

# GONADOTROPIC ACTION OF MEDICATION ADMINISTERED IN VARIOUS DOSES TO SYNCHRONISE THE OESTRUS OF ANOESTRAL SHEEP\*\*

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**Abstract:** The experiment included 20 ewes of the Tsigai breed, allocated in two groups of 10 animals each. Inducing of oestrus synchronisation was performed during anoestral season (April – May) through vaginal sponges containing gestagens, and administration of Gravohormon (Vetbiopharm, Bulgaria) - 500 UI in group 1 ewes and 1000 UI in group 2 ewes. Macromorphological study of the ovaries was carried out by means of laparoscopy after the adopted methods – on the 56<sup>th</sup> hour, 72<sup>nd</sup> hour and 5<sup>th</sup> day following sponge withdrawal and injection of gonadotropin. The levels of the steroid hormones progesteron and 17 $\beta$ -oestradiol were recorded in three ewes per each group at different times according to the experiment scheme. The histological structure of the ovaries was determined in seven animals subjected to ovariectomy.

**Key words:** ewes, synchronisation oestrus, anoestral season, steroid hormones, macromorphological structure ovaries.

## Introduction

The level of ovulatory response is the main parameter that determines the effectiveness of applied serum gonadotropins for the purpose of inducing synchronous oestrus. This is largely true for anoestral livestock when under the influence of diverse factors (most probably seasonal changes of the light exposure) the cyclic nature of sexual processes is upset, and consequently the normal follicle genesis does not end in follicle maturation with subsequent ovulation. As a result of the negative correspondence between the oestrogenes synthesized in the ovary and the structures of the hypothalamus – hypophysis axis, an inhibiting effect occurs, which is demonstrated by the lack of LH peak

and ovulation to follow it. The ever-increasing interest in breeding ewes during anoestrus has necessitated optimization of the schemes for inducing of synchronous oestrus.

In this particular study our goal was to examine the gonadotropic activity of Gravohormon (Vetbiopharm, Bulgaria) applied in different doses, with regard to the functional activity and the dynamic processes in the ovaries macro- and micro-flora.

## Material and Methods

The experiment was conducted during anoestrus (May – June) of 20 ewes of Tsigai breed, aged 5<sup>th</sup> – 6<sup>th</sup> lambs, reared on the experimental farm of RIMSA – Troyan. Two trial groups per 10 animals each were established. The ewes were similar in live weight and lambing time. They were treated with vaginal sponges containing Cronolone - 30 mg (Intervet, Holland) for 12 days and then injected with 500UI and 1000UI of Gravohormon (Vetbiopharm, Bulgaria), respectively.

The macromorphology of ovaries was examined by laparoscopy on the 56<sup>th</sup>, and 72<sup>nd</sup> hour and the 5<sup>th</sup> day after the application of medication. The objects examined were taken picture of by mirror-reflective camera Ricohn (Ricoh Company, Japan) and TTL computer flash (manufactured by Karl-Storz-Endoskope, Germany). The microstructure of ovaries was determined after ovariectomy on the 5<sup>th</sup> day following the exogenous hormone introduction. The ovaries were fixed in formalin and subjected to preliminary processing, and subsequently put to routine methods of dehydration, brightening, inclusion into paraffin, cutting and staining. The 5-7  $\mu\text{m}$  thick cuts were stained by haematoxiline and eosin for histological assessment of the ovarian organoids. The ovaries of five ewes were used in the group receiving 500UI Gravohormon, and in the group treated with 1000UI - the ovaries of two ewes.

The functional activity of ovaries in three ewes of each group was determined by the plasma contents of steroid hormones – progesteron and 17- $\beta$ -oestradiol. This was performed after a schedule – immediately after removal of vaginal sponges, on the 24<sup>th</sup>, 36<sup>th</sup>, 48<sup>th</sup>, 60<sup>th</sup> and 72<sup>nd</sup> hour and the 5<sup>th</sup> day.

## Results and Discussion

The available ovarian structures found by laparoscopic methods at different times after treatment with the gonadotropic activity medical preparation are listed in table 1.

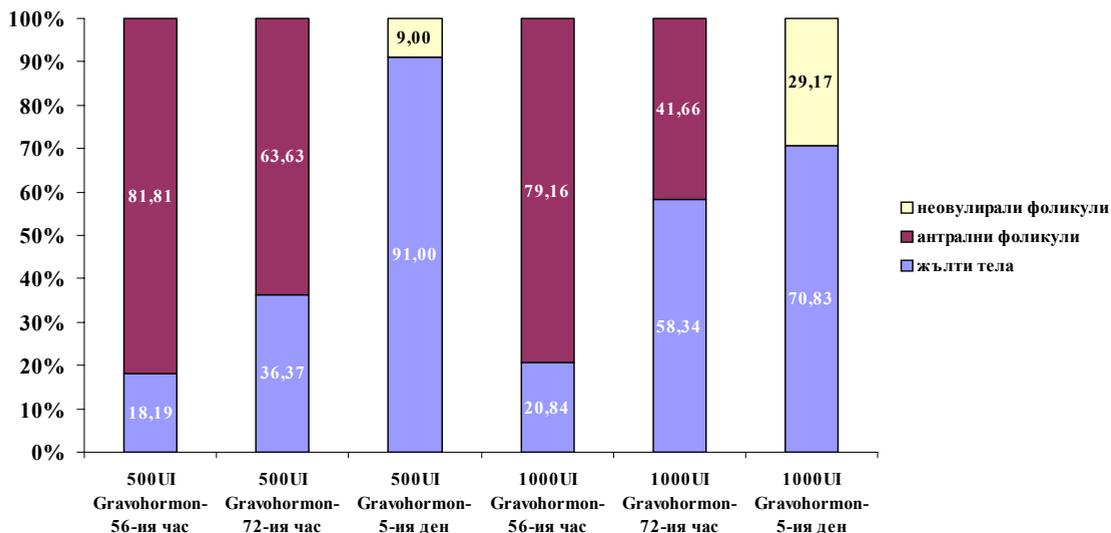
**Table 1. Macro morphology of the ovarian structures of ewes treated with 500UI and 1000UI Gravohormon****Tabela 1. Makro morfologija structure jajnika ovaca tretiranih sa 500UI i 1000UI Gravohormona**

| №    | Gravohormon x 500UI   |         |                       |         |                     |         | №  | Gravohormon x 1000UI  |         |                       |         |                     |    |         |
|------|-----------------------|---------|-----------------------|---------|---------------------|---------|----|-----------------------|---------|-----------------------|---------|---------------------|----|---------|
|      | 56 <sup>th</sup> hour |         | 72 <sup>nd</sup> hour |         | 5 <sup>th</sup> day |         |    | 56 <sup>th</sup> hour |         | 72 <sup>nd</sup> hour |         | 5 <sup>th</sup> day |    |         |
|      | F                     | CL      | F                     | CL      | NF                  | CL      |    | F                     | CL      | F                     | CL      | NF                  | CL |         |
| 1    | 1                     |         |                       | 1       |                     | 1       | 11 | 0                     | 2       |                       |         | 2                   |    | 2       |
| 2    | 1                     |         |                       | 1       |                     | 1       | 12 | 1                     |         |                       |         | 1                   |    | 1       |
| 3    |                       | 2       |                       | 2       |                     | 2       | 13 | 2                     |         | 1                     | 1       |                     |    | 2       |
| 4    |                       |         |                       |         |                     |         | 14 | 2                     |         | 1                     | 1       |                     |    | 2       |
| 5    | 1                     |         | 1                     |         |                     | 1       | 15 | 3                     |         | 1                     | 2       |                     |    | 3       |
| 6    | 1                     |         |                       | 1       |                     | 1       | 16 | 2                     | 1       | 2                     | 1       |                     | 2  | 1       |
| 7    | 2                     |         | 2                     |         | 1                   | 1       | 17 | 3                     |         |                       |         | 3                   |    | 3       |
| 8    |                       |         |                       |         |                     |         | 18 | 3                     |         | 2                     | 1       |                     | 2  | 1       |
| 9    | 2                     |         |                       | 2       |                     | 2       | 19 | 1                     | 2       | 1                     | 2       |                     | 1  | 2       |
| 10   | 1                     |         | 1                     |         |                     | 1       | 20 | 2                     |         | 2                     |         |                     | 2  |         |
| общо | 9                     | 2       | 4                     | 7       | 1                   | 10      |    | 19                    | 5       | 10                    | 14      | 7                   |    | 17      |
| x ±  | 0,9±0,2               | 0,2±0,2 | 0,4±0,2               | 0,7±0,2 | 0,1±0,1             | 1,0±0,2 |    | 1,9±0,3               | 0,5±0,2 | 1,0±0,2               | 1,4±0,2 | 0,7±0,3             |    | 1,7±0,3 |
| Sx   |                       |         |                       |         |                     |         |    |                       |         |                       |         |                     |    |         |

*F – antral follicles, CL – yellow body, N.F. – non-ovulated follicles.*

The quantitative percentage of the different ovarian structures is shown in figure 1.

**Figure 1. Quantitative percentage of the different ovarian structures**  
**Slika 1. Kvantitativni procenat različitih struktura jajnika**

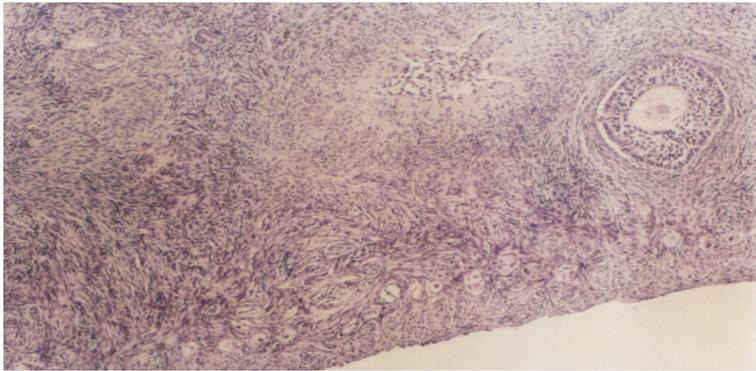


Increase was found in the average number of ovulated follicles observed on the 56<sup>th</sup>, and 72<sup>nd</sup> hour and on the 5<sup>th</sup> day with both doses of Gravohormon applied, namely:  $0.2 \pm 0.2$ ,  $0.7 \pm 0.2$ ,  $1.0 \pm 0.2$  и  $0.5 \pm 0.2$ ,  $1.4 \pm 0.2$ ,  $1.7 \pm 0.3$ . The number of antral follicles found on the 56<sup>th</sup> hour was greater in ewes treated with 1000UI Gravohormon than in those treated with 500UI, the corresponding values being  $1.9 \pm 0.3$  and  $0.9 \pm 0.2$ . With increase of the medication dose resulted we found rising percentage of non-ovulated follicles - 9.00% with a dose of 500UI and 29.17% with 1000UI.

The ovaries of ewes where 500UI PMSG was applied had normal histological structure. Three of the ovaries had structure patterns characteristic of the follicle stage, and two ovaries – of the luteal stage. Ovaries in their follicle stage show no signs of atresia of antral follicles, while those in the luteal stage have mature yellow bodies and atresia of the antral follicles (figure 2).

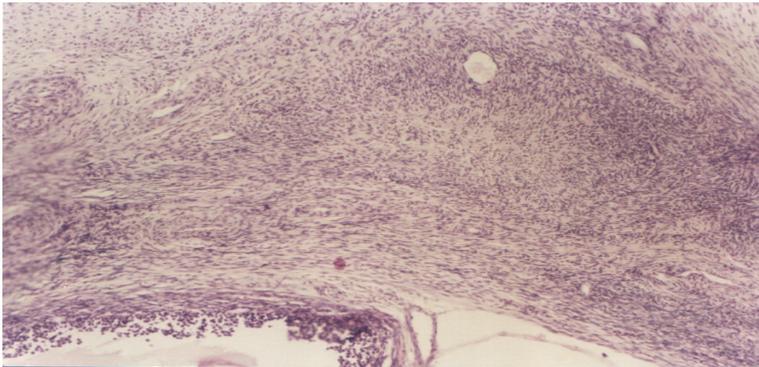
Our findings included a total of 18 antral follicles, 2 yellow bodies and 14 antral follicles in atresia. With the latter (antral follicles with atresia) no cystic mutations were observed. In the ewes where 1000UI PMSG were applied, the ovaries were found to be in their luteal stage (with mature yellow body), in the available part of the cuts. Follicles in atresia were found at secondary or immature level.

**Figure 2. Atresia of antral follicle**  
**Slika 2. Atrezija antralnog folikula**



The antral follicles were anovulatory and highly enlarged, while some of them had cystic mutations (figure 3).

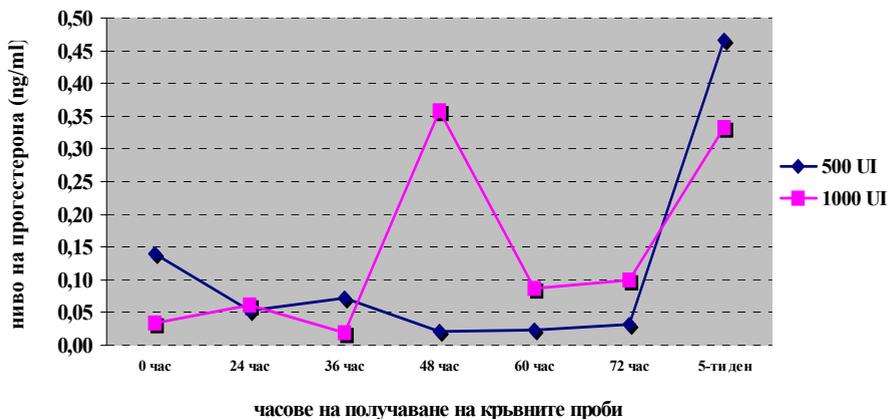
**Figure 3. Cystic mutations of antral follicle**  
**Slika 3. Cistične mutacije antralnog folikula**



The plasma content of steroid hormones (progesteron and 17 $\beta$ -oestradiol) is demonstrated in figures 4-5.

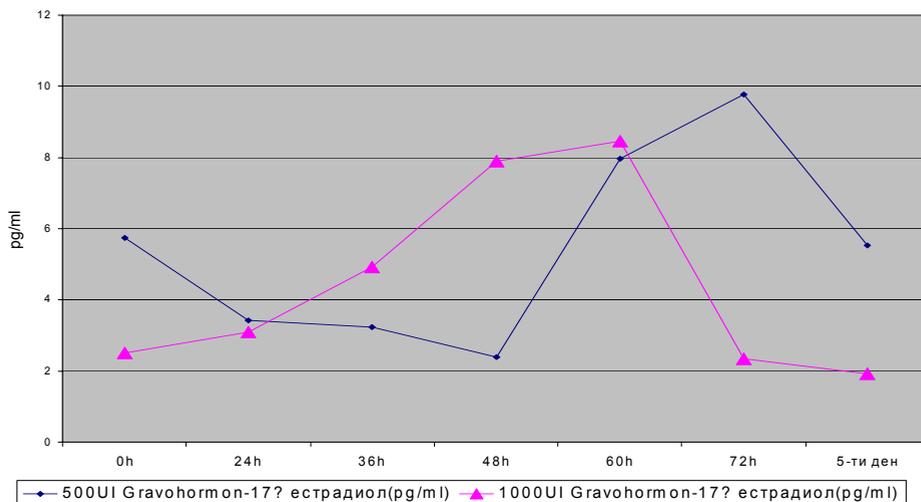
**Figure 4. Dynamics of the progesteron plasma levels in ewes treated with 500UI and 1000UI Gravohormon**

**Slika 4. Dinamika nivoa progesterona u plazmi kod ovaca tretiranih sa 500UI and 1000UI Gravohormona**



In ewes where 500UI were applied the average values of progesteron ranged from  $0.022 \pm 0.008 \text{ ng/ml}$  to  $0.467 \pm 0.088 \text{ ng/ml}$ , while in those with 1000UI the values ranged from  $0.019 \pm 0.006 \text{ ng/ml}$  to  $0.333 \pm 0.128 \text{ ng/ml}$ . There was a growing trend of the progesteron level after the 72<sup>nd</sup> hour, and the highest values were read on the 5<sup>th</sup> day in both trial groups. The high level of the hormone found on the 5<sup>th</sup> day indicates available formed and functioning yellow body.

**Figure 5. Dynamics of the oestradiol plasma levels in ewes treated with 500UI and 1000UI of Gravohormon**



**Slika 5. Dinamika nivoa estradiola u plazmi kod ovaca tretiranih sa 500UI i 1000UI Gravohormona**

In the trial ewes treated with a dose of 500 UI the average values of  $17\beta$ -oestradiol ranged from  $2.40 \pm 1.07$  pg/ml to  $9.77 \pm 0.30$  pg/ml, and in ewes receiving 1000UI the values ranged from  $1.93 \pm 0.52$  pg/ml to  $8.47 \pm 1.38$  pg/ml. The plasma level of  $17\beta$ -oestradiol was the highest on the 72<sup>nd</sup> hour in trial group 1 (500UI), and on the 60<sup>th</sup> hour in group 2 (1000UI). The elevated  $17\beta$ -oestradiol levels can be defined at the time of ovulation, which in group 1 is on the 72<sup>nd</sup> hour and in group 2 – on the 60<sup>th</sup> hour.

The differences found were not significant due to the small number of ewes in the group.

## Discussion

The results we obtained correspond to those reported by *Stankov* (1983) and *Karsch et al.* (1980) for the levels of both steroid hormones.

According to *Cahill et al.* (1981), the  $17\beta$ -oestradiol level before ovulation is within 2.5-5.0 pg/ml.

The dose of the applied medication is one of the main factors that influence the effectiveness of the applied exogenous gonadotropins for inducing of

oestrus, especially in anoestral season. A good number of investigations have been conducted on this issue.

Their assumption, as well as ours stated in some other studies, is that hormonal preparations stimulate the development and growth of follicles, but the problem is that not all of them ovulate. This fact finds confirmation also in the evidence on macro morphology of ovaries in this experiment.

We assume that the reason for that is the dose of the gonadotropins applied. The dose increase of Gravohormon from 500UI to 1000UI resulted in considerable growth of non-ovulated follicles (from 9.00% to 29.17%). By laparoscopic methods for ovaries it is possible to count non-ovulated follicles only on the 5<sup>th</sup> day following treatment. However, this method cannot assess the morphological and functional status of non-ovulated follicles that could be antral follicles in a state of atresia or with cystic mutations. This gave us good reason to examine the microstructure of sheep ovaries in this study.

The inconsistent growth of follicles due to the application of gonadotropins is attributed to their long-lasting term of half life *Armstrong et al.*(1983).

Similar hypothesis seems acceptable, but we should also consider some characteristics of sheep follicle genesis.

According to *Findlay et al.* (1987), *Hirshfield* (1991) and *Scaramuzzi* (1993), the follicle genesis proceeds in several stages – initial, pre-antral, antral, ovulatory and atresia stages. The first three stages are largely autonomous and are not affected by the hypophysis gonadotropins, irrespective of whether they were naturally secreted or introduced exogenously. The gonadotropic activity is manifested only in the final two stages.

This assumption could not be confirmed or discarded through the methods utilized in our experiment. The data are documental by nature, which classifies the study as having importance both for research and for practical application.

## Conclusions

1. Any changes in the ovary morphology and functions under the effect of exogenously introduced gonadotropins, following a schedule for inducing of synchronous oestrus in anoestral period, are responses of idiosyncratic nature.

2. The dose increasing of the serum gonadotropins from 500UI to 1000UI resulted in increasing the number of antral follicles to  $0.9 \pm 0.23$  and  $1.9 \pm 0.31$ , correspondingly, but there was a simultaneous increase in the number of anovulatory follicles to 9.00% and 29.17%.

3. The increase of the dose of exogenously introduced serum gonadotropins did not produce improvements in the follicle genesis, while posing risks of cystic mutations.

## **Gonadotropsko dejstvo različitih doza leka korišćenog za sinhronizaciju estrusa kod anestralnih ovaca**

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### **Rezime**

U ogledu su korišćene ovce rase Cigaja, podeljene u dve grupe od po 10 grla. U toku anestralne sezone rađena je indukcija sinhronizacije estrusa (april – maj) korišćenjem vaginalnih sundera koji su sadržavali gestagens, i preparata Gravohormon (Vetbiopharm, Bulgaria) - 500 UI u grupi 1 i 1000 UI u grupi 2. Makromorfološka analiza jajnika je rađena laparoskopijom po usvojenoj metodi – 56. sata, 72. sata i 5 dana nakon uklanjanja sundera I injekcije gonadotropina. Nivoi steroid hormona progesterona i 17 $\beta$ -estradiola su zabeleženi kod tri ovce u svakoj grupi u različitim vremenima prema shemi oglada. Histološka struktura jajnika je određivana kod sedam životinja koje su bile podvrgnute odstranjivanju jajnika.

**Ključne reči:** ovce, sinhronizacija estrusa, anestralna sezona, steroidni hormoni, makromorfološka struktura jajnika

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