VETERINARSKI ARHIV 82 (3), 239-249, 2012

Anovulatory estrus in dairy cows: treatment options and the influence of breed, parity, heredity and season on its incidence

Robert Zobel*, Ivana Pipal, and Vlatka Buić

Vetmed d.o.o., Stružec, Gornje Selo, Croatia

ZOBEL, R., I. PIPAL, V. BUIĆ: Anovulatory estrus in dairy cows: treatment options and the influence of breed, parity, heredity and season on its incidence. Vet. arhiv 82, 239-249, 2012.

ABSTRACT

The aim of the study was to establish the anovulatory estrus incidence in different breeds and reproductive ages of dairy cattle in Croatia, and to assess the effectiveness of hormone therapy as a treatment option. A total of 820 cows of different breeds and ages were involved in the study over two years. Anovulatory estrus was recorded in 25.61% of the observed cows and heifers. The highest anovulatory estrus ratio was recorded in heifers and the lowest in cows after the 6^{th} pregnancy ($P<0.05$). Regarding the breed, the highest anovulatory estrus ratio was diagnosed in crossbreeds and the lowest in the Simmental breed ($P<0.05$). The highest anovulatory estrus incidence was recorded during the summer months (III quartile of the year) ($P<0.05$). Heredity of anovulatory estrus was found to be statistically significant. Therapy success was measured by the number of days from the day of anovulatory estrus diagnosis confirmation until the day of the first artificial insemination with confirmed ovulation. The best results were achieved in the group T animals who were treated twice with a 20 day interval with Gonadotropin Releasing Hormone analogues ($P<0.05$). Vitamin (A, D, E) and mineral (Selenium) supplements in the group A cows also improved therapy results if compared to the control (untreated) group C ($P<0.05$), although this was less significant when compared to group T ($P<0.05$). We conclude that anovulatory estrus is a hereditary condition and related to a lack of Gonadotropin Releasing Hormone, as well as a lack of vitamins and minerals.

Key words: anovulatory estrus, breed, cattle, heredity, quartile, treatment

Introduction

As reported by ESPEY (1994), ovulation is initiated by an increase of LH surge, which results in follicle rupture and the release of the ovum. According to HAFEZ and HAFEZ (2000), a syndrome associated with the conditions that lead to both true anovulatory estrus or to Cystic Ovarian Disease is ovulation failure. The exact cause of ovarian cysts is not

*Corresponding author:
Robert Zobel, DVM, Vetmed d.o.o., Stružec 59, 44317 Popovača, Croatia, Phone +385 44 643 700; E-mail: robert.zobel@sk.t-com.hr

ISSN 0372-5480
Printed in Croatia

presently known, but it appears that an important component in the pathogenesis of this condition is the inappropriate or lack of release of the Gonadotropin Releasing Hormone (GnRH) at the time of estrus (JOHNSON et al., 1966; ROBERTS, 1971; LÓPEZ-GATIUS et al., 2002). Sometimes, however, a follicle does not regress but, having reached its maximum size of 2 - 2.5 cm in diameter, the wall becomes luteinised. This structure functions in the same way as a corpus luteum, either regressing after 17 - 18 days, or frequently much earlier, so that the cow returns to estrus for a shorter than normal interval. Such a structure will be <2.5 cm in diameter and fluid-filled, with a rim of luteal tissue lining the follicle and with no evidence of a point of ovulation. In these cases, a diagnosis of anovulatory estrus can only be made retrospectively by finding a follicle that persists longer than expected (WATSON and HARWOOD, 1984; PARKINSON, 2001; WILTBANK et al., 2002).

There are many factors that influence the time to first ovulation, including breed, parity, heredity, season, BCS, peripartum disease, a variety of nutritional effects and an extended period of high ambient temperature (WALSH et al., 2007).

In the present study, anovulatory estrus was confirmed using ultrasonography and transrectal palpation, after finding a follicle <2.5 cm in diameter, with unchanged morphology of the examined ovary for 7 consecutive days after the estrus onset.

There is no information on the incidence of this condition in dairy cows in Croatia. To fill this gap, our aim was to establish the incidence of anovulatory estrus (AE) in dairy cows in Croatia in relation to breed, age, heredity and quartile (season) of the year. Since ovarian cysts and anovulatory follicles have the same origin, we speculated that the cause could also be the same (the lack of GnRH at the time of estrus). Further, another objective of this study was to assess the efficacy of GnRH analogue as a treatment modality of anovulatory estrus, compared with vitamin/mineral supplements and a control (untreated) group.

Materials and methods

Animals. A total of 820 dairy cows were included in the study over 2 years (2007 and 2008 inclusive) in the central part of Croatia (Moslavina region) with a continental climate. The group included 138 heifers and 682 cows of the following breeds: 538 Simmentals (65.61%), 140 Holstein Friesian (17.07%) and 142 Crossbreeds (17.32%).

The animals were housed in 9 commercial dairy farms, with 40 to 80 cows per farm with similar management and feeding systems. Lactating cows, dry cows and heifers were housed separately. The cows were milked twice daily (morning - evening milking schedule) and during the warm period of the year (March until November) they were released into pasture during the day. Starting from November until March, the cows were kept inside the tie stall barns, with free access to the backyard for a few hours daily. The animals were fed hay *ad libitum* and a concentrate ration, consisting of oat, corn meal, soybean and barley. Grass silage, corn silage, and propylene glycol were added to the rations according to the age, stage of lactation and milk yield. Starting in June, fresh alfalfa and clover were added. The average annual milk yield of the Holstein Friesian cows was 8250 ± 639 kg, and 6990 ± 312 kg in Crossbreeds and 5600 ± 420 kg in the Simmental breed. Concerning parity, 138 (16.8%) were heifers, 155 (18.9%) primiparae, 129 (15.7 %) after their 2nd pregnancy, 92 (11.2%) after their 3rd pregnancy, 106 (12.9%) after their 4th pregnancy, 58 (7.1%) after their 5th pregnancy, 71 (8.7%) after their 6th and 71 (8.7%) after their 7th pregnancy.

Experimental design. The total sample pool of cows/heifers involved in the study was 820. Onset of estrus was detected by experienced farm managers, who monitored the cows throughout the day (three times daily, for 15 minutes each) for changes in behavior and characteristic signs of estrus. Veterinary clinical examination techniques followed and included vaginoscopy, ultrasound using a linear rectal probe 7 MHz (Draminski, PROFI L, Poland), and transrectal palpation. The first examination was performed 50 to 60 days post partum in cows or at the age of 13 to 14 months in heifers. Findings in the genital tract that included vaginal content (mucus, fluid, color, consistency and smell) and the morphology of the cervix (open, closed, color, exudate, location, orifice morphology and position) were observed and recorded. Following vaginoscopy, transrectal palpation of the reproductive tract was performed to determine the diameter, location (abdominal or pelvic), consistency, and symmetry of the uterine horns. The uterus was examined by ultrasonography for the presence of fluid in the lumen and/or thickening of the uterine mucosa. The size and location of the ovaries and the presence of the ovarian structures (Graafian follicle, corpus luteum, cyst, luteinised follicle, scars, or no visible or palpable structures) were also recorded. When in heat, the cows and heifers were examined daily until transrectal confirmation of ovulation (disappearance of the dominant follicle) or for at least 7 consecutive days when AE diagnosis was established. Exclusion criteria included retention of fetal membranes after a previous birth, body condition score <2.5 (EDMONSON et al., 1989), the presence of corpus luteum on the ovary, cystic ovarian disease, endometritis, periovarian and uterine adhesions, pyometra, gravidity, hypoplastic ovaries and contagious reproductive diseases (Q-fever, Leptospirosis, TBC, Lysteriosis, Campylobacteriosis and Brucellosis). Blood samples and vaginal swabs were collected in order to test for sexually transmitted diseases. All the laboratory tests were performed at the Croatian Veterinary Institute in Zagreb, Croatia.

The diagnosis of anovulatory estrus was established after finding the same follicle <2.5 cm in diameter on the same ovary at least for 7 consecutive days following the estrus onset, with the absence of corpus luteum. Cows with AE diagnosis were further randomly divided into three groups, based on the last two ear-tag numbers (group C: both even numbers; group T: both odd numbers; group A: last odd and penultimate even number; last even and penultimate odd number). Each group was treated differently.

After the exclusion criteria were applied, a group of 210 animals was created.

Artificial insemination was performed using thawed frozen semen (thawed in water at a temperature between 35˚C and 37˚C for 30 seconds). Semen was deposited into the uterine horn ipsilateral to the ovary bearing the dominant follicle. One of the three experienced veterinarians from the same practice performed all the artificial inseminations and ultrasound examinations each time.

Based on the results obtained from the Croatian Agriculture Agency (HPA), data were compared to cows with diagnosed AE. The list included data about the genealogy of four generations of each cow/heifer with diagnosed AE.

The year was divided into four quartiles: I (including January, February, March); II (April, May, June); III (July, August, September) and IV (October, November, December).

Therapy protocols. group C (n = 50) was the control group and no treatment was applied. The cows were examined at each observed estrus noticed by the owner or at least once a week. The animals were examined as described until ovulation occurred spontaneously, or until corpus luteum was found on the one of the ovaries, or until 180 days postpartum when the research ended. The number of days from establishing the diagnosis of AE until the first AI with confirmed ovulation was recorded for further data analysis.

Group T (n = 150) received GnRH analogue (0.05 mg gonadorelin, 1 mLDepherilin Gonavet Veyx®) intramuscularly, twice in an interval of 20 days. Treatment began on the day of confirmation of the diagnosis of AE and was repeated 20 days later. Three days following the administration of the second GnRH dose, the cows and heifers were examined in order to confirm the estrus onset. In cases where no signs of estrus were found (absence of a dominant follicle and estrus mucus, a closed cervix, no clinical signs of estrus), the cows and heifers were examined weekly until estrus was detected or corpus luteum found on one of the ovaries. Examinations continued once a week until 180 days postpartum when the study ended.

Group A (n = 60) received A, D, E vitamins with Selenium intramuscularly on the day of the confirmation of the diagnosis of AE . The animals were examined weekly until ovulation was confirmed, corpus luteum was found on one of the ovaries, or until 180 days postpartum (the end of the research).

Ovulation in all cows and heifers was confirmed after the disappearance of the dominant follicle during the observed estrus or after finding of corpus luteum on one of the ovaries. AI was performed at the next estrus and the data (the number of days from the day of the diagnose establishment until the first AI with confirmed ovulation) recorded.

Statistical analysis and data processing. The success of the therapy was evaluated by the number of days from the AE diagnosis to the first AI with confirmed ovulation. The incidence of AE was expressed as the percentage of total number of cows examined. Possible risk factors (breed, heredity, parity and quartile) for cows diagnosed with AE were initially examined using the Kolmogorov Smirnof test. Multivariable logistic regression was used to compare results between variables: breed vs. incidence of AE; parity vs. incidence of AE; quartile vs. incidence of AE; incidence of AE vs. number of days from AE diagnosis until the first AI with confirmed ovulation; treatment vs. number of days from the AE diagnosis until the first AI with confirmed ovulation. The results were found to be significant when P<0.05.

Results

From the total of 820 cows and heifers observed, AE was diagnosed in 210 animals: 47 heifers (22.38%) and 163 cows (77.62%).

The AE incidence was found to be the highest in the Crossbreeds (54.93%), followed by the Holstein Friesian breed (38.57%) and the lowest (14.50%) in the Simmental breed (P<0.05).

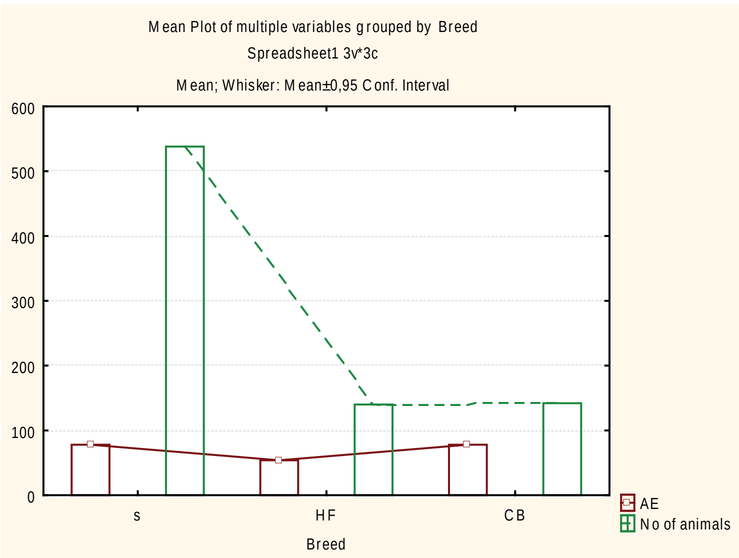

Fig. 1. Relationship between breed and anovulatory estrus incidence

AE = number of cows/heifers with anovulatory estrus diagnosis; S = Simmental breed; HF = Holstein Friesian breed; CB = Crossbreeds; No. of animals = number of animals of respective breed

The highest AE incidence was recorded in heifers, followed by cows after the 4th pregnancy, while the lowest AE incidence was recorded in cows after the 6th pregnancy, in all breeds of animals (P<0.05).

Table 1. Incidence of anovulatory estrus in terms of parity

Breed	n	%	Parity	n	AE	%
Simmental	538	65.61	heifers	96	28[a]	5.20
			1	97	9[b]	1.67
			2	102	11[b]	2.04
			3	48	3[c]	0.56
			4	58	14[b]	2.60
			5	37	11[b]	2.04
			6	50	0[d]	0
			7	50	2[c]	0.37
Total				538	78[1]	14.50
HF	140	17.07	heifers	21	8[a]	5.71
			1	11	9[a]	6.43
			2	31	4[b]	2.85
			3	22	6[a]	4.29
			4	24	14[c]	10.00
			5	10	10[a]	7.14
			6	11	0[d]	0
			7	10	3[b]	2.14
Total				140	54[2]	38.57
CB	142	17.31	heifers	21	11	7.75
			1	29	13[a]	9.15
			2	14	6[b]	4.22
			3	22	9[b]	6.34
			4	24	18[c]	12.68
			5	11	6[c]	11.27
			6	10	5[d]	0
			7	11	10[b]	3.52
Total				142	78[3]	54.93
Total	820			820	210	25.61

n = number of observed animals; AE diagnosis = number of animals with anovulatory estrus diagnosis; HF = Holstein Friesian breed; CB = Crossbreeds; % = percentage of animals with AE diagnosis in respective subgroup; parity = number of partus. [a,b,c,d,e,f] Values in the AE column marked with different letter in superscript differ significantly (P<0.05). [1, 2, 3] Values in the "total" column marked with different number in superscript differ significantly (P<0.05).

Anovulatory estrus was recorded in 61 mothers, 42 daughters, 32 granddaughters and 20 animals of the 4th generation (P<0.05). Anovulatory estrus was also diagnosed in 55 other cows (P<0.05), not genetically connected (P<0.05).

Table 2. Heredity of anovulatory estrus

	No.	%
Mother	61[a]	29.05
Daughter	42[b]	20
3rd gen.	32[c]	15.23
4th gen.	20[d]	9.52
Other cows	55[a]	26.19
Total	210	

Other cows = cows with anovulatory estrus diagnosis not genetically connected. [a, b, c, d] Values in the total column marked with different letter in superscript differ significantly (P<0.05).

The highest incidence of AE was recorded during the 3rd quartile in both years of the research, and the lowest during the 4th quartile (P<0.05).

Table 3. Influence of the quartile on AE incidence

Year	Quartile	AE	%
2007	I	16[a]	7.62
	II	13[a]	6.19
	III	68[b]	32.38
	IV	10[c]	4.76
2008	I	14[a]	6.67
	II	14[a]	6.67
	III	64[b]	30.48
	IV	11[c]	5.24
Total		210	

AE = number of animals with the anovulatory estrus diagnosis; % = percentage of animals with AE diagnose. [a, b, c] Values in the AE column marked with different letter in superscript differ significantly (P<0.05).

The lowest number of days from the AE diagnosis to the first AI with confirmed ovulation was recorded in group T, with 24 days less than the average (131). At the same time, 41 and 30 more days were recorded in groups C and A respectively, if compared to group T (P<0.05).

Table 4. Therapy success in different groups of cows measured by days from the anovulatory estrus diagnosis to the first artificial insemination with confirmed ovulation

Group	Total	C	%	T	%	A	%
No of animals	210	50	23.81	150	71.43	60	28.57
Days	344	135[a]		94[b]		115[c]	
Av. days	116	+21[a]		-21[b]		-1[c]	

C = control group; T = group receiving GnRH analogues; A = group receiving vitamins and minerals; days = number of days from the first examination and AE diagnosis to the first AI with confirmed ovulation; Av. days = average days for all three groups with expressed number of days from the first examination to the first AI with confirmed ovulation (less (-) or more (+) from the average); [a,b,c] Values in each row marked with different letter in superscript differs significantly ($P<0.05$)

Discussion

The diagnosis of anovulatory estrus was recorded in 25.61% (210/820) of cows and heifers observed which is in accordance to the findings of GUMEN et al. (2003) who reported this condition in 20.52% of cows and heifers, and WILTBANK et al. (2002) with 26% of animals having anovulatory estrus.

The AE incidence was found to be the highest in heifers of all breeds. The highest incidence of AE in heifers was also reported by WILTBANK (2006) and WALSH et al. (2007). According to this finding, we speculate that AE is more closely associated to parity than to milk yield performance in dairy cows.

Concerning the breed, the highest incidence of AE was recorded in Crossbreeds (Simmental/Holstein-Friesian) and twofold less frequently in Holstein Friesian breed ($P<0.05$). The lowest AE incidence was recorded in the Simmental breed ($P<0.05$). We speculate that the high AE incidence in Crossbreds is an important reason against unreasonable cross breeding. The lowest AE incidence was noticed in the Simmental breed, probably due to the adaptation of the breed to the climate and management. Our data are supported by the results of BARTLET (1984) and ERB (1984), who reported the highest incidence of AE in the Holstein Friesian breed of cows. Our results suggest a significant connection between the AE ratio and the breed, with Crossbreeds and Holstein Friesians being the most affected.

A strong connection between the AE incidence and the quartile of the year (ambient temperature) was recorded. Our results show that the highest incidence of AE was recorded during the 3rd quartile (July, August and September) when the ambient temperature is high, due to the summer period in our continental climate. High ambient temperature resulted in a higher AE incidence if compared to the other quartiles of the year ($P<0.05$). Our results are supported by the findings of WALSH et al. (2007).

Anovulatory estrus is highly hereditary ($P<0.05$) since the offspring of cows with AE diagnose were also burdened with the same condition in a high percentage even to the 4th generation. These data are also supported by the findings of WALSH et al. (2007).

The best therapy success was achieved in group T, using GnRH analogues in a 20 day interval, which resulted in the lowest number of days from the AE diagnosis to the first AI with confirmed ovulation at the end of heat. Anovulatory estrus can, thus, be successfully treated using GnRH analogues as previously suggested by GUMEN et al. (2003). Our results, therefore, support the findings of JOHNSON et al. (1966), ROBERTS (1971), and LÓPEZ-GATIUS et al. (2002), that an important component in the pathogenesis of anovulatory estrus is the inappropriate or lack of release of GnRH at the time of estrus.

At the same time, there was significant difference concerning therapy success (the number of days from the first examination to the first AI with confirmed ovulation) between groups C and A. Following the administration of vitamins and minerals, cows and heifers in group A showed a significant decrease in the number of days from AE diagnosis to the first AI with confirmed ovulation when compared to the control group C. We speculate that hypovitaminosis and the lack of minerals (most probably vitamins A and E and Selenium) could be another cause of AE. This opinion is supported by the findings of WALSH et al. (2007) showing that a variety of nutritional effects can influence the time to first ovulation, including a higher incidence of anovulatory estrus.

Conclusion

We conclude that the anovulatory estrus rate in dairy cattle is closely connected to parity, breed, and quartile of the year. We suggest that the condition is highly hereditary. It may also be concluded that inappropriate release of GnRH at the time of estrus, coupled with hypovitaminosis (A, D, E) and lack of minerals (Selenium) are the most significant causes of this condition. Once diagnosed, anovulatory estrus can be successfully treated using GnRH analogues.

Further investigations should be directed toward genetic herd improvement, heat stress avoidance and research into hypovitaminosis and lack of minerals in affected animals, in order to prevent this condition.

References:

BARTLETT, P. C. (1984): Cystic follicular disease in Michigan Holstein-Friesian cattle: Incidence, descriptive epidemiology and economic impact. Prev. Vet. Med. 4, 15-33.

EDMONSON, A. J., I. J. LEAN, L. D. WEAVER, T. FARVER, G. WEBSTER (1989): A body condition scoring chart for Holstein Dairy Cows. J. Dairy Sci. 72, 68-78.

ERB, H. N. (1984): High milk production as a cause of cystic ovaries in dairy cows: Evidence to the contrary. Comp. Cont. Edu. 6, 215-219.

ESPEY, L. L. (1994): Current status of the hypothesis that mammalian ovulation is comparable to an inflammatory reaction. Biol. Repr. 50, 233-241.

GUMEN, A., J. N. GUENTHER, M. C. WILTBANK (2003): Follicular size and response to ovsynch versus detection of estrus in anovular and ovular lactating dairy cows. J. Dairy Sci. 86, 3184-3194.

HAFEZ, E. S. E., B. HAFEZ (2000): Reproductive failure in females. In: Reproduction in Farm Animals. 7th Edition. Lippincot Williams and Wilkins, Philadelphia. pp. 242-289.

JOHNSON, A. D., J. E. LEGATES, L. C. ULBERG (1966): Relationship between follicular cysts and milk production in dairy cattle. J. Dairy Sci. 49, 865-868.

LÓPEZ-GATIUS, F., P. SANTOLARIA, J. YANIZ, M. FENECH, M. LÓPEZ-BEJAR (2002): Risk factors for postpartum ovarian cysts and their spontaneous recovery or persistence in lactating dairy cows. Theriogenology 58, 1623-1632.

PARKINSON, T. J. (2001): Infertility in the cow. In: Arthur's Veterinary Reproduction and Obstetrics. (Noakes, E. D., T. J. Parkinson, G. C. W. England, Eds.), 8th ed., Saunders Company, USA. pp. 383-472.

ROBERTS, S. J. (1971): Veterinary obstetrics and genital diseases (Theriogenology), 2nd ed., Ann Arbor, Mich: Edward Brothers Inc. pp. 421-435.

WALSH, R. B., D. F. KELTON, K. E. LESLIE, J. S. WALTON, S. J. LEBLANC (2007): Prevalence and risk factors for postpartum anovulatory condition in dairy cows. J. Dairy Sci. 90, 315-324.

WATSON, E. D., D. J. HARWOOD (1984): Clinical and endocrinological investigation of possible ovulatory failure in a dairy cow. Vet. Rec. 114, 424-425.

WILTBANK, M. C. (2006): Changes in reproductive physiology of lactating dairy cows due to elevated steroid metabolism. Theriogenology 65, 17-29.

WILTBANK, M. C., A. GUMEN, R. SARTORY (2002): Physiological classification of anovulatory conditions in cattle. Theriogenology 57, 21-52.

Received: 30 April 2011
Accepted: 19 September 2011

ZOBEL, R., I. PIPAL, V. BUIĆ: Anovulatorni estrus u mliječnih krava: liječenje i utjecaj pasmine, dobi, nasljednosti i temperature okoliša. Vet. arhiv 82, 239-249, 2012.

SAŽETAK

Cilj rada bio je utvrditi pojavnost anovulatornog estrusa u mliječnih krava središnjega dijela Hrvatske, različitih pasmina i dobi te utvrditi učinkovitost hormonalne terapije kao jedne od mogućnosti liječenja ovoga stanja. Istraživanjem tijekom dvije godine bilo je obuhvaćeno ukupno 820 životinja različitih pasmina i dobi. Dijagnoza anovulatornog estrusa postavljena je u 25,61% pregledanih krava i junica. Najviša pojavnost anovulatornog estrusa utvrđena je u junica, a najniža u krava nakon šestoga graviditeta (P<0,05). U pogledu pasmine, najviša pojavnost anovulatornoga estrusa utvrđena je u križanih pasmina, a najniža u goveda simentalske pasmine (P<0,05). Utvrđena je statistička značajnost nasljednosti ovoga stanja (P<0,05). Najviša incidencija anovulatornoga estrusa utvrđena je tijekom trećega tromjesečja (vrući ljetni mjeseci) (P<0,05). Uspjeh liječenja mjeren je brojem dana od dana postavljanja dijagnoze do prvoga umjetnoga osjemenjivanja s potvrđenom ovulacijom. Najbolji uspjeh liječenja postignut je u skupini T koja je primila analogne pripravke Gonadotropin Releasing Hormona (P<0,05). Aplikacija vitamina A, D i E te selena (skupina A) također je smanjila broj dana od dana postavljanja dijagnoze anovulatornoga estrusa do prvoga osjemenjivanja s potvrdom ovulacije (P<0,05) u odnosu na skupinu C (kontrolna, neliječena skupina), no ipak značajno manje u odnosu na skupinu T. Možemo zaključiti kako je pojavnost anovulatornoga estrusa u mliječnih krava u sprezi s nedostatkom "Gonadotropin Releasing Hormona", ali i nedostatkom vitamina i minerala.

Ključne riječi: govedo, anovulatorni estrus, liječenje, pasmina, nasljednost