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REPRODUCTIVE PERFORMANCE IN ALPINE GOATS ACCORDING TO THE APPLICATION OF A SIMPLIFIED PROTOCOL FOR INDUCING ESTROUS INTO THE REPRODUCTIVE OUT OF SEASON

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Abstract

Studies in the field of goat breeding have shown that the most widely used and effective way to induce and synchronize estrus in the breeding season is the hormonal method with Chronogest sponges and the administration of Folligon and a prostaglandin. In the present study, we looked at the reproductive performance of a batch of 148 Alpina goats synchronized in April with Chronogest sponges maintained intravaginally for 11 days and the injection of 400 IU Folligon without prostaglandin administration. Artificial insemination with frozen semen was performed 43 +/- 2 hours after the removal of the sponges and the following reproductive aspects were established: the degree of the cervix opening at the time of artificial insemination, ultrasound diagnosis of 50 days gestation after artificial insemination with the identification of cases of pseud-gestation (4.05%), the rate of calving (56.76%) and prolificacy (310.71%). In farm conditions, specific to our country, the exclusion of prostaglandin administration decreases the risk of abortions by lysis of luteal bodies in possibly pregnant goats at the time of treatment and insemination.

Key words*: estrous, goats, reproduction indices, synchronization.*

INTRODUCTION

The trend of the last decades has been to increase the goat herd throughout Europe and to establish development strategies in order to make this species profitable. In our country, too, the development of the goat sector has taken on a special scale in accordance with the requirements of the internal and external market. Thus, the studies published by ANICAP France show that at the end of 2017 Romania ranks among the top three countries in Europe in terms of goat herds, along with Greece and Spain (https://agriculture.gouv.fr). According to Eurostat, Romania ranks 3rd in Europe in terms of goats in 2021 (https://ec.europa.eu/eurostat/databrowser/view/apro_mt_lsgoat/default/table?lang=en).

The subsequent evolution was not characterized by a numerical increase but by the improvement of the present herds in relation to the direction of exploitation imposed by the internal market, respectively in the direction of milk production. Goats of specialized breeds were imported for milk production and breeding programs were developed for purebreds for both imported and domestic breeds. (https://www.caprirom.ro/rg). Taking into account the seasonal reproduction of goats and the need to improve the purebred herds, in order to ensure genetic progress in the shortest possible time, it was necessary to resort to reproductive biotechnologies (Arredondo et al., 2015). Thus, the collection of semen from males with high genetic value and its preservation in refrigerated or frozen form have ensured the artificial insemination of a large number of females, from several farms, at a distance. The estrous induction and synchronization programs made it possible to remove the reproductive seasonality and perform artificial inseminations at any time of the year and also, by staggering calvings according to a commercial plan allowed continuity in the consumer market of dairy and goat meat products. The use of artificial inseminations has as its main purpose the improvement of goat herds by infusing valuable genes from tested males and the rapid diffusion of genetic progress (Leboeuf et al., 1998, 2008). In the case of specialized goat breeds imported for the milk production, a number of bucks needed for natural breeding have been

purchased, but in order to rotate the pairs and avoid inbreeding, it is more efficient to import frozen semen produced in the bucks' testing centers. In the economic conditions of our country, of the semi-intensive system of exploitation of goats, in the absence of capital infusion and conservatism specific to goat owners, it is necessary to adopt the most efficient and economical measures in order to apply scientific methods of genetic progress. The method of artificial inseminations with frozen semen is a viable solution, reducing the costs of acquiring and maintaining males throughout the year, avoiding the spread of disease and reducing the stress of adaptation. Moreover, by applying the programs of induction and synchronization of estrous, artificial inseminations can be made in the non-breeding season, each farm managing to constitute two lots of goats with autumn and spring calving and ensuring a continuity of milk and meat production during the whole year, which will allow them a better sale on the internal and external market (Fatet et al., 2011). The aim of this study is to reduce the costs of estrous synchronization and artificial insemination by reducing labor on females and avoiding possible abortions by suppressing prostaglandin F2α, provided that local goat owners practice is grazing the entire herd, including males, for about 300 days / year, and unplanned and unobserved pregnancies may occur. In the case of owners of imported specialized goat breeds, they have adopted the specific operating conditions, respectively the intensive system with permanent stabulation but with the maintenance in the same shelter, in separate boxes for females and males, which can lead to unplanned pregnancies.

MATERIALS AND METHODS

The research was carried out on 200 Alpine goats with normal reproductive activity. Estrous synchronization and induction were performed in April 2021, at least 90 days after the previous calving date, all goats having a single calving registered in November-December 2020. Of the 200 goats, 52 represented the reference batch 1 and 148 goats constituted experimental batch 2. In the control batch 1, was applied the hormonal method of induction and synchronization of estrous recommended by INRA - Capgenes (France) and the most internationally used. The hormonal protocol INRA - Capgenes consists in the intravaginal application of Chronogest sponges, impregnated with 20 mg of fluorogeston acetate and maintained for 11 days. On day 9, 400 IU PMSG (Folligon commercial product) and 0.2 ml Roflavol (0.05 mg prostaglandin F2α) were administered. The role of Chronogest sponges is to block the estrous cycle for 11 days, through the action of progestogen, and at the time of their withdrawal the sexual activity resumes synchronously for all goats that have undergone treatment. The Folligon, that has as active substance Equine chorionic gonadotrophin, has a synergistic action of FSH type but also LH, achieving both the growth and maturation of ovarian follicles and follicular dehiscence by reaching the ovulatory peak of LH. The dose of Folligon can range between 400 and 700 IU being determined by the level of milk production. The goats monitorized in the experiment, being at the first unfinished lactation, registered milk productions from 2,200 kg up to 3,300 kg milk / day, which is why the minimum recommended dose of 400 IU / goat was used. Goats often have persistent luteal bodies that prevent the onset of pregnancy, which is why the prostaglandin F2α is used in order to perform their lysis.

At an interval of 12, 24, 36 hours after the removal of the sponges, the estrous was detected with test bucks, provided with an apron, to determine the goats that responded to the hormonal treatment and manifested estrous. In the present experiment, we performed artificial insemination with frozen semen at 43 hours after the sponges were removed from all goats, regardless of the clinical manifestation of estrous. In experimental batch 2 consisting of 148 Alpine goats, the protocol for inducing and synchronizing estrous was simplified by giving up the administration of prostaglandin F2α, and the injection of Folligon was done at the same time with the withdrawal of sponges and we gave up detecting the goats in estrous. In this case, the insemination was performed at a fixed point, respectively at 43 +/- 1 hour after the sponges were removed. Artificial insemination of goats was performed with frozen semen (MSC) imported from France.

Thus, we reduced the interventions performed on animals and the trips of the insemination team to 3, with a significant reduction of the workforce but also of the stress on the animals.
In order to perform the insemination of the goats from the experimental batch 2 in an optimal time, it was divided into 3 batches of females of 48/50/50 females that entered the experiment at an interval of 3 days, so that the actions performed at one batch should not overlap with other batches. All actions were performed by the same team of researchers and artificial insemination was done by a single operator, to remove as much as possible the factor of manipulation and execution.
Artificial insemination was performed with the vaginal speculum provided with its own light that allowed the visualization of the cervical ostium and the placement of the insemination pipette in the cervix. Depending on the appearance of the involted flora and the degree of penetration of the insemination pipette, the degree of opening of the cervix and implicitly the level at which the semen was deposited was assessed. When the cervix is closed the semen is deposited intravaginally, at the level of the involted flora, when the pipette penetrates 1-2 cm into the cervix, the insemination is intracervical and when the cervix is open, the pipette crosses the entire cervix and the artificial insemination is done intrauterine, similar to laparoscopic insemination.
Artificial insemination was performed with frozen semen imported from CAPGENE-France, kept in containers with liquid nitrogen at -196^0C. The preparation of the semen for insemination went through the following stages: 3 sequins of 0.25 ml were thawed, by immersion for 1 minute, in the thermostat defroster, the water having a temperature of $+ 38^0$C, then the sequin was wiped, the insemination pipette was loaded, the laboratory stopper was cut, a drop was placed on a heated slide for microscopic examination and it was kept at a temperature of about $36\text{-}37^0$C for 2-3 minutes, until the moment of insemination.
50 days after the artificial insemination, the diagnosis of ultrasonographic gestation was established by using the portable ultrasound WED 3100 with the multifrequency convex probe 2.5 / 3.5 / 5 MHz. Transabdominal ultrasound was performed on the animal in a four-legged position, with a slight neck restraint. The ultrasound probe was set to 3.5 MHz and placed in the groin, at the base of the breast, the area with reduced hair, and oriented to the area of the flank opposite the place of election. Ultrasound images showed the three physiological states in which the goats were, respectively non-gestation, gestation with highlighting the chains of uterine caruncles and the embryo or the state of pseudo-gestation with highlighting the accumulation of fluid in the embryonic vesicle and lysis of the embryo and placentomes. At 50 days after artificial insemination, the sensitivity of the transabdominal ultrasonographic diagnosis is estimated to be 99-100% (Traore et al., 2019a, 2019b, Gonzales et al., 2004), confirmed in the present experiment, when no abortion was recorded and the calving rate coincided with the ultrasound result.
Based on the observations from the artificial insemination, respectively from the level of the vaginal ostium and on the ultrasound data, we established the efficiency of applying the simplified protocol of induction and synchronization of estrous following the gestation rate recorded in the two groups of Alpine goats.
During October, the calvings were registered and the prolificacy was established following the artificial insemination in the counter-season of reproduction, on hormone-induced estrous.

RESULTS AND DISCUSSIONS

Of the 52 goats in the 1st control batch, only one did not show estrous, respectively did not accept the male tester within 12-36 hours after the withdrawal of the Chronogest sponges, and at the time of insemination it was found that the cervix was closed. According to studies published by Camacho (2020), the state of estrous manifests itself in the range from 16 up to 72 hours, with an average of 33 hours after the withdrawal of sponges. All the goats were artificially inseminated, and when the semen was deposited, the degree of penetration of the insemination pipette and the opening of the uterine cervix were observed. These degrees of cervical opening and artificial insemination, respectively, were correlated with the physiological condition of the goats assessed by ultrasound (Table 1).

Table 1. The result of the ultrasound gestation diagnosis compared to the degree of opening of the cervix in goats in batch control 1

	CI		CD1		CD2		IU		Total	
	N	%	N	%	N	%	N	%	N	%
P	2	50	9	56.25	16	64	7	100	34	65.38
PP	1	25	0	0	0	0.	0	0	1	1.92
NP	1	25	7	43.75	9	36	0	0	17	32.69
Total	4		16		25		7		52	

P- pregnant, PP- pseudo-pregnant, NP- non pregnant
CI- closed cervix, CD1- open cervix 1 degree,
CD2- open cervix 2-degree, IU- intra-uterine

The goats in experimental batch 2 (n = 148 goats) were artificially inseminated without detecting estrous, only with the assessment of the degree of opening of the cervix and its correlation with the reproductive status of goats determined by ultrasound, data presented in table 2.

Table 2. The result of the ultrasound gestation diagnosis related to the degree of opening of the cervix in goats in experimental batch 2

	CI		CD1		CD2		IU		Total	
	N	%	N	%	N	%	N	%	N	%
P	2	40	20	45,45	48	57,83	14	87,50	84	56,76
PP	1	20	2	4,55	3	3,61	0	0,00	6	4,05
NP	2	40	22	50,00	32	38,55	2	12,50	58	39,19
Total	5		44		83		16		148	

P- pregnant, PP- pseudo-pregnant, NP- non pregnant
CI- closed cervix, CD1- open cervix 1 degree,
CD2- open cervix 2-degree, IU- intra-uterine

Figure 1 shows that 7.69% of the control goats had a closed cervix with the deposition of intravaginal semen, at the level of the involved flora (intravaginal insemination), 30.77% had an open cervix 1 degree, a situation in which the tip of the pipette penetrates and the seminal material is partially repressed intravaginally.

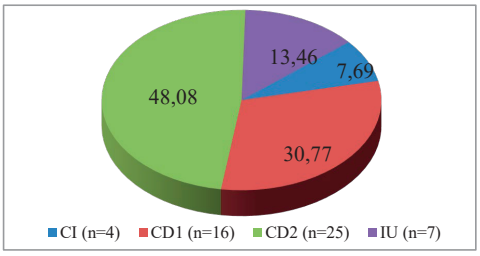

Figure. 1. The percentage representation of the cervical modification response to hormone treatment in batch witness 1

In 48.08% of goats the cervix was opened 1 degree, the insemination pipette penetrating the cervix path, without intravaginal discharge (cervical insemination) and in 13.46% of goats the pipette slips very easily, it completely crosses the cervical opening and the administration of the seminal material was done intrauterine.

In experimental batch 2, the assessment of the degree of opening of the cervix (Figure 2) showed that the percentage of goats with open cervix (intrauterine insemination) was lower than in control batch 1, respectively 10.81%, while the percentage of goats with closed cervix (intravaginal insemination) was 3.38% compared to 7.69% in control batch 1. Intracervical insemination was performed in 85.81% of goats in experimental batch 2 (29.73% - open cervix 1 degree and 56.08% open cervix 2 degree) compared to 78.85% in control batch 1.

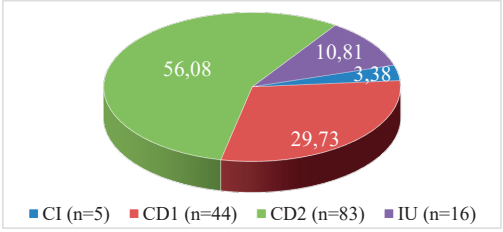

Figure 2. The percentage representation of the cervical modification response to hormone treatment in experimental batch 2

The estrous response expressed by the stages of cervical opening (CD1, CD2, IU) at 43+/-1 hours after the interruption of hormone treatment of 92.31% in control batch 1 and 96.62% in experimental batch 2 is similar to data reported by Hashemi & Sofdarian (2017), respectively 94.7% and is lower than the estrous response expressed by the 100% clinical manifestation reported by Dogan et al. (2004), 98.2% reported by Freitas et al (1997) and respectively 97% reported by Motlomelo et al. (2002).

The ultrasound examination established the gestation rate on each batch and calculated the fertility distribution according to the degree of opening of the cervix at the time of insemination. In the case of intravaginal insemination, the fertility was 40% in the experimental batch and 50% in the control batch, including the goat that did not show estrous and had a closed cervix at the time of insemination. The incidence of pseudo-gestation was 25% in the control batch and 20% in the experimental batch. Among the

goats with closed cervix at the time of insemination, 25% of the control batch and 40% of the experimental batch were pregnant, which indicates the presence of estrous at intervals of more than 36-48 hours, knowing that ovulation occurs at the end of the heat (Figure 3).

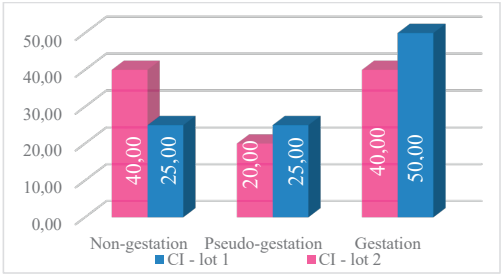

Figure 3. The representation of the gestational condition of artificially intravaginally inseminated goats

In the case of the open cervix (Figure 4), the gestation rate was 60.98% in intracervical insemination in control batch 1 and 53.54% in experimental batch 2, from which it is observed that in case of discharge the gestation rate is lower, respectively 56.25% compared to 64% when the insemination is intracervical in the 1st control batch and 45.45% (intracervical insemination with vaginal discharge) compared to 57.83% (without vaginal discharge) in the experimental one.

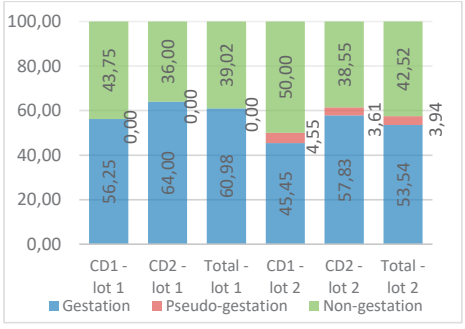

Figure 4. The representation of the gestational status of artificially intracervical inseminated goats

In the control batch to which the classic hormonal treatment of estrous induction was applied, with the use of prostaglandin no false pregnancies were found, while in the experimental batch 2 the pseudo-gestation rate is 3.94% with a higher incidence in case of intracervical insemination with seminal material, of 4.25% compared to 3.61% in the case of open cervix 1degree.

The highest percentage of non-gestation was obtained in experimental batch 2 in which the insemination was performed intracervical with vaginal discharge of the seminal material, of 54.55% of which 4.55% are represented by pseudo-gestations.

The picture of the physiological condition of the goats in the two groups confirms the incidence of pseudo-gestations in the absence of Prostaglandin F2α administration between 0.6% and 4.6 ^ in the Alpine breed following artificial insemination (Duquesnel et al., 1992). Studies conducted by Bousquet (2005) highlighted the higher incidence of pseudo-gestation as a result of the application of artificial insemination, reporting over 10% goats with pseudo-gestation in 16.7% of the farms studied.

At intrauterine insemination, a gestation rate of 100% was obtained in control batch 1 and experimental batch 2 registered 87.5% pregnant and 12.5% non-pregnant females (Figure 5).

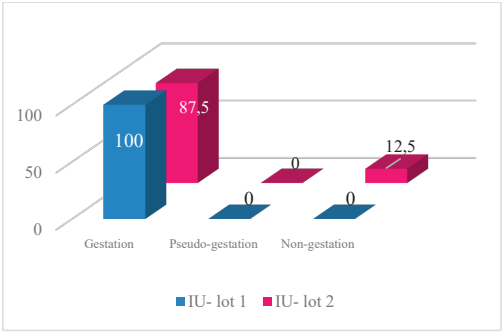

Figure 5. The representation of the gestational status of artificially intrauterine inseminated goats

If we consider the artificial insemination with frozen semen, on estrous induced by the two hormonal methods, in the counter-season of reproduction, regardless of the observations found in the vagina and cervix, it is observed that the gestation rate is 65.38% in the control batch 1 and 56.76% in experimental batch 2 (Figure 5). The results obtained in both groups are superior to those obtained by Dogan et al. (2004) which in the Saanen breed obtained a fertility of 50% or by Motlomelo et al. (2002) which registered a fertility of 47%. In 1997 Freitas reported a fertility rate of 75% after artificial insemination with frozen semen on

synchronized estrous of goats that showed clinical estrous.

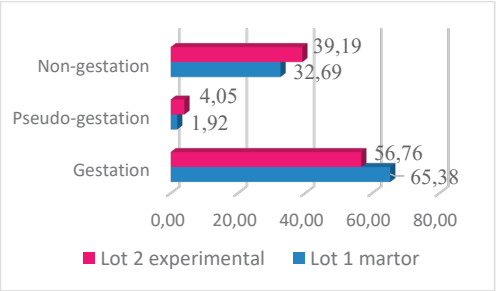

Figure 6. Representation of the state of gestation of artificially inseminated goats

Following the registration of calvings, all the goats diagnosed by ultrasound as pregnant gave birth, without any abortion. In experimental batch 2, out of the 84 goats, 261 kids were obtained, respectively a prolificacy of 310.71% and of the 34 goats in the 1st control batch, 107 kids were obtained, obtaining a prolificacy of 114.71%, values that fall specific to the breed described by CAPRIA-CAPGENE.

CONCLUSIONS

Artificial insemination with frozen semen using the simplified protocol, respectively excluding the administration of prostaglandin to protect against accidental gestations and gonadotropin administration with the interruption of progestogen treatment has recorded lower fertility values than the control batch but is included in international research reports. This estrous induction and synchronization protocol can be extended under our country specific conditions, in which goat owners have adopted a semi-intensive breeding and reproduction technology.

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