

Fertility issues in Simmental cows in Central Croatia: a 5-year study

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Abstract: The purpose of this study was to establish the fertility trends in dairy cows in Central Croatia and compare the trends and causes of infertility and subfertility during a 5-year period. A total of 12,060 Simmental cows and heifers were included in the study. The animals were examined by vaginoscopy, transrectal palpation, and ultrasonography on a regular basis. Fertility problems that included infertility and subfertility were diagnosed in 56.61% of all observed animals. The total number of animals diagnosed with subfertility increased and almost doubled by the end of the study period. Subfertility issues associated with anovulatory oestrus, delayed ovulation, and endometritis were 2 to 3 times more frequent at the end of the study period compared to the first year of the study. The number of animals with the repeat breeder syndrome diagnosis remained constant throughout the study. Anoestrus and cystic ovarian disease occurrence declined over time and were more than 50% reduced by the end of the study. Infertility was present in less than 1% of cows and did not change over time. In this study we recognised a rising trend of subfertility in Simmental cows in Central Croatia, primarily related to anovulatory oestrus, delayed ovulation, and endometritis.

Key words: Cattle, infertility, subfertility, Croatia

1. Introduction

According to Darwash et al. (1), fertility in dairy cows is defined as the ability of the animal to conceive and maintain pregnancy if inseminated at the appropriate time. As reported by Parkinson (2), sterility is an absolute inability to reproduce while infertility is either considered to be synonymous with sterility or may imply a delayed or irregular ability to produce offspring during a regular reproductive cycle in cows. However, the term "subfertility" is considered as a more appropriate term for the latter reproductive problems. One of the greatest challenges facing veterinarians in dairy industry and dairy farm management today is the decline in dairy cows' fertility and the considerable economic losses associated with this condition (1).

In this worldwide trend in declining fertility of dairy cows, subfertility is the most common reason for culling, second only to mastitis (2). In a study of 43 dairy herds in California conducted by Gardner et al. (3), 24.8% of the cows were culled each year with reproductive failure as the most common cause. In a survey of reproductive performances in New Zealand dairy herds performed by Xu and Burton (4), 13.6% of cows were culled during each season. Of these, 42.5% were culled for failure to conceive, and 4.4% were culled for other reproductive problems.

In this research we divided reproductive disorders into true infertility (mostly absolute and incurable infertility; sterility) and subfertility (delayed and irregular fertility or conception, mostly responsive to therapy). Infertility included congenital and developmental degenerative changes in the genital tract: freemartinism, cervix duplex, aplastic ovaries, aplastic uterus, periovarian adhesions (peribursitis fibrosa), and uterine adhesions (perimetritis fibrosa). Subfertility included mostly medically treatable conditions: anovulatory oestrus, anoestrus, cystic ovarian disease, repeat breeder syndrome, endometritis, and delayed ovulation. The aim of this study was to establish the prevalence and the trends of infertility and subfertility in the Simmental cows in Central Croatia during a 5-year period.

2. Materials and methods

2.1. Animals

The study included a total of 12,060 of Simmental cows and heifers, housed in 98 commercial dairy farms with an average of 54 cows per farm (range 30 to 180). Approximately 2500 cows and heifers were examined each year during the 5 years of the research period. The average milk yield was 4448 ± 300 kg/year and ranged from 3968 to 4828 kg/year depending on the season.

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Farms were located in the central part of Croatia with a moderate continental climate and were selected for similar management practices and husbandry systems. Simmental cows included in the study ranged from 10 months to 8 years of age. The study covered the 5-year period from 2004 to 2008, inclusive.

Animals were housed in tie stall barns, secured with collars with access to grazing during the day, and milked twice daily (morning and evening). In the warm period of the year (March to November), the animals were on a pasture 8–10 h per day, and during the cold season (November to March), cows were housed in stables with free access to the dry lot for a few hours daily. The feeding regimen included hay ad libitum on pasture and in the stables, and a concentrated mix containing oat, corn meal, soybean, barley, and mineral supplements offered before milking. Grass silage, corn silage, and propylene glycol or glycerol were provided for the lactating cows depending on the age, stage of the lactation, and milk yield. Starting in May fresh cut alfalfa and clover grass were added to the feeding regimen. The feeding regimen was adjusted monthly, based on the laboratory results provided by the Croatian Agriculture Agency (HPA) considering fat content, protein, dry matter, fat-protein index, and urea nitrogen in the milk samples from individual animals.

2.2. Experimental protocol

Exam at distance and physical exam were performed on all cows. All animals were tested for the presence of the following diseases: brucellosis, leptospirosis, and tuberculosis. A 5-mL sample of whole blood (collected via coccygeal vein venepuncture) from each cow was submitted to the Croatian Veterinary Institute for comprehensive diagnostic testing. Exclusion criteria applied in data analysis included cows testing positive for the previously mentioned diseases; cows with a previous caesarean section; animals with polyarthritis, laminitis, or extreme hind limb deformities; cows with recurrent mastitis (occurring more than once during previous or current lactation) or chronic mastitis; and cows with increased somatic cell count (>200,000) for more than 3 months during the lactation.

Onset of oestrus was detected and documented by an experienced farm manager who monitored cows throughout the day (minimum of 3 times daily, for 30 min each) for changes in behaviour and characteristic clinical and gynaecological signs of oestrus. Veterinary clinical examination techniques followed and included vaginoscopy, ultrasound, and transrectal palpation. The same veterinarian performed all clinical assessments and artificial inseminations (AI).

Animals were clinically examined during routine weekly visits to the farms. Cows were first examined between 50 and 60 days postpartum and then reexamined

depending on the clinical status until reliable diagnosis was established. The first examination of the heifers took place at the age of 11 to 15 months of age. All animals were examined using vaginoscopy, transrectal palpation, and ultrasonography with a 7-MHz rectal linear probe (Draminski, Profi L, Olsztyn, Poland). For consistency, the first author performed all clinical examinations. For vaginoscopy, a separate sterilised metal vaginoscope was inserted into the vagina of each cow up to the level of the external cervical orifice. Findings in the genital tract that included vaginal content (mucus, fluid, colour, consistency, and smell) and morphology of the cervix (open, closed, colour, exudate, location, orifice morphology, and position) were observed and recorded. Following the 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, no visible or palpable structures) were also recorded. AIs were performed by 3 experienced veterinarians from the same veterinary practice. When in heat, cows and heifers were inseminated daily until transrectal confirmation of ovulation (disappearance of dominant follicle). Data regarding the diagnosis and causes of infertility or subfertility were collected and recorded.

2.3. Diagnostic criteria

2.3.1. Infertility

Freemartinism was diagnosed in heifers by finding of an abnormally large clitoris, markedly short vagina (less than 5 cm), and the absence of ovaries and/or uterus with additional history of being born a co-twin to a bull calf (5,6). The diagnosis was confirmed postmortem at the local slaughterhouse.

According to Parkinson (2), cervix duplex was diagnosed by vaginoscopy and ultrasound findings of 2 cervical orifices and 2 corresponding distinct cervical canals. The diagnosis was confirmed postmortem at the local slaughterhouse.

Aplastic uterus in heifers was diagnosed due to the absence of uterine body and/or horns and finding of small ovaries that seem to “float” in the abdomen without any presence or connection to other reproductive organs (7,8). According to Peter et al. (9), aplastic/hypoplastic ovaries were diagnosed based on the complete absence or finding of very small ovaries. The diagnosis was confirmed postmortem at the local slaughterhouse.

Extrauterine adhesions (perimetritis fibrosa) were diagnosed based on the findings of fibrous connective tissue attachments between uterus and adjacent organs

(ovaries, rumen, urinary bladder, and rectum). History data revealed that these animals suffered from the retention of foetal membranes and/or were treated with an unknown amount of irritating solutions (e.g., 3% solution of Lotagen, Schering-Plough Animal Health, in distilled water) during the early puerperium. These animals were treated with parenteral antibiotics and a uterine flush done by veterinarians from other practices or by owners as instructed by veterinarians.

According to Zemjanis (10), periovarian adhesions (peribursitis fibrosa) were diagnosed based on the findings of firm fibrous attachments between the ovarian bursa and ovaries, uterus, and/or urinary bladder. In these cases, during rectal palpation, the ovaries were found fixed in place, without the possibility of any movement or an opportunity for a proper palpation (2). The oestrous cycle in these animals was present at regular intervals (17 to 23 days), but inseminations were unsuccessful, and animals were culled due to the infertility. The diagnosis was also confirmed postmortem at the local slaughterhouse.

2.3.2. Subfertility

According to the reports of Lagerlöf and Boyd (7), Zemjanis (10), and Peter (11) that provided descriptive guidelines for diagnosing anoestrus, we confirmed anoestrus for all cows/heifers presented with smooth, round to oval, and small ovaries (size of a pea) that failed to come into heat by 100 days postpartum or 15 months of age. Animals were examined in 20- to 30-day periods. Ovaries had no palpable structures and ultrasound examination showed only scars. Heifers diagnosed with anoestrus were eventually culled due to infertility. The findings were confirmed postmortem at the local slaughterhouse.

Anovulatory oestrus is typically associated with a rare finding of a Graafian follicle that does not regress, but becomes luteinised after reaching a maximum size of 2 to 2.5 cm in diameter. This structure then functions as a corpus luteum that regresses before or after 17 to 18 days of the oestrus cycle, and the cow returns to oestrus in a shorter period than expected (12). In these cases a diagnosis of anovulatory oestrus can only be made retrospectively by finding a follicle that persists longer than expected (11). Similarly, anovulatory oestrus was confirmed by ultrasonography and transrectal palpation after finding the follicle of unchanged morphology on a given ovary 7 days after the onset of oestrus and/or AI.

According to Peter (13), an ovarian cyst is defined as an anovulatory fluid-filled follicle-like structure, usually more than 24 mm in diameter, that persists for 7 to 10 days with the absence of corpus luteum. For the purpose of the study, cystic ovarian disease (COD) was diagnosed when 1 or more fluid-filled structures were found on ovaries unilaterally or bilaterally measuring more than 3 cm in diameter and persisting for 10 or more days with

the absence of corpus luteum (13–18). The diameter was estimated by transrectal palpation and measured using ultrasonography. For the purposes of this study, ovarian cysts were not further classified as luteal and follicular cysts.

According to Trimberger (19) and Walker et al. (20), duration of normal oestrus in cows is between 2.5 and 18 h and onset of ovulation occurs approximately 28 to 32 h after the onset of oestrus. Furthermore, Roelofs et al. (21) reported that average duration of oestrus is 18 h with ovulation occurring 30 h after the onset of oestrus, or 10 to 15 h after the end of clinical signs of oestrus. According to Van Rensburg and de Vos (22) and Zobel et al. (23), delayed ovulation is considered in instances when the ovulation occurs 3 to 7 days after the onset of oestrus. Based on these data, dairy managers observed animals as previously described. Delayed ovulation in this study was diagnosed for cows/heifers when ovulation occurred 48 h or later after the first signs of oestrus. Ultrasound examination of ovaries in these cases was performed daily for up to 7 consecutive days in order to determine the exact location of a dominant follicle and to confirm ovulation. If a cow ovulated by day 7, it was diagnosed with a delayed ovulation. However, if animal failed to ovulate by this time, it was diagnosed with anovulatory oestrus.

Repeat breeder syndrome (RBS) is considered in cows that fail to conceive after 3 to 4 services/inseminations with a confirmed ovulation. As reported by Parkinson (2) and Allen (24), these cows typically exhibit normal signs of oestrus every 18 to 24 days, and they return to service repeatedly after being bred to a fertile male or after AI. In our study, RBS was diagnosed in cows/heifers that failed to conceive after the fourth AI with confirmed ovulation and were rebred within 18 to 23 days. RBS diagnosis was further confirmed by the lack of reproductive organ pathologies and negative diagnostic tests for bovine diseases (see above).

Despite its substantial impact on reproductive performance, controversy exists concerning case definition and treatment options of endometritis in cattle. In addition, different diagnostic techniques (transrectal uterine palpation, vaginoscopy, examination of uterine content, uterine biopsy, and uterine cytology) as well as different postpartum sampling times contribute to reported variations and scientific controversies reported by Kinsel (25), Studer and Morrow (26), LeBlanc et al. (27), Bretzlaff (28), and Bonnett et al. (29). In our study, endometritis was diagnosed based on the findings of cloudy viscous mucus or pasty (purulent) exudates in the vagina or cervix during vaginoscopy, and/or by ultrasound findings of a thickened uterine wall with fluid filled lumen during oestrus or metestrus. Other criteria also included a cervix size of >7 cm, uterine diameter of 5 to 8 cm, and a purulent

or mucopurulent discharge visible through the speculum (28,29). In severe cases discharge was visible at the level of the anterior vagina, spread over the tail, or present on the skin of hind limbs. In these cases, endometritis was confirmed by ultrasonography. As reported by Bretzlaff (28) and Bonnett et al. (29), cows considered healthy (sound) had a cervix size of <7 cm, uterine diameter of <5 cm, and no abnormal discharge or fluid in the uterine lumen. Based on these criteria, cows were placed in 2 groups: cows with endometritis and sound cows.

2.4. Statistical analysis

Data were analysed by Statistica 8 software (StatSoft Inc., Tulsa, OK, USA). Models were created with a multivariate logistic regression. All data were collected and processed initially by the Kolmogorov–Smirnov test for the variable groups. Infertility and subfertility were studied by means of 2 statistical analyses: frequencies were compared by means of a chi-square test using a variable (diagnosis) for the study, and regression study was performed by linear regression between the percentages of different diagnoses. Due to a nonregular distribution of data, the chi-square test

was used and set on the level of the statistical significance $P < 0.05$.

3. Results

Infertility and subfertility were diagnosed in 56.61% of all animals in the study (Table 1). All diagnoses of infertility occurred in less than 1% of the 12,060 animals across all 5 years of the study. The most common cause of infertility was peribursitis fibrosa (0.41%), which varied by year (Table 2), being lowest in 2007 (0.19%) and highest in 2005 (0.85%). The second most common cause of infertility was perimetritis fibrosa, which averaged 0.15% across all 5 years of the study and showed similar year-to-year variation as peribursitis fibrosa.

Freemartinism, cervix duplex, aplasia of the uterine horn, and aplasia of the ovary all occurred at low frequencies, averaging 0.02% or less across all 5 years of the study.

Causes of subfertility averaged 55.91% for all diagnoses reported (Table 1). Anovulatory oestrus, delayed ovulation, and endometritis represented the most common causes

Table 1. Summarised causes of infertility and subfertility in Simmental cows over a 5-year study in Central Croatia.

Diagnosis	No. of animals	%
	(n = 12,060)	
F	3 ^a	0.02
C D	1 ^a	0.01
A U	2 ^a	0.02
A O	9 ^a	0.02
Perim F	19 ^a	0.15
Perib F	50 ^a	0.41
Subtotal -infertility	84 ¹	0.69
A E	2199 ^a	18.23
COD	508 ^b	4.21
D O	2214 ^a	18.36
RBS	556 ^b	4.61
AnE	369 ^b	3.06
E	897 ^c	7.44
Subtotal -subfertility	6743 ²	55.91
Total	6827	56.61

Legend: F = Freemartinism; C D = cervix duplex; A U = aplastic uterus; A O = aplastic ovary; Perim F = perimetritis fibrosa; Perib F = peribursitis fibrosa; A E = anovulatory oestrus; COD = cystic ovarian disease; D O = delayed ovulation; RBS = repeat breeder syndrome; AnE = anoestrus; E = endometritis; grey shading = causes of infertility; no shading = causes of subfertility; P = level of statistical significance.

^{a, b, c}: Values with different superscripted letters within a category (subtotal infertility and subtotal subfertility) differ significantly ($P < 0.05$).

^{1, 2}: Values in the rows “Subtotal - infertility” and “Subtotal - subfertility” differ significantly ($P < 0.05$).

Table 2. Causes of infertility and subfertility per year in Simmental cows of Central Croatia.

Diagnosis	2004, n = 1989	%	2005, n = 2118	%	2006, n = 2298	%	2007, n = 2666	%	2008, n = 2989	%
F	1 ^a	0.05	0 ^a	0.00	2 ^a	0.08	0 ^a	0.00	0 ^a	0.00
CD	0 ^a	0.00	1 ^a	0.04	0 ^a	0.00	0 ^a	0.00	0 ^a	0.00
AU	1 ^a	0.05	1 ^a	0.04	0 ^a	0.00	0 ^a	0.00	0 ^a	0.00
AO	3 ^a	0.15	1 ^a	0.04	0 ^a	0.00	2 ^a	0.07	3 ^a	0.10
Perim F	3 ^a	0.15	8 ^a	0.38	0 ^a	0.00	3 ^a	0.11	5 ^a	0.16
Perib F	6 ^a	0.30	18 ^b	0.85	12 ^a	0.52	5 ^a	0.19	9 ^a	0.30
A E	159 ^a	7.99	317 ^b	14.96	476 ^c	20.71	580 ^c	21.75	667 ^c	22.31
COD	151 ^a	7.59	77 ^b	3.63	101 ^b	4.39	85 ^b	3.19	94 ^b	3.14
D O	107 ^a	5.38	412 ^b	19.45	481 ^b	20.93	548 ^b	20.55	666 ^b	22.28
RBS	99 ^a	4.97	113 ^a	5.33	90 ^a	3.92	98 ^a	3.67	156 ^a	5.22
AnE	140 ^a	7.03	56 ^b	2.64	56 ^b	2.44	62 ^b	2.32	55 ^b	1.84
E	41 ^a	2.06	102 ^b	4.81	190 ^b	8.27	243 ^c	9.11	321 ^c	10.74
Total	711 ^a	35.75	1106 ^b	52.22	1408 ^c	61.27	1626 ^c	60.99	1976 ^c	66.10

Legend: F = Freemartinism; CD = cervix duplex; AU = aplastic uterus; AO = aplastic ovary; Perim F = perimetritis fibrosa; Perib F = peribursitis fibrosa; A E = anovulatory oestrus; COD = cystic ovarian disease; D O = delayed ovulation; RBS = repeat breeder syndrome; AnE = anoestrus; E = endometritis; n = number of examined animals in each year; Total = number of animals with infertility and subfertility diagnosis for each year during the study period with expressed percentages; grey shading = causes of infertility; no shading = causes of subfertility.

^{a,b,c}: Values within a row marked with different superscripted letters differ significantly ($P < 0.05$).

of subfertility and showed an increasing trend over time (Figure). COD (4.21%), RBS (4.61%), and anoestrus (3.06%) occurred at lower frequencies. The frequency of RBS remained relatively constant over all 5 years, whereas COD and anoestrus declined from 2004 to 2008.

While the causes of infertility remained constant, subfertility significantly increased over time. Three categories of subfertility, anovulatory oestrus, delayed ovulation, and endometritis, were notably increased over the 5-year period as presented in the Figure. This trend showed a statistically significant ($P < 0.05$) cumulative increase from 15.43% in 2004 to 55.33% in 2008.

4. Discussion

Subfertility averaged 55.91% over the 5 years of the study and showed an increasing trend over time. Chagas et al. (30) reported declining fertility in dairy cattle worldwide and attributed the decline to single trait selection for milk production. The cows in this study averaged 4448 kg annual milk production, which is in concordance with the national average of 4459 kg reported by Perišić et al. (31) for Simmental cows in Croatia. Our data also confirm subfertility as the second most common reason for culling in Croatian dairy cows, right after mastitis, as previously reported by Zobel et al. (32). It is interesting to observe that these data concerning declining fertility in Simmental

cows of this region with moderate milk yield and of dual purpose follow the worldwide trend recognised in breeds with high milk yield.

Anovulatory oestrus and delayed ovulation were the 2 most common causes of subfertility in our study and increased dramatically over the 5 years. These findings may well represent a disturbance in the hypothalamic-pituitary-ovarian axis or in the somatotrophic axis, as suggested by Chagas et al. (30), and may represent an antagonism between milk production and reproductive performance. Ovarian follicles in cows diagnosed with anovulatory oestrus persisted for 7 to 10 days after the onset of oestrus, based on previously established criteria by Watson and Harwood (12). Delayed ovulation was, in our study, more frequent and more important as a cause of subfertility in comparison to previous reports in dairy cattle from the Netherlands, as reported by Van Rensburg and de Vos (22) and Roelofs et al. (21). Data previously reported by Zobel et al. (23) showed a delayed ovulation within 48 to 72 h in 35% of 1088 observed cows, ovulation within 72 to 96 h in 3.68% cows, and ovulation within 96 to 108 h after the onset of oestrus in 3.11% of cows in the Moslavina region of Croatia. Findings in the present research are in accordance with the previous ones, suggesting that delayed ovulation is not a sporadic occurrence, but is becoming a rather serious problem for the fertility in the

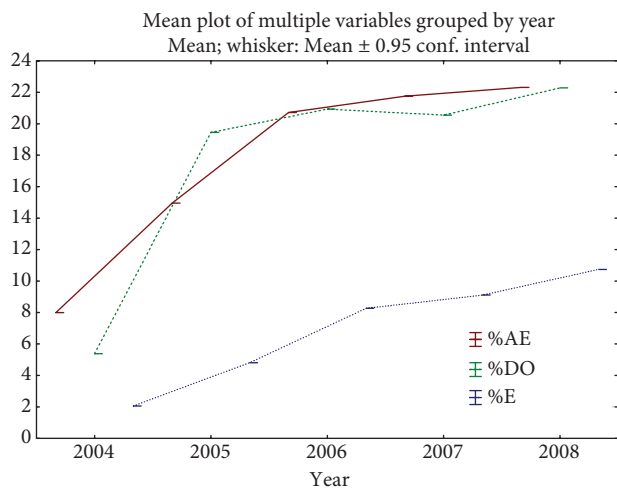


Figure. Subfertility associated with anovulatory oestrus, delayed ovulation, and endometritis in Simmental cows in Central Croatia. **Legend:** AE = Anovulatory oestrus; DO = delayed ovulation; E = endometritis.

Simmental dairy cows in Central Croatia. These findings also suggest a need for frequent rectal examinations in cows as they are coming back to oestrus after AI and a need for daily inseminations until the transrectal detection of ovulation is confirmed. In this aspect, we suggest that the single insemination (1-day insemination) will be soon considered a past trend in the dairy cattle reproduction practices in this area. Cows should be reexamined the day following AI in order to confirm the onset or failure of ovulation, especially in the rebreeding cows/heifers. As suggested, it is possible that genetic selection for higher milk or meat production may lead to the lower fertility via disturbance in the somatotrophic axis.

Endometritis was the third most common cause of subfertility, present in almost 1/10 of observed animals and with a steady increase over the 5 years of study. This is in accordance with reports of LeBlanc et al. (27) with endometritis in 15.3% of animals, as well as with the results of Gautam et al. (33) and Markusfeld (34), but in the contrast to those of Kim and Kang (35). It can be speculated that management, including feeding regimen, milk yield, and inheritance, are factors contributing to the increased incidence of endometritis, as proposed by Opsomer et al. (36) and Bruun et al. (37).

Our findings indicate peribursitis fibrosa and perimetritis fibrosa as the most common causes of infertility, most probably associated with traumatic events during previous dystocia, aggressive treatments for endometritis, and retained placenta during early puerperium in these cows. This hypothesis is supported by previous reports of Parkinson (2) that the uterine irrigation with irritating therapeutic and nontherapeutic solutions can cause severe damage in the uterine wall and

other portions of the genital tract, causing tissue necrosis, ulceration, and leakage of the solution in the surrounding tissues, resulting in a strong inflammatory response in the affected areas. Medical records containing detailed diagnostic and treatment history for these cows were not available since the procedures were performed by other veterinary practitioners. However, since Lotagen solution is recognised as an aggressive irrigation substance, widely used in Croatia for treatment of endometritis and retained placenta, our findings suggest that use of Lotagen as a treatment option for in utero irrigation should be avoided.

Aplastic uterus and freemartinism were found only in a small number of animals and are not considered as a significant cause of infertility in our region.

One of the heifers included in the study was diagnosed with cervix duplex and gave birth to a live calf without assistance, as also reported by Gračner et al. (38). Parkinson (2) reported earlier that animals are able to conceive and give birth in spite of cervix duplex, but usually there are medical problems during the parturition, and these cows should be removed from the breeding program. In addition, the condition is hereditary, thus another reason for removing them from the breeding program.

Low prevalence of freemartinism is probably a result of a good cattle breeder's education, since almost all female calves twinned with a bull calf were sold to the slaughterhouse without veterinary intervention. Our findings regarding aplastic ovaries as a cause of infertility are consistent with the reports of Lagerlöf and Boyd (7) in Swedish Highland cattle.

Contrary to the previous reports by López-Gatius (16) and López-Gatius et al. (17,18) of COD as a common disorder and a significant cause of reproductive failure in dairy cows in Spain, COD is in the fifth place as a cause of subfertility in cows from Central Croatia and has decreased 2-fold over a 5-year period. We speculate that the mentioned differences can be attributed to the breed and milk yield since studies in Spain included the high-producing Holstein-Friesian breed while our study included cows of the Simmental breed of dual purpose with lower milk production. Hereditary factors should also be taken into consideration regarding the fertility issues among different breeds.

As reported by Zemjanis (10) and Gustafsson and Emanuelson (39), RBS is defined in cows as a failure to conceive in 3 or more regularly spaced services/AIs with the absence of detectable clinical abnormalities, and it represents a significant loss for the dairy industry worldwide. RBS was found consistently in 5% of observed animals in our study. Previous reports by Zemjanis (10) and Bartlett et al. (40) indicated that RBS was diagnosed in 10.1% of Swedish dairy cattle and in 24% of Holstein-

Friesian dairy cows in the United States. These both represent much higher percentages when compared to our data and the trends may be attributed to genetic diversity, geographical location, and different management practices.

Anoestrus is the last on our list of causes for subfertility, and it had showed a 3-fold decrease at the end of the study. Its prevalence in our region is similar to the trends in Spain as presented by López-Gatius (16), most probably due to the changes in feeding regimen, management, and genetic improvement in dairy cattle, as suggested by Peter et al. (41).

In summary, subfertility and infertility diagnoses combined show an increasing trend over the 5 years in our field study. At the same time, the number of animals diagnosed with subfertility was also on a rise, with almost a 2-fold increase by the end of the study.

The number of cows with anovulatory oestrus almost doubled over a course of 5 years, the number of cows with delayed ovulation increased 4 times, and the number of cows with endometritis increased 5 times. The number of animals with RBS remained almost constant throughout the study. On the other hand, a positive trend was recognised in the number of animals diagnosed with COD and anoestrus that presented with a constant decline over the 5-year study period and a significant reduction in total numbers at the end of the study. These data suggest a need for a more thorough clinical approach to the diagnosis, treatment, and use of animals diagnosed with subfertility, since this problem has a direct economic impact on dairy industry.

Finally, our research shows an increasing trend in both infertility and subfertility. True infertility was present in 0.63% of all animals, and, as expected, it represents a small and insignificant segment of the fertility problems in general. However, within this category, perimetritis and peribursitis fibrosa were present in higher numbers, suggesting the need for a change in veterinary practices when it comes to treatment options for complications during puerperium that are considered in etiology for these pathological findings. Subfertility showed an almost 2-fold overall increase by the end of our 5-year study, with anovulatory oestrus, delayed ovulation, and endometritis recognised as main underlying causes associated with this trend. The number (percentage) of animals with RBS remained almost constant throughout the study, but the number of animals presented with COD and anoestrus declined.

This almost double increase in subfertility during the 5-year study represents an alarming trend. This trend is suggesting that relative to cost-effectiveness of dairy operations in Central Croatia, subfertility is becoming, along with mastitis, the most important consideration for culling. Veterinary expertise plays a key role in diagnosis and decision-making strategy in Croatian dairy industry. Therefore, we recommend a wide-scale intervention program that should include a thorough scientific, educational, and regulatory approach to fertility in Simmental dairy cows in Croatia, with the emphasis on client education and good relationships between dairy industry and veterinary practice (including academia).

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