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Comparative Survey of the Effectiveness of Hormonal Treatment with PGF2-Alpha and Gnrh, in the Cows With Sub-Estrus of some Farms in the Polog Region

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Abstract

Sub-estrus is a special form of temporary infertility in cows. It delays their pregnancy and makes it difficult to realize the economic objective; a cow, a calf, a year. Cows with sub-estrus possess active ovaries, develop cycles, but do not develop sexual excitement during estrus (fervor). In essence, sub-estrus is a neuro-hormonal disorder related to a variety of factors. The fact that sub-estrus often meets even in cow families, makes you think that the diagnosis in question is also due to the little care that is usually shown on the cow's observation and detection of fervor. According to contemporary concepts, quite a deficiency, objective or subjective, which are conditioning the high incidence of sub estrus, can be overcome through early diagnosis, hormonal therapy and programmed insemination of cows with sub estrus, in fixed days and hours. The purpose of the study is related to the definition: The efficacy of two methods of hormone treatment with anti lutein (PGF2-alpha) and gonadorelin (GnRH), in enhancing sexual excitement and the fast pregnancy of cows affected by sub-estrus and to rate the pregnancy of cows with sub-estrus, which are treated with hormonal standards and inseminated at fixed time. The importance of the study lies in the fact that it provides for the cows a method of treatment and insemination programmed for cows with sub-estrus, in order to increase their annual fertility.

Keywords: Sub-estrus; infertility; PGF2-alpha; GnRH.

1. Introduction

It is well-known the sign that the cow has finally and completely reached the stage of estrus or of fervor is its "unstable attitude" to the jumping of a bull or another cow of the herd.

This means that the cow that leaves the bull or the cow is not in fervor or the fervor is over and the next phase begins (met-estrus). Posture reflexes, as the basic sign of fervor on the cows, lasts about 18 hours [1][21] or about 24 hours according to [2]. The unilateral impression should not be created, as the fervor lasts continuously and in all cows on equal time, 18 hours or 24 hours. Studies show that it can fluctuate significantly, ranging from 8 to 30 hours (according to [21]) or 4 to 24 hours [1]. Other sources also refer to about 2-4 hours, which in some batches/herds constitute after calving period, 20-23% of all developed fervors [17]. It can be guessed of how much can be discovered the fervor of single cow in connected state to the manger, when it lasts only 2-4 hours and more in the night hours. This becomes even more convincing if it is considered that the reflex of the cow's attitude or immobilization best suits after morning milking, after being released out of the cot and in the presence or sexual interest of at least 4 to 5 other cows [19]. Regarding the main factors influencing the length of the fervor on cows, it can be said that the tropical and subtropical areas affect negatively, in the first place, the hot climate and in the second the amount of not proper nutrition [2]. This statement suggests that in other areas in the first plan come the amount of not proper nutrition and not the environmental temperature.

Temperatures above 30 ° C become a fertility inhibitory factor, especially when associated with the high relative humidity of the environment [16]. In the hot climate that cows pass the process of postpartum and develop estrus cycles that cause shortening of the estrus, therefore the discovery of fervor during the day is difficult.

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The purpose of the study is related to the definition:

a. The efficacy of two methods of hormone treatment with *anti lutein* (PGF2-alpha) and *gonadorelin* (GnRH), in enhancing sexual excitement and the fast pregnancy of cows affected by sub-estrus.
b. To rate the pregnancy of cows with sub-estrus, which are treated with hormonal standards and inseminated at fixed time.

The study has two main tasks, they are: Determination of sub-estrus density in cows with "anestrus" anamnesis and Comparison of the cow's pregnancy rate treated with two hormonal methods: PGF2-alpha + PGF2-alpha and GnRH + PGF2-alpha + GnRH.

2. Materials and Methods

The study was conducted in the family cows of Pollog region, Macedonia, during 2016-2017.

Methodical attention is focused on:

- Selection of animal studies.
- Gynecological examinations of ambulatory cows.
- Formation of cow's groups in the study.
- Use of certain hormones to study.

In this study were included 51 cows, Holshtein and Simental breeds, and aged 3-6, who had not manifested sexual excitement 60-90 days after calving. The cows were selected according to the principle of coincidence and gynecologist examination. The coincidence was relied on the farmer's arrival at the veterinary clinic and complaints (anamnesis) about the lack of on his cow fervor.

Gynecological examination was based on comprehensive/general inspection, vaginal inspection and rectal palpation:

General Inspection

This examination was focused on some external animal indicators, such as:

- *Hair Brightness:* Non-glitter is rated as one of the energy deficit signs.
- *Sprightliness of the sight:* Non-sprightliness is considered as one of the acidosis signs.
- *Perinatal cleanliness:* The unclean appearance of the perineal region is considered as contamination by vaginal discharge and as one of the signs of genital infection.
- *Articular condition:* The presence of arthritis or laminitis is considered as a sign of heavy acidosis and the existence of a strong pain.

Vaginal Inspection

This examination was focused on some indicators of the vagina, such as:

- *Color of mucous:* The lightness of the vagina's mucous is one of the anestrus and dioestrus signs.
- *Integrity of the mucous:* Encroachment of the vaginal mucus integrity (presence of lesions) is a sign of pain.
- *Mucus of the mucous:* The slightly and thick mucus in the vagina space is a sign of anthrax, while the presence of abundant and transparent mucus is a sign of estrus, and so on.
- *Vaginal bleeding:* The presence of a slight bleeding from the vagina, in most of the cows is the sign of being in the fervor 2-3 days earlier.

3. Results and Discussions

To identify the density of sub-estrus, i.e. of secret fervor, we examined 51 cows; incomes randomly for ambulatory visit and then met the conditions of the study methodology. From conversation with the owners it turned out that according to them, all of them were lacking in fervor. They argued this with the fact that their cows had never wanted to *copulation*, even though they had calve for more than two months. The cows of this group underwent gynecological examination, through inspection, vaginal examination and rectal palpation, as described in the methodology. Examination in question revealed three diagnoses of barrenness (Table 1).

In Table 1 , it is seen that in the group of our cows with the anamnesis "anestrus" there are three barrenness diagnosis that have prevented their pregnancy, even though they were over 60 days after calving. The author [15], points out that between functional and structural defects of ovaries in dairy cows, of first hand importance are: ***Sub-estrus; Anestrus and Ovary with cyst.*** By comparing the density of our three diagnoses within the cow group in the study, with the incidence of literature in the common herds, a relative correlation is observed (Table 2).

Table 1 Results of gynecological examination in cows with anamnesis "Anestrus of transplantation".

Examined Cows	Cows in true anestrus (Without TV in the ovary)	Cows in untrue anestrus(With TV in the ovary)	Cows in anestrus *progesterogenic* (With cyst in the ovary)
51	12 (23.53%)	25 (49.02%)	14 (27.45%)

The relative correlation in Table 2, between ambulatory diagnosis and annual incidence of major anestrus, is more apparent in the untrue anestrus or sub-estrus. Regardless to the percentages, the study and literature data in Table 2 are completely consistent with the fact that in both cases, the untrue anestrus (sub-estrus) stands in the first place. In the setting of the first experiment, the PGF2-alpha + PGF2-alpha scheme was used, whereas under the second experiment the GnRH + PGF2-alpha + GNRH scheme was used.

By confirming the fervor during the insemination of the treated cows, we first recognized the degree of their reaction to the hormones in both experiments (Table 3).

Table 2 Comparison of anestrus diagnosis density, as evidenced by the studying ambulatory conditions and literature [2] under cot conditions.

Data source	**After calving time**	**True anestrus**	**Untrue anestrus**	**Progestrogenic anestrus**
Study	Over 60 days	23. 53 %	49.02%	27.5%
Literature*	40- 60 days	10%	60-90%	2-7%

Table 3. Rate of cow reaction to hormonal treatment

Hormones used	Treatment Time After Calving	Cows treated		Rate of reaction to hormonal treatment
		Heads	With fervor on the day of insemination	
First experiment: **PGF2-alfa+PGF2-alfa***	60- 90 days	25	25 (clearly)	100%
Second experiment: **GnRH + PGF2 alfa +GnRH****	60-90 days	22	22 (clearly)	100%

**Sinhrogal: Analog Pharmaceutical of PGF2-alpha uterine.*

**Enagon: Analog Pharmaceutical of GnRH Hypothalamycin.*

Table 4 proves that cows who underwent hormonal treatment reacted biologically well, even on equal rate for both experiments (100%). We say that the hormones reacted biologically well because, in all treated cows (No. 47) were observed during the time of insemination: The graphite follicle in the palpable ovary; Sufficient vaginal secretions; The cervix easily passable.

We think that this reaction is relevant in two directions: *First*, because it shows that the hormones used by us were biologically active and *Second*, that the cows were with TV in the ovary, i.e. were really with sub-estrus.

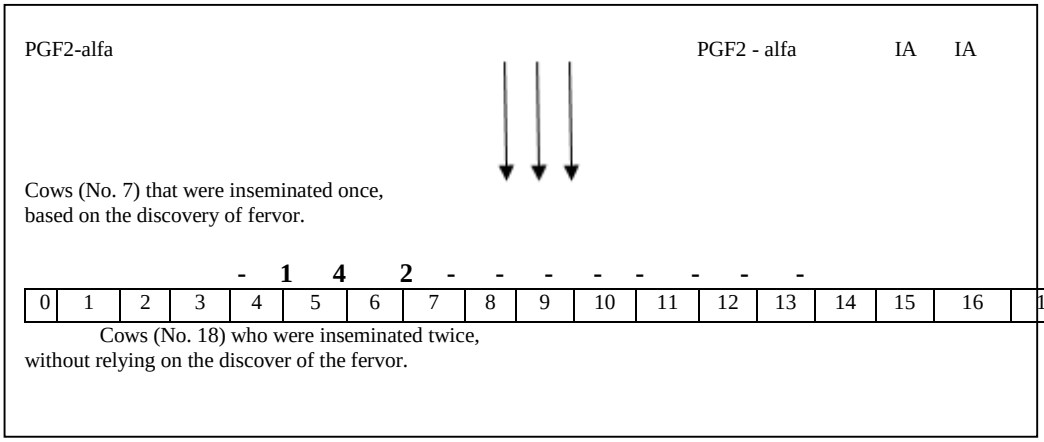
It is also said that PGF2-alpha is a typical luteolytic substance that *regression* TV in the ovary, lowering near zero the P4 in the blood and as a result the animal exhibits sexual excitement, estrus or fervor. The good biological activity of GnRH proves the programmed arrival of fervor in cows where it was used, combined with PGF2-alpha. Through methodical insemination during the fervor, a good pregnancy of the problem cows was obtained in both experiments (Table 4).

As seen in Table 4, some cows and exactly 7 heads did not "take" the second PGF2-alpha because they came up on fervor after the first PGF2-alpha. Although they were injected with the same dose of Sinhrogal, the fervor did not appear at an uneven interval after injection (Table 5).

Table 4 General data and results overview in the group of cows with sub-estrus of the first experiment.

No.	Cow No.	Clinic Diagnosis	Day 0: PGF_2-alfa	Day 13: PGF_2-alfa	IA1	IA2	Res.
1.	661/9	Sub-estrus (with TV)	06.04.'15	19.04.'15	22.03.'15	23.03.'15	+
2.	678/1	Sub-estrus (with TV)	27.04.'15	10.05.'15	14.05.'15	15.05.'15	–
3.	378/0	Sub-estrus (with TV)	13.09.'15	26.09.'15	29.09.'15	30.09.'15	+
4.	237/0	Sub-estrus (with TV)	08.01.'16	21.01.'16	24.01.'16	25.01.'16	+
5.	160/8	Sub-estrus (with TV)	23.03.'16	/	26.03.'16	/	+
6.	769/2	Sub-estrus (with TV)	03.05.'16	16.05.'16	19.05.'16	20.05.'16	+
7.	768/2	Sub-estrus (with TV)	03.05.'16	16.05.'16	19.05.'16	20.05.'16	+
8.	418/5	Sub-estrus (with TV)	13.05.'16	26.05.'16	29.05.'16	30.05.'16	+
9.	163/3	Sub-estrus (with TV)	01.06.'16	/	04.06.'16	/	+
10.	169/1	Sub-estrus (with TV)	29.05.'16	09.06.'16	12.06.'16	13.05.'16	+
11.	961/1	Sub-estrus (with TV)	22.06.'16	05.07.'16	08.07.'16	09.07.'16	+
12.	108/3	Sub-estrus (with TV)	04.07.'16	/	08.07.'16	/	+
13.	131/7	Sub-estrus (with TV)	04.07.'16	17.07.'16	20.07.'16	21.07.'16	–
14.	323/0	Sub-estrus (with TV)	17.08.'16	30.08.'16	02.09.'16	03.09.'16	+
15.	320/0	Sub-estrus (with TV)	17.08.'16	30.08.'16	03.09.'16	04.09.'16	+
16.	379/6	Sub-estrus (with TV)	25.09.'16	08.10.'16	11.10.'16	12.10.'16	+
17.	118/2	Sub-estrus (with TV)	27.09.'16	10.10.'16	13.10.'16	14.10.'16	+
18.	/	Sub-estrus (with TV)	27.09.'16	11.10.'16	14.10.'16	15.10.'16	+
19.	739/9	Sub-estrus (with TV)	13.12.'16	/	16.12.'16	/	+
20.	270/9	Sub-estrus (with TV)	24.01.'17	06.02.'17	09.02.'16	10.02.'17	+
21.	431/8	Sub-estrus (with TV)	31.01.'17	/	01.02.'17	/	–
22.	296/3	Sub-estrus (with TV)	21.02.'17	/	25.02.'17	/	-
23.	294/9	Sub-estrus (with TV)	21.02.'17	06.03.'17	09.03.'17	10.03.'17	+
24.	353/7	Sub-estrus (with TV)	03.03.'17	16.03.'17	19.03.'17	20.03.'17	+
25.	043/8	Sub-estrus (with TV)	24.04.'17	/	27.04.'17	/	-

Table 5. Distribution by days of cows' number in fervor, after the first injection of PGF2-alpha.



The phenomenon of some cows that exhibiting fervor 2 days after the first injection of PGF2-alpha, while some others 3 days, 4 days or 5 days after that injection, is related to the fact of how much follicular wave is needed for different cows to realize the cycle, and how big or small is one of the ovaries follicles at the injection time of PGF2-alpha [1][13]. As it was also stated in the methodology, we had predicted that some cows could show fervor after the first PGF2-alpha (day 0) and only need to be inseminated once. The table above (Tab.3.5) indicates that only 7 cows with sub-estrus or 28% of the first experiment group came into fervor after the first PGF2 alpha.

The prognosis was based on estrus cycle biology, which states that in a group of cows in the cycle, some of them carry an active or functional TV (aged 6-15 days) in the ovary, which is *regression* 2-5 days after first injection of the PGF2-alpha. Regarding this, the literature states that in a group of cows in the cycle, approximately 2/3 of them should be in dioestrus for a certain time. This means that most of the herd heads should react with estrus after the first injection of PG, which occurs 2-5 days after its injection [13]. We actually expected more than 28% of cows to exhibit a distinct or discoverable fervor after the first injection of PGF2-alpha. We say this because even the cows with sub-estrus are in the cycle. As such, at a certain time such as the day of treatment, most of them should be in the luteal phase of the cycle, during which PGF2-alpha acts actively.

Regarding this, we think that it is quite possible that after the first injection of PGF2-alpha, even the other cows have been in fervor, but they have not been discovered by their owners. This is acceptable because the discovery requires time and skill [13]. In an in-depth study with cows, after the first injection of PGF2-alfa, 43.4 % of treated cows or about 15% more than in our study resulted with a noticeable fervor.

Regardless of this, cows with sub-estrus that did not respond to the first PGF2-alpha received the second PGf2-alpha (day 13) and were insemination twice during the fervor (at fixed hours and regardless of the fervor signs). The obtained results are reflected in the following (Table 6).

Table 6 demonstrates that despite the fact that the fervor in the cows is a fervor stimulated with an external hormone, they can be fertilized at normal levels with an insemination; it's enough for it to be done when the signs of the fervor are clinically distinguished. The fact which proves this is that with an insemination remained pregnant > 57% of the cows. Of course, it would be nice for cows with an insemination to have a greater number of heads, which would be a study object in the future for us.

Table 6 shows that cows with sub-estrus inseminated at fixed time after the second injection of PGF2-alpha remained pregnant with > 88%. Under these conditions it can be said that the treatment of cows with PGF2 alpha and two frontal inseminations (regardless of the fervor signs) offers very good pregnancy levels, about 88%. Our results have a good correlation with those of literature (Table 7).

Table 6 The result of the first experiment with one / two insemination during the fervor

The time of insemination after hormonal treatment	***Hormonal treatment***	***No. of heads***	***Result***
IA (Fervor signs - based)	PGF2-alfa* (Day 0)	7 (28 %)	4/7 (57.2 %)
IA1-72 hours IA2-96 hours (Fixed time - based)	PGF2-alfa + PGF2-alfa* (Day 0 + Day 13)	18 (72 %)	16/18 (88.8 %)
Overall	**-**	**25(100%)**	**20/25 (80.0 %)**

** Sinhrogal: Analog Pharmaceutical of PGF2-alpha uterine.*

Table 7 Some comparative results between study and literature, on the fertility of cows with sub-estrus, after treatment with PGF2-alpha.

The time of insemination after hormonal treatment	***Hormonal Treatment***	***Comparison***	***No. of heads***	***Result***
IA (Fervor signs – based)	PGF2-alfa* (Day 0)	Study	7	4 (57.2 %)
		Literature*	**51**	**33 (64.7 %)**
IA1-72 hours IA2-96 hours (Fixed-time – based)	PGF2-alfa +PGF2-alfa* (Day 0+Day 13)	Study	18	16 (88.8 %)
		Literature	**13**	**7 (53.8 %)**

Approximation seems to the fact that, the average level of pregnancy of cows with sub-estrus treated with PGF2-alpha in study and literature, results in over 60% (respectively 80% and 62.5%). The level of the pregnancy at first *copulation* is around 60%, is a coveted indicator for normal cows with natural fervor. It is even said that, the pregnancy of 45-55% of the cows with normal reproductive health and with PGF2-alpha synchronized fervor if fully accessible [13].

From our study it follows that the method of the first experiment has enabled that within 17 days, to get pregnant 80% of the cows treated (Table 7). This result is important, keeping in mind that:

- We are dealing with problematic cows (over 60 days after calving without fervor).
- Unfertilized cows in the hormonal fervor (experimental) are fertilized in the next natural fervor.

The fact that in this case, the estrus cycle has resulted in 17 days and not 21 days, indicates that cows with sub-estrus are normally reproduced even in the conditions of the shortest estrus cycle.

Abbreviation of the cycle is the result of the dioestrus abbreviation after the action of PGF2-alpha. The result 80% is well-matched even with the conclusion of the other authors, that dioestrus abbreviation by the prostaglandin does not affect the fertility of the cows that are inseminated in the estrus that occurs after their injection [13]. Meanwhile, it is said that, sub-estrus treatment with one or two injections of PGF2-alpha is a very effective protocol, because it strengthens sexual excitement and causes a distinct estrus in about 85% of the treated cows and therefore, it can be a factor of fertility growth, especially in the farms where is being worked well for the discovery of heat (Mialot et al., 1999). Moreover, the author Jeager of b / p (1995) points out that how much later after calving PGF2-alpha is injected, then the better it affects the shortening of interval between calving and fervor after calving.

According to this author, cows' treatment with PGF2-alpha on the 40th day after calving provided the following 91.4% pregnancy, compared to 72.9% that reached the control group. Injecting a PGF2-alpha dose is positive not only on cows with the yellow bodies in the ovary to precede the appearance of a fervor with normal sexual excitement but also in cows with anestrus that are without yellow bodies in the ovary [12]. They think that the presence of PGF2-alpha in ovarian tissues is important not only for the *regression* of the TV, but also for the normal development of steroidogenesis in the dominant follicle and the meiosis of *ovocyte* within it.

Table 8. General overview of data and results in the group of the cows with sub-estrus of the second experiment.

No.	*No. Matriculation*	Clinical diagnosis	Day 0: GnRH	Day 7: PGF_2-alfa	Day 9: GnRH	Day 10: IA	Result
1.	431/8	Sub-estrus (TV)	31.01.'17	06.02.'17	/	7.02.'17*	–
2.	183/7	Sub-estrus (TV)	21.02.'7	28.02.'17	02.03.'17	03.03.'17	+
3.	161/7	Sub-estrus (TV)	20.03.'17	27.03.'17	29.03.'17	30.03.'17	–
4.	186/3	Sub-estrus (TV)	29.03.'17	04.03.'17	06.03.'17	07.03.'17	–
5.	043/8	Sub-estrus (TV)	24.04.'17	30.04.'17	/	1.05.'17*	+
6.	678/1	Sub-estrus (TV)	27.04.'17	03.05.'17	05.05.'17	06.05.'17	+
7.	131/7	Sub-estrus (TV)	04.05.'17	11.05.'17	13.05.'17	14.05.'17	+
8.	152/3	Sub-estrus (TV)	04.05.'17	11.05.'17	13.05.'17	14.05.'17	+
9.	782/7	Sub-estrus (TV)	10.05.'17	17.05.'17	19.05.'17	20.05.'17	+
10.	550/9	Sub-estrus (TV)	11.05.'17	18.05.'17	20.05.'17	21.07.'17	+
11.	687/3	Sub-estrus (TV)	13.05.'17	21.05.'17	22.05.'17	23.05.'17	+
12.	296/3	Sub-estrus (TV)	18.05.'17	25.05.'17	/	26.05.'17*	+
13.	141/6	Sub-estrus (TV)	30.05.'17	05.06.'16	07.06.'17	08.06.'17	–
14.	482/1	Sub-estrus (TV)	31.05.'17	06.06.'17	08.06.'17	09.06.'17	+
15.	834/3	Sub-estrus (TV)	08.06.'17	15.06.'17	/	15.06.'17*	+
16.	873/2	Sub-estrus (TV)	03.06.'17	10.06.'17	12.06.'17	13.06.'17	+
17.	614/0	Sub-estrus (TV)	17.06.'17	/ **	/	22.06.'17	+
18.	/	Sub-estrus (TV)	29.06.'17	04.07.'17	/	06.07.'17*	+
19.	/	Sub-estrus (TV)	16.07.'17	23.07.'17	25.07.'17	26.07.'17	–
20.	380/7	Sub-estrus (TV)	16.07.'17	23.06.'17	25.07.'17	26.06.'17	–
21.	383/1	Sub-estrus (TV)	22.08.'17	29.08.'17	31.08.'17	01.09.'17	+
22.	386/2	Sub-estrus (TV)	29.08.'17	05.09.'17	07.09.'17	08.09.'17	–

* Cows that came in fervor after PGF2-alpha and were inseminated without the second GnRH.

** The cow that came in fervor without taking PGF2-alpha.

Normal steroidogenesis follows with proper sexual excitement and distinctness of fervor in the cows. The second experiment, based on the sub-estrus treatment with the GnRH + PGF2-alpha + GnRH hormonal scheme, included 22 cows. The main indicators of this experiment are shown in Table 8.

Also for the second experiment, we had predicted that some cows could show fervor after PGf2-alpha (Day 7), without the need to "get" the second GnRH injection. In fact, 6 heads or 27.3% of the second group cows displayed the fervor before the expected time, which was only insemination once. One of them failed to receive not only GnRH but PGF2-alpha on the 7th day (Table 9).

Table 9 shows that 6 cows or 27.3% of the heads of the second experiment have exhibited fever (and are inseminated) before the 10th day. This phenomenon was also found by foreign authors.

We think that the cow 614/0 (Table 8 and Table 9) that showed fervor 6 days after the first injection of GnRH or without PGF2-alpha appeared to have been in the phase of natural *luteolysis* and GnRH of day 0 stimulated the growth and ovulation of the dominant follicle. The other cows, the 3 heads of the 8th day and the 2 heads of the 9th day, should have been at the beginning of *luteolysis* and PGF2 alpha of the 7th day has just accelerated it. Other authors emphasize cows in the scheme that have shown the fervor and have been inseminated not on the 10th day, but 11th, 12th and 13th days [10]. Based on this fact, the author in question calls the GnRH + PGF2-alpha + GnRH scheme as a protocol that does not provide systematic or constant ovulation synchronization within an 8-hour period of 10th day as published for cows with normal estrus cycle [10]. In total, 11-14% of cows treated with GnRH + PGF2-alpha + GnRH had discoverable fervor at another time, before and after the 10th day [10]. After the insemination of 22 cows of the second experiment (with a programmed insemination of 72.7% of fervor and with an unprogrammed insemination 27.3%), most of them could be pregnant (Table 10).

Table 9. Distribution by days of cow's number income in fervor, after the first injection of GnRH.

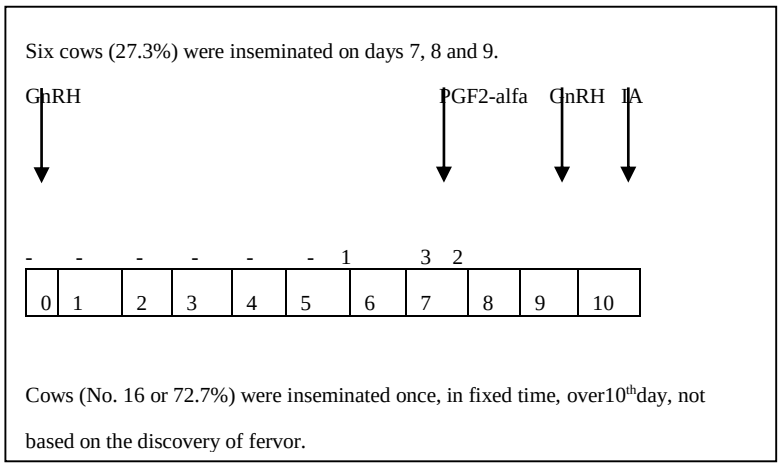


Table10. Result of cows with sub-estrus of the second experiment, with one insemination in fixed time.

The time of insemination after hormonal treatment	***Hormonal Treatment***	***No. of heads***	***Result***
IA (Fervor-signs-based)	GnRH + PGF2-alfa (Without the second GnRH)	6	5 (83.3 %)
IA (Fixed-time-based)	GnRH + PGF2-alfa + GnRH* (Day 0 + Day 7 + Day 9)	16	10 (62.5 %)
Overall	**-**	**22 (100%)**	**15 (68.2 %)**

** Enagon: Analog Pharmaceutical of GnRH Hypothalamycin.*

Table 10 proves that cows with sub-estrus that are treated with the standard GnRH + PGF2-alpha + GnRH scheme can be fertilized at normal levels even with a single insemination. The fact that only by a single insemination was fertilized on average 68.2% of treated cows. Under these conditions it can be said that treatment of cows with the standard GnRH + PGF2-alpha + GnRH scheme, by a single insemination and regardless of the fervor's signs, offers pretty good pregnancy rates, about 62%.

This scheme seems to be worthwhile, especially for rural families, having a cow and not being able to discover the fervor in their cows. We say this because we also found the discoverable fervor in about 27% of the heads. According to [10], the scheme in question produces completely clear fervor in less than 37% of the cows with sub-estrus, at a time when the PGF2-alpha + PGF2 alpha scheme, this indicator of about 85%, as we mentioned in the first experiment. It is also noted that 20% of cows inseminated on 10th day had such concentration of P4 (> 2 ng / ml) in milk, which does not match with that of estrus (<1.5 ng / ml). Our results have a good approximation to those of literature (Table 11).

Table 11 shows a 14.4 % lower difference in cows with sub-estrus of foreign authors. We think that the difference in question is related to the fact that the cows of their study, as for PGF2-alpha and GnRH, belonged to about 8,000 l / milk in lactation. Perhaps for this reason, the authors emphasize that the protocol Ov-Sink (GnRH + PG + GnRH) with fixed-time insemination results with good synchronization of ovulation and good fertilization level in cows with sub-estrus [10].

In the individual cards of our cows we had recorded the maximum daily milk production, to calculate indirectly the total production of each cow during lactation. It results that our cows belonged to the capacity of 4000-6000 l milk in lactation. From some processing, it results that we had no correlation between the level of productivity and the level of pregnancy of the cows with sub-estrus after their hormonal treatment. We recognize and acknowledge the impact of the level of production on the results of reproduction studies in cows, but this may be more clearly seen in those with production above 7000 l. If we compare our two experiments, we see simultaneously the contributions of each experiment and of the study as a whole (Table 12).

Table 11. Some comparative results between study and literature on fertilityof cows with sub-estrus, after treatment with GnRH + PGF2-alpha + GnRH.

The time of insemination after hormonal treatment	***Hormonal treatment***	***Comparison***	***No. of heads***	***Result***
IA (Fixed-time-based)	GnRH + PG + GnRH (Day 0+7+ 9)	Study	22	**15 (68.2 %)**
		Literature*	**93**	**50 (53.8 %)**
		Difference	**-**	**14.4 %**

** Mialot J.P., Laumonnier G., Ponsart H., Barassin E. etc.*

Table 12 Comparison of the pregnancy results of cows, treated with two hormonal schemes.

Hormonal treatment	***No. of heads***	***Result***
PGF2-alfa + PGF2-alfa	25	20/5 (80.0 %)
GnRH+PGF2-alfa+GnRH	22	15/7 (68.2 %)
Overall	**47**	**35/12 (74.5 %)**

Table 12 proves that the first experiment has produced 11.8% more pregnancy *(shq.barrsëri)* than the second experiment. Both experiments have made it possible for a total of 74.5% of cows with sub-estrus, to be pregnant for 10 to 17 days, set in the hormonal treatment defined in the methodology.

We think that in these results, among other things, there was a good influence on the *(shq. "flashing")* that farmers applied in experimental cows, in the two weeks of hormonal treatment and in the four weeks after the insemination, to provide a positive balance of metabolic energy. For this matter, the literature states that cows placed in the hormonal treatment in question must have a positive energy balance, i.e. A dynamic addition to body weight: cows 350 g / day and heifers 450 g / day [13].

It was also said in the introductory part of the study that its main objective was; Recognition of the effectiveness of two hormonal schemes for the strengthening of sexual excitement and the fast pregnancy of cows affected by sub-estrus, in the benefit of their fertility, on the farms of the Tetovo municipality and beyond. Table 4.10 shows that this objective has been achieved quite well. Standard PGF2-alpha + PGF2-alpha or GnRH + PGF2-alpha + GnRH therapy in cows with sub-estrus is as efficient as in normal cows.

We think that, at the close of our analysis and discussion, we can also reflect the approximate cost of a cow with sub-estrus in one or the other hormonal method (Tab.13a, b)

Table 13a. The cost of methodical treatment for each cow possibly pregnant,according to the study (experiment 1)

The standard treatment method	The cost of a PG dose on the market & Service (Euro)	Cost of sperm & IA (Euro)	Observation and discovery by owner (1 PG + 1 IA) (Euro)	Without observation & discovery by the owner. (2 PG + 2 IAs) (Euro)	Control (Euro)	The sum for a pregnant cow (Euro)*
PGF2-alfa + PGF2-alfa	5	10	**15**	**-**	**5**	20
	5	10	-	**30**	**5**	35

Table 13b. The cost of methodical treatment for each cow possibly pregnant, according to the study (experiment 2)

The standard treatment method	The cost of three hormonal doses & Service (Euro)	Cost of sperm dose & IA (Euro)	Without observation and discovery by the owner, with one IA (Euro)	Control (Euro)	The sum for a pregnant cow (Euro)*
GnRH+PGF2-alfa+GnRH	15	10	**25**	**5**	30

**The total amount does not include the case of non-pregnancy, but spending on this case is still beneficial because, the non-hormonal fervor develops normal and this increases the chance of pregnancy after three weeks.*

4. Conclusions

Cow's families with barrenness problems and over 60 days after calving appearing with anestrus in veterinary clinics over a year may result from a gynecological examination with three different diagnoses and densities:

- Real anestrus (without yellow body in the ovary) about 23%.
- Unreal anestrus (with yellow body in the ovary: sub-estrus) about 49%.
- Progestogenic anestrus (with lutein cyst in the ovary); about 27%.

The standard PGF2-alpha and GnRH-based therapy consist of the use of two hormonal study schemes (Es-Sink: PGF2-alpha + PGF2-alpha) (Ov-Sink: GnRH + PGF2-alpha + GnRH), results in bio-clinical action at 100% mass of cows with sub-estrus. For 2-5 days after the first injection of PGF2-alpha (Day 0), they show a clear fervor and can be detected by the family members 28% of the treated cows and only insemination once (non-fixed time) and remained pregnant 57.2% of them. Cows with sub-estrus that did not react to the first injection of PGF2-alpha (Day 0) and received their second injection (Day 13) were inseminated all without based on fervor detection, twice, fixed time (IA1- 72 hours & IA2-96 hours after PGF2-alpha) and remained

88.8% of them. The first experiment method (Es-Sink: PGF2-alpha + PGF2-alpha) has enabled within 17 days 80% of the treated cows with sub-estrus to remain pregnant. Standard Therapy with the Ov-Sink Scheme: GnRH + PGF2-alpha + GnRH, enables the fervor and only one synchronization insemination at fixed time (Day 10: 24 hours after the second GnRH), to 72.7% of the cows with sub-estrus, while 27.3% showed the fervor and were inseminated earlier (Day 7, 8, 9), without taking the second injection of GnRH, with 62.5% fertilization and 83.3% respectively. The second experiment method (Ov-Sink: GnRH + PGF2-alpha + GnRH) has enabled within 10 days to get pregnant 68.2% of the treated cows with sub-estrus.

5. References

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