HEXESTROL DIACETATE-INDUCED FOLLICULAR ATRESIA IN IMPUBER SHEEP

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Summary

An experimental group formed out of 5 ewes, 3 months old, was inoculated subcutaneously (S.C.) with 0.2 ml Sintofolin/animal, and the other served as control group. After 30 days, the ovaries were harvested through oophorectomy for histopathological examination. There was a large number of atretic follicles in the harvested ovaries from the experimental group, and the primordial follicular store was reduced by 50%. We can observe that the hexestrol diacetate initiates and maintains the follicular atresia in young impuber sheep, with the drastic reduction of the follicular store, compromising the female reproductively.

Key words: Sintofolin, follicle, atresia, apoptosis, granulosa.

Introduction

Female domestic mammals have 60,000 to 200,000, or even more, primordial follicles (with differences from one species to another) in the ovaries at birth. They contain sex cells that will be gradually consumed until exhaustion (Senger, 1997; Hafez and Hafez, 2000). The ovaries of an adult ewe contain 12,000-86,000 primordial follicles (Hafez and Hafez, 2000; Groza and Muntean, 2002), out of which, the female actually utilizes a very small number (under 0.5%) for reproduction. This small amount end up ovulating, between puberty and climacterium (Groza, 2006; Tabarowski et al. 2005; Sharma and Bhardwaj, 2009). Only a few hundred follicles reach maturity in a lifetime. All the other follicles are gradually eliminated from the ovaries through follicular atresia, the process which consumes the sex cell store (Bhardwaj and Sharma, 2011). The follicles can undergo atresia at any developmental stage (Carson et al., 1978).

Besides physiological follicular atresia, there is a pathological one, initiated by certain agents, among which, we can name the following: inappropriate hormonal treatments (Paraipan, 2001), myco or phytoestrogens consumption etc.

Material and methods

10 impuber animals, 3 months old, were used in this study. They were raised on traditional conditions pasture. Two groups were formed: experimental and control. Animals from the experimental group were inoculated subcutaneously with a therapeutic dose of Sintofolin, precisely 0.2 ml/animal. After 30 days from the beginning of the experiment, ovaries were harvested through oophorectomy from animals in both groups, for histopathological examination. The harvested samples were fixed in 10% formaline and embedded in paraffin. Serial sections, 5 micrometers thickness, were made and they were dyed with tricrom Goldner staining.

Results and discussion

In animals from the control group, the ovary had a certain activity at the age of 4 months, in the sense that besides the large
number of primordial follicles at the periphery of the cortical region (Fig. 2), there were also a few follicles engaged in evolution/involution processes (stages II-IV) (Fig. 1). There was no yellow body, which demonstrates that the ovarian activity does not end with ovulation, at this age. This means that besides primordial follicles, all the other follicles engaged, will undergo atresia.

In the Sintofolin treated group, the situation is entirely different in comparison to the control group, in the sense that the number of follicles engaged in evolution/involution processes is clearly bigger. Among the experimental group there are relatively big differences from one animal to another (and even between the ovaries of the same animal) concerning the intensity of follicular atresia and especially the number of the engaged follicles, which in some cases are at an impressive level, practically occupying the entire section area of the ovary (Fig. 3).

The primordial follicle store existing in the peripherical area of the ovarian cortical region is 50% lower than the one from the control group (Fig. 4).

There are follicles in different development and especially involution stages and no yellow body, which demonstrates that none of the follicles engaged in the follicular maturation processes end up ovulating, but rather undergo atresia. The time when the follicular maturation settles in, is different from one follicle to another. In antral follicles, the granulosa layer appears to be thickened at some point and even wavy sometimes, due to the excessive multiplication of the granulosa cells (Fig. 5).

Notably, a relatively large number of large antral follicles have folds of different heights in the granulosa layer. These folds can be simple or branched (Fig. 6).

Both simple and branched folds, have a connective tissue core, containing small blood vessels, which demonstrates that they are formed out by both granulosa and theca interna folliculi.

Then the granulosa cells are dissociated by intercellular oedema (Fig. 7) and gradually undergo atresia, forming various apoptotic bodies that can either be observed in the granulosa layer, on its surface or in the follicular liquid in the antrum (Fig. 8).

The process continues until the granulosa layer completely disorganizes and the basement membrane appears discontinuous. Fibroblasts, from the theca interna folliculi, enter the follicular antrum through these discontinuities. At first there are a few fibroblasts, but they gradually invade the whole follicular antrum (Fig. 9).

Finally, the connective tissue replacing the former follicle (Fig. 10) will coalesce with the neighbouring stroma.

Although they are impuber, the animals from the control group have a certain ovarian activity, namely a small number of follicles initiate the follicular maturation process, develop through one or two stages after which they undergo atresia. The process is not wide spread such as to significantly reduce the follicular store. None of the follicles that initiate developmental processes end up ovulating, meaning that the female does not have an actual reproductive activity at this age.

The presence of atretic follicles in ovaries of the females treated with Sintofolin in large numbers compared to those in the control group, suggests that hexestrol diacetate initiates and maintains the atretic process. The follicles are gradually affected by atresia, therefore they can appear in incipient to advanced stages of atresia (fibrosis). Concerning its progress, the atresia initiated by hexestrol diacetate follows approximately the same stages as physiological atresia, except for some aspects appear to have a higher intensity. First signs of atresia are present in the oocyte, that degenerates and disintegrates, and due to its disappearance, zona pellucida appears collapsed. Immediately, or even simultaneously,
changes appear in the granulosa layer. Initially, it hypertrophies through cell proliferation which dispose in more layers than usually. In some follicles, the multiplication of the granulosa cells is so intense that it determines the appearance of simple or branched folds, thus the follicular antrum appears more or less wavy (an aspect that is not normally seen). At a certain point, the granulosa cells are dissociated by intercellular oedema and their massive apoptosis is initiated, until the disappearance of the granulosa layer. The participation of apoptosis in the morphological changes in follicles undergoing atresia, was highlighted by other authors as well, who claimed that apoptosis is the main mechanism through which the cellular loss takes place during the follicular degeneration (Hirshfield, 1991; Tilly, 1996; Yu et al., 2004).

After the appearance of discontinuities in the basement membrane, the follicular antrum is invaded by fibroblasts that synthesize collagen. Gradually the follicle transforms in connective tissue and finally coalesces with the neighbouring stroma, along with the disappearance of the former follicle. In this manner, after the administration of a single therapeutic dose of Sintofolin, in 30 days’ time it comes to the consumption of over 50% out of the follicular store of the females and the process is still in progress, meaning the percent will grow. This aspect highlights on the one side the substance’s action and the consequences of using it in impuber sheep, and on the other side the increased susceptibility of the female sex cells to certain agents that can initiate and maintain follicular atresia. By consumption of most of the follicular store in a relatively short time it comes to the situation in which the female is actually reproductively compromised. The high susceptibility of the female sex cells, makes the incorrect utilisation of hormonal formulations or the consumption of forages that contain estrogen like compounds, one (if not the main) of the causes of infertility in sheep.

**Conclusions**

A single therapeutic dose of hexestrol diacetate administered in impuber sheep induced massive follicular atresia, thus in one month’s time more than 50% of the follicular store was consumed.

The drastic decrease of the follicular store in an impuber animal illustrates the particular susceptibility of the female sex cells towards the utilized formulation and leads to compromising the female reproductively.

The incorrect utilisation of some hormonal formulations and the consumption of forages that contain estrogen like compounds, can represent major causes of infertility in sheep.

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Fig. 1. Control group – entire ovary (Tricrom Goldner, ob. 2)

Fig. 2. Control group – primordial follicles at the cortical periphery (Tricrom Goldner, ob. 10)

Fig. 3. Experimental group - entire ovary (Tricrom Goldner, ob. 2)

Fig. 4. Experimental group - primordial follicles at the cortical periphery (Tricrom Goldner, ob. 10)

Fig. 5. Experimental group – thickened and wavy granulosa layer (Tricrom Goldner, ob. 10)

Fig. 6. Experimental group – branched fold (Tricrom Goldner, ob. 10)

Fig. 7. Experimental group – intercellular oedema in granulosa layer (Tricrom Goldner, ob. 20)

Fig. 8. Experimental group – apoptotic bodies (Tricrom Goldner, ob. 40)

Fig. 9. Experimental group – fibroblasts in follicular antrum (Tricrom Goldner, ob. 20)

Fig. 10. Experimental group – Follicular fibrosis (Tricrom Goldner, ob. 40)