

Research on the use of monoclonal antibodies regarding to the study of swine's testicular parenchyma

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Abstract

The use of the reproduction biotechnology requires knowledge of the functional structures of testicular parenchyma in boars of different ages by obtaining new data on the evolution of seminal postnatal cell line, Sertoli cells and Leydig interstitial gland cells. Testicles boars of several breeds have been studied: Duroc, Big White, Landrace and Synthetic Line 345 – Periș from SC Romsuintest Peris. Immunohistochemical studies were performed for a panel composed of the following monoclonal antibodies: Leu 7, Inhibin, PLAP-placental alkaline phosphatase. The use of monoclonal antibodies in studies of testicular parenchyma may have a positive effect for early diagnosis of abnormalities or disorders of spermatogenesis for the purpose of using the reproduction biotechnologies in order to obtain a quality seminal material.

Keywords: testicular parenchyma, seminiferous tubules, monoclonal antibodies: Leu 7, Inhibin, PLAP-placental alkaline phosphatase.

Introduction

Reproduction biotechnology requires knowledge of the functional structures of testicular parenchyma in boars of different ages by obtaining new data on the evolution of seminal postnatal cell line, Sertoli cells and Leydig interstitial gland cells. The use of monoclonal antibodies in studies of testicular parenchyma may have a positive effect for early diagnosis of abnormalities or disorders of spermatogenesis for the purpose of utilising the reproduction biotechnologies in order to obtain a seminal material of high quality.

The development of the testes includes changes in cell morphology and endocrine levels that are essential for the maturation of males. A large number of novel proteins are expressed throughout testis development and play important roles in spermatogenesis. Finally, a large proportion of these differentially expressed proteins are involved in cellular (25%) and metabolic (22%) processes. Identifying these differentially expressed proteins should be valuable for exploring developmental biology and the pathology of male reproduction. (HUANG, 2010 [16]). For instance, osteopontin (OPN) is detected in the majority of germ cells and is involved in spermatogenesis in boar testis, being one of decapacitation factors to prevent premature activation of sperm mobility. Intense OPN immunostaining was seen in the residual bodies and acrosomes in the spermatids while, occasionally, OPN immunostaining was seen in spermatogonia and various stage of

spermatocytes but in few Sertoli cells in the seminiferous tubules. In addition, Leydig cells in adult boars are weakly immunostained with OPN (KIM, 2007 [11]).

Expression of certain proteins is clearly associated with immaturity or maturity of the Sertoli cell, and study of the immunoexpression of such markers can potentially aid interpretation of various seminiferous tubule phenotypes that may present. The studies might indicate whether there has been a fundamental failure of maturation of Sertoli cells and include identification of markers such as: Wilms' tumour gene (WT1) provides a stable marker of Sertoli cells, against which other protein markers can be compared; anti-Mullerian hormone (AMH) is one of the first genes to be switched on in Sertoli cells after their differentiation in fetal life, and expression continues while Sertoli cells remain immature; aromatase (P450 enzyme) is expressed in fetal or neonatal Sertoli cells but expression is downregulated during maturation, such that in the adult rat testis, aromatase is expressed mainly in Leydig cells and in certain germ cells; M2A, an unidentified antigen that is recognized by a monoclonal antibody and it is expressed only by immature and not mature Sertoli cells in humans and its expression may persist in patients in whom disorders of spermatogenesis are present. Moreover, nuclear immunoexpression of androgen receptor (AR) is a feature of mature Sertoli cells, though expression varies according to the stage of the spermatogenic cycle in rats and humans. (SHARPE, 2003 [14]). Immunohistochemical and *in situ* hybridization studies have shown that AR is present not only in Sertoli cells, peritubular myoid cells in seminiferous tubules, but possibly in germ cells and there are distinct mechanisms controlling AR concentrations in Leydig and Sertoli cells during the development of the testis. (SHAN, 1997 [4]). The function of AR in the Leydig cells has primarily been linked to the postnatal development of Leydig cell progenitors and immature Leydig cells. The presence of AR in Leydig cells can suggest a certain feedback mechanism for androgen secretion that is operative in 6-month-old boars, whereas the presence of AR in Sertoli and peritubular cells can indicate that in sexually mature boars androgens control Sertoli cell function both directly through own AR or indirectly through AR localized to surrounding peritubular cells. (KOPERA, 2008 [12])

In human testes with various disorders, some, but not all, Sertoli cell-only (SCO) tubules express one or more markers of immature Sertoli cells such as AMH, cytokeratin 18 and M2A, and may not express antigens such as AR that are normal features of mature Sertoli cells. In some men with impaired spermatogenesis, especially those who exhibit focal SCO with morphologically immature Sertoli cells, focal expression of AMH and cytokeratin 18 and absence of AR expression are evident, consistent with failure of maturation of Sertoli cells in these regions. (SHARPE, 2003 [14])

Another marker used is the monoclonal C11H antibody useful in investigations of transcription and translation and their regulation during spermatogenesis because it recognizes acrosin that is first expressed in haploid cells coincident with the onset of nuclear elongation and cessation of RNA transcription. In boar spermatozoa the antibody reacted, in immunoblotting analysis, with polypeptides of Mr 55,000 and 53,000. The changes in the distribution pattern suggest that acrosin may be modified by Sertoli cells. (KALLAJOKI, 1986 [10]). Also expression of the progesterone receptor (PR) was monitored in testes of groups of five boars by using monoclonal antibody (KOHLENER, 2007 [13]).

GnRH-agonist (gonadotropin-releasing hormone) deslorelin is a useful tool to study testicular function at the cellular level and, subsequently, the mechanisms of endocrine control of reproduction in boars. In this case luteinizing hormone receptors (LHRs) and (3 β -hydroxysteroid dehydrogenase) 3 β -HSD immunostainings were confined to the cytoplasm of

Leydig cells. A weak staining for LHR or no staining observed in Leydig cells could be explained by a lack of LH release as a consequence of desensitization of the pituitary gonadotropes induced by deslorelin implant. Similarly, a lack of the staining for 3 β -HSD that reflects the lack of the key enzyme in testosterone biosynthesis can explain the suppression of testicular steroidogenesis in Leydig cells of deslorelin-treated males. (KOPERA, 2008 [12]).

The aim of this study is to provide a method of detecting evolution of seminal postnatal cell line, Sertoli cells and Leydig interstitial gland cells of swine testis using monoclonal antibodies.

Materials and methods

To perform experimental research, testicles boars of several breeds have been studied: Duroc, Big White, Landrace and Synthetic Line 345 – Periş from SC Romsuintest Peris. Specimens were monitored based on distinguishing marks sheets, in which data such as the ascendants, descendants, breeding activity and genital development were recorded. Immuno-histochemical studies were performed for a panel composed of the following monoclonal antibodies: Leu 7 Inhibin, PLAP-placental alkaline phosphatase.

To highlight immuno-histochemical tissue antigens, an indirect method was used, based on two stages polymerized dextrane (DAKO ENVISION).

Tissue fragments were included in paraffin and sectioned to 4 micrometers thickness. Sections were displayed on slides treated with poly-L-lysine, dewaxed, hydrated and followed by inhibition of endogenous peroxidase with a 3% hydrogen peroxide solution. An enzymatic pretreatment with pronaze and nonspecific binding sites of type FC was achieved by adding normal serum.

Diaminobenzidine splitted by free peroxidase from the streptavidin-biotin complex, produces a brown precipitate accurately locating by the antigen either in the cytoplasm or in the nucleus. In this way, a counter coloring of the nuclei with Mayer hemalaun was achieved, followed by dehydration, clearing and mounting in a synthetic organic medium.

Many tissues contain endogenous alkaline phosphatase which should be blocked by pretreatment with levamisole, when using alkaline phosphatase as a marker.

In order to prevent unwanted binding of endogenous biotin to avidin in the case of using the avidin-biotin detection system, a step of blocking is necessary to pre-treat the tissue through a pre-treating process of non-conjugated avidin, saturating it with biotin.

Autofluorescence or fluorescent lighting exists in some tissues and it may cause background problems when the fluorescent markers are used in experiments. The easiest test is viewing the tissue sections through fluorescence microscopy before any antibody incubation.

Antigen	Clone	Specificity	Manufacturer	Dilution
Leu 7	TB01	Germinal Cells	DAKO CARPINETRIA CA,USA	1:50
Inhibin	policlonal	Interstitial Cells	NOVOCASTRA NEW CASTLE Upon Tyne UK	1:50
PLAP Placental Alkaline Phosphatase	policlonal	Embryonic Cells Testicular Cells	DAKO	1:50

Results and discussions

Testicular parenchyma is a tissue with a well-known positivity for control. Special controls should be performed to test protocols and the specificity of the used antibodies .

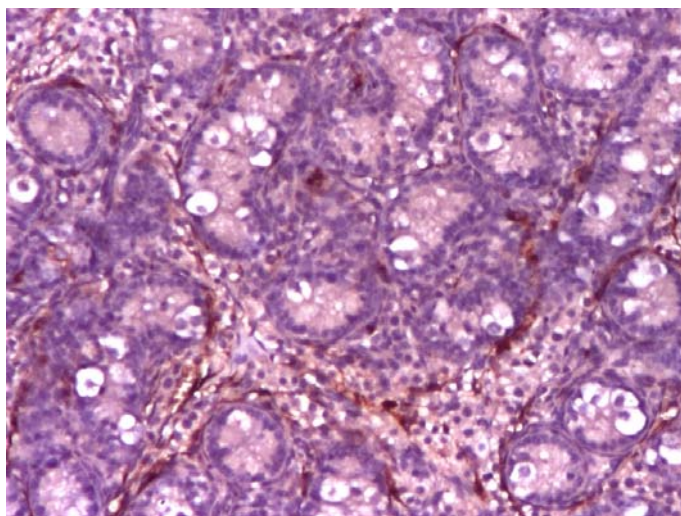


Fig.1 Swine testicle at birth – Leu 7 Ob 20x
Positive reaction in the basal layer and interstitial cells

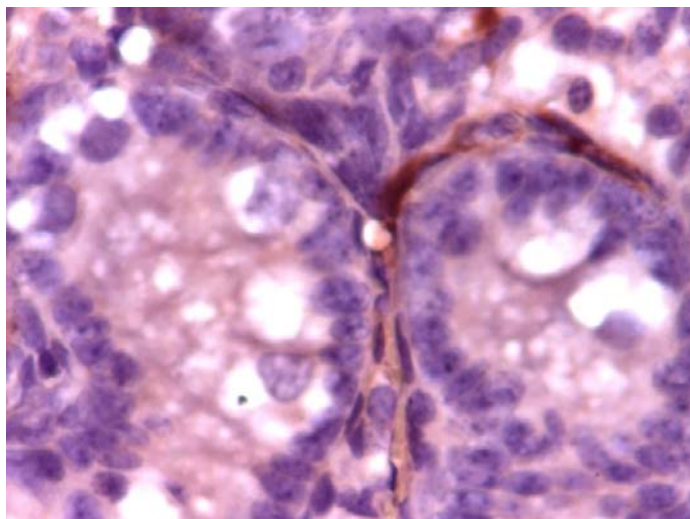


Fig. 2 Swine testicle at birth – Leu 7 Ob 40x
Positive reaction in the basal layer of peritubular cells.

As it can be observed in figures 1, 2 and 3 presenting sections of testicular parenchyma at birth, the lumen of the seminiferous tubules is bounded by a simple germinal epithelium consisting of Sertoli cells and spermatogonia located on a basement membrane. The basal membrane shapes the seminiferous tubules lumen (JUNQUEIRA, 2003 [9]).

This is linked to the collagen and elastic fibers in peritubular cells forming a blanket made up of 1-5 layers (CORNILA, 2001 [4]).

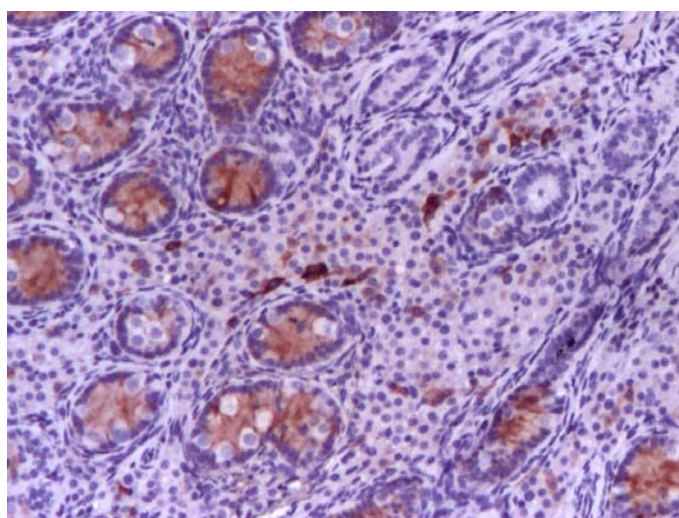


Fig. 3 Swine testicle at birth – inhibin Ob 10x
Positive reaction to peritubular cells.

Mitotic and meiotic divisions and cytological changes of the cellular elements of epithelium take place in a regular and timely way, leading to different associations, characteristic to histological picture of the seminiferous tubules. By using monoclonal antibody Leu 7, a positive reaction is observed in the basal layer and interstitial cells (Fig. 4).

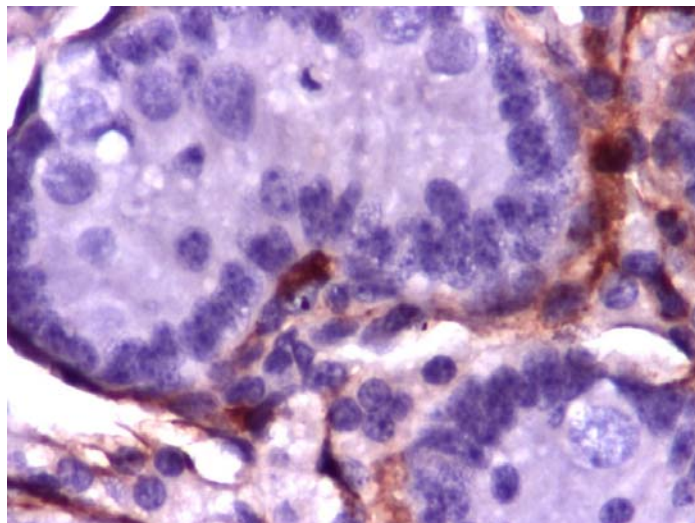
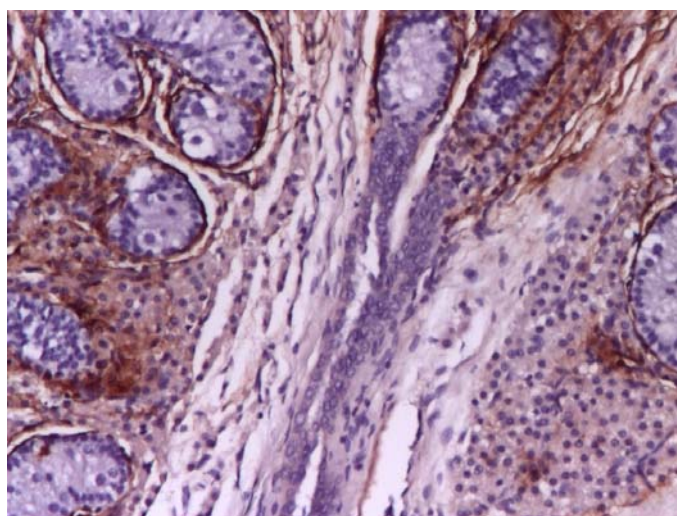


Fig. 4 Testicles of swines at weaning – Leu 7 Ob 40x

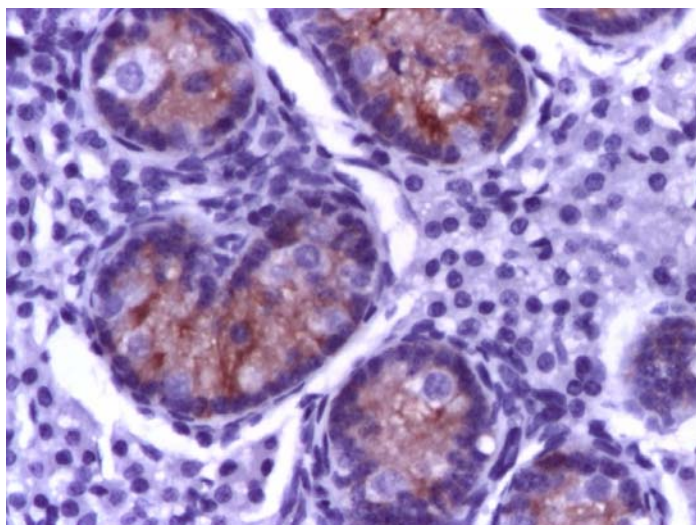
Positive reaction in the basal layer and interstitial cells.

A positive reaction in the basal layer of the epithelium semen was observed also for placental alkaline phosphatase (Fig. 5). They can be considered suitable markers to identify stem cells at this level .



**Fig. 5 Testicles of swines at weaning - PLAP -Placental Alkaline Phosphatase
Ob. 10 Positive reaction in Sertoli cells.
Interstitial cells with positive reaction.**

During the final stages of maturation of spermatids into sperm, residual cytoplasm and organelles are removed to a cytoplasm body attached to the caudal portion of the developing spermatid. These cytoplasmic wastes are called residual bodies and they are phagocytosed by Sertoli cells and degraded in lysosomes cell. As regards the mature testicle, the studies with inhibin monoclonal antibody showed a positive reaction of seminiferous tubules and negative in the interstitial cells. (Fig. 6).



**Fig. 6 Testicles of swines at weaning – Inhibin Ob 20x
Positive reaction to sperm cells.**

Synchronous appearance of seminiferous epithelium cells requires directed spermatogenesis under the influence of the timing or coordination factors. These factors, along with the neurohormone, have a role in directing the proper development of the spermatogenesis processes.

The synchronisation factor provides a new mitosis of basal spermatogonia when the meiosis of primary spermatocytes of the previous generation of the same cell occurs (BOGDAN, 1999 [3]). Also this factor is involved in meiosis processes that culminates with spermatids metamorphosis.

In the process of spermatogenesis, coordination factors occur (BERGMANN, 2006 [2]). Without their participation, testicular cell populations would not find the same stage of development.

In histological sections the concurrent spermatogonial divisions were highlighted as well as the coexistence of cell populations at different stages of development. The first coordination factor is involved in the organization on the cross section of the seminiferous tubules. The second factor coordinates stages of the seminiferous epithelium cycle along the small canal of the tissue, in fact of the spermatogenic wave to achieve a constant time difference between early divisions of the different sperm populations (DANACU, 2001 [6]). In the mature testicles, the study shows that a positive reaction of the placental alkaline phosphatase is imminent at the basal layer of the epithelium (Fig. 7). This can be considered suitable for the identification of stem cell markers at this level.

During puberty, sustentacular cell differentiation is accompanied by a morphological transformation and loss of mitotic capacity (the adult Sertoli cells stop dividing). Sustentacular are pyramidal cells with irregular outline; Sertoli cell contour is not visible due to the presence of numerous germ cells observing that sustentacular cells emit large branches around and between germ cells making intimate contact with them (GROZA, 2006 [7,8]). Sperm stem cell renewal and spermatogonial multiplication is performed in the basal compartment, in which intertubular tissue fluid has a relatively free access. Hemo-testicular barrier selectively prevents the entrance of many substances in the adluminal compartment where vital processes of meiosis and spermiogenesis take place in a controlled microenvironment (BERGMANN M 2006 [2]). Early spermatocytes pass through these intercellular junctions without interruption physiological hemo-testis barrier (Fig. 8).

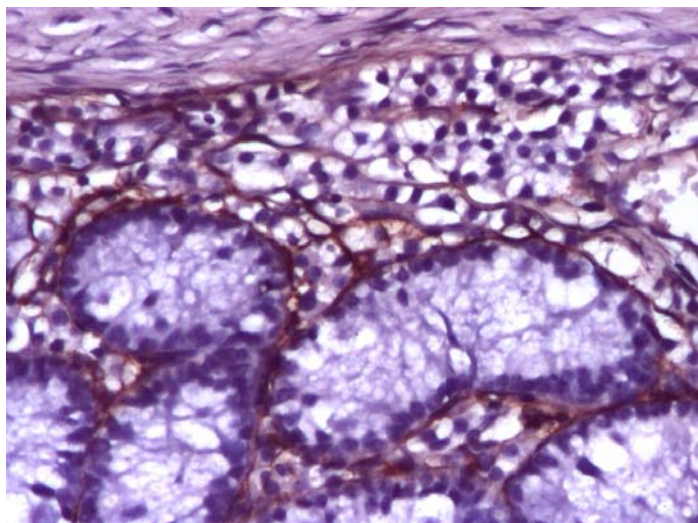


Fig 7 Swines testicles at maturity - PLAP -Placental Alkaline Phosphatase.Ob 20x Positive reaction in the basal layer.

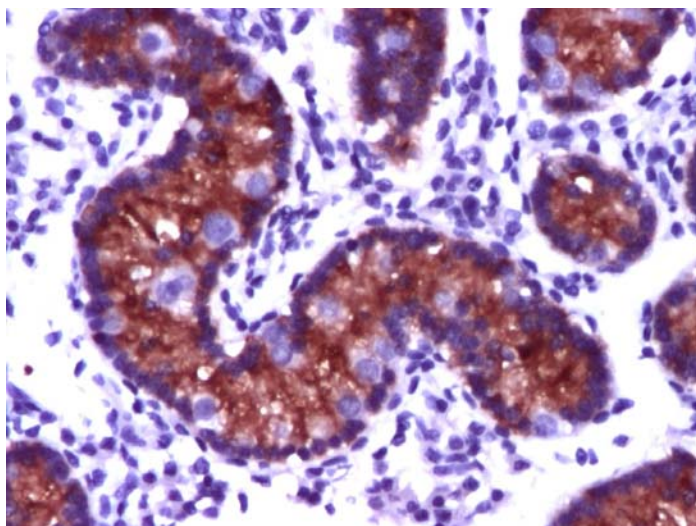


Fig 8 Swines testicles at maturity – Inhibin Ob 20x Positive reaction in seminiferous tubules and negative reaction to the interstitial cells.

An effective barrier could be observed in tubules that contained early spermatids and in which primary spermatocytes were still inside the opened compartment. Such barrier formation has been correlated with the development of haploid germ cells. Complete partition of the seminiferous tubules, leaving only spermatogonia within the opened compartment, was performed in tubules that contained elongated spermatids of the maturation phase (Fig. 9).

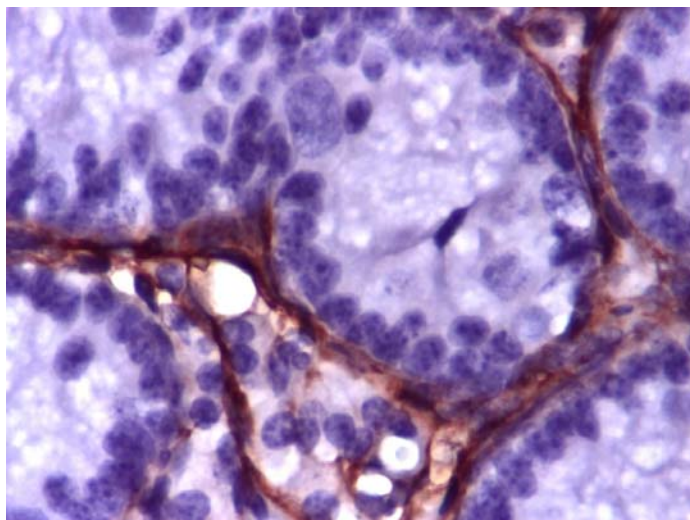


Fig 9 Swines testicles at maturity – Leu 7 Ob 40x
Positive reaction in the basal layer. Positive reaction in the interstitial cells.

Conclusions

Various testicular parenchyma cells in different development stages were detected by the presented method. By using monoclonal antibody Leu 7, a positive reaction is observed in the basal layer and interstitial cells in swine testicle at birth. In the mature testicle, by using inhibin monoclonal antibody, a positive reaction at the seminiferous tubules layer and a negative reaction at the interstitial cells layer were observed. Also, by studies on swine testicle, a positive reaction for placental alkaline phosphatase was observed in the basal layer of the epithelium, which could be considered a suitable marker to identify stem cells at this level.

The study proved that the use of monoclonal antibodies in analysis of testicular parenchyma may have a positive effect for early diagnosis of abnormalities or disorders of spermatogenesis for the purpose of using the reproduction biotechnologies in order to obtain a quality seminal material.

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