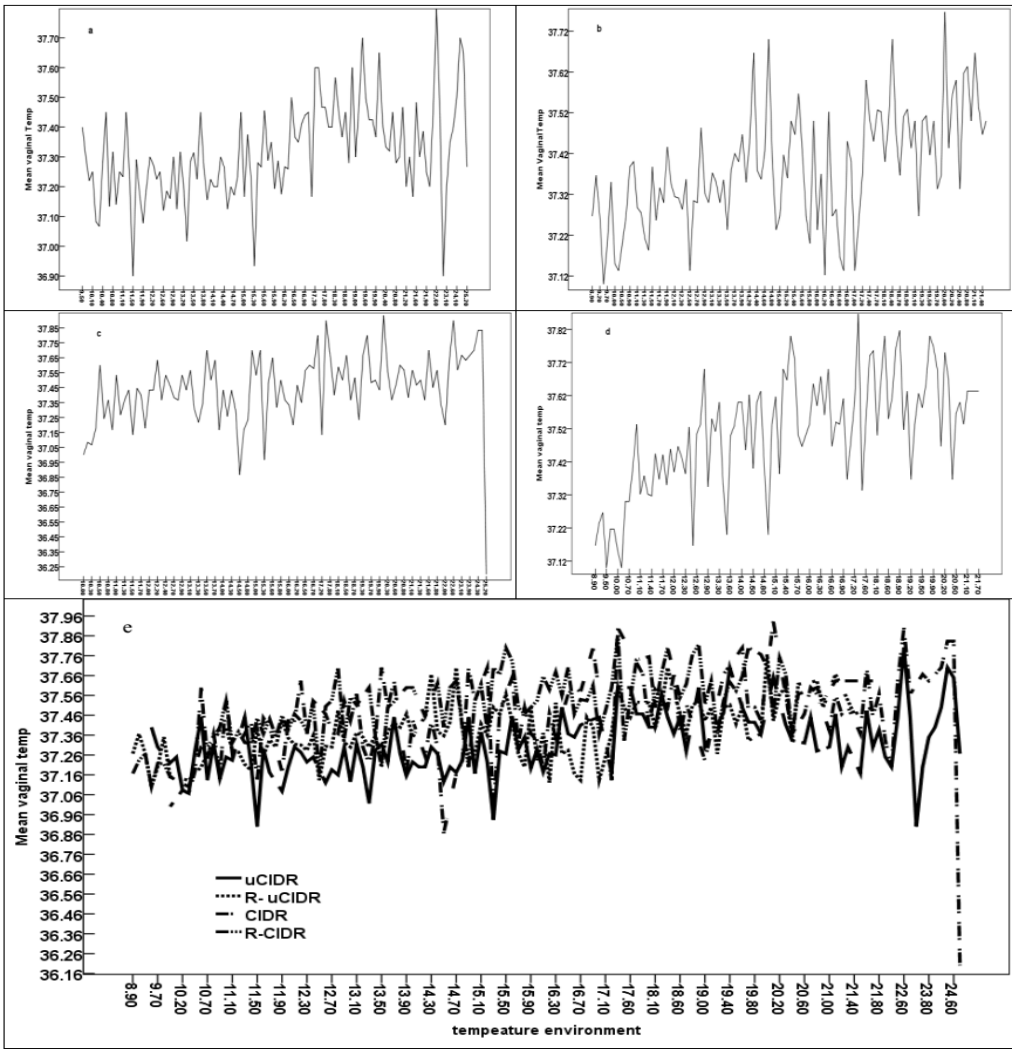

Fig. 2. The relationship between the vaginal temperature and ambient temperature in camels treated with uCIDR (a), R-uCIDR (b), CIDR (c), R-CIDR (d), and all treatments (e)

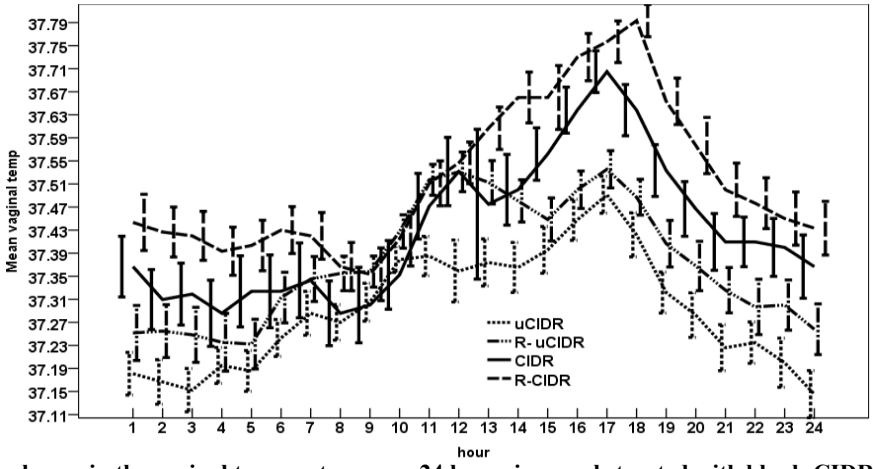

Fig. 3. The mean change in the vaginal temperature over 24 hours in camels treated with blank-CIDR and CIDR with error bars

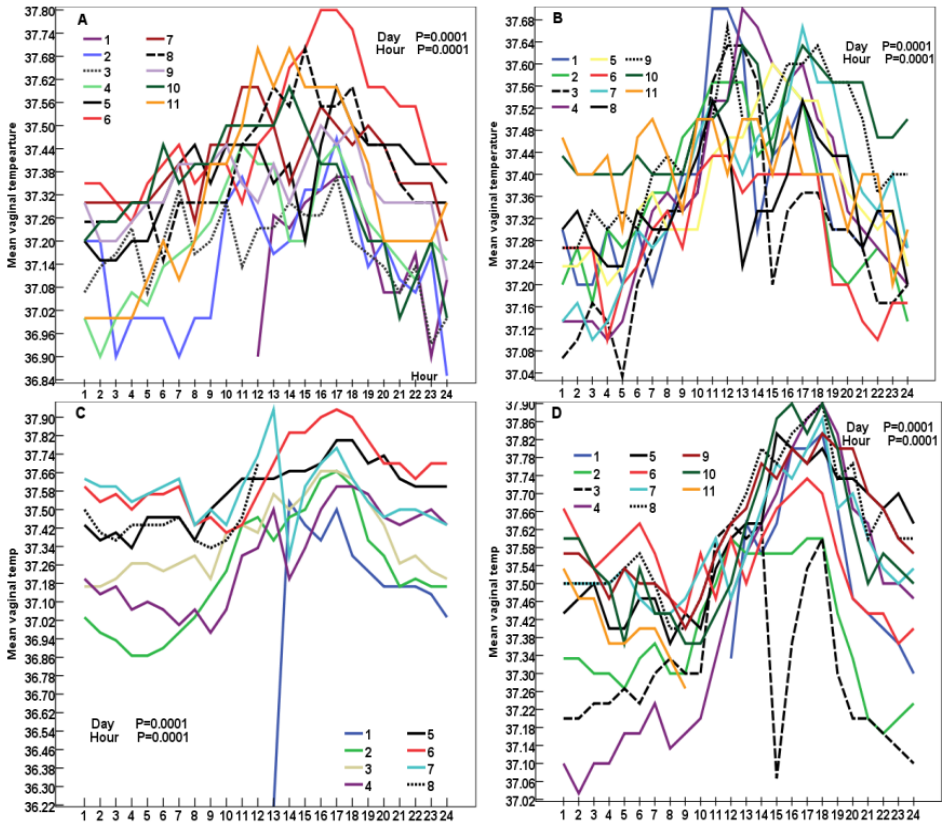

Fig. 4. Mean hourly and daily fluctuations of vaginal temperature / °C in camels treated with blank-CIDR (A), R blank-CIDR (B), CIDR (C), and R CIDR (D)

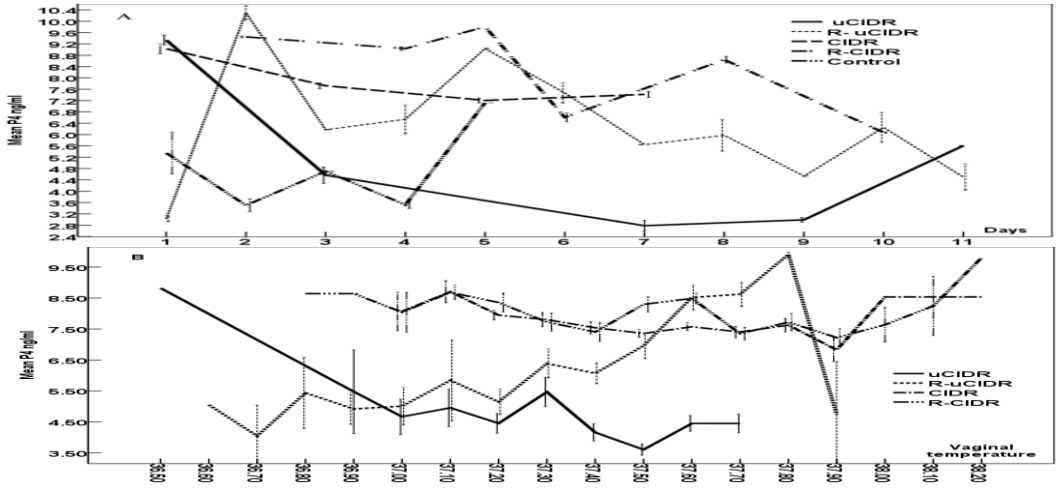

Fig. 5. Mean progesterone (P4 ng/ml) in relation to days after insertion (A) and different vaginal temperatures (B) in camels treated with uCIDR, R- uCIDR, CIDR, R-CIDR, and control with error bars

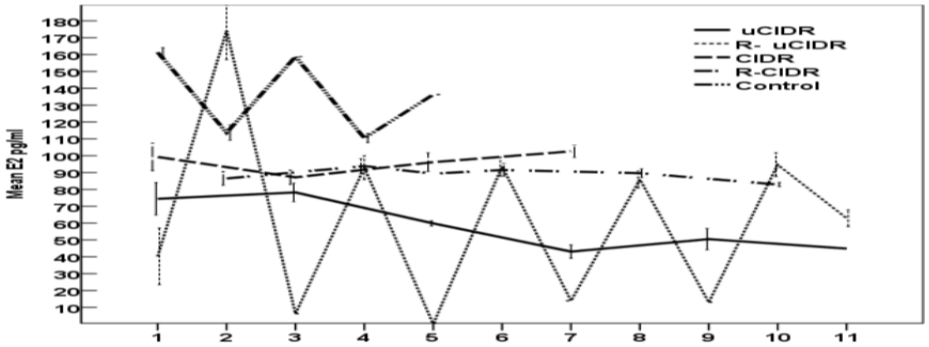

Fig. 6. Mean estradiol (E2 pg/ml) in the camels treated with uCIDR, R- uCIDR, CIDR, RCIDR, and control with error bars

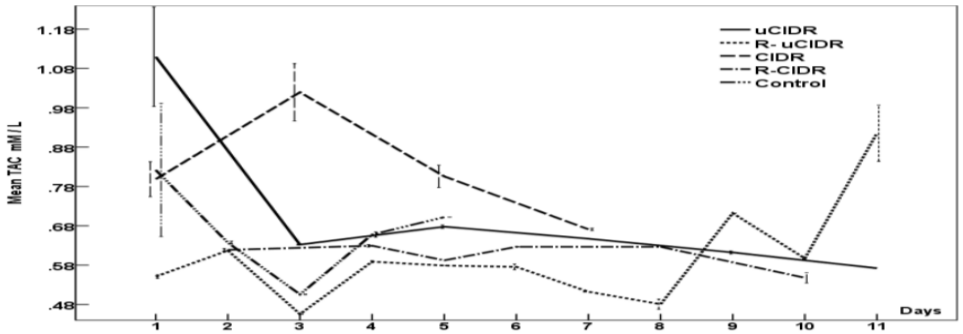

Fig.7. Mean total antioxidants capacity (TAC mM/L) in the camels treated with uCIDR, R- uCIDR, CIDR, R-CIDR, and control with error bars

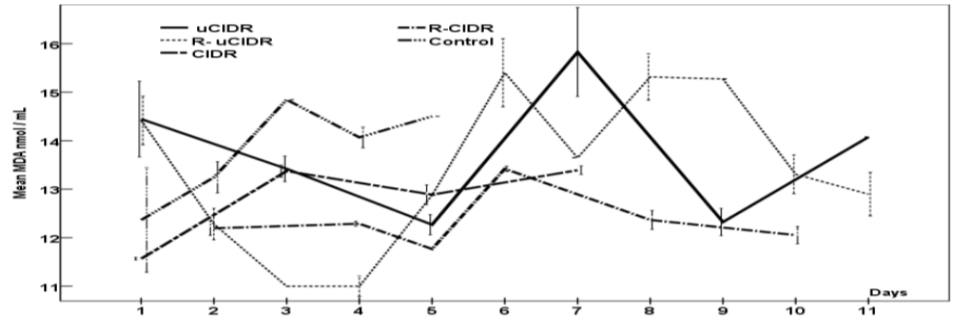

Fig. 8. Mean Malondialdehyde (MDA nmol/mL) in the camels treated with uCIDR, RuCIDR, CIDR, R-CIDR, and control with error bars

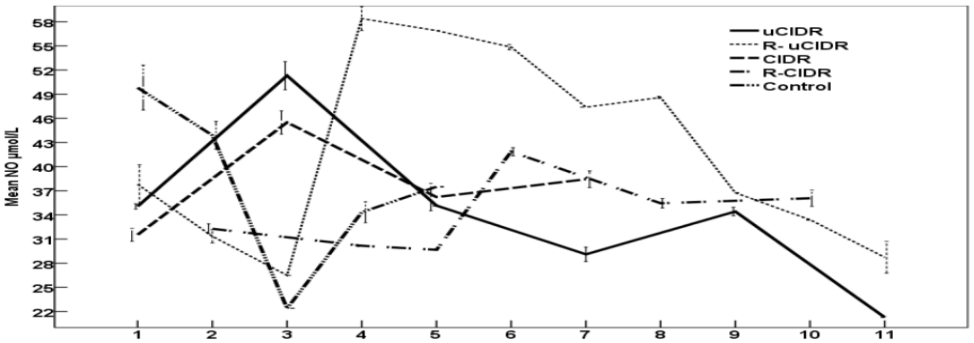

Fig. 9. Mean Nitric oxide (NO µmol/L) in the camels treated with uCIDR, R- uCIDR, CIDR, R-CIDR, and control with error bars

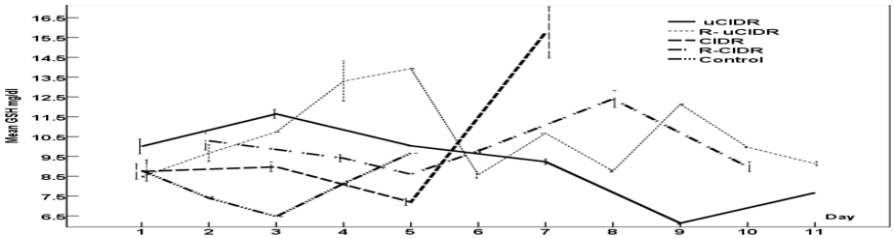

Fig. 10. Mean glutathione reduced (GSH mg/dL) in the camels treated with uCIDR, RuCIDR, CIDR, R-CIDR, and control with error bars

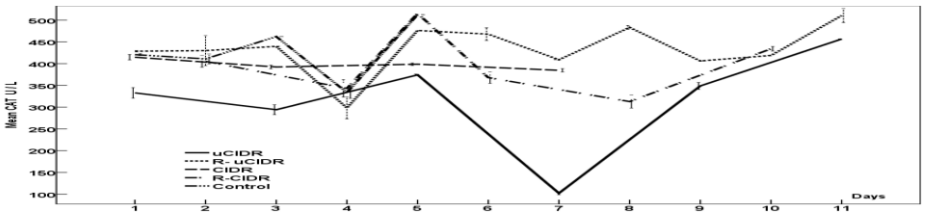

Fig. 11. Mean catalase (CAT U/L) in the camels treated with uCIDR, R- uCIDR, CIDR, RCIDR, and control with error bars

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التغيرات في درجة حرارة المهبل و الاجهاد التأكسدي و مضادات الاكسدة و الهرمونات الاسترويدية للابل في استجابة لهرمون البروجيستيرون الخارجي اثناء درجات الحرارة المنخفضة

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الملخص

لتحديد درجة حرارة المهبل و المؤكسدات –مضادات الاكسدة و هرمونات المبيض اثناء استخدام السيدر المتعدد في الجمال وحيدة السنام تم تقسيم عشة من النوق لمجموعتين متساويتين و تم ادخال سيدر سبق استعمالها مرة واحدة من قبل ملتصق بها جهاز رصد درجة حرارة الجسم كل ساعة لمدة 11 يوم و تم ادخال سيدر جديدة بها 1.35مجم بروجيستيرون لمدة 8 ايام و تم اخراج السيدر لمدة 5 ايامع اعادة ادخالهم بعد تنظيفهم لمدة 11 يوم و تم تجميع عينات دم لقياس هرمون الاستروجين و البروجيستيرون و قدرة مضادات الاكسدة الكلية و المالون ثنائي الالدهيد اكسيد النيتريك و الجلوتاسيون المختزل و الكتاليز. و اظهرت النتائج زيادة معنوية في درجة حرارة المهبل بعد ادخال السيدر الجديدة مرة ثانية. كما انخفض مستوى هرمون البروجيستيرون و الجلوتاسيون المختزل بينما ذادت مستويات الاستروجين و المالون ثنائي الالدهيد لااعلى مستوى في الفترة التي تم نزع السيدر خلالها. كما ان فترة ادال السيدر و اعادة ادخالها تميزت بزيادة في درجات حرارة المهبل و البروجيستيرون و الاستروجين و انفاض كل من المالون ثنائي الالدهيد و اكسيد النيتريك مقارنة بالفترة التي تم ادخال السيدر سابقة الاستعمال. و اظهر التحليل الاحصائي للتباين الخطي ان درجة حرارة المهبل تأثرت بنوع السيدر (المعاملة) و ايام ادخالها و ساعة اليوم و المعاملة –اليوم و المعاملة – الساعة. كما ارتبطت درجة حرارة المهبل بدرجة حرارة الجو اثناء ادخال السيدر سابقة الاستعمال و اعادة ادخالها و السيدر الجديدة و اعادة ادخالها و نستنتج من هذه الدراسة ان درجة حرارة الجو و ايام وجود السيدر بالمهبل و الساعة من اليوم و نوع السيدر اثر على درجة حرارة المهبل كما تاثرت هرمونات المبيض و المؤكسدات- مضادات الاكسدة حسب نوع السيدر و فترات ادخالها و فترات ما بعد نزعها.

الكلمات الدالة: درجة حرارة الجسم ، هرمونات المبيض ، المؤكسدات و مضادات الاكسدة ، الجمال وحيدة السنام.