Reproductive Performance and Progesterone Profile in Dairy Sheep after GnRH Administration on Day 4 Post Artificial Insemination

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Abstract. *The current study aimed to determine the reproductive performance and progesterone profile in East Friesian sheep after GnRH administration on day 4 post artificial insemination (AI). The experiment was carried out with 67 sheep subjected to estrus synchronization and artificial insemination. The animals were divided in two groups: I (control group, n = 30) and II (GnRH group, n = 37), treated by GnRH on day 4 after AI. Rams were introduced on day 12 and removed on day 20 after AI. Ultrasound pregnancy check was on days 20 and 60 after AI, and sheep were separated on the basis of their reproductive status (RS). Blood progesterone (P4) concentrations were measured by ELFA on days 4, 12 and 20 after AI, and a likehood for early embryonic mortality was estimated. The percentages of non-pregnant and pregnant sheep in both groups on day 20 were close; however, there were significantly (P < 0.05) more pregnant sheep in the GnRH group on day 60 compared with day 20. The investigated factors affected the P4 concentrations independently of each other. Analysis of the main effects of GnRH and RS showed a significant (P < 0.005) influence on the progesterone profile. The day after AI had a strong positive effect on the P4 concentration, with significant (P < 0.001) differences between mean P4 values measured during the different days. The estimated likehoods of early embryonic mortality (EEM) for both groups was 25.4%. In conclusion, GnRH injection on day 4 after artificial insemination and the introduction of ram on day 12 did not have a direct effect on the pregnancy rate, but led to improvement of the reproductive performance at the flock level. Gonadotropin releasing hormone treatment, reproductive status and day after AI affected the progesterone concentrations in East Friesian sheep irrespective of each other, and had a significant (P < 0.005) effect on the hormonal profile. The treatment by GnRH on day 4 after AI tended to reduce early embryo mortality, but future investigations are needed to clarify this effect.*

Introduction

The main goal in dairy sheep farming is breeding sheep with a high milk yield and fertility resulting in production of more milk (Pollott & Gootwine, 2004; Tzanidakis et al., 2017). An achievement of good results requires optimal reproductive performance, an introduction of artificial insemination and different hormonal interventions (Valergakis et al., 2010; Gibbons et al., 2019; Hameed et al., 2021; Didarkhah & Vatandoost, 2022). In this aspect, most protocols for estrus synchronization include a combination of intravaginal progesterone releasing devices with equine chorionic gonadotropin (eCG) (Ataman et al., 2013; Hashem et al., 2015; Fernandez et al., 2018, 2019; Hajibemani et al., 2022). Although they provide an acceptable conception rate, the reproductive performance at a farm level is limited by embryonic and fetal losses (Diskin & Morris, 2008; Chundekkad et al., 2020). An early gestational period includes several events such as attachment of conceptus, implantation, placentation and initiation of fetal and placental growth, which are critical for maintenance of pregnancy (Bairagi et al., 2018; Reynolds et al., 2019). The embryonic mortality is an important factor leading to low reproductive efficiency and significant economic losses (Dixon et al., 2007; Moraes et al., 2009; Rickard et al., 2017). In sheep, the cases registered up to 18–20 days after fertilization are defined as early embryonic mortality, and from day 20 to day 45 as late embryonic loss (Thatcher et al., 2001; Dixon et al., 2007). Most of the losses in sheep are related to embryonic mortality, as 30% to 40% of fertilized ova are lost during the pre-implantation period and 70% to 80% of losses are during the first 3 weeks post-mating (Wilkins, 1997; Michels et al., 1998). The majority of embryonic losses occur during pre- and peri-implantation between days 12 and 17 of gestation (Rickard et al., 2017). Detection of early embryonic mortality is very difficult because of a lack of reliable diagnostic methods. Moreover, sheep with early embryo loss until gestation days 11–12 return to estrus between days 15 and 19 after mating (Edey, 1967).

A measurement of progesterone concentration on day 12 and transrectal ultrasound examination between days 10 and 17 after AI have been used for

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pregnancy diagnosis and retrospective determination of early embryonic mortality in sheep (Vinoles et al., 2012; Rickard et al., 2017), but still there are no detected accurate diagnosis methods.

A few studies report that administration of gonadotropin releasing hormone (GnRH) or human chorionic gonadotropin (hCG) after AI of synchronized sheep lead to a decrease of embryonic loss and increased pregnancy rate (Cam & Kuran, 2004; Hashem et al., 2015; Farrag et al., 2017; Zonturlu et al., 2018; Nikbakht et al., 2022). However, other authors have determined no effect on the pregnancy rate after AI and recommended future investigations to explore the effect of gonadotropin treatments according to day post mating, season and breed of sheep (Fukuy et al., 2001; Fernandez et al., 2018, 2019). A single gonadotropin injection between days 4 and 12 after mating has been shown to improve the activity of the corpus luteum (CL) or the formation of accessory corpora lutea, and to increase angiogenic factors in pregnant sheep (Beck et al., 1996; Coleson et al., 2015). As a result, an increase in the P4 concentration has beneficial effects on early embryonic development and placentation, and decreased pregnancy loss (Cam & Kuran, 2003; Khan et al., 2007; Hashem et al., 2015). In contrast, Fernandez et al. (2018) have observed no increase in the concentration of serum P4 in GnRH generated accessory corpora lutea in sheep and a lack of effect on pregnancy results. Most of the studies in the last years (Hashem et al., 2015; Farrag et al., 2017; Nikbakht et al., 2022) have reported a positive influence of the treatment with luteotrophic agents after mating on the pregnancy maintenance, but the information evaluating the effect of GnRH injection during the early luteal phase is controversial and still studied (Hajibemani et al., 2022).

The current study aimed to determine the reproductive performance and the progesterone profile in East Friesian sheep after GnRH administration on day 4 post artificial insemination. In addition, a hypothesis for determination of the early embryonic mortality on the basis of progesterone concentrations measured on day 12 after AI was tested.

Materials and methods

Animals

The experiment was carried out with 67 sheep from East Friesian breed at the end of lactation, reared in dairy sheep farms, located at *latitude* 42° 28' 59.99" N and *longitude* 26° 01' 0.01" E. Animals were aged 2.5 ± 0.5 years, body weight was 67.5 ± 2.5 kg, and the housing technology was in group boxes. The animals' feeding (total mixed ration included hay, concentrate containing mineral supplements and corn silage) was in agreemnt with the requirements for dairy breed, age and stage of lactation, and water intake was *ad libitum*. The experiment was performed during the breeding season (from September throughout October). All procedures were in accordance with the minimum requirements for protection and welfare of experimental animals and requirements for use, rearing and/or their delivery included in Bulgarian legislation (Ordinance No 20/1.11.2012).

Estrus synchronization, artificial insemination and ram mating

All sheep were subjected to estrus synchronization by intravaginal sponges containing 30 mg of flurogeston acetate (Syncro-part® 30, Ceva Sante Animale, France) for 12 days and an intramuscular injection of 500 UI eCG (Folligon®, MSD Animal Health, USA) on day of a sponge removal. Artificial insemination with fresh-diluted semen was performed from 52 to 58 h after the sponge removal. Fresh-diluted semen of 0.2 mL with 300 x 10^6 motile sperms was introduced deep cervically by an experienced operator. The animals were divided in two groups: I (control group, n = 30) and II (GnRH group, n = 37). Group I was not treated and Group II received 50 µg of gonadorelin as diacetate (Ovarelin®, Ceva Sante Animale, France) on day 4 after AI. Rams with proven fertility in a ratio male/female 1/6 were introduced in each group on day 12 and removed on day 20 after AI.

Ultrasound examination and reproductive performance registration

Ultrasound pregnancy check was conducted on days 20 and 60 by ultrasound equipment SonoScape S2 Vet and multifrequency (7–12 MHz) linear transducer (SonoScape Medical Corporation. Shenzhen, China). On day 20, all sheep were subjected firstly to a transabdominal ultrasonography. In case of negative pregnancy diagnose, a transrectal ultrasonography was performed. On the basis of ultrasound results, the sheep were recoded non-pregnant and pregnant. A positive pregnancy diagnose on day 20 was given in visualization of an echogenic embryo, located in the anechogenic uterine lumen. On day 60, a fetus with visible cardiac activity and placentomas were observed. Pregnant animals with an ultrasound picture different from those observed on days 20 and 60 were recorded as the sheep *conceived after mating by ram (SCAMR)*. The reproductive status (non-pregnant and pregnant sheep) based on the data on day 20 and (non-pregnant, SCAMR and pregnant sheep) based on the data on day 60 and a total value of pregnant and non-pregnant animals in both groups were calculated and presented in percentages.

Progesterone assay

Blood samples for P4 assay in all the sheep were collected on days 4, 12 and 20 after artificial insemination. After the sample collection, the blood plasma was separated by centrifugation (3000 g for 15 min) and stored in a sterile tube at –20°C until analysis. Progesterone concentrations were measured by automated quantitative-enzyme-linked fluorescent immunoassay (ELFA, VIDAS, ImmunoDiagnostic Assay System, bioMerieux, France) and VIDAS®

Progesterone kit imprecision within and between runs of 5.7–3.8% and 6.2–3.8%, respectively. The progesterone concentrations according to group, reproductive status, and days after AI were calculated and compared.

Statistical analysis of the progesterone concentration in all animals in both groups on day 12 showed significantly ($P < 0.003$) higher P4 concentrations in pregnant sheep compared with non-pregnant sheep. For exclusion of a possible influence of the number of embryos and gonadotropin treatment, the lowest progesterone concentrations for animals in the control and the GnRH group on day 12 determined as pregnant by ultrasound on day 20 were accepted as indicative for pregnancy on day 12 after AI. The assumption was that the ewes recorded as non-pregnant by ultrasound on day 20 but with progesterone concentrations above the indicative for pregnancy on day 12 underwent embryo loss. The subgroups of pregnant, non-pregnant sheep and animals with EEM from each group were formed after the pregnancy check. The embryonic mortality was calculated as a percentage of animals determined with embryo loss compared with all sheep in each group.

Statistical analysis

Values of non-pregnant and pregnant animals between different groups and total values were compared by the non-parametric method for comparison of proportions with small samples. The effects of GnRH treatment, reproductive status of animals and day after AI on the P4 concentrations were determined by ANOVA based on the Wilks-lambda test. The progesterone values (mean ± standard deviation) of non-pregnant animals, sheep *conceived after mating by ram and* pregnant animals according to group and day after AI were compared by ANOVA and the post-hoc Tukey-test. Differences were considered significant at $P < 0.05$ level.

Results

The percentages of non-pregnant sheep in both groups on day 20 were close (Table 1). On the same day, the percentage of pregnant sheep in the GnRH group tended to be higher than in the control group, but there was no statistical difference ($P > 0.05$). Six sheep (37.5%) from the non-pregnant animals in group I and 10 (62.5%) from these in group II on day 20 were recorded as pregnant during the second ultrasound examination for pregnancy. The percentages of non-pregnant and pregnant animals in both groups on day 60 also did not differ significantly. However, there were significantly ($P < 0.05$) more pregnant sheep in the GnRH group on day 60 compared with day 20. The total value of pregnant animals in both groups was higher than the recorded one of non-pregnant sheep ($P < 0.05$).

Factorial analysis showed that the investigated factors affected the P4 concentrations independently of each other (*GnRH x RS, observed power* = 0.26, $P = 0.65$). The progesterone profiles of non-pregnant sheep in the control and the GnRH treated group did not differ considerably from day 4 to day 20 after artificial insemination (Fig. 1). In contrast, additional analysis of the main effects of GnRH and the reproductive status showed a significant influence on the progesterone profile (*Wilks-lmbda GnRH* = 0.80, $P = 0.0045$; and *RS* = 0.94, $P = 0.0000$) (Fig. 2AB). The mean progesterone concentrations of the non-pregnant sheep in both groups on day 12 were higher ($P < 0.05$), compared with P4 concentrations measured on day 4 (Table 2). After that, they started to decrease rapidly as on day 20 they were close to the concentrations of P4 registered on day 4, and significantly ($P < 0.05$) lower than those on day 12.

A similar enhancement in P4 between days 4 and 12 and a drop on day 20 after AI were observed in the sheep conceived after mating by ram. However, the mean concentration of P4 in SCAMR on day 20 was higher ($P < 0.05$) in the GnRH compared with the control group. It should be noted that regardless of a lower progesterone concentration in the GnRH group, compared with the control group on day 4 (3.03 ± 0.88 ng/mL vs 3.82 ± 1.95 ng/mL), the concentration of P4 on day 12 was relatively

Table 1. Non-pregnant and pregnant sheep in control and GnRH groups according to time of the ultrasound examination

Day after AI	Groups			
	Non-pregnant		Pregnant	
	Control n = 30	GnRH n = 37	Control n = 30	GnRH n = 37
	% (n)	% (n)	% (n)	% (n)
20	53.3 (16)	43.2 (16)	46.7 (14)	56.8 (21)[1]
60	30.8 (10)	16.2 (6)	69.2 (20)	83.8 (31)[2]
Total	23.9 (16/67)[a]		76.1 (51/67)[b]	

AI, artificial insemination; GnRH, gonadotropin-releasing hormone
Percentages within a column marked with different numbers differ at $P < 0.05$.
Percentages within a row marked with different superscripts differ at $P < 0.05$.

higher in the GnRH group (7.81 ± 2.44 ng/mL vs 6.50 ± 2.11 ng/mL), as on day 20, it remained higher than in the control group (3.20 ± 0.99 ng/mL vs 1.97 ± 0.88 ng/mL; P<0.05).

In the pregnant animals from the different groups, a strong positive effect of the day after AI on the concentration of P4 (Fig. 1) was determined with significant ($P < 0.05$) differences between the mean values of P4 measured during the different days (Table 2). These data supported the previous result about a more powerful effect on the reproductive status, in comparison with the effect of GnRH treatment on the progesterone concentration (*observed power 1 vs 0.74, respectively*). On day 4 after AI, the P4 level was relatively lower in group II compared with group I, but on days 12 and 20, it tended to be higher in GnRH treated sheep, compared with non-treated sheep.

A retrospective analysis of the progesterone concentration measured on day 12 after AI showed a likehood of an early embryonic mortality of 33.3% and 24.3% in the control and the GnRH treated sheep, respectively, but a significant difference was not registered ($P = 0.19$). A total value of 25.4% for both groups was estimated (Table 3).

Table 2 Progesterone concentration (Mean ± SD, ng/mL) of sheep in control and GnRH groups according to day after AI and reproductive status based on ultrasound results

Day after AI	Control group (n = 30)			GnRH group (n = 37)		
	Non-pregnant (n = 10)	SCAMR (n = 6)	Pregnant (n = 14)	Non-pregnant (n = 6)	SCAMR (n = 10)	Pregnant (n = 21)
4	2.45 ± 1.01^{a1}	3.82 ± 1.95^{ab1}	3.74 ± 0.73^{b1}	2.56 ± 0.79^{a1}	3.03 ± 0.88^{bc1}	3.45 ± 0.98^{b1}
12	5.82 ± 1.65^{a2}	6.43 ± 2.52^{ab1}	7.53 ± 1.09^{bc2}	6.50 ± 2.11^{ab2}	7.81 ± 2.44^{bc2}	8.38 ± 0.91^{c2}
20	1.91 ± 0.95^{a1}	1.97 ± 0.88^{a2}	9.47 ± 1.96^{c3}	1.93 ± 0.43^{a1}	3.20 ± 0.99^{b1}	10.26 ± 1.69^{c3}

AI, artificial insemination; GnRH, gonadotropin-releasing hormone; *SCAMR,* sheep *conceived after mating by ram*
Mean values within a column marked with different numbers differ at $P < 0.05$.
Mean values within a row marked with different superscripts differ at $P < 0.05$.

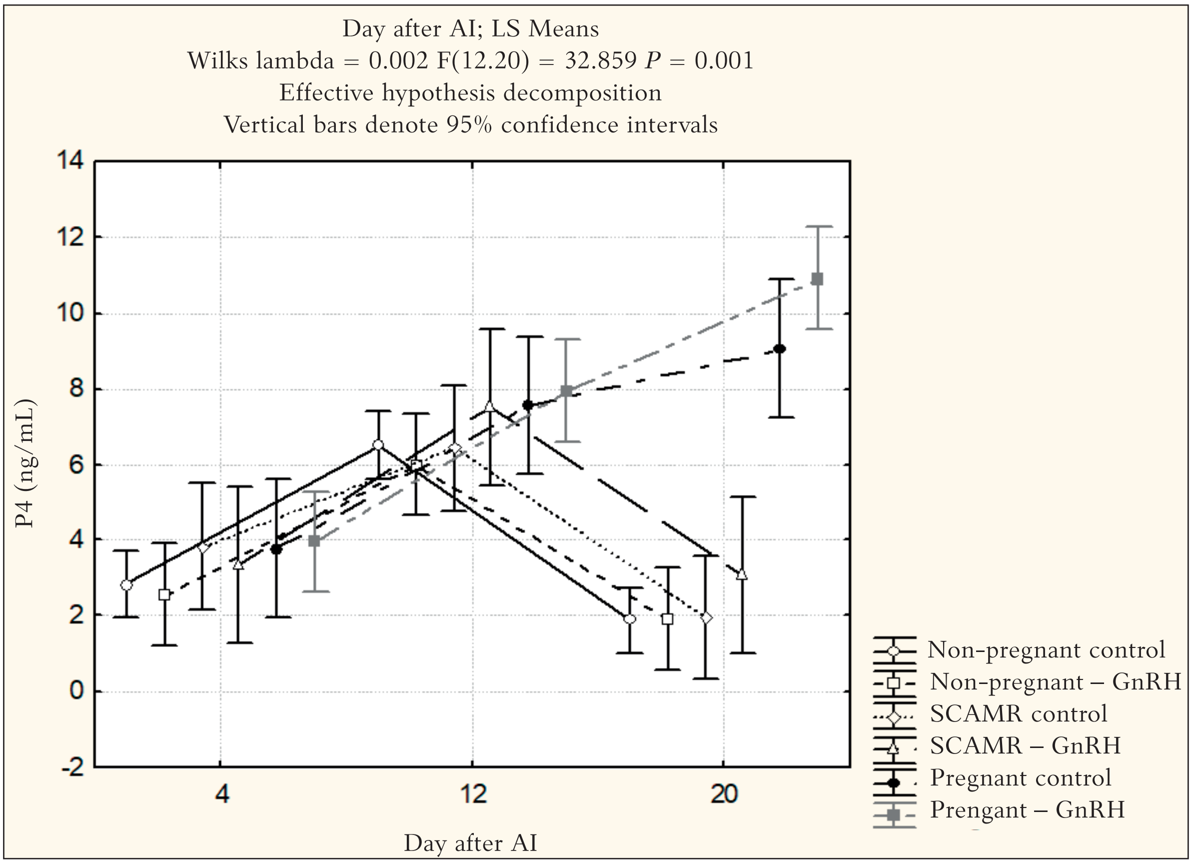
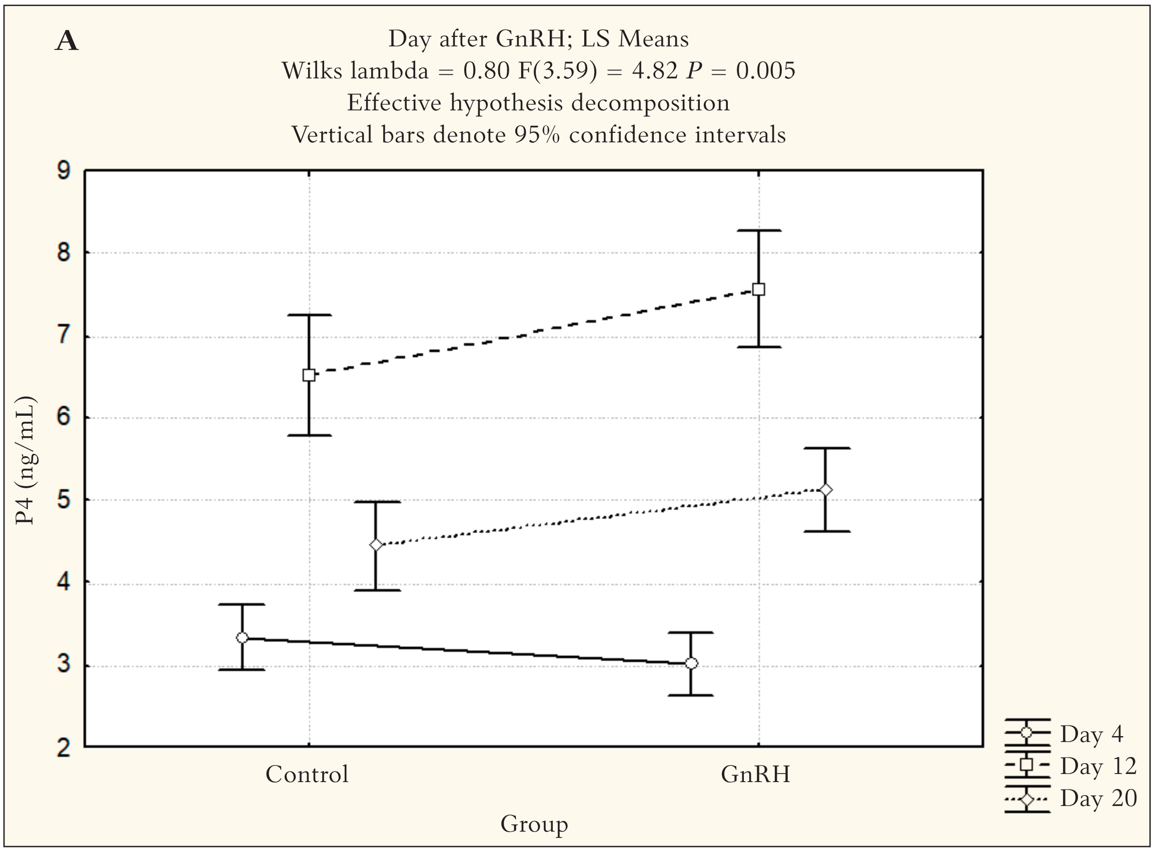


Fig. 1. Effect of day after artificial insemination on progesterone (P4) concentrations during the different days (ANOVA one way effects)
AI, artificial insemination; GnRH, gonadotropin-releasing hormone; *SCAMR,* sheep *conceived after mating by ram*



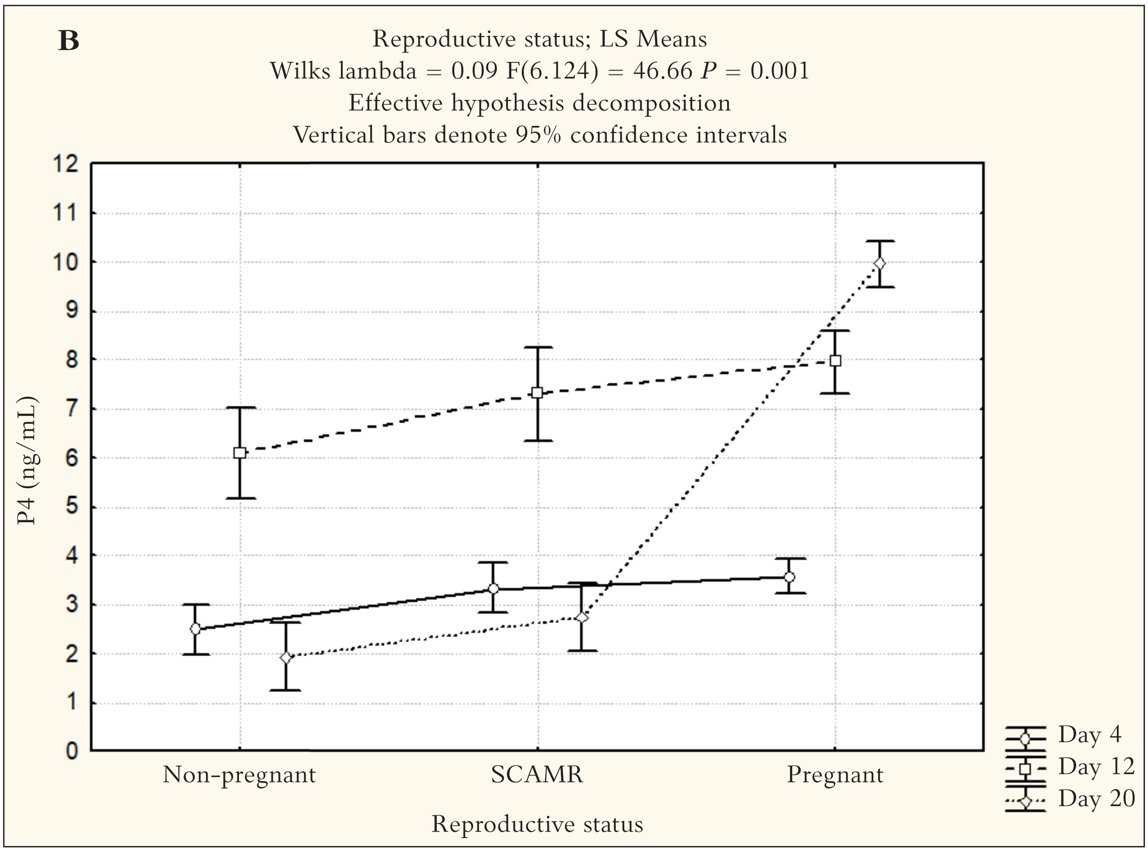


Fig. 2. Effect of GnRH (A) and reproductive status (B) on progesterone concentrations during the different days (ANOVA main effects)
AI, artificial insemination; GnRH, gonadotropin-releasing hormone; *SCAMR,* sheep *conceived after mating by ram*

Table 3. Early embryonic mortality (EEM) in the different groups based on minimal progesterone (P4) concentrations for pregnant animals measured on day 12 after artificial insemination

	Indicative P4 concentrations for pregnancy	
	(P4 > 5.08 ng/mL)	*(P4 > 6.60 ng/mL)*
	Control group (n = 30)	GnRH group (n = 37)
	% (n)	% (n)
EEM	33.3 (10)	24.3 (9)
Total	25.4 (17)	

Discussion

Administration of GnRH agonists has been used on the day of mating or days 1, 2, 4, 7 and 12 after that and showed to improve the reproductive performance in sheep (Cam & Kurran, 2004; Mirzaei et al., 2014; Hashem et al., 2015; Fernandez et al., 2019; Didarkhah & Vatandoost, 2022; Nikbakht et al., 2022). The obtained results are rather various and the information about the effect of GnRH treatment during the first several days after AI on the progesterone production, pregnancy rate and early embryonic mortality in dairy sheep is limited and sometimes controversial.

The information about close percentages of non-pregnant and pregnant sheep in both groups on day 20 after AI was indicative of an insignificant direct effect of the gonadotropin treatment on the pregnancy rate. It was in agreement with the results of other studies using GnRH administration on day 4 (Fernandez et al., 2019) or days 2 and 5 post AI (Nikbakht et al., 2022). However, the registration of significantly higher ($P < 0.05$) cumulative pregnancy in the GnRH group on day 60 was an indicator of an indirect positive influence of the GnRH treatment on the pregnancy results. It was supported by the fact that more animals from group II recorded as non-pregnant on day 20 were returned to estrus before day 20 after AI and were conceived *after mating by ram*. A possible reason for this finding could be a rapid increase of the P4 level after the GnRH treatment resulting in the attainment of the P4 level required to lysis of corpus luteum and earlier resumption of regular estrus activity in the treated sheep. In ruminants, oxytocin, progesterone, and estradiol regulate the uterine secretion of prostaglandin F2a that causes luteolysis (Silva et al., 1991; Goff, 2004). Regardless of a lower percent of sheep conceived after mating by ram in group I, the significant ($P < 0.05$) difference between the total value of pregnant and non-pregnant animals indicated that a combination of GnRH treatment on day 4 after AI and an introduction of fertile rams on day 12 may improve the reproductive performance on the flock level. Zonturlu et al. (2018) have also reported an increase of the pregnancy rate (up to 96.6%) after GnRH treatment on days 0 and 9 post-mating and an introduction of ram into the flock.

The information about the progesterone profile of the sheep treated by GnRH after mating or artificial insemination is contradictory. Hashem et al. (2015) injected 4.2 μg of buserelin on days 0, 7 and 0+7 post-mating in non-lactating Rahman ewes. The greatest overall mean P4 concentration was observed for GnRH 7 and GnRH 0+7 groups, followed by GnRH 0 and the control group. An investigation in Lake-Ghashghaei ewes during the spring season revealed a significantly higher serum progesterone concentration in all groups receiving GnRH (25μg as diacetate) on days 1, 2, 5, 7 and 12, compared with the control group (Nikbakht et al., 2022). However, Ayaseh et al. (2020) have reported that an injection of hCG or 4.2 μg of buserelin on days 0 and 5 after did not increase serum P4 concentrations in Karakul ewes during the non-breeding season.

The present study showed a similarity in the progesterone profiles of sheep with the same reproductive status in the control and the gonado-tropin treated group. This result does not exclude fully a beneficial effect of GnRH treatment on the luteal function, followed by an increase in endogenic progesterone production. It was in agreement with the analysis of the main effect of the GnRH on the P4 concentration. The rapid increase of the P4 concentration in *SCAMR between days 4 and 12* after AI, in spite of a lower baseline of P4 on day 4, compared with the control group, could be accepted as evidence for enhanced luteal activity in the GnRH treated sheep. It is known that many factors (age, breed, body condition and lactating status of sheep, season, level of feeding, etc.) affect the progesterone concentration in the blood circulation of ruminants (Nawito et al., 2015; Kamil, 2019; Garcia-Baccino et al., 2022). Moreover, an adequate response of the animals to gonadotropin treatment depends on the type of a GnRH agonist and a dose (Mirzaei et al., 2011; Picard-Hagen et al., 2015). Mirzaei et al. (2011) have treated cross-bred fat tailed ewes with a low (4.2 μg) dose and a high (8.4 μg) dose of buserelin on days 5 and 19 after ram introduction, respectively. On day 12 after treatment, the high dose GnRH resulted in significantly lower plasma P4 concentrations ($P < 0.05$), compared with the treatments by the low dose GnRH and without GnRH. All aforementioned may explain the discrepancy of the current results with the data of previous authors for different P4 profiles in non-treated and GnRH treated sheep. Probably the response of the East Friesian sheep to the used GnRH treatment was lesser compared with other sheep breeds. Additional detailed investigations with a large number of animals may clarify the question about a dose of the used GnRH agonist and the time for treatment after AI that can provide a significantly higher increase in P4 of East Friesian sheep at the end of lactation.

The reproductive status had a significant effect on the progesterone profiles of the animals with a close pattern in non-pregnant and SCAMR between days 4 and 20 after AI. A similar elevation of P4 until day 12 and a decrease until day 18 were established by Garcia et al. (2022) in non-pregnant sheep. Although the factorial analysis did not show a simultaneous influence of all factors, there was a tendency ($P = 0.057$) for P4 concentration to increase in SCAMR and pregnant animals after GnRH administration. The abovementioned subgroups had relatively lower baseline P4 levels, compared with the same non-treated subgroups on day 4, but their progesterone levels were relatively higher on days 12 and 20. The same effect on the mean plasma progesterone concentration was observed by Farrag et al. (2017) in adult Barki ewes.

The pregnancy affected the progesterone profile expressing in a gradual rise between days 4 and 12 and a rapid increase until day 20 after AI. This result was in unison with the data reported by Ganaie et al. (2009) in pregnant Corriedale ewes. They determined a significant increase in the mean plasma progesterone concentration from day 0–6 to day 16–30, while the progesterone level dropped to less than 1.0 ng/mL in the case of ewes that returned to estrus. The significant differences ($P < 0.05$) between the progesterone concentrations of non-pregnant and pregnant sheep in groups I and II on day 20 indicated that the measurement of P4 can be used for pregnancy detection, but only in a lack of ram introduction after artificial insemination. Otherwise, there is a risk of a large number of false negative diagnoses, as a result of re-conception by ram of non-pregnant but returning to estrus sheep. It was confirmed by the registration of pregnant animals on day 60 coming from those recorded as non-pregnant on day 20 after AI.

According to Vinoles et al. (2012), the pattern and concentrations of progesterone were affected by the pregnancy as the values were higher on day 12 in pregnant than non-pregnant ewes from all groups ($P = 0.01$). The P4 concentrations decreased in non-pregnant ewes between days 12 and 17, the expected time of return to estrus. The same effect was estimated in our study, and on this base, the likehood of early embryonic mortality in different groups was calculated. Regardless of the insignificant differences between EEM in gonadotropin treated and non-treated animals, the obtained value in the GnRH group was 9% lesser, compared with the controls. Related to this, the significantly ($P < 0.05$) higher mean P4 concentration in treated than non-treated SCAMR on day 20 supported indirectly the hypothesis for a positive influence of the gonadotropin administration on the early embryonic mortality, conditioned by stimulation of the P4 production from the corpus luteum or formation of accessory corpora (Cam & Kuran, 2004). Progesterone predominantly exerts an indirect effect on the conceptus via the endometrium to regulate blastocyst growth and conceptus elongation (Spencer, 2013; Coleson et al., 2015). The absence of a sufficiently developed conceptus to signal maternal pregnancy recognition results in the genes implicated in uterine receptivity being "turned off" as luteolysis ensues, P4 concentrations decline, and the animal returns to estrus for another opportunity to mate (Spencer, 2013). The aforementioned information was in accordance with the obtained result for more sheep returning to estrus after GnRH treatment and determined as pregnant during the second ultrasound examination. An effective prevention of the embryonic death in ewes has been achieved by the application of GnRH or FGA on days 4 and 12 after mating (Ataman et al., 2013). The total value of EEM in our study was close to the registered embryo loss of 27% between days 10 and 17 and 30 ± 13% between ovulation and preimplantation in Merino ewes (Vinoles et al., 2012; Rickard et al., 2017). On the other hand, the absence of a statistical difference in EEM between non-treated and GnRH treated sheep implies an influence of additional factors different than progesterone on the embryo loss in the earliest gestational phase. Hoskins et al. (2021) suggest that while progesterone may accelerate conceptus development, its role in the mechanisms of implantation and pregnancy is complex and requires further research to investigate its therapeutic properties in livestock reproduction.

Conclusion

Administration of GnRH on day 4 after artificial insemination and an introduction of ram on day 12 do not have a direct effect on the pregnancy rate, but lead to improvement of the reproductive performance on the flock level by an increase of the number of sheep conceived after mating by ram. Gonadotropin releasing hormone treatment, reproductive status and day after AI affected the progesterone concentration in East Friesian sheep irrespective of each other, and had a significant (*Wilks-lambda tests,* $P < 0.005$) effect on the hormonal profile. The treatment by GnRH on day 4 after AI tended to reduce early embryo mortality, but future investigations are needed to clarify this effect.

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