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**Immunohistochemical Localization of Progesterone Receptors  
in the Non-pregnant One-humped Camel Uterus**

Abd-Elnaeim M. M. M., K. A. Al-kheraije

*Department of Veterinary Medicine, Faculty of Agriculture and Veterinary Medicine, Qassim University, KSA.*

(Received 14 May 2012/ Accepted 4 Juli 2012)

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**Abstract**

The current study aimed to localize immunohistochemically the cellular progesterone receptors in the non-pregnant one-humped camel uterus. Uterine tissue specimens from 10 she-camels during estrous were collected from local slaughterhouses and prepared for indirect immunoperoxidase staining using primary antibody for human progesterone receptor PR (clone 10A9). Nuclear signals for progesterone receptors were clearly observed in the uterine surface epithelium, glandular epithelium, myometrium, uterine stroma and in walls of some large blood vessels. The staining intensity was variable at different uterine tissue regions. Strong signals were demonstrated in the superficial gland zone, in the connective tissue stroma and in the myometrium. However, Nuclear signals of moderate intensity were noticed in the surface epithelium, deep gland zones and in the walls of the uterine arteries. No cytoplasmic signals could be detected. The current study concluded that nuclear signals for progesterone receptors were found in uterine surface epithelium, uterine glands, myometrium, uterine blood vessels and stroma.

*Keywords:* Camel; progesterone receptors; uterus

**Introduction**

The diverse effects of progesterone on female reproductive tissues are mediated by the progesterone receptor (PR), a member of the nuclear receptor family of ligand-dependent transcription factors. Thus, PR is an important therapeutic target in female reproduction (Leonhardt *et al.*, 2003). The uterus is influenced by ovarian steroid hormone progesterone, which induces glandular and stromal differentiation as well as cell proliferation (Graham and Clark, 1997). A stimulatory effect by progesterone on uterine protein secretion has been shown in several studies (Adams *et al.*, 1981; Roberts *et al.*, 1987; Vallet *et al.*, 1998). During pregnancy progesterone has an important role in preparations for uterine reception of the early embryos and for the maintenance of pregnancy (Sukjumlong *et al.*, 2005). The effects of progesterone are mediated by interaction of the hormone with specific intracellular progesterone receptors (PR). Absence of PR was associated with uterine release of the luteolytic prostaglandin F<sub>2</sub>alpha (Spencer *et al.*, 2004). Prog-

esterone receptors have been isolated and sequenced in several animal species like cattle (Robinson *et al.*, 2001), dogs (Dhaliwal *et al.*, 1997), goats (Flores *et al.*, 2001), pigs (Geisert *et al.*, 1994). However, in camels only one article was found dealing with the effect of the reproductive status, ovariectomy and sex steroid administration on estrogen and progesterone receptors (Homedia *et al.*, 2010). Therefore, the current study was conducted to evaluate the exact cellular site of progesterone receptors in the cyclic one-humped camel uterus.

**Materials and methods**

Uterine tissue specimens from 10 animals during the stage of follicular growth (presence of large number of growing follicles on the surface of the ovary) were collected directly after slaughtering and fixed in 10% formalin solution. Indirect immunoperoxidase staining was carried out using human PR (clone 10A9; Dianova-Immunotech, D-20354 Hamburg) as primary antibody. Blocking serum, biotinylated secondary antibody against mouse IgG and avidin-biotin complex were taken from the Vectastain Elite ABC Kit 6102 and used

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\*Corresponding author: Abd-Elnaeim M. M. M

*E-mail address:* naeim61@yahoo.com

under the instructions from the manufacturers (Vector Laboratories, Burlingame, CA, USA).

Tissue sections (ca. 5  $\mu$ m) were mounted on Super-Frost-Plus slides (Menzel Glaeser, D-38116 Braunschweig), deparaffinized by two 4 min changes of xylene, rehydrated in graded ethanol and washed under running tap water for 5 min. For antigen retrieval, the rehydrated sections were pre-incubated in 10 mM citrate buffer pH 6.0 for 5 min prior to three 5-min microwave irradiations in pre-heated citrate buffer in an oven run at 560 W. After a 20-min cooling period, the slides were washed under running tap water for 5 min followed by a treatment with 0.3% hydrogen peroxide in methanol for 30 min in order to quench endogenous peroxidase activity. They were then washed with IHC buffer (phosphate-buffered saline/0.3% Triton X pH 7.2) for 5 min and covered with 10% inactivated blocking serum in IHC buffer to block non-specific binding sites. After draining the blocking reagent, the respective primary antibody diluted in IHC buffer (10A9: dilutions 1:100) was applied and the slides were incubated for 20 h in a humid chamber at 4C. They were then washed with IHC

buffer, covered with biotinylated secondary antibody, diluted 1:200 in IHC buffer and incubated for 30 min at room temperature. Following the draining of excess antibody, the sections were washed for 5 min with IHC buffer, then covered with streptavidin-peroxidase complex and incubated for 30 min. After washing with IHC buffer (5 min), the sections were incubated with substrate (AEC substrate kit for peroxidase, Vector Laboratories). The slides were then washed under running tap water for 5 min, counterstained with haematoxylin and mounted in Kaisers glycerol gelatin (Merck KgaA, D-64293 Darmstadt). Negative controls were established by replacing the primary antibodies with incubation buffer.

## Results

Indirect immunoperoxidase staining for progesterone receptors by using human PR (clone 10A9) as primary antibody demonstrated nuclear signals with varying intensities for progesterone receptors in most of the uterine tissues (table 1.).

Table 1. Staining intensity of the different uterine tissues for progesterone receptors.

	Surface epithelium	Superficial gland zone	Middle gland zone	Deep gland zone	Uterine stroma cells	Uterine musculature	Uterine blood vessels
Staining intensity	++	+++	+++	++	+++	+++	++

Staining intensity: 0 = negative, + = weak, ++ = moderate, +++ = strong

Nuclear signals with moderate intensity were detected in the uterine surface epithelium (Fig. 1B), in the deep gland zone (Fig. 1D) and in the myoepithelial cells of large uterine arteries (Fig. 1F). Strong nuclear signals were observed in the uterine connective tissue cells (Figs. 1B, C, D), in the superficial gland zone (Fig. 1B), in the middle gland zone (Fig. 1C) and in the smooth muscle cells of the uterine musculature (Fig. 1E). However, cytoplasmic signals for progesterone receptor could not be observed.

## Discussion

Steroid hormones are important regulators of reproductive physiology in female animals. Both estrogen and progesterone mediate dramatic changes in bovine reproductive tissues during oestrous cycle and early pregnancy. Progesterone, synthe-

sized and secreted by the corpus luteum, is one of the major regulators of the reproductive cycle in mammals. It exerts its effects on the growth and differentiation of ovarian structures and renders the endometrium receptive to the implantation of the embryo. Progesterone acts on its target tissues after binding to a specific intracellular progesterone receptors (Pinter *et al.*, 1996; Delmann and Eurell, 1998). These receptors are located in the nuclei of cells (Defranco, 2002). The ligand-receptors complexes serve as transcription factors that interact with DNA directly to regulate gene expression (Clarke, 1990). The exact cellular site of progesterone action has not been exactly evaluated in camel according to the available literature. Thus, the present immunohistochemical study was designed to assess the distribution of the progesterone receptors in the non pregnant one-humped camel uterus.

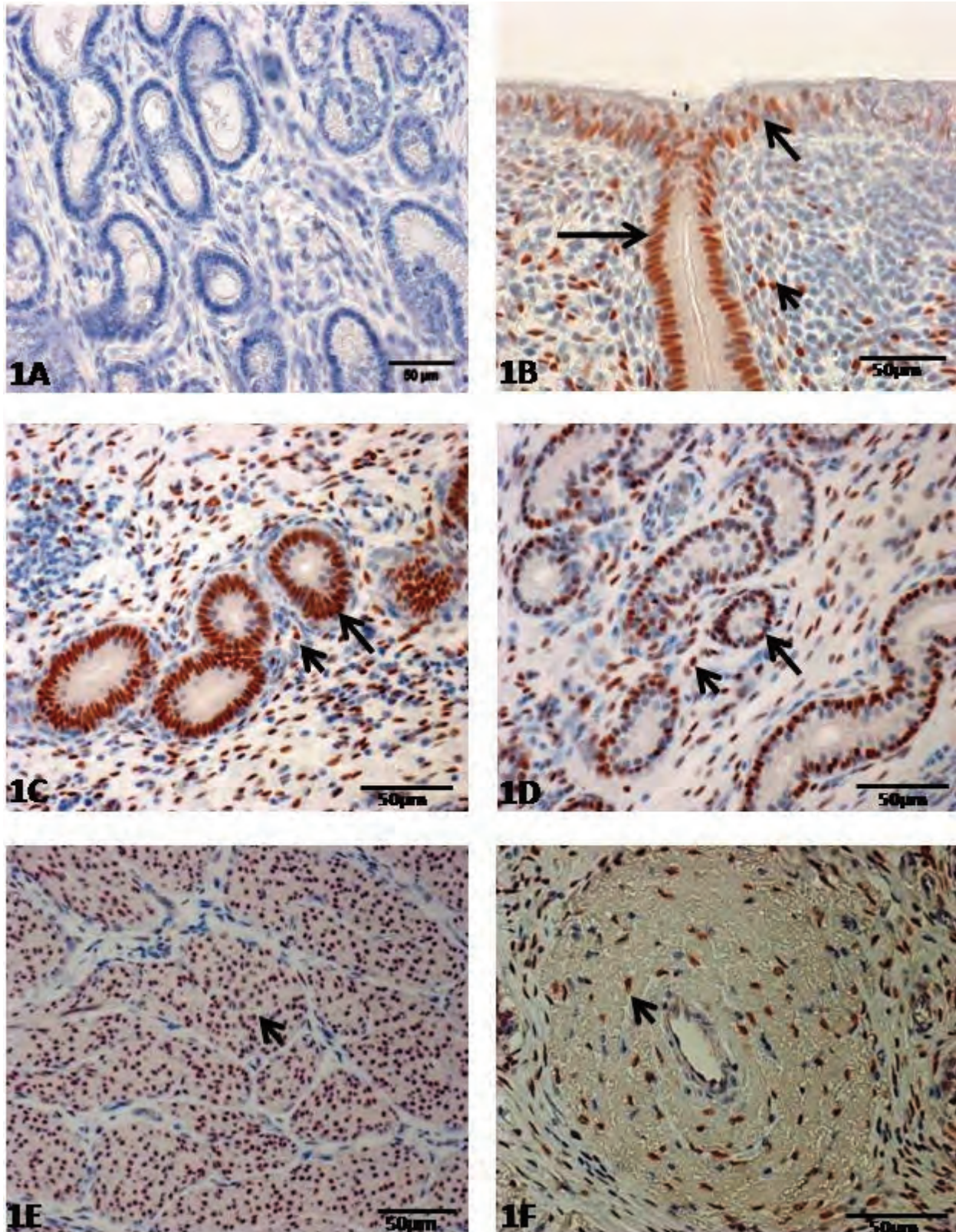


Fig. 1. showing negative controls for progesterone receptors (1A), Nuclear signals of progesterone receptor (brown-red staining) in the uterine surface epithelium (short arrow), superficial gland zone (long arrow) and connective tissue stroma (arrow head) 1B, Strong nuclear signals in the middle gland zone (arrow) and uterine stroma (arrow head) 1C, Nuclear signals of moderate intensity in the deep gland zone (arrow) and uterine stroma (arrow head) 1D, Strong nuclear signals of progesterone receptor in the uterine musculature (arrow head) 1E and nuclear signals of moderate intensity in the wall of large uterine blood vessel (arrow head) 1F.

In the present study positive immunohistochemical staining for progesterone receptors was exclusively found in the nuclei of all uterine cell type and no cytoplasmic staining was observed. This is

in accordance with the finding of Homeida *et al.* (2010) in the same species. Similar results were described in the non pregnant sow uterus by Sukjumlong *et al.* (2005). However, in cattle Berna *et al.*

have been reported that progesterone receptors were obviously strong in the uterine epithelia and myometrium during estrous. The present study demonstrated strong nuclear signals for progesterone receptors in the glandular tissues especially the superficial and middle gland zones in addition to the uterine myometrium and most of the uterine stroma cells. Strong nuclear signals of the stroma cells than the other parts of the uterus was also reported by Homeida *et al.* (2010) in the same species. Moreover, they mentioned that the nuclear signals were moderate in the glandular epithelium and myometrium which was on contrary of the results of the current study because moderate to weak nuclear signals were only detected in the deep gland zones.

In bovine uterus, Berna *et al.* (2009) reported that no positive staining for progesterone receptors was ever observed in the walls of the endometrial blood vessels and red staining in the cytoplasm of the epithelial cells was observed occasionally and they considered these staining to be nonspecific. Here, the situation in the one-humped camel uterus of the present study is different whereas nuclear staining were clearly observed in the walls of the endometrial blood vessels and no cytoplasmic staining in the uterine epithelium could be detected. In conclusion, this study demonstrates the expression of progesterone receptors in uterine surface epithelium, glandular epithelium, connective tissue stroma and myometrium of the one-humped camel uterus during the follicular phase.

## Acknowledgement

The authors are very thankful to Prof. Dr. G. Schuler, Justus Liebig University, Giessen, Germany for providing the antibodies.

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