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Effect of Ovarian Status on Reproductive Performance in Dairy Heifers Inseminated with Sexed Semen

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ABSTRACT

***Background*:** The intensive reproductive management in the dairy farms requires inclusion of a large group of replacement heifers in the breeding program for a shorter period. In this aspect, a creation of effective estrus synchronization protocols with timed artificial insemination (TAI) by sexed semen and optimization of the current ones have a crucial role for obtaining high pregnancy rate. These protocols are beneficial, because they led to reduced interval to first AI, lack of need for estrus detection, and allow obtaining a large group of female calves for on time. Because of limited fertilizing potential of sexed spermatozoa this type of semen is applied mainly for heifers, as fertility is higher compared to lactating cows. The main objective of the present study was to investigate the effect of ovarian status on the reproductive performance in dairy heifers subjected to estrus synchronization and timed artificial insemination with sexed semen.

***Materials, Methods & Results*:** Forty-eight Holstein healthy heifers separated in 2 groups were subjected to PGF2α-GnRH pre-treatment and Ovsynch or PRID-5-day estrus synchronization protocol and timed artificial insemination (TAI) with sex-sorted semen, staring 6 days after end of the hormonal pre-treatment. The ovarian status (presence of follicles with or not a corpus luteum) of the heifers at the begin of the treatment, on day of TAI and pregnancy rate in different groups were determined and compared. Additionally, the ovarian status at the begin of the treatment, ovarian status and size of preovulatory follicle (PF) on day of TAI and total values for both groups according to reproductive performance (pregnant or non-pregnant) were also analyzed. On day of TAI the animals with PF and a lack of corpus luteum (CL) in both groups were more that those with PF and CL (39.3% and 30% vs. 60.7 % and 70%), with significant (P < 0.05) difference in PRID-5-day group. The pregnancy rate tended to be higher in PRID-5 day than Ovsynch treatment (65% vs. 35.7%). A higher percentage (100% and 67.9%) of the pregnant animals in both treatments had not CL on day of TAI, and the size of the PF (1.58 ± 0.12 cm and 1.64 ± 0.13 cm) was increased (P < 0.05). Similar effects of the ovarian status on reproductive performance were obtained after a comparison of the total values between pregnant and non-pregnant animals. The percentage of heifers with observed PF without CL on day of TAI was significantly greater (P < 0.05) in pregnant compared to non-pregnant group (91.3% vs. 40%). The opposite dependence was determined for the parameter presence of follicles and corpus luteum (8.7% vs. 60%; P < 0.05). Furthermore, the size of the PF measured immediately before TAI was increased in animals became pregnant (1.60 ± 0.12 cm vs. 1.34 ± 0.17 cm; P < 0.05).

***Discussion*:** The analysis of the obtained results showed that the ovarian status on day of TAI affects the reproductive performance in dairy heifers subjected to estrus synchronization and timed artificial insemination with sexed semen. Hormonal pre-treatment with onset of PRID-5-day protocol 6 days later and TAI with sex-sorted semen ensure acceptable pregnancy rate. The greater preovulatory follicle and a lack of corpus luteum before insemination provide significantly (P < 0.05) more pregnant animals, compared to the cases when CL is presented. Ultrasound determination of the ovarian function before insemination can be used in selection of heifers for TAI with sex-sorted semen.

Keywords: heifers; estrus synchronization; sexed semen, pregnancy.

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INTRODUCTION

The effective dairy farm management is associated with optimization of reproductive process and fast production of replacement heifers [15,19,22]. Estrus synchronization and artificial insemination with sexed semen are very important tools for achieving better reproductive performance [5,13,26]. Because of limited fertilising potential of sexed spermatozoa this type of semen is applied mainly for heifers, as fertility is higher compared to lactating cows [8,31]. The economic benefit and the genetic gain are greater, due to obtaining of replacement heifers from genetically superior animals [9,32]. A creation of effective estrus synchronization protocols with timed artificial insemination (TAI) by sexed semen and optimization of the current ones have a crucial role for obtaining high pregnancy rate [11,17]. Additionally, these protocols are beneficial, because they led to reduced interval to first AI, lack of need for estrus detection, and allow obtaining a large group of female calves for on time [27]. Different studies [5-7,17] report various pregnancy rate after estrus synchronization and TAI with sexed semen in dairy heifers. The ovarian responses of dairy heifers to GnRH given at different stages of the estrous cycle were similar to those observed in lactating dairy cows [20], but information about the association between stage of the estrous cycle at the start of the protocol and pregnancy rate in dairy heifers is still debatable.

The aim of the present study was to evaluate the effect of the ovarian status on the reproductive performance in dairy heifers subjected to different estrus synchronization treatments and timed artificial insemination with sexed semen.

MATERIALS AND METHODS

Animals

This study was carried out in a commercial dairy farm in Bulgaria, located at latitude of 42.183 N, longitude 25.567 E. The experimental animals included 48 clinically healthy Holstein heifers between 18 and 21 months of age, weighing 380-400 kg, housed in free-stalls with free access to water, and fed a total mixed ration. The daily diet comprised corn silage (14 kg), Triticale haylage (10 kg), straw (4 kg), soybean meal 46% (1.3 kg) medium-coarse grain soft wheat (0.7 kg), corn grain (0.5 kg), mineral and vitamin supplement (0.02 kg) and a natrium chloride (0.03 kg). The investigation was conducted between June and August. All procedures were conducted in accordance with the guidelines for welfare and animals protection showed in Bulgarian legislation.

Experimental design

Initially, a reproductive ultrasound examination was performed and ovarian status of the heifers was registered. It was characterized through detection of small and medium follicles with corpus luteum (CL) or only follicles (small, medium and large). A transrectal ultrasonography was done by ultrasound scanner SonoScape S2 Vet[1] and multifrequency (7-12 MHz) linear transducer. The evaluation of follicular size (the largest diameter in cm) was measured using the in-built scale provided with the ultrasound.

The animals were divided into 2 groups according to the ovarian status and the hormonal treatments. The estrus synchronization protocols were designed to start on day 6-7 of the estrous cycle by hormonal pre-treatment (Figure 1). For this purpose, all animals were primary treated with PGF2α [Enzaprost T - 25 mg dinoprost tromethamine, 5 mL, i.m.][2] on day of the first ultrasound and GnRH [Ovarelin - 100 µg gonadorelin as diacetate, 2 mL, i.m.][2] 2 days later. Day 6th after the end of the hormonal pre-treatment was accepted as Day 0 of the estrus synchronization protocols. The first group was subjected to Ovsynch treatment including 100 µg GnRH on days 0 and 9 and 25 mg PGF2α on day 7. The artificial insemination was 18 h after the last GnRH injection. The heifers in the second group were enrolled in PRID-5-day protocol. Progesterone releasing intravaginal device [PRID Delta - containing 1.55 g progesterone][2] was inserted into the vagina on day 0, followed by intramuscular administration of 100 µg GnRH. PRID removal with 25 mg PGF2α injection were made 5 days later. The second prostaglandin was placed 24 h after the first one. Seventy-two h after PRID withdrawal, the heifers were injected again with 100 µg GnRH and artificially inseminated.

Ultrasonographic evaluation of the ovarian status and measurement of the size of the largest follicle (preovulatory follicle - PF) were carried out immediately before insemination. Timed artificial insemination of all animals was performed by experienced operator, as a frozen sex-sorted semen in straw of 0.25 cm^3 form the same bull with proven fertility was deposited deeply inside the cervix. Pregnancy check was made by transrectal ultrasonography 30 days after the TAI and pregnancy rate was calculated. A positive pregnancy diagnosis was given in clear visualization of an embryo located into fluid-filled uterine lumen.

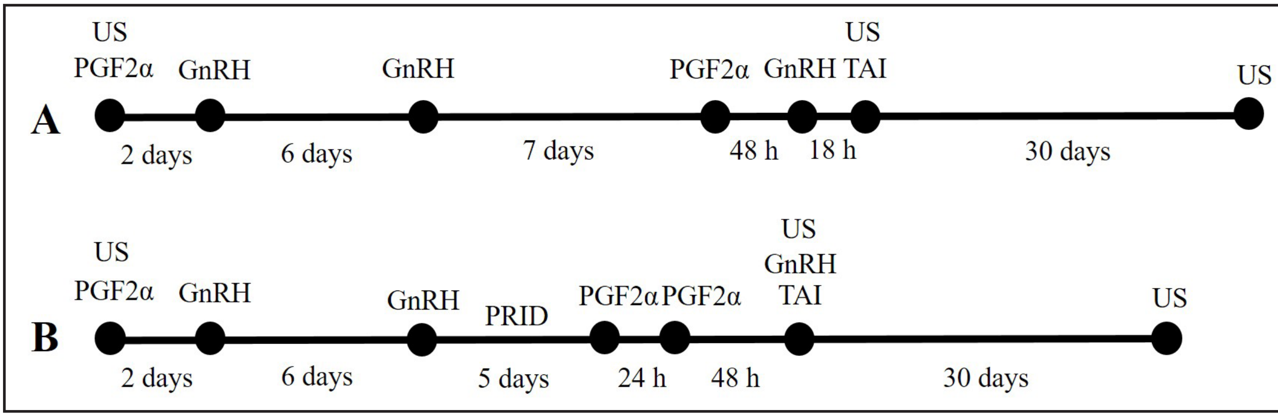

Figure 1. Schematic diagram of hormonal pre-treatment, Ovsynch (A) and PRID-5 day (B) estrus synchronization protocols and all activities in Holstein dairy heifers [US - ultrasound examination; PGF2α - 25 mg i.m. of dinoprost tromethamine; GnRH - 100 µg i.m. gonadorelin as diacetate; PRID - progesterone releasing intravaginal device with 1.55 g progesterone; TAI - timed artificial insemination with sex-sorted semen].

Statistical analysis

The percentages of heifers bearing follicles with or not presence of a corpus luteum at the begin of the treatment, on day of TAI and pregnancy rate between groups with different hormonal treatments were subjected to comparative analysis. In addition, the parameters ovarian status of heifers at the begin of the treatment, ovarian status and size of preovulatory follicle (mean ± standard deviation) on day of TAI and total values for both groups according to reproductive performance (non-pregnant or pregnant) were also compared.

The results were processed by a computer program Statistica version 7.0 (Stat-Soft. - 1984-2000)[3]. The values of the parameters between different groups were compared by non-parametric analysis for comparison of proportions and mean values, using Student's t-criterion. Differences were considered significant at the $P < 0.05$ level.

RESULTS

During the first ultrasonographic examination the most animals had small and medium follicles with corpus luteum located in 1 of the ovaries (78.6 % for Ovsynch and 70% for PRID-5-day group) [Table 1]. Follicles without presence of corpus luteum (Figure1B) were registered in 21.4% and 30% of the animals for first and second group, respectively. The significant differences ($P < 0.05$) between a percentage of the animals with different ovarian status within the groups and insignificant differences between the groups were indicators for uniformly distribution of the heifer according this parameter at the begin of the hormonal pre-treatment. However, the ovarian status on the day of TAI was different. A preovulatory follicle with visible corpus luteum was determined in a low percent of animals (39.3% for Ovsynch and 30% for PRID-5 day group; Figure 2A), while a preovulatory follicle without luteal structure (Figure 2B) was recorded in 60.7 % and 70% of the cases. This result was clearly expressed in PRID-5-day group, where the percentage of heifers without presence of CL on day of TAI were increased ($P < 0.05$). Nevertheless, the values for both evaluated parameters in different groups were close. The pregnancy rate (65%) in the heifers treated with PRID-5-day protocol was higher compared to this (35.7%) after Ovsynch treatment, but not differed significantly.

The comparative analysis of the reproductive performance after booth hormonal treatments (Table 2) showed, that the values for non-pregnant and pregnant heifers presenting follicles with CL or only follicles at the begin of the treatment are close. In both group, the cases with detection of CL were more (72.2% and 90% vs. 27.8% and 10% in Ovsynch; 71.4% and 69.2% vs. 28.6% and 30.8% PRID-5 day). A significance ($P < 0.05$) was recorded in the Ovsynch group.

On day of TAI, a preovulatory follicle without CL was presented in 100% and 67.9% of the heifers became pregnant in first and second group, respectively. A significant ($P < 0.05$) differences in the ovarian status between non-pregnant and pregnant group was registered in Ovsynch treatment. In PRID-5-day treatment only 23.1% of the animals with registered corpus luteum on day of TAI were pregnant. The average sizes of PFs were significant ($P < 0.05$) greater in pregnant (1.58 ± 0.12 cm and 1.64 ± 0.13 cm) than non-pregnant (1.32 ± 0.17 mm and 1.37 ± 0.19 mm) heifers.

The data from analysis of the total values between non-pregnant and pregnant animals was indicative for a similarity of the ovarian status at the begin of the experiment. In the contrary, the ovarian status of the groups on day of TAI was totally different. The percentage of heifers with observed PF without CL. on day of TAI was significantly greater ($P < 0.05$) in pregnant compared to non-pregnant group (91.3% vs. 40%). The opposite dependence was determined for the parameter presence of follicles and corpus luteum (8.7% vs. 60%; $P < 0.05$). Furthermore, the size of the preovulatory follicles measured immediately before TAI was increased in animals became pregnant (1.60 ± 0.12 cm vs. 1.34 ± 0.17 cm; $P < 0.05$).

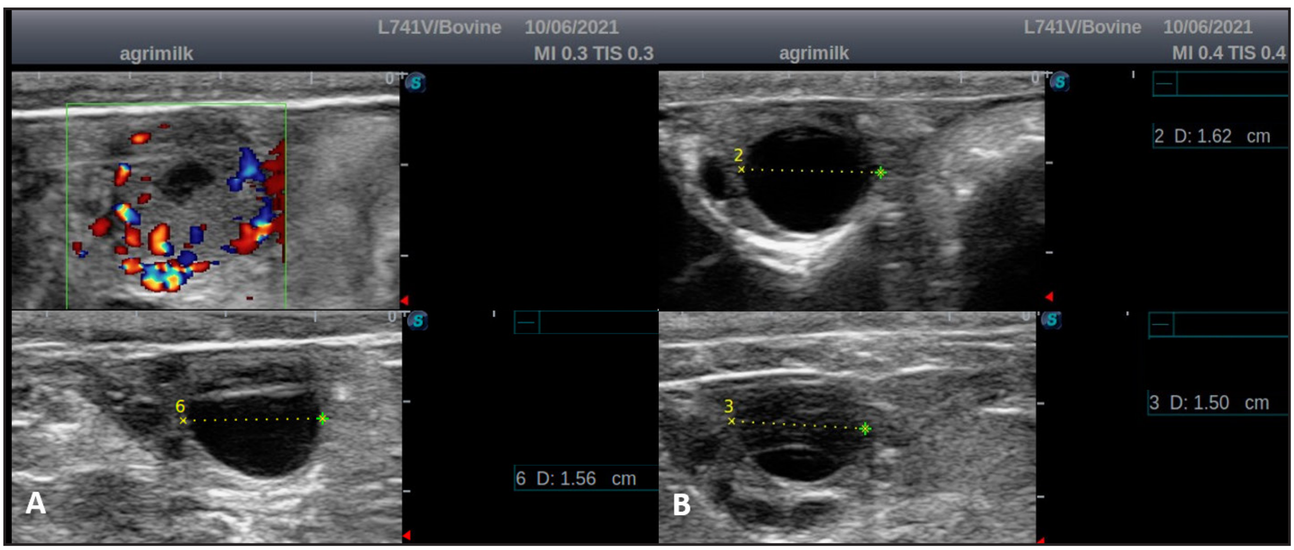

Figure 2. Ultrasound image of the ovarian status in heifers on day of TAI. A- Follicles with corpus luteum located in one of the ovaries. B- Preovulatory follicles without corpus luteum.

Table 1. Ovarian status at the begin of the treatment, on day of TAI and pregnancy rate in different groups according to used estrus synchronization protocol.

Parameters	Ovsynch group (n = 28)	PRID-5 group (n = 20)
	Treatments % (n)	
Follicles with corpus luteum	78.6 (22)[1]	70 (14)[1]
Follicles without corpus luteum	21.4 (6)[2]	30 (6)[2]
Ovarian status on day of TAI		
Preovulatory follicle with corpus luteum	39.3 (11)	30 (6)[1]
Preovulatory follicle without corpus luteum	60.7 (17)	70 (14)[2]
Pregnancy rate	35.7 (10)	65 (13)

The values in a column marked with different number differ each other at $P < 0.05$.

Table 2. Ovarian status at the begin of the treatment and on day of AI, size of preovulatory follicle (mean ± SD and total values in different groups according to reproductive performance.

Parameters	Ovsynch group (n = 28)		PRID-5 group (n = 20)	
	Non pregnant (n = 18)	Pregnant (n = 10)	Non pregnant (n = 7)	Pregnant (n = 13)
	Treatments % (n)			
Follicles with corpus luteum	72.2 (13)^{1}	90 (9)^{1}	71.4 (5)	69.2 (9)
Follicles without corpus luteum	27.8 (5)^{2}	10 (1)^{2}	28.6 (2)	30.8 (4)
Ovarian status on day of TAI				
Preovulatory follicle with corpus luteum	61.1 (11)^{a}	0 (0)^{1b}	57.1 (4)	23.1 (2)
Preovulatory follicle without corpus luteum	38.9 (7)^{a}	100 (10)^{2b}	42.9 (3)	67.9 (11)
Size of preovulatory follicle (cm)	1.32 ± 0.17^{a}	1.58 ± 0.12^{b}	1.37 ± 0.19^{a}	1.64 ± 0.13^{b}

	Total for both groups	
	Non pregnant (n = 25)	Pregnant (n = 23)
Parameters	Treatments % (n)	
Follicles with corpus luteum	72 (18)^{1}	78.3 (18)^{1}
Follicles without corpus luteum	28 (7)^{2}	21.7 (5)^{2}
Ovarian status on day of TAI		
Preovulatory follicle with corpus luteum	60 (15)^{a}	8.7 (2)$^{1\,b}$
Preovulatory follicle without corpus luteum	40 (10)^{a}	91.3 (21)^{2b}
Size of preovulatory follicle (cm)	1.34 ± 0.17^{a}	1.60 ± 0.12^{b}

The values in a column marked with different number differ each other at $P < 0.05$. The values in a row marked with different superscript differ each other at $P < 0.05$.

DISCUSSION

The estrus synchronization and the artificial insemination with sexed semen are successfully introduced for improvement of reproduction in dairy herds, but the obtained results are rather various. Mean reasons for less than expected reproductive performance after AI with sexed semen are inadequate estrus detection with artificial insemination out of the optimal period and number of sorted spermatozoa per inseminating dose [4,31]. It is widely accepted that the unsatisfactory pregnancy rate after using of sex-sorted semen may not be overcome by simply increasing the number of spermatozoa per dose [8,33]. In large dairy farms, these problems can be partly reduced by implementation of effective estrus synchronization programs with timed artificial insemination. Many authors report to an influence of different factors (type of protocols and used hormones, start of the treatments, ovarian function at the begin of protocols, age and body condition score of the animals) on the pregnancy results [5,6,26,34,35]. Regardless of all, this topic is still obscure and the investigations are in progress.

The current study reveals an information about effect of the ovarian status on the reproductive performance of heifers after using of Ovsynch and PRID-5-day estrus synchronization protocols and artificial insemination with sexed semen in a fixed time. An evidence for uniformly distribution of animals with different ovarian status before the start of the experiment was the lack of the significant difference in the percentage of animals with follicles with or not CL between the groups. The prostaglandin injection in hormonal pre-treatment aimed to induce luteolysis of the luteal structure available into the ovaries and to stimulate follicular growth. Administration of PGF2α during the luteal phase of the estrous cycle leads to estrus expression with or not ovulation, usually from day 2 to 5 days after treatment [14,18]. Because of that, GnRH injection is needed to induce synchronized follicular wave emergence within 1.6 to 2.5 d after injection [24,30]. Treatment with GnRH ensures increased magnitude of LH surges and decreased intervals to ovulation and animals can ovulate ≤ 30 h after onset of estrus [12,16]. In animals with follicles greater than 12 mm GnRH treatment may induce successful ovulation [10].

Thus, we expected that the treated animals to be on day 6 or 7 of estrus cycle at the begin of estrus synchronization protocol. A positive effect of the same hormonal pre-treatment before the start of Ovsynch protocol in lactating dairy cows was determined [1]. The correct determination of effects of ovarian status of the heifers on the success of the hormonal pre-treatment need of additional investigations.

The increased values of heifers without CL on day of TAI in both groups was as indicator for effective estrus synchronization rate of the used hormonal treatments. Despite, start of Ovsynch protocol on days 6-7 of the estrus cycle not all of them (39.3%) were with good estrus synchronization response on day of TAI. The significant difference ($P < 0.05$) in the ovarian status of the animals in PRID-5-day group was a proof that 5 days progesterone (P4) exposition, followed by two PGF2α injections 24 h apart results in effective luteolysis and more animals with preovulatory follicles able to ovulate. This confirmation was supported by the observed tendency to increase of the pregnancy rate after PRID-5-days, compared to Ovsynch treatment (65% vs. 35.7%). A possible reason for low pregnancy rate after Ovsynch treatment could be earlier or delayed ovulation after the second GnRH injection, related to fixed time of insemination. The Ovsynch protocol led to adequate synchronization of the ovulation and allows TAI without the need of heat detection in lactating dairy cows. However, the response of dairy heifers to Ovsynch and TAI is poor due to the number of follicular waves or inconsistent follicular wave emergence [21,25]. Close pregnancy rate (38%) after treatment of animals with modified the Ovsynch protocol by administering PGF2α 6 days after the first GnRH, and the second GnRH injected 48 h after PGF2α together with TAI have was recorded [28]. A significant higher pregnancy value (51.45%) than the obtained in this study was recorded in another study [7]. However, they used double Ovsynch and TAI with sexed semen in younger dairy heifers. This differences can be attributed to better pre-synchronization of their protocol and influence of the animals age.

Inclusion of progesterone releasing device into the estrus synchronization protocol suppress ovulation and heat during its presence into the vagina, thereby allows 100% submission rate for TAI without affecting fertility [29]. In this regard, other authors who are subjected the heifers to 5-days Co-synch plus PRID protocol and TAI with sex-selected semen, were reported acceptable (63.2%) pregnancy rate [6]. The effect of early or late insemination with sexed semen of the pregnancy rate were investigated in some research [5]. The authors concluded that after use of CIDR-5 day program and insemination with sex-sorted semen this parameter is not improved when insemination is delayed by approximately 12 h [5].

In agreement with all abovementioned, were the results about reproductive performance on day of TAI according to type of the used estrus synchronization protocol. Almost all pregnant animals (90%) in Ovsynch group are been cyclic (presence of a CL with a dominant follicle in 1 of the ovaries) at the start of the hormonal treatment that means they were subjected to a progesterone influence from the functional luteal structure. Their response to the pre- and Ovsynch treatments was significantly better due to a complete luteolysis and confirmed by the significant differences ($P < 0.05$) between the parameters related to the ovarian status on day of TAI. The similar situation (increased percentage of heifers without CL on day of TAI) in the PRID-5-day group, additionally supported the confirmation for close relationship between achievement of effective luteolysis in the estrus synchronization programs, follicular development and pregnancy status. It was in accordance with significantly ($P < 0.05$) greater sizes of preovulatory follicles in pregnant than non-pregnant animals for both groups on day of TAI, because in most of them CL was not recorded.

The detection of animals with a corpus luteum on day of TAI, in spite of PGF2α administration, can be explained with incomplete luteolysis. Probably, the presence of the luteal structure during this time is associated with increased progesterone level, leading to delayed growth of the preovulatory follicle with absence of ovulation in optimal time. It also can be accepted as a reason for smaller size of the preovulatory follicles at the time of insemination in the non-pregnant animals. A problem with the Ovsynch protocol is that heifers can come in heat between the first GnRH and PGF2α administration, associated with failure to synchronize ovulation in all heifers subitted to TAI protocol [28]. On the other hand, not all animals have adequate response to GnRH treatment. In one study, that authors were used 100 μg GnRH in dairy cows and determined in 95% of the cases release of LH, but only 45% were with ovulation and formation of a CL after that [10].

The same explanation can be given for increased percentage (42.9%) of the cases with CL on day of TAI in non-pregnant animals treated with progesterone based protocol. Even after the second PGF2α treatment in PRID-5-day protocol, a considerable number of cows remained with high progesterone (> 1 ng/mL) at AI and with no decrease of P4 concentration between the 2 measurements (at the time of the first PGF2α treatment and at AI) [3].

We assume, a combination of inadequate progesterone level in incomplete luteolysis on day of TAI and insufficient estrogen production from PFs with decreased size may affect ovulatory process and distribution of spermatozoa after their deposition in the uterus. A follicle diameter had a positive relationship with peak concentrations of estradiol, but only among cows that exhibited standing estrus, and estradiol increased earlier in cows that exhibited estrus compared with cows that did not [23]. In addition, the complete maturation of the PF in the heifers with observed CL on day of TAI could be temporary suppressed from higher P4 production, regardless of GnRH treatment. It is also probable reason for asynchronous ovulation and low pregnancy results, especially after insemination with sex-selected semen in a fixed time. According to some researchers, ovulatory response to GnRH treatment in heifers could be infuenced by diversity in the maturity of preovulatory follicles [2]. They found that ovulating follicles smaller than 15 mm ovulated signifcantly later than larger follicles [2].

The pregnancy registration in 23.1% of the animals in PRID-5-day group, bearing CL immediately before insemination assumed that not always observation of a luteal structure can be attributed to high progesterone level in blood circulation and failed ovulation. The total data for non-pregnant and pregnant animals in both groups was also indicative for clearly expressed effect of the ovarian status on day of TAI on the reproductive performance of animals. It was represented by the significantly ($P < 0.05$) higher percentage of pregnant heifers (91.3%) with no observed CL before insemination and larger size (1.60 ± 0.12 cm; $P < 0.05$) of their preovulatory follicle.

The final analysis indicated that application of PGF2α-GnRH pre-treatment with start of Ovsynch or PRID-5-day protocol 6 days later, can be utilize for estrus synchronization and TAI with sex-sorted semen in dairy heifers. The above mentioned hormonal treatments resulted in adequate luteolysis in more than 60% of animals on day of TAI with acceptable pregnancy rate after using of the presented PRID-5-day protocol. The ovarian status on day of TAI has significant ($P < 0.05$) effect on the pregnancy results after artificial insemination of dairy heifers with sexed semen. Animals presenting a complete luteolysis and PF with a size ≥ 1.50 cm in diameter immediately before TAI have significantly ($P < 0.05$) greater chance to get pregnant. The information in the current study can be beneficial in a selection of animals for artificial insemination with sex-selected semen by primary determination of their ovarian status on day TAI. Future experiments in a larger number dairy heifers and evaluation of the steroid hormones profile might clarify some debatable questions.

CONCLUSIONS

In conclusion, the ovarian status on day of TAI affects the reproductive performance in dairy heifers subjected to estrus synchronization and timed artificial insemination with sexed semen. Hormonal pretreatment with onset of PRID-5-day protocol 6 days later and TAI with sex-sorted semen ensure acceptable pregnancy rate. The greater preovulatory follicle and a lack of corpus luteum before insemination provide significantly ($P < 0.05$) more pregnant animals, compared to the cases when CL is presented. Ultrasound determination of the ovarian function before insemination can be used in selection of heifers for TAI with sex-sorted semen.

MANUFACTURERS

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***Ethical approval*.** The experiment was approved by the National Ethics Committee for animals in accordance with the Bulgarian legislation (Ordinance No 20/1.11.2012) on the minimum requirements for protection and welfare of experimental animals and requirements for use, rearing and/or their delivery.

***Declaration of interest*.** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of paper.

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