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Research Article

Evaluation of Infrared Thermography Findings in Pseudopregnant Rabbit

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ABSTRACT

In the present study, the diagnostic efficiency of infrared thermography (IRT) implementation in pseudopregnant rabbits, the change of these images in the pseudopregnancy process, and its relationship with serum progesterone (P4) levels were examined. Fourteen healthy female rabbits of New Zealand breed were divided into two study groups. Pseudopregnancy induction was performed in the first study group with GnRH injection (0.2 ml Buserelin acetate, intramuscularly) on the 0th day of the study (Group 1, n=7). The second group (Group 2, n=7) was given placebo (0.2 ml 0.9% NaCl, intramuscularly). Rectal temperature was taken from all rabbits on days 0-5-10 and 15; eye, nasal tip, and vulvar IRT were applied; and serum P4 values were measured by Enzyme-linked immunosorbent assay (ELISA) method. In the pseudopregnant group, the temperatures of the eyes, nose, and vulva were higher on the 10th day ($P<0.05$). While time-dependent eye, nose, vulva, and rectal temperature changes were observed in the pseudopregnant group ($P<0.05$), it was not observed in the control group ($P>0.05$). The increase in rectal temperature was only on the 15th day in the pseudopregnant group ($P<0.001$). Serum P4 value was high on day 0 in Group 1 ($P<0.05$), but no change was observed in both groups over time ($P>0.05$). A significant correlation was observed between the serum P4 value and rectal temperature and the groups ($r=-0.32$, $P<0.05$; $r=0.35$, $P<0.01$). A significant difference was determined between nasal temperature and rectal temperature ($r=0.28$, $P<0.05$). As a result, IRT implementation in rabbits; It was determined that it is useful in noninvasive and rapid monitoring of body temperature and can determine the temperature increase in the eyes, nose and vulva on the 10th day of pseudopregnancy.

Keywords: Progesterone, pseudopregnancy, rabbit, thermography

Yalancı Gebe Tavşanlarda İnfrared Termografi Bulgularının Değerlendirilmesi

ÖZET

Sunulan çalışmada yalancı gebe tavşanlarda infrared termografi (IRT) uygulamalarının tanısal etkinliği, bu görüntülerin yalancı gebelik sürecindeki değişimi ve serum progesteron (P4) düzeyi ile olan ilişkisi incelendi. Yeni Zelanda ırkı, 14 adet sağlıklı dişi tavşan iki adet çalışma grubuna ayrıldı. Birinci çalışma grubuna çalışmanın 0. gününde (Grup 1, n=7) GnRH enjeksiyonu (0,2 ml Buserelin asetat, im) ile yalancı gebelik indüksiyonu yapıldı. İkinci gruba ise (Grup 2, n=7) plasebo (0,2 ml %0,9 NaCl, im) verildi. Tüm tavşanlardan 0-5-10 ve 15 günlerde rektal sıcaklık alındı;göz, burun ucu ve vulvar termografi uygulandı; Enzyme-linked immunosorbent assay (ELISA) yöntemi ile serum P4 değerleri ölçüldü. Yalancı gebe grubunda göz, burun ve vulvar bölge sıcaklığı 10. günde daha yüksekti ($P<0,05$). Zamana bağlı göz, burun, vulva ve rektal sıcaklık değişimi yalancı gebe grubunda görülürken ($P<0,05$) kontrol grubunda gözlenmedi ($P>0,05$). Rektal sıcaklık artışı ise yalancı gebe grubunda sadece 15. günde belirlendi ($P<0,001$). Grup 1'de serum P4 değeri 0. günde yüksekti ($P<0,05$) ancak her iki grupta zaman içinde değişim görülmedi ($P>0,05$). Serum P4 değeri ve rektal sıcaklık ile gruplar arasında anlamlı bir ilişki gözlendi ($r=-0,32$, $P<0,05$; $r=0,35$, $P<0,01$). Burun sıcaklığı ile rektal sıcaklık arasında anlamlı bir farklılık belirlendi ($r=0,28$, $P<0,05$). Sonuç olarak tavşanlarda IRT uygulamaları noninvaziv ve hızlı şekilde vücut sıcaklığının izleminde yararlı olup, yalancı gebeliğin 10. gününde göz, burun ve vulvadaki sıcaklık artışını belirleyebileceği belirlendi.

Anahtar kelimeler: Progesteron, tavşan, termografi, yalancı gebelik

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Introduction

Rabbits have been bred for meat production and experimental animals in scientific research for a long time, but they have been frequently seen as pet animals in veterinary clinics. Although their reproductive physiology depends on day length, environmental temperature, nutrition, and social factors (Bell, 1999), the rabbits bred in the laboratory environment reflect the symptoms more stable. By the neurohumoral pathway due to mating, jumping movements, or carrying stress, ovulation stimulates (Dal Bosco et al., 2011; Donnelly, 2013; Carter et al., 2016). Within 1-2 hours after stimulation, serum LH level reaches its peak level and reaches 8-10 ovulation takes place within hours. However, spontaneous ovulation can also be seen under good care and feeding conditions (Bekyürek, 2010). In each ovulation, an average of 6-10 oocytes reaches the oviduct (McNitt et al., 1996). In the condition of non-fertilization, pseudopregnancy lasting 15-18 days may be encountered (McNitt et al., 1996; Donnelly, 2013). There is no difference in serum progesterone levels until luteal regression begins in pregnant and pseudopregnant rabbits (Ptaszynska, 2001). Corpora lutea that occurs in pseudopregnancy persists for 10-12 days and causes external deposits such as pregnancy behaviors and nest preparation for birth with the effect of estrogen, P4 and prolactin hormones. Observed behavioral changes also disappear with luteal regression (Donnelly, 2013).

Infrared thermography (IRT) is a real-time, non-invasive, and side-effect-free auxiliary diagnostic tool (Huynh, 2019). In addition, the compatibility of eye temperature with rectal and vaginal measurements was also investigated (Sykes et al., 2006; Willard et al., 2006). Its non-invasive usage has made it more accessible to detect ovulation and pregnancy in Asian elephants, rhinos, and giraffes (Durrant et al., 2006; Hilsberg-Merz, 2008). Moreover, the distinction between pregnant and pseudopregnant pandas could be performed (Durrant et al., 2006). It is also effective in detecting estrus and determining the appropriate insemination time with IRT in the farm animals, dogs, and chinchillas (Osawa et al., 2004; Sykes et al., 2006; Talukder et al., 2015; Olğaç et al., 2017; De Freitas et al., 2018; Façanha et al., 2018).

Although many IRT results belong to different species in literature, limited data are seen about the rabbits. Therefore, this study was aimed to evaluate the diagnostic efficiency of IRT taken from different body parts of rabbits undergoing pseudopregnancy.

Materials and Methods

In the study, 14 New Zealand breed female rabbits, 1-3 years old, housed in an individual cage setup and known to be healthy in general, and gynecological examinations were used. All animals were housed in individual wire mesh cages under controlled temperature (18-24°C) and light conditions during the study. They were fed ad-libitum water and pellet calf rearer feed. Animals were randomly divided into two study groups (Group 1 and 2). During the study, general care and all clinical practices were carried out according to the regulations of Aydın Adnan Menderes University Experimental Animals Application and Research Center and in line with the relevant ethics committee decision (64583101/2019/007).

In the study, rabbits with pseudopregnancy induction were included in Group 1 (n=7), while rabbits without any induction were considered Group 2 (n=7). Accordingly, on the 0^{th} day of the study, 0.8 mcg buserelin acetate (Buserin, Alke®, Turkey) was injected intramuscularly to stimulate pseudopregnancy in Group 1. In Group 2, intramuscularly placebo injection (0.2 ml of 0.9% NaCl) was used on the same day. All IRT imaging from different body points, measuring rectal temperature with degrees, and collecting venous blood samples are included in the 0-5-10-15 days of the study. The procedures were repeated 70 times for all animals on the respective days, 280 times.

Taking IRT Samples

A mobile thermal camera (Flir E6, Flir Systems AB®-Sweden) system recorded thermal images. Attention was paid to keeping the ambient conditions (temperature change, airflow, humidity, etc.) for the images' standardization. During the measurements, uniformity was ensured in the images under the environment's temperature, in which the device was single and center oriented. During the IRT, care was taken to ensure an average of 1 meter between the camera and the rabbit. During thermal imaging, the rabbits were taken from their cages not to cause stress, and they were rested on the table on which the shooting would take place for about 10 minutes. At the end of the waiting period, thermal images of the eye, nose tip, and vulvar region were taken from the rabbits in both study groups. In eyeshots, images were taken from the lateral line, regardless of the direction, while the animals were not stressed. In nasal tip extractions, the nasal tip was fully visualized (upright/slightly oblique), taking into account the stress conditions. In the vulvar region shots, the rabbits were taken in restraint by lying on their backs not to cause stress. Finally, the tail tip was directed ventrally, the vulva and perineum region were visualized, and the shooting was taken. All measurements were completed in approximately 15 minutes for each rabbit.

Serum Progesterone Measurements

Venous blood samples were taken to measure serum progesterone value from the ear vein (Vena auricularis) after the images were taken on the days (0-5-10-15. days) when images were taken with a thermal infrared camera. The serum extracted from the samples were stored at -20 degrees until the progesterone measurement was made. At the end of the study, serum P4 levels were determined from the serum samples by the Enzyme Linked Immunosorbent-Assay method (ELISA) using the commercial Rabbit (PROG) ELISA test kit (Shanghai Sunred Biological Technology) was used.

Body temperature values measured on IRT images were compared between Group 1 and Group 2, and the curves of change over time for each group were extracted. The obtained data were evaluated using the SPSS 22.0 (SPSS Inc. Chicago, IL, USA) package program. Shapiro-Wilk test was used to analyze whether the data met normal distribution assumptions. Pairwise comparisons were evaluated with the Independent Groups t-test. Repeated Measures ANOVA was used to detect time-dependent changes within the group. Pearson Correlation Analysis was used to determine the relationship between variables. The tables, graphics, and results are given as mean±standard error of mean (SEM). Statistical significance was accepted as P < 0.05.

Results

After the intramuscular injection of 0.2 ml of GnRH, no complications were encountered to induce pseudopregnancy in rabbits. Imaging was performed smoothly and quickly thanks to the elimination of stress factors. It was observed that around 12-15 days, pseudopregnant rabbits pulled their underbelly wool to a corner of the cage as if they were going to give birth, and exhibited the behavior of preparing for birth.

At the end of the study, the average temperature values are taken from the eye, nose, and vulva region with the help of a thermal camera are presented in Table 1, Table 2, Table 3, Figure 1, Figure 2, Figure 3. Accordingly, on the 10th day of pseuopregnancy, a significant temperature increase was observed in the IRT images taken from the eye, nose, and vulva region (P <0.05), especially from the eye (P <0.01). At the end of the study, the rectal temperatures by digital thermometer are presented in Table 4 and Figure 4. Accordingly, a significant difference was detected between the body temperatures taken on the 15th day (P <0.05). The serum P4 levels of the rabbits in the pseudopregnant group were 135.57±19.05 pmol/L on day 0, 122.43±14.95 pmol/L on Day 5, 125.29±9.61 pmol/L on Day 10, and was measured as 120.00±10.11 pmol/L on the 15th day (Table 5, Figure 5). There was no statistical difference in serum P4 concentrations between

Table 1. The mean eye temperatures of the study groups (°C).

Day	n	Pseudopregnant	Control	P
		$\bar{X} \pm S_{\bar{x}}$	$\bar{X} \pm S_{\bar{x}}$	
0	7	34.34±0.29^{ab}	33.95±0.30	0.382
5	7	34.22±0.32^{ab}	34.51±0.26	0.509
10	7	35.12±0.14^{a}	34.18±0.23	**0.006**
15	7	33.87±0.22^{b}	34.05±0.12	0.487
P_{ANOVA}		**0.016**	0.407	

^{a,b}: Different letters at the same column show statistical differences
P<0.05: Significant difference between study groups

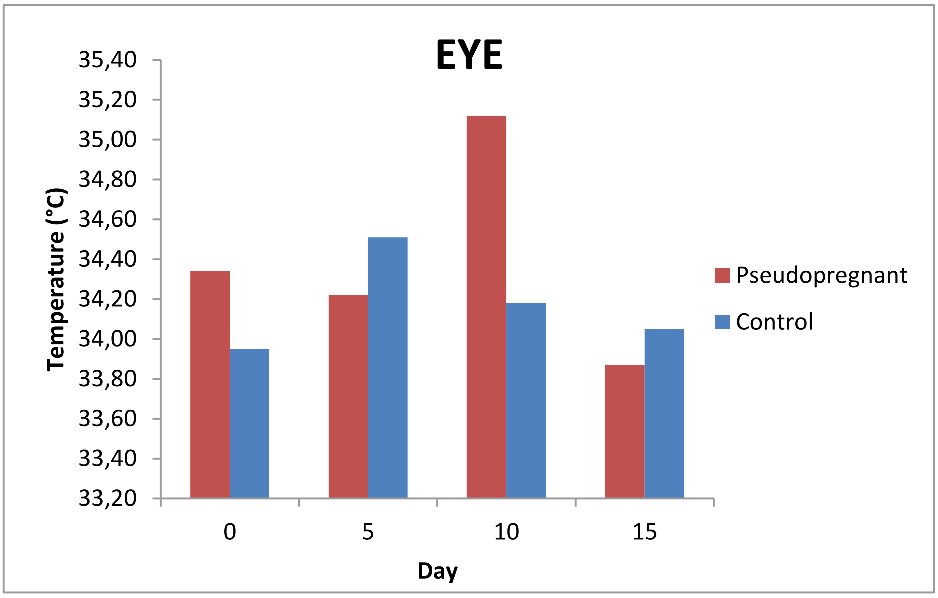

Figure 1. The mean eye temperatures of study groups (°C).

Table 2. The mean nasal temperatures of study groups (°C).

Days	n	Pseudopregnant	Control	P
		$\bar{X} \pm S_{\bar{x}}$	$\bar{X} \pm S_{\bar{x}}$	
0	7	30.77±0.68[ab]	30.84±0.61	0.940
5	7	28.84±0.96[b]	28.88±0.96	0.975
10	7	31.80±0.37[a]	30.20±0.59	**0.041**
15	7	30.35±0.67[ab]	30.12±0.63	0.809
P_{ANOVA}		**0.050**	0.299	

[a,b]: Different letters at the same column show statistical differences
P<0.05: Significant difference between study groups

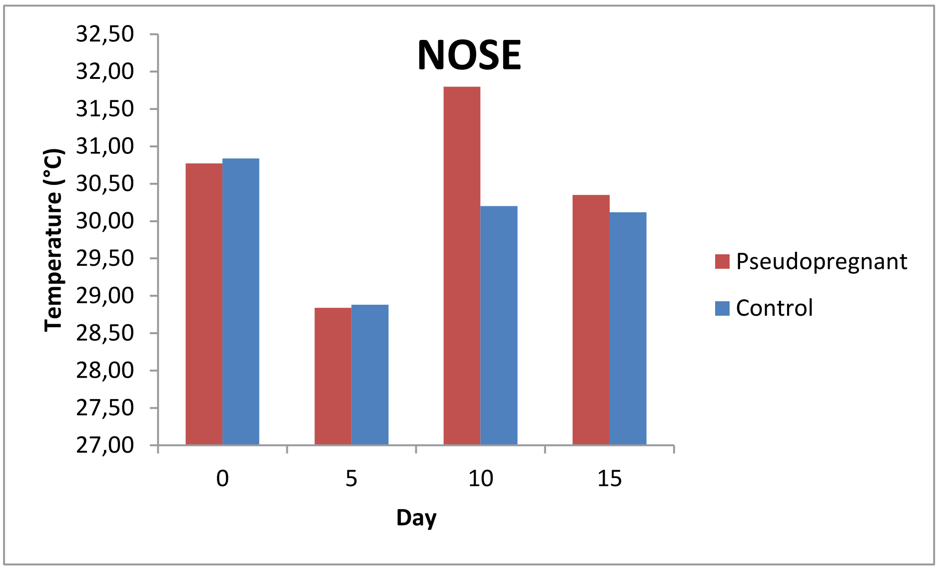

Figure 2. The mean nasal temperatures of study groups (°C).

Table 3. The mean vulvar temperatures of study groups (°C).

Day	n	Pseudopregnant	Control	P
		$\bar{X} \pm S_{\bar{x}}$	$\bar{X} \pm S_{\bar{x}}$	
0	7	30.82±1.16[b]	32.38±0.54	0.249
5	7	32.61±0.68[ab]	32.11±1.06	0.699
10	7	34.58±0.38[a]	31.41±1.01	**0.012**
15	7	32.17±1.02[ab]	32.61±0.52	0.707
P_{ANOVA}		**0.041**	0.756	

[a,b]: Different letters at the same column show statistical differences
P<0.05: Significant difference between study groups

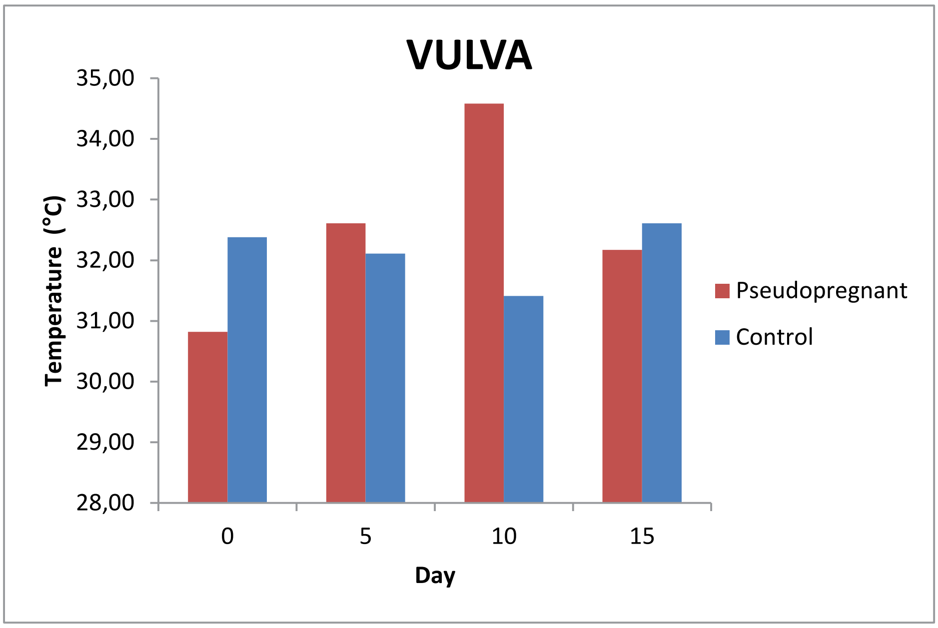

Figure 3. The mean vulvar temperatures of study groups (°C).

Table 4. The mean rectal temperatures of study groups (°C).

Day	n	Pseudopregnant	Control	P
		$\bar{X} \pm S_{\bar{x}}$	$\bar{X} \pm S_{\bar{x}}$	
0	7	38.35±0.30[b]	38.14±0.11	0.530
5	7	37.88±0.24[b]	38.22±0.19	0.287
10	7	38.80±0.30[ab]	38.20±0.20	0.125
15	7	39.50±0.15[a]	37.62±0.37	**0.001**
P_{ANOVA}		**0.002**	0.276	

[a,b]: Different letters at the same column show statistical differences
P<0.05: Significant difference between study groups

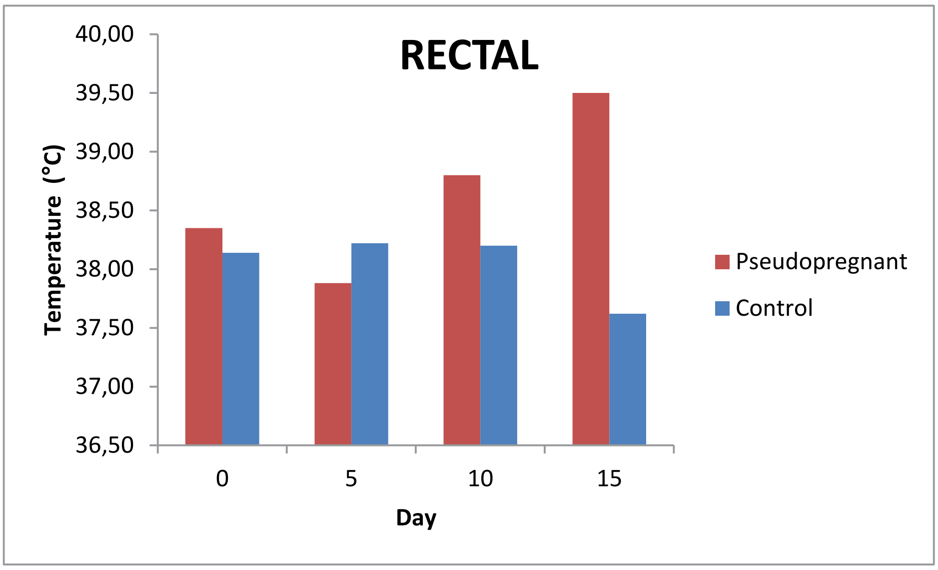

Figure 4. The mean rectal temperatures of study groups (°C).

Table 5. The mean progesterone values of study groups (pmol/L).

Day	n	Pseudopregnant $\bar{X} \pm S_{\bar{x}}$	Control $\bar{X} \pm S_{\bar{x}}$	P
0	7	135.57±19.05	83.57±6.02	**0.023**
5	7	122.43±14.95	105.71±3.84	0.300
10	7	125.29±9.61	109.71±15.77	0.416
15	7	120.00±10.11	116.29±12.94	0.825
P_{ANOVA}		0.867	0.189	

[a,b]: Different letters at the same column show statistical differences
P<0.05: Significant difference between study groups

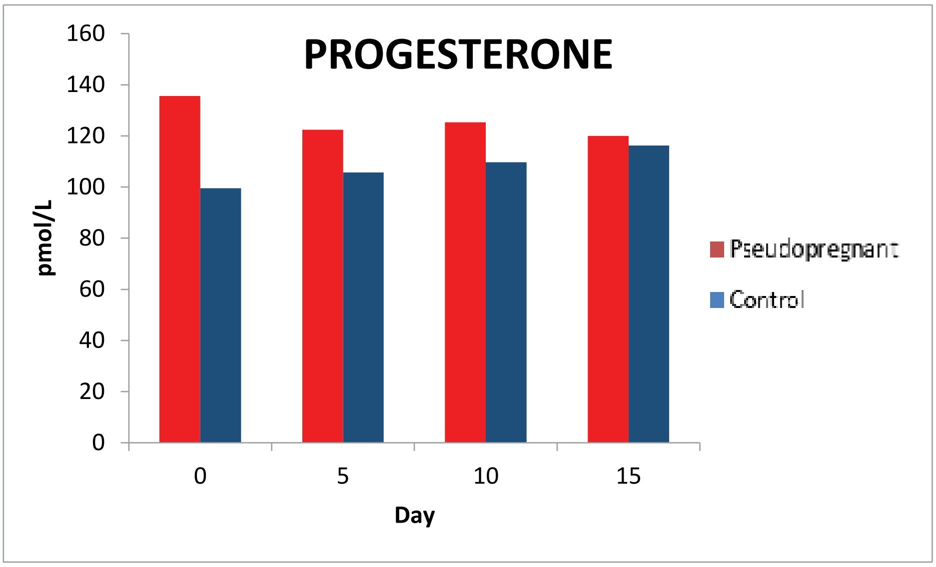

Figure 5. The mean progesterone values of pseudopregnant and control groups (pmol/L)

days (P>0.05). The serum P4 levels of the rabbits in the control group were 83.57±6.02 on day 0, 105.71±3.84 on Day 5, 109.71±15.77 on Day 10, and 116.29±12.94 on Day 15. In addition, there was no statistical difference in serum P4 levels between days in the control group (P >0.05). On the 0th day of the study, serum progesterone value was significantly higher in the pseudopregnant group (P<0.05).

There is a low (+) positive correlation between progesterone level and day (0.09). A low negative relationship (-0.08, -0.16, -0.03, -0.04, respectively) was observed between progesterone and eye, nose, vulva, and rectal temperature. There was a significant (P<0.05) moderate negative (-0.32) relationship between progesterone and the group. There was a significant (P<0.01) positive moderate (0.35) relationship between the rectal temperature and the group. There was a low positive correlation (0.18, 0.04, 0.08, respectively) between rectal temperature and day, eye, and vulva temperature. A positive and moderate (0.28) relationship was found between the rectal and nasal temperature (P<0.05). There was a low positive correlation (0.09, 0.14, 0.23, 0.13, respectively) between the measured from the vulva with a thermal camera and the group, day, eye, and nose temperatures. There was a low positive correlation between nose temperature and group, day and eye temperatures (0.10, 0.02, 0.10), respectively. Considering the eye temperature, the group is positively low (0.14); there was a low negative correlation with the day (-0.04) (Table 6).

Discussion

The main purpose of this study is to use non-invasive and easy-to-apply IRT images to diagnose pseudopregnancy in rabbits. Although the rate of pseudopregnancy is expected to be relatively low in female rabbits kept in individual cages and raised away from the presence of males, spontaneous ovulation and pseudopregnancy can be seen for different reasons (Bekyürek, 2010; Donnelly, 2013; Carter et al., 2016). Despite the use of drugs compatible with the literature for induction (0.2 ml Buserelin, intramuscularly); it is noteworthy

Table 6. Correlations between temperatures and serum progesterone values in all rabbits

	Group	Day	Eye	Nose	Vulva	Rectal	Progesterone
Group	-	-	-	-	-	-	-
Day	0.00	-	-	-	-	-	-
Eye	0.14	-0.04		-	-	-	-
Nose	0.10	0.02	0.10		-	-	-
Vulva	0.09	0.14	0.23	0.13	-	-	-
Rectal	**0.35****	0.18	0.04	**0.28***	0.08	-	-
Progesterone	**-0.32***	0.09	-0.08	-0.16	-0.03	-0.04	-

*P<0.05; **P<0.01: Showing significant correlations

that the difference between the P4 values of the two study groups was statistically seen only on the 0th day, and the presence of a numerical increase in the pseudopregnant group on the other days, which did not make a difference. Although fur plucking and nest-building behaviors were observed during the study, this suggests the existence of possible individuals with spontaneous ovulation on different days. It has been reported that 23% of pseudopregnancy are seen even in rabbit populations in individual cages (Carter et al., 2016). Pseudopregnancy could be observed in 23% of rabbits housed in groups and in hierarchical order (Rommers et al., 2006). Rabbits that remain passive by leaving the group and switching to a single cage system may also have pseudopregnancy (Carter et al., 2016). Researchers have provided the induction of ovulation by mechanical stimulation of the vagina, mating with a vasectomized male or injections of GnRH, LH, and hCG to investigate ovulation and corpus luteum (CL) physiology (Zavos et al., 1998). The hormone measurements made to determine the success of the induction process were made with ELISA at the end of the study, instead of using the rapid P4 kit during the study, caused this situation to be noticed later. Again, this situation can be investigated with more animals in the future. In future studies, it will be helpful to confirm that all material is not in the luteal phase using perineal inspection, vaginal cytology, and rapid hormone measurements. However, the possibility that the physician can stimulate ovulation during manipulations during this clinical evaluation should also be considered. Pregnancy behaviors can be observed in pseudopregnant rabbits under the influence of estrogen, progesterone, and prolactin hormones (Donnelly, 2013). Among all these physiological changes, it is expected that the diagnostic efficiency will be limited since the temperature changes due to pseudopregnancy will occur in a narrower temperature range. The diagnosis of pseudopregnancy in the use of IRT in the reproductive examinations of domestic and wild animals is a new application, and a similar application has been investigated using abdominal scans in pandas and dogs (Durrant et al. 2004; 2006). At the end of this research, it was seen that especially the shots in pregnant pandas were more sensitive than dogs.

In thermographic studies in rabbits, it is known that the most suitable attraction areas are the eyes and ears (Redaelli et al., 2014). However, the study aimed to investigate alternative regions, and the nasal tip and vulvar region were added to this. Additionally, hiding abdomen postures in the cage also arose the need for these measurements to be focused on the face, and the measurements were carried out considering these conditions in the present study. Measurements of vulvar temperature changes can be challenging, mainly since they cover the perineum with their tails. Unlike facial imaging, it can be thought that there are manipulations that can trigger stress and affect the results, taking the animals out, tilting them, and pulling the tail to make the region visible. In order to prevent such negativities, it will be effective to monitor the animals by the same person and handle them duly without causing stress.

When this study is evaluated within the data obtained with the number of animals available, the most sensitive period for the eye, nose and vulvar region thermal shots in terms of diagnostics is the 10th day. In pseudopregnancy, the CL is functional for 16-18 days, and thus the CL of pseudopregnant rabbits begins to regress due to increased PGF-2α luteal response (Dugré et al., 1989). Considering that the CL formed in pseudopregnancy persists for 10-12 days (Donnelly, 2013) and that increased P4 activates the catabolic metabolism and raises the basal body temperature (Regidor, 2014), this situation is compatible with the literature. It was determined that the significant temperature increase observed on the 10th day in the pseudopregnant group was mainly in the eyes, followed by the vulva and nasal region. A mature CL will respond to luteolytic agents in the relevant period. The high correlation between eye temperature and the rectal temperature has been detected in different species, and it is known to facilitate remote thermal evaluations (Redaelli et al., 2014), additionally, to measure stress response (Ludwig et al., 2007).

An increase of more than 2.33 ^{0}C was found in thermal images made due to hyperemia in the vulva in Chinchillas during the estrus period (Polit et al., 2018). Similarly, IRT can determine the vulvar temperature increase during estrus in rabbits and other livestock (Osawa et al., 2004; De Freitas et al., 2018; Façanha et al., 2018). However, it was also stated that perivulvar temperature measurements were not successful in pregnancy diagnosis in cows (Radigonda et al., 2017). Vulvar hyperemia is seen in estrus in rabbits (Bekyürek, 2010); however, it is not among the pseudopregnancy findings. Despite this, in the presented thesis study, a temperature increase of 3.17 ^{0}C was detected on the 10th day of pseudopregnancy compared to the control group. Although there is no hyperemia or cyanosis in the vulva, this temperature increase is expected to be in parallel with the measurements in the eyes and nose, but the limited literature on the subject creates difficulties in interpretation. Due to the excessive hairiness of the perineal region in vulvar measurements, the application is not practical, and this situation is similar to previous studies (Polit et al., 2018). When measuring in future studies, it should be kept in mind that the hairs in the area, the fecal residue found on the rectum's outer surface and cannot be cleaned entirely, can create artifacts and prevent temperature measurement.

When the rectal temperature measurements taken in the study were examined, it was seen that they did not show similar curves with the IRT results. A significant temperature increase in the pseudopregnant group was observed on the 15th day. As shown in the graph in Figure 4, the temperature increase on the 10th and 15th days in the pseudopregnant group was evident only on the 15th day ($P<0.001$). It is noteworthy that temperature changes were detected in two different methods on different days. Although rectal temperature measurement is an effortless application, the reason for this incompatibility may be that the temperature is in contact with feces in the rectum and not enough in contact with the mucosa. In addition, different from far-IRT, it was thought that the stress caused by manipulations in rectal degree measurement might have affected the results. Another problem is the risk of ovulation stimulation during rectal temperature measurements. Another possible reason for the control group's P4 values to be close to the pseudopregnant group during the study is the uncontrolled stimulation during these measurements.

By decreasing of the P4 level after the Days 10-12, as seen in Table 6, the eye, nose, and rectal temperature continue to increase with the decrease in days showing a negative correlation between progesterone and eye, nose, and rectal temperature. The negative correlation between the P4 level and the groups is a finding parallel to the fact that the P4 level in the pseudopregnant group increased until the 10th day and then decreased. Regarding P4 measurements made in similar studies, it has been reported in the literature that serum progesterone levels in rabbits are high during pseudopregnancy, and the upper limit values measured are around 30 ng/ml. However, with the regression of the corpus luteum, there is a rapid decrease in progesterone, and it has been stated that it falls below 0.6 ng/ml (Orstead et al., 1988; Maranesi et al., 2018). Therefore, generally, it is seen that the P4 measurement unit is ng/ml in literature studies (Maranesi et al., 2018). However, due to the kits used in the presented study, calculations were made at the pmol/L level. Since this unit is mentioned in the previous literature (Fragalà et al., 2015), the current measurement unit is ng/ml (Orstead et al., 1988; Maranesi et al., 2018) can be challenging to discuss the findings.

Conclusion

As a result, temperature rise can be easily determined by IRT taken from different body parts on the 10th day of pseudopregnancy rabbits. This condition should be considered a preliminary diagnosis finding to distinguish it from other individuals and should be evaluated with other clinical / laboratory measurements. Depending on the species, the findings should be carefully examined against the possibility of pseudopregnancy even in separate breedings with ovulation stimulation during handlings. Although IRT measurements are a helpful method like a rabbit, they are open to new regulations and improvements. Therefore, investigating optimum conditions and making preliminary preparations will benefit more precise and practical measurements.

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Conflict of interest

The authors declare that they have no conflict of interest in this study.

References

Bekyürek, T. (2010). Laboratuvar Hayvanlarında Üreme ve Sorunları. In: Alaçam E (ed), Evcil Hayvanlarda Doğum ve İnfertilite (pp. 355-381). Ankara: Medisan.

Bell, D.J. (1999). The European wild rabbit. In: Poole T (ed). The UFAW Handbook on the Care and Management of Laboratory Animals 7th edition (pp. 389-394). Oxford: Blackwell Publishing.

Carter, C.L., Adams, J.K., Czarra, J.A., & Coan, P.N. (2016). An incidence of pseudopregnancy associated with the social enrichment of rabbits (Oryctolagus cuniculi). Journal of the American Association for Laboratory Animal Science, 55(1), 98-99.

Dal Bosco, A., Rebollar, P.G., Boiti, C., Zerani, M., & Castellini, C. (2011). Ovulation induction in rabbit does: current knowledge and perspectives. Animal Reproduction Science, 129(3-4), 106-117. https://doi.org/10.1016/j.anireprosci.2011.11.007

De Freitas, A.C.B., Vega, W.H.O., Quirino, C.R., Junior, A.B., David, C.M.G., Geraldo, A. T., & Dias, A.J.B. (2018). Surface temperature of ewes during estrous cycle measured by infrared thermography. Theriogenology, 119, 245-251.

https://doi.org/10.1016/j.theriogenology.2018.07.015

Donnelly, T.M. (2013). Pseudopregnancy. J. Mayer (Ed.): Clinical Veterinary Advisor Birds and Exotic Pets (pp. 411-412). Saint Louis: W.B. Saunders.

Dugré, F.J., Lambert, R.D., Bélanger, A. & Fortier, M.A. (1989). Relationship between steroid levels in peripheral serum and uterine tissue during pseudopregnancy in rabbit. Theriogenology, 31(2), 353-360. https://doi.org/10.1016/0093-691X(89)90541-4

Durrant B.S., Schwede T., & Spady T.J. (2004). The potential utility of thermography to differentiate pregnancy and pseudopregnancy in bears. Proceedings, 15th International Conference on Bear Research and Management. San Diego, CA.

Durrant, B.S., Ravida, N., Spady, T., & Cheng, A. (2006). New technologies for the study of carnivore reproduction. Theriogenology, 66(6-7), 1729-1736. https://doi.org/10.1016/j.theriogenology.2006.02.046

Façanha, D.A.E., Peixoto, G.C.X., Ferreira, J.B., de Souza, J.E.R., Paiva, R.D.M., & Ricarte, A.R.F. (2018). Detecting estrus in Canindé goats by two infrared thermography methods. Acta Veterinaria Brasilica, 12(2). https://doi.org/10.21708/avb.2018.12.2.7243

Fragalà, S., Medica, P., Grande, F., Vazzana, I., & Fazio, E. (2015). Evaluation of seasonal changes of serum and plasma estradiol-17β, progesterone and testosterone in dolphins (Tursiops truncatus) by chemiluminescence. Veterinary World, 8(8), 977. doi: 10.14202/vetworld.2015.977-982

Hilsberg-Merz, S. (2008). Infrared thermography in zoo and wild animals. In Fowler, M.E. & Miller, R.E. (eds) Zoo and Wild Animal Medicine. Current Therapy, 6th edn. Philadelphia: Saunders.

Huynh, M. (2019). Smartphone-based device in exotic pet medicine. Veterinary Clinics: Exotic Animal Practice, 22(3), 349-366. https://doi.org/10.1016/j.cvex.2019.05.001

Ludwig, N., Gargano, M., Luzi, F., Carenzi, C., & Verga, M. (2007). Applicability of infrared thermography as a noninvasive measurements of stress in rabbit. World Rabbit Science, 15(4). https://doi.org/10.4995/wrs.2007.588

Maranesi, M., Petrucci, L., Leonardi, L., Piro, F., Rebollar, P.G., Millán, P., & Zerani, M. (2018). New insights on a NGF-mediated pathway to induce ovulation in rabbits (Oryctolagus cuniculus). Biology of Reproduction, 98(5), 634-643. https://doi.org/10.1093/biolre/ioy041

McNitt, J.I., Lukefahr, S.D., Cheeke, P.R., Patton, N.M. (2013). Rabbit Reproduction, 9th ed In: Rabbit Production (pp. 144-159). USA.

Orstead, M. K., Hess, D.L., & Spies, H.G. (1988). Pulsatile patterns of gonadotropins and ovarian steroids during estrus and pseudopregnancy in the rabbit. Biology of Reproduction, 38(4), 733-743. https://doi.org/10.1095/biolreprod38.4.733

Olğaç, K.T., Akçay, E., Çil, B., Uçar, B.M., & Daşkın, A. (2017). The use of infrared thermography to detect the stages of estrus cycle and ovulation time in Anatolian Shepherd dogs. Journal of Animal Science and Technology, 59(1), 1-6. https://doi.org/10.1186/s40781-017-0146-4

Osawa T., Tanaka M., Morimatsu M., Hashizume K., & Syuto B. (2004). Use of infrared thermography to detect the change in the body surface temperature with estrus in the cow. Proceedings from the 2004 SFT/ACT Annual Conference & Symposium. Kentucky-USA.

Polit, M., Rząsa, A., Rafajłowicz, W., & Niżański, W. (2018). Infrared technology for estrous detection in Chinchilla lanigera. Animal Reproduction Science, 197, 81-86. https://doi.org/10.1016/j.anireprosci.2018.08.012

Ptaszynska, M. (2001). Reproduction in the Rabbit. In: Ptaszynska (Ed). Compendium of Animal Reproduction (pp. 243-256). International Intervet Publisher.

Radigonda, V.L., Pereira, G.R., da Cruz Favaro, P., Júnior, F.A.B., Borges, M.H.F., Galdioli, V.H.G., & Júnior, C. K. (2017). Infrared thermography relationship between the temperature of the vulvar skin, ovarian activity, and pregnancy rates in Braford cows. Tropical Animal Health and Production, 49(8), 1787-1791.

Redaelli, V., Ludwig, N., Cosat, L.N., Crosta, L., Riva, J., & Luzi, F. (2014). Potential application of thermography (IRT) in animal production ad for animal welfare. A case report of working dogs. Annali dell'Istituto Superiore di Sanita, 50(2), 147-152.

Regidor, P.A. (2014). Progesterone in peri-and postmenopause: a review. Geburtshilfe und Frauenheilkunde, 74(11), 995. doi: 10.1055/s-0034-1383297

Rommers, J.M., Boiti, C., De Jong, I., & Brecchia, G. (2006). Performance and behaviour of rabbit does in a group-housing system with natural mating or artificial insemination. Reproduction Nutrition Development, 46(6), 677-687. https://doi.org/10.1051/rnd:2006038

Sykes, D., Chromiak, A., Couvillion, S., Gerard, P., Crenshaw, M., Willard, S., & Ryan, P. (2006). Estrus detection in gilts using digital infrared thermal imaging. Journal of Animal Science, 84, 1.

Talukder, S., Thomson, P.C., Kerrisk, K.L., Clark, C.E.F., & Celi, P. (2015). Evaluation of infrared thermography body temperature and collar-mounted accelerometer and acoustic technology for predicting time of ovulation of cows in a pasture-based system. Theriogenology, 83(4), 739-748. https://doi.org/10.1016/j.theriogenology.2014.11.005

Willard, S.T., Vinson, M.C., & Godfrey, R.W. (2006). Digital infrared thermal imaging of the eye as correlated to rectal and vaginal temperature measurements in the ewe. Journal of Animal Science, 84, 434-434.

Zavos, P.M., Correa, J.R., Panayota, N,. Zarmakoupis-Zavos, M.D. (1998). Assessment of a tablet drug delivery system incorporating nonoxynol-9 coprecipitated with polyvinylpyrrolidone in preventing the onset of pregnancy in rabbits. Fertility and Sterility, 69, 4,768-773. https://doi.org/10.1016/S0015-0282(98)00004-1