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Effect of rectal temperature on efficiency of artificial insemination and embryo transfer technique in dairy cattle during hot season

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Abstract: This study aimed to evaluate the effects of rectal temperature (RT) as well as artificial insemination (AI) technique on pregnancy rates in Holstein cows and artificial insemination or embryo transfer (ET) techniques in Holstein heifers during summer. The experiments were conducted on five dairy farms in Hungary, where 1 631 data were examined. The RT was measured immediately before reproductive techniques using a digital thermometer. The effects of animal group and month on RT were analysed. The enhancement of RT until September can be seen in heifers, but the highest RT of cows occurred during July. In line with the enhancement of RT the pregnancy rate decreased in all groups; the lowest (cows) and the highest (heifers) value was observed in July. Moderate to high negative coefficients of correlation were detected between rectal temperature and pregnancy rate for particular groups (r = −0.3 – −0.7). More than 92% of ET, 48% and 16% of inseminated heifers and cows were pregnant up to the category of 39.1 °C of RT, above this it did not change considerably in the inseminated groups. In inseminated animals with RT higher than 39.1 °C significantly higher services per conception could be observed contrary to ET heifers. ET may become a more effective strategy to improve pregnancy success in heifers compared to AI during summer.

Keywords: Holstein; summer period; heat stress; pregnancy rate

The elevation of ambient temperature especially in summer is a critical issue in livestock productivity. Heat stress may be defined as an environmental condition that affects body temperature to increase it above a set-point temperature (Hansen 2009). The heat stress has a great effect on cattle reproductive performance. Since 1990 it has been reported that the pregnancy rate by artificial insemination in dairy cattle dramatically decreased in the summer (De Rensis 2017). The elevation of maternal body temperature is considered a major reason for this effect, which has detrimental impacts on the repro-

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ductive capabilities of dairy cows due to the direct effects on the ovaries, follicular growth, oocyte quality, steroidogenesis, oestrus behaviour, uterine environment, and embryo health (Sakatani 2017). A study conducted by Lopez-Gatius et al. (2004) found that the pregnancy loss rate for cows inseminated in the warm period was 3.7 times higher compared to animals inseminated in the cold season. Ultimately, the conception rates in summer months can decrease 20–30% when compared to winter months (De Rensis and Scaramuzzi 2003).

Bovine embryos become more resistant to heat stress during their development (Ealy et al. 1993) and can be successfully transferred into recipient cows without detected oestrus (Al-Katanani et al. 2002). There is some evidence suggesting that the embryo can develop thermotolerance to the increased environmental temperature, enabling more resistance to future thermal stress episodes (Hansen 2009). When compared to timed artificial insemination, fresh *in vitro* embryos improve pregnancy rates during heat stress conditions (Al-Katanani et al. 2002), especially in high-producing animals that are already at risk of lower conception rates (Demetrio et al. 2007). At the same time Stadnik et al. (2014) confirmed the effect of the season on the yield and quality of bovine *in vivo* recovered embryos, they found that significantly lower embryo yield as well as rate of transferable embryos were found during the summer months.

During the period of high heat load, increases in core body temperature become a function of heat accumulated and dissipated between the animal and environment, therefore body temperature is considered to be a reliable indicator of an animal's thermal status (Lees et al. 2019). Several technologies (reviewed by Godyn et al. 2019) can be used to continuously monitor body temperature in cattle, such as rumen boluses, thermistors implanted in the ear canal or udder, sensors on milking equipment, infrared thermography, rectal thermometers, and vaginal loggers. Rectal temperature is the most common sampling method for collecting body temperature due to the ease of collection, low cost of thermometers, and physiological accuracy to core body temperature and it is a good measure of heat tolerance in animals. Kou et al. (2017) found that rectal temperature closely correlated with the automated measurement of cattle surface temperature and results showed a significant seasonal effect on both measured parameters.

The purpose of this study was to evaluate the relationship between rectal temperature and pregnancy rate during the summer period in dairy cattle to determine the suitability of measuring rectal temperature as an estimation of successful embryo transfer (in heifers) and artificial insemination (in heifers and in cows).

MATERIAL AND METHODS

Location, animals and diet

This study was conducted on five commercial dairy farms in different regions of Hungary. The herds were composed of purebred Holstein dairy cows and heifers. Cows produced over 10 000 kg milk/lactation on average. The cows were kept in a loose housing system, receiving a total mixed ration (TMR) composed of maize silage, haylage, and they were supplemented with concentrate (wheat, maize, barley, rapeseed and sunflower) and minerals. The TMR was balanced to meet or exceed minimum nutritional requirements for dairy cows. Cows were mechanically milked three times per day. The average services per conception for cows (n = 18 559) and for heifers (n = 13 980) on farms varied from 5.3 to 9.7 and from 2.0 to 2.4 between July and September of 2017 and 2018. The pregnancy rates of artificially inseminated cows and heifers as well as embryo transferred heifers were 27.08 ± 17.54%, 27.67 ± 14.61% and 56.06 ± 15.00% during the above-mentioned period, respectively. Contrary to these results, between October and December of 2017 and 2018 the average pregnancy rates of artificially inseminated cows and heifers as well as embryo transferred heifers on farms were 37.22 ± 12.30%, 40.00 ± 11.31% and 88.50 ± 20.41%, respectively.

During the study period of current work which occurred between July and September of 2017 and 2018, a total of 1 631 (heifer AI: 282, cow AI: 1 181, heifer ET: 168) assisted reproductive techniques were evaluated. The minimum and maximum number of animals on farms was distributed as follows: for the artificially inseminated heifer group it varied from 17 (in July) to 22 (in September), for artificially inseminated cows it varied from 75 (in August) to 81 (in September) and for embryo transferred heifers it varied from 11 (in all months) to 13 (in September) animals. The weather

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of Hungary is classified by temperate climate zone, while of this region the moderately warm, moderately dry climate zones are typical (OMSZ 2019). The average temperatures in the examined period ranged between 15.5 °C (September, 2017) and 23.3 °C (August, 2018).

Measurement of rectal temperature

Rectal temperature was measured three times immediately before each insemination or embryo transfer using a digital thermometer (AET-F101; Alicn Medical Co., Ltd., Shenzhen, P.R. China). The mean rectal temperature was calculated. The assisted reproductive techniques were done between 9:00 and 11:00 in the morning.

Reproductive program and pregnancy diagnosis

Detection of the oestrous cycle of AI animals on all farms was evaluated via ultrasound diagnosis (Easy-Scan 4; IMV, IBEX Go, Loveland, Colorado, USA). The same protocol, so called Double-Ovsynch presynchronization protocol, was used on farms (T. Zubor personal communication). It was used in the period of 41 DIM (days in milk). This protocol uses 4 injections of GnRH (75 µg of gonadorelin *i.m.* injection; Ceva, Libourne, France) at a specified time interval followed by 2 injections of PGF2α (500 µg of cloprostenol *i.m.* injection, MSD Animal Health,Uxbridge, United Kingdom). The last GnRH injection followed after 12 h from TAI (timed artificial insemination). For the treatment of anoestrus cows intravaginal progesterone implants were involved (1.38 g of progesterone implant; Zoetis, São Paulo, Brasil) (Figure 1A). All the inseminations were observed following this protocol (described above as first inseminations).

Detection of oestrus was performed by the observation method (heifers) as well as by an automated activity monitor (cows) and those in oestrus were inseminated. Heifers are inseminated after the live weight of 360 kg and height of 127 cm at the withers are reached. In heifers there is no synchronization, but animals showing no signs of oestrus are treated individually either with prostaglandin (in the presence of the *corpus luteum*) or GnRH (day 0) + prostaglandin (day 7). The recipient cows were treated in the luteal status only by one intramuscular injection of PGF2α (500 µg/animal cloprostenol; MSD Animal Health, Uxbridge, United Kingdom) (Figure 1B). Standing heat was detected by MontaPlus® heat detector or visual appraisal, after that data were transferred to a computer. On each farm a single professional technician performed all procedures.

In this study, biopsied and genome evaluated embryos were used, which were produced by the Ovum Pick Up-In Vitro Production (OPU-IVP) technique via SEMEX Alliance. The quality of embryos was grade 1 quality as categorized by the International Embryo Technology Society

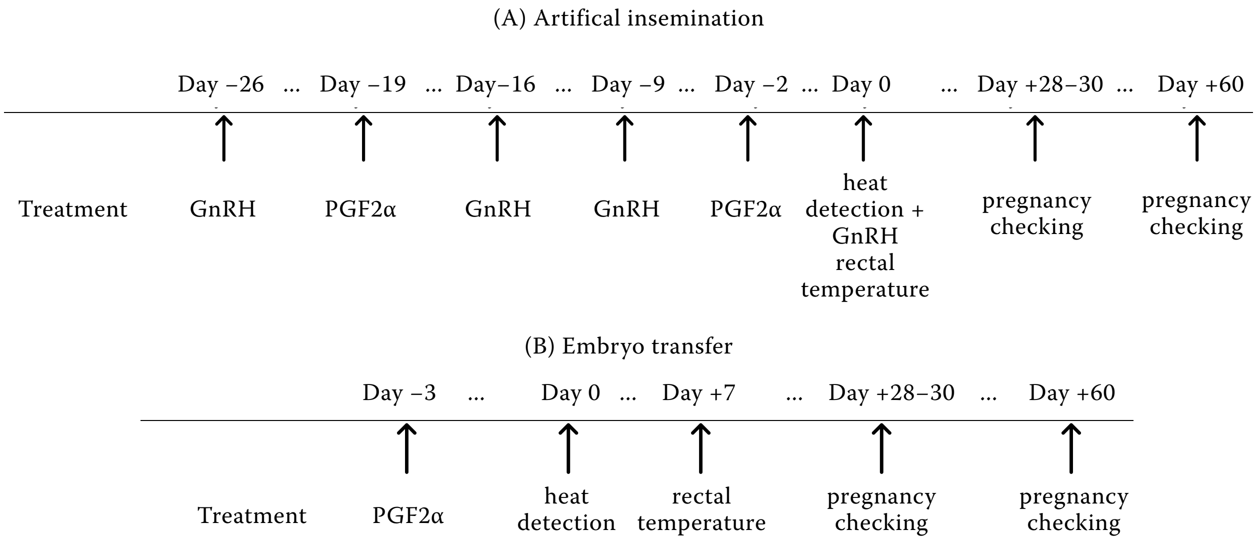

Figure 1. Schematic view of protocols

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(IETS), whilst the stage of embryos varied from morula up to blastocyst-stage embryos. In all cases, frozen embryos (0.25 cc straws) were transferred into recipient animals on day 7 after oestrus, using ultrasound and manual *corpus luteum* detection. Embryos were transferred into the uterine horn ipsilateral to the ovary of the *corpus luteum*. On each farm a single professional technician performed all procedures.

Pregnancy diagnosis was performed between 28 and 30 days post AI or ET by ultrasonography examination, using ultrasonography equipment with a rectal linear transductor of 5–7 MHz (Easy-Scan 4; IMV, IBEX Go, Loveland, Co, USA). Pregnant animals were re-examined by palpation per rectum of the uterus in 60 days. On each farm a single professional technician did the ultrasonic procedures.

Statistical analysis

For statistical analysis rectal temperatures of cows and heifers were evaluated and classified as by degrees (0.2 °C). The farm fixed effect was not included in the model, because previous analyses indicated that it was not significant for these variables (data not shown).

For the evaluation of reproductive performance in dairy herds we used the following formula: the total number of inseminations (embryo transfers) was given for all cows (heifers) divided by the number of pregnant animals.

The effects of month and animal groups (heifers, cows) on rectal temperature were analysed separately by a GLM test using the SAS program (v9.4, SAS Institute, Inc., Cary, NC, USA), whilst the effect of month and procedure on fertility was analysed separately by logistic regression (PROC GENMOD, LOGIT model). Means and standard deviations as well as standard error of the mean were estimated. The Pearson correlation coefficient (PROC CORR) between rectal temperature and fertility was calculated for all study periods and rectal temperature categories of each group. Statistical paired significance was defined as $P < 0.05$.

RESULTS

The average rectal temperature in artificially inseminated (AI) heifers and cows was 38.3 °C and 38.8 °C, respectively, whereas in heifers subjected to embryo transfer (ET) it was 38.8 °C (Table 1). AI heifers had significantly lower body temperature compared to those values measured in the other two groups. The enhancement of rectal temperature due to the temperature elevation until August can be seen in heifer groups. In both groups a similar tendency can be observed, the highest values

Table 1. Rectal temperature (RT) and pregnancy rate (PR) of artificially inseminated (AI) and embryo-transferred (ET) females according to months

Item	Heifer (AI)			Cow (AI)			Heifer (ET)		
	n = 282			n = 1 181			n = 168		
	RT (°C)		PR (%)	RT (°C)		PR (%)	RT (°C)		PR (%)
	mean ± SD	SE	mean	mean ± SD	SE	mean	mean ± SD	SE	mean
July Tmean: 21.85 °C	$38.19 \pm 0.31^{a,A}$	0.048	54.5^{A}	$39.10 \pm 0.60^{a,B}$	0.056	$10.4^{B,**}$	$38.49 \pm 0.39^{a,A}$	0.095	65.2^{A}
August Tmean: 23.02 °C	$38.36 \pm 0.45^{b,A}$	0.039	44.1^{A}	$38.84 \pm 0.56^{b,B}$	0.025	$19.2^{B,*}$	$39.21 \pm 1.03^{b,C}$	0.25	52.4^{A}
September Tmean: 16.5 °C	$38.32 \pm 0.38^{b,B}$	0.042	42.7^{A}	$38.63 \pm 0.46^{c,A}$	0.023	$18.9^{B,**}$	$38.76 \pm 0.33^{ab,A}$	0.096	53.8^{A}
Mean	38.3 ± 0.4^{A}	0.023	$47.1^{A,+}$	38.76 ± 0.56^{B}	0.016	16.7^{B}	38.80 ± 0.50^{B}	0.04	$57.1^{A,++}$
Coefficient of correlation RT-PR	−0.58 (P = 0.42)			−0.72 (P = 0.02)			−0.34 (P = 0.37)		

$^{A–C}$Values in rows with different letters differ significantly (P < 0.000 1); $^{a–c}$Values in columns with different letters differ significantly (P < 0.000 1); $^{+,++}$Values in rows with different letters differ significantly (P < 0.05); *,**Values in columns with different letters differ significantly (P < 0.05) were measured in August, followed by temperatures of September and July. Contrary to this, the highest average values of AI cows occurred during July, followed by August and September. In July the rectal temperature in AI cows was significantly higher than that in the other two months. Concerning the pregnancy rate, it was significantly lower in AI cows (mean value: 16%), especially in July (10%), whereas in heifers the lowest values can be seen in the other two months (AI: 43% and ET: 54%). Rectal temperature in AI heifer group increased by 0.2 °C from 38.2 °C resulting in a 10% reduction in the pregnancy rate from 54.5%. In AI cow group about 9% reduction in pregnancy rate could be seen, if the rectal temperature elevated by 0.3–0.5 °C. The highest rectal temperature value was measured in ET heifer group, and the highest variation of data could be observed in this group. Similarly like in the other two groups, the higher rectal temperature of ET heifers negatively influenced pregnancy rate; the pregnancy rate in September decreased by 12% when compared to the value measured in July. This reduced pregnancy rate was also found out in AI heifers (from 55% to 43%). On the other hand, it should be emphasized that the worst pregnancy rate of ET heifers (53%) was like the best pregnancy rate of AI heifers (54%). It can be stated that in line with an increase of rectal temperature there was a decrease in the pregnancy rate in all groups. The correlations between rectal temperature and pregnancy rate were stronger in cows when compared with the values for heifer groups. Values for ET heifer group were much lower than those measured for the other two groups.

The percentage of insemination/embryo transfer as well as the cumulative percentage of pregnant animals according to rectal temperature categories can be seen in Table 2. The cumulative percentage of pregnant animals calculates the percentage of the cumulative frequency within each category. Up to 39.1 °C more than 92% of ET heifers were pregnant, at the same time this value was nearly half in AI heifers (48%) and close to a sixth in cows (16%). Moreover, it was shown that over this rectal temperature category the pregnancy rate did not considerably change in AI groups.

Figure 2 shows the services per pregnancy according to rectal temperature categories in particu-

Table 2. Percentage of pregnant females according to rectal temperature categories

RT category (°C)	Percentage of insemination/embryo transfer (%)			Cumulative percentage of pregnant animals (%)		
	heifer (AI)	cow (AI)	heifer (ET)	heifer (AI)	cow (AI)	heifer (ET)
37.7–37.9	13	4	0	5.67	0.51	0
38–38.2	28	12	33	19.15	3.72	34.31
38.3–38.5	31	21	38	35.46	8.45	70.59
38.6–38.8	20	25	13	45.39	12.76	83.33
38.9–39.1	6	16	9	47.52	16.23	92.16
39.2–39.4	2	11	3	47.52	17.75	94.12
39.5–39.7	0	6	1	47.52	18.34	96.08
39.8–40	0	2	3	47.52	18.43	99.02
40.1–40.3	–	2	0	–	18.60	–
40.4–40.6	–	1	–	–	18.68	–

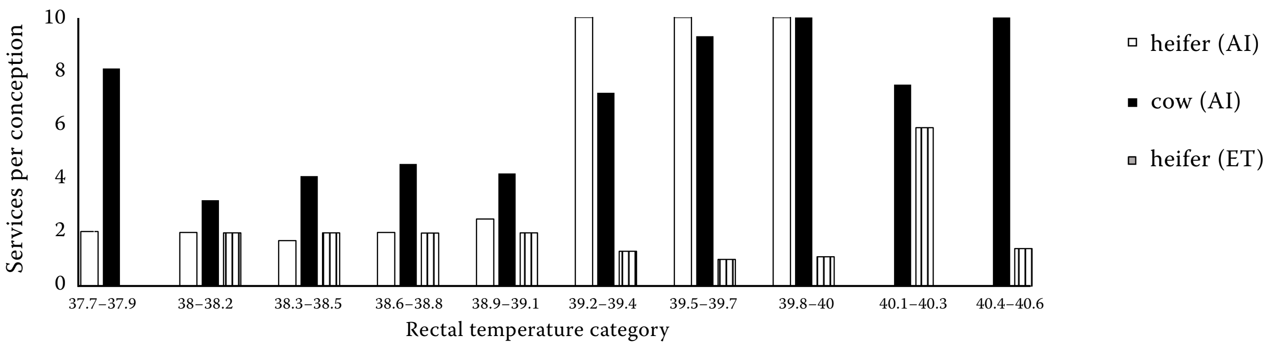

Figure 2. Distribution of services per conception according to rectal temperature categories

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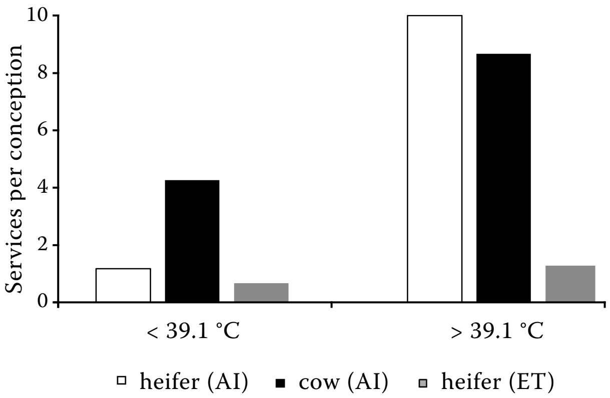

Figure 3. Distribution of services per conception according to rectal temperature below and above 39.1 °C

lar groups. This index was strongly associated with rectal temperature, in the category above 39.1 °C significantly higher values can be seen in AI groups, except in ET heifers. Animals that had the rectal temperature lower than 38 °C were more infertile; especially cows with rectal temperature of 37.7–37.9 °C had a higher number of services per conception. Services per conception in AI heifers, cows and ET heifers with rectal temperature lower than 39.1 °C were 2, 4.8 and 1.6, respectively. Above this category services per conception were considerably higher in AI cows (8.8), whereas the number of services in ET heifers was 2.1 (Figure 3).

DISCUSSION

In the present study we evaluated the effects of rectal temperature and reproductive management techniques on the pregnancy rate in dairy cows and heifers. It is well known that the body temperature is influenced by heat stress in cattle (Hahn 1999; Kadzere et al. 2002) and responds earlier than dry matter intake. Rectal temperature may be used as an indicator of physiological adaptation to a hot environment, once its increase indicates that the mechanisms of heat release became insufficient to maintain homeothermy (Hemsworth et al. 1995). According to the data of Hungarian Meteorological Service (OMSZ 2019) the mean temperature in July, August and September of the examined years was 21.85 °C, 23.02 °C and 16.5 °C, respectively. The average monthly temperature higher by 0.8–2.7 °C compared to the average temperature was measured between the years 1981 and 2010. In previous studies, the mean value of rectal temperature obtained in lactating cows changed from 38.4 °C (Suthar et al. 2013) to 38.8 °C (Amer et al. 2016). Within the present study the mean ambient temperature was higher (20.5 °C) than in Suthar's study (17.2 °C). Previously, Hegedusova et al. (2009) demonstrated that the optimal period for conception in dairy cows was in the temperature range from 16 °C to 20 °C because of the potential adverse effect of heat stress in high-yielding cows.

The large amount of heat produced as a lactation makes it difficult for the lactating female to regulate its body temperature, especially in the hot season (Nabenishi et al. 2011). Moreover, heifers have a lower sensitivity to heat stress compared to cows (Sartori et al. 2002), cows showed a greater increase in body temperature in response to increases in the environmental temperature than it was observed in heifers. In our study it was also confirmed that the highest maximum rectal temperature values were measured in cows during July, however their values did not differ from those data which were measured in August in ET heifer group. In heifer groups, the elevation of rectal temperature followed a month later than in cows. In AI heifer group the lowest rectal temperature values were observed during the whole examined period indicating their better thermoregulatory capabilities contrary to cow group.

First, Ulberg and Burfening (1967) reported an about 25% reduction in pregnancy rate for every 1 °C elevation in body temperature. The negative correlation between the body temperature on the day of artificial insemination and conception rate was reported by Nabenishi et al. (2011). According to their results the vaginal temperature increased by 0.6 °C from 38.7 °C, resulting in a 11.6% reduction in the conception rate from 40.5%.

Our study supports the findings by Mellado et al. (2014), who found that the worst pregnancy rate of heifers and cows differed during the year, the worst pregnancy rate per artificial insemination of heifers was in September, contrary to cows who had the poorest value in July. In recent study, Novotni-Danko et al. (2017) showed that in line with our results the cow's pregnancy rate in Hungary decreased from May and it was the worst in July and August.

Research has shown that cows with a higher body temperature on the day of embryo transfer had a lower probability of pregnancy and higher probability of embryonic loss (Vasconcelos et al. 2006).

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The results of the present study supported our hypothesis, we found that there was a reduction in pregnancy rate in line with the elevation of rectal temperature, however different tendencies can be seen for ET and AI heifers and cows during summer. It was previously demonstrated (Hansen 2009) that heat stress for 20–26 days before oestrus alters the follicular function, so the reduced fertility of heifer groups in autumn can be explained by this phenomenon.

Polsky et al. (2017) concluded that body (vaginal) temperature higher than 39.1 °C is the most functional measurement to represent heat stress. Temperature thresholds of body temperature > 39.2 °C and > 39.7 °C (Smith et al. 1998; Wenz et al. 2011) have been recommended to distinguish between healthy cows and cows suffering from an infectious disease. According to our findings females with rectal temperature categories lower than 38 °C and higher than 39.1 °C showed a lower pregnancy rate compared to the cumulative percentage of pregnant females with rectal temperature between 38 and 39.1 °C. However, it can be mentioned that the success of techniques in different groups was unequal. In line with previous foreign findings (Stewart et al. 2011) embryo transfer improves pregnancy rates during summer because embryos are less sensitive to heat stress. Our results confirmed that the decline in fertility was seen in cows and heifers treated with artificial insemination with rectal temperature above 39.1 °C, but it did not occur in heifers subjected to embryo transfer.

We found that the insemination number was considerably higher than the number of embryo transfers in heifers especially in the rectal temperature category higher than 39.1 °C. Novotni-Danko et al. (2017) observed a similar index for heifers 1.6–2.5 to our values measured in the group with rectal temperature lower than 39.1 °C.

In summary, the rectal temperature and pregnancy rate of high-yielding lactating cows and heifers were inversely affected by heat stress during the examined hot period. According to our results, cows had the highest rectal temperature at the beginning of the experimental period (in early summer), although the ambient temperature continued to rise they could adapt in one month, as proved by our decreasing rectal temperature data and progressive pregnancy results. Contrary to cows, during summer heifers were exposed only to environmental heat shock. In line with the ambient and rectal temperature elevation, the decline of pregnancy rate at the end of the second month (August) indicated the adverse effect of heat stress on both heifer groups. The degree of temperature changes in heifers of AI group was much lower than the value of heifers in ET group; so it seems that higher rectal temperature leads to a higher degree of temperature change. Despite the higher rectal temperature value of ET heifer group, in this group the higher pregnancy rate could be observed compared to the artificially inseminated heifer group.

CONCLUSION

The present findings demonstrate that with the enhancement of rectal temperature the pregnancy rate decreased in all groups; however different trends can be seen in heifers and cows during the examined period. It seems that embryo transfer has the capability of providing more pregnancies in heifers over artificial insemination during the summer season. Selection criteria based on the rectal temperature lower than 39.1 °C could lead to higher fertility rate and lower services per conception in heifers and lactating cows.

Conflict of interest

The authors declare no conflict of interest.

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