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EXPRESSION OF ARYLHYDROCARBON (DIOXIN) RECEPTOR IN CHICKEN GENITAL SYSTEM DURING PRELAYING AND LAYING PERIODS

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ABSTRACT

The present immunohistochemical study was carried out in the chicken genital tract as an attempt to understand the regulation of the Ah receptor expression.Two groups of chickens were investigated;namely, immature chickens, mature chickens (hens) in laying period .The monoclonal Ah receptor antibody omitted in control sections, was diluted 1: 1000 and applied according to the manufacture's instructions. In the ovaries, the Ah receptor was expressed in the interstitial cells in all studied birds in addition to stromal elements in the laying hens. The expression in the oviduct varied according to the physiological state, indicating a devel-opmental as well as endocrine control. In immature chickens, the receptor was not expressed. In the laying hens both cytoplasmic and nuclear staining was evident in the luminal epithelial cells. Nuclear localization is an obligatory process for activation and transcription of this receptor. Our results indicated that the Ah receptor is involved in the process of egg production in the laying hens and its expression is greatly influenced by the endocrine function of the chicken ovary.

INTRODUCTION

Xenobiotics such as dioxins are widespread environmental and food chain contaminants.The toxic effects of dioxins are mediated by an intra-

cellular receptor called arylhydrocarbon (Ah) or dioxin receptor. The Ah receptor is a ligand activated transcription factor regulating a broad spectrum of genes.The physiological function of Ah receptor is still unknown. Information on the expression of the Ah receptor in farm animals are scarce. The AhR is expressed in various mammalian tissues with highest levels of protein constantly found in the lung ***(Carver et al., 1994)***. It is also expressed in liver, thymus,kidney,spleen, placenta***(LI et al.,1994)***,human uterus ***(Kuechenhoff et al., 1999b)***, rabbit uterus **(Tscheudschilsuren et al., 1999; Hasan and Fischer, 2001)**, rabbit ovary **(Hasan and Fischer, 2003)**, mouse urinary tract **(Bryant et al.,1997)**and male rat reproductive tract **(Roman et al., 1998)**.

The arylhydrocarbon receptor (AhR) is a ligand - activated transcription factor ***(Bock 1993 ; Schmidt and Bradfield, 1996)*** found in the cytosol as part of a protein complex ***(Denison et al.,1986; Cuthill et al., 1987)***. Its molecular weight in different species varies between 94 KD (kilodalton) in the rabbit ***(Takahashi et al., 1996)*** and 124 KD in the hamster ***(Schmidt and Bradfield, 1996)***. The unligand AhR resides in the cytosol and has a half life of about eight hours ***(Hannah et al., 1981; Denison et al., 1986; Gudas et al., 1986; Pollenz et al., 1994; Swanson and Perdew, 1993)***. Upon ligand binding, the AhR is activated to translocate to the nucleus where it heterodimerize with the arylhydrocarbon nuclear translocator then bind a DNA response element termed xenobiotic responsive element (XRE) to induces a variety of genes, the so called AhR gene battery ***(Nebert et al., 1993; Schmidt and Bradfield, 1996)***. Regarding reproductive organs, AhR expression in human endometrial epithelium seems cyclically dependent and was maximally expressed at the time of ovulation ***(Kuechenhoff et al.,1999b).***In the human placenta, AhR has been shown to be abundantly present ***(Manchester et al., 1987;***

Dolwick et al., 1993) while in the rabbit placenta, it is specifically expressed in decidua cells ***(Tscheudschilsuren et al.,1999b)***.

A detailed study of Ah receptor expression in the chicken genital tract at different physiological stages (immature chicken - laying hens) still missing. The localisation of the AhR and the potential relationship between ovarian steroid hormones and Ah receptor expression in the chicken genital tract is the aim of the current study.

MATERIALS AND METHODS

For this study,10 Lohmann brown chickens were used.The chickens were divided into two groups,6 laying hens one year old as first group and 4 immature chickens two months old as second group; the hens begins to lay eggs at 6 month old.Chickens were raised in Ah receptor agonists free environment and not treated before use. Birds were sacrificed by cervical dislocation. Fresh specimens were fixed in Bouin's fixative (18 hours), embedded in paraffin and 5um sections were used for immunohistochemistry and fresh ovarian and oviductal tissues from laying hens were frozen in liquid nitrogen and then stored at - 80°C for western blot analysis.

Western blot analysis:

Ovarian and oviductal tissues from laying hens were homogenised in 62.5 mM Tris-HCL buffer (pH 6.8) containing 10% (w/v) saccharose and 2% (w/v) sodium dodecyl sulfate(SDS); then 30 µg of protein was heated at 90°C in water bath for 5 minutes and run on a 8% sodium dodecyl sulfate-polyacrylamide gel (1 hour at 150 V) then transferred onto nitrocellulose membrane (Amersham, Braunschweig, Germany). Non specific binding was blocked by overnight incubation with 5%(w/v)dry skim milk in phosphate-buffered saline containing 0.1% (v/v) Tween 20 (PBST) at 4°C. The blot was incubated with a mouse monoclonal AhR antibody

(PA3-513; Dianova, Hamburg, Germany) diluted at 1:1000 in 3% (w/v) bovine serum albumin (BSA) in PBST at room temperature for 1 hour. Then the blot was washed 2 X 5 minute and 3 X 15 minute with PBST; after that, the blot was incubated with a peroxidase-conjugated goat anti-mouse antibody (118-036-003; Dianova), diluted 10,000 in 3% (w/v) bovine serum albumin in PBST at room temperature for 1 hour followed by 2 X 5 min and 3 X 15 min washes in PBST and 1 X 5 min in PBS. Specific immunostaining was visualised with an enhanced chemiluminescence kit (ECL, Amersham).

Immunohistochemistry:

The sections were dewaxed and endogenous peroxidases were blocked by incubation in 3% (v/v)H2O2 in methanol at room temperature for 25 minutes.Unspecific reactions were blocked by incubation with two types of sera. The first, rabbit serum, 20% (v/v) in PBST was added for 25 minutes followed by three times washing with PBS-T for 5 minutes. The second, goat serum, 10% (v/v) in PBST, was given for 25 minutes. The primary antibody against the AhR (PA3-513; Dianova, Hamburg, Germany) was applied at a dilution of 1:1000 in PBST at 4°C overnight. Sections were washed three times with PBS-T for 15 minutes and incubated with the secondary antibody, a peroxidase-conjugated goat anti-mouse antibody (118-036-003; Dianova) at a dilution of 1:250 in PBS-T for 1 hour. After three times washing with PBST for 15 minutes, immunostaining was visualized by 10% (v/v) diaminobenzidine using the Immunopure metal-enhanced diaminobenzidine substrate kit (34062; Pierce, Rockford, IL, USA) for 2 minutes then haematoxylin was used as a counter stain. Controls omitting the primary antibody were included in each experiment. Finally, the sections were covered in entellan (Merck, Darmstadt, Germany) and examined under bright-field or Nomarski

interference contrast microscopy with an AH-3 microscope (Olympus, Hamburg, Germany).

RESULTS

Western blot analysis:

Analysis of 30µg extracted proteins from ovarian and oviductal tissues of laying hens and incubated with monoclonal anti-AhR antibody revealed a protein band in the expected molecular weight of 101 KDa in all specimens; as recorded in this species ***(Hahn, 1998)*** (Fig. 1).

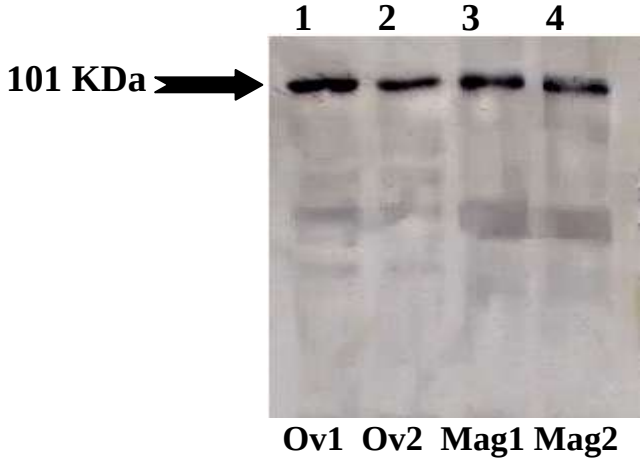

Fig. (1): Western blot analysis for AhR (from left to right) ovary1(ov1), ovary 2 (ov2), magnum1(Mag1), magnum2 (Mag2) detected a single 101 KDa band in each lane. The X-ray film has been computer-scanned for presentation.

Immunohistochemistry:

The ovaries of all the examined birds were immunopositive for AhR, on the other hand, the oviducts of the laying hens were immunopositive while that of the immature chickens were immunonegative.

The ovary in laying hens was large punch like and contain follicles of varying sizes and developmental stages.The receptor was expressed in the interstitial cells which were in the cortex near the follicles and between the medullary elements.The reaction was restricted to the cytoplasm of the stained cells. Stromal cells were immunopositive and strongly expressed AhR; inaddition, the walls of the ovarian blood vessels were immunopositive.

The ovary in immature chickens was bean size with homogenous granular appearance. AhR was expressed only in the interstitial cells while all the other ovarian tissues were immunonegative.

The oviduct in laying hens was long, thick walled and sometimes contain eggs inside it. The luminal epithelium of the whole oviduct was expressing AhR showing diffuse cytoplasmic and nuclear staining.Glands of the oviduct which were immunonegative and were filled with secretions. Tunica muscularis was always immunopositive for AhR along the different parts of the oviduct beginning with the infundibulum, magnum, isthmus, uterus(shell gland)and finally the vagina. Walls of the blood vessels were also immunopositive.

The oviduct in immature chickens was short and thin walled. It was not differentiated into segments and along its length, it was immunonegative expressing no AhR.

DISCUSSION

The expression pattern of the AhR showed specific changes during the development of the chicken genital tract.In the oviduct of the immature chickens the AhR was not expressed; comparable findings were found in the uterus of juvenile rabbits as the receptor was very weakly expressed ***(Hasan and Fischer, 2001)***. A marked high regulation in AhR was observed in both ovary and oviduct of the laying chickens indicating that the receptor expression was dependent on the physiological activity of the

genital tract under effect of ovarian steroids (estrogen and progesterone hormones)which are secreted by the ovary during egg laying;in the rabbit ovary,the receptor was expressed in the endocrine interstitial cells,stromal cells and corpora lutea during the preimplantation period ***(Hasan and Fischer, 2003)***.

Regarding the ovary, in both immature and laying hens the receptor was expressed in the interstitial cells which are endocrine cells and it is said to secrete ovarian steroids, similar results were obtained in the rabbit ovary in both non-pregnant and pregnant ones ***(Hasan and Fischer, 2003)***; inaddition, it was reported that the AhR system may play a role in mouse folliculogenesis such as formation of primordial follicles and regulation of antral follicle numbers ***(Benedict et al 2000)***. Unlike the ovary of immature chickens in which the receptor was expressed only in the interstitial cells, the ovary of the laying hens showed widespread presence of the AhR not only in the interstitial cells but also in stromal cells and walls of the ovarian blood vessels. ***(Hasan and Fischer, 2001)*** proved that the AhR could be high regulated after treatment of juvenile rabbits with estrogen and progesterone hormones, Although AhR is not a member of the steroid hormone receptor superfamily, its cross- talk with steroid hormone receptors is not uncommon ***(Burbach et al, 1992; Ema et al, 1992; Wormke et al, 2000)***; moreover, ***(Lee et al, 1998)*** suggested that the steroids could bind the cytosolic AhR. The perceding finding indicate the involvement of the AhR in the ovarian activity and its relation to the ovarian steroids synthesis.

In chicken oviduct, it is worthy to state that this organ showed its maximal activity during the egg formation under effect of ovarian steroids. The oviduct of the immature chickens expressed no AhR while that of the laying hens was strongly expressing the receptor in the form of cytoplasmic and nuclear staining inaddition to staining of the smooth muscle cells of tunica muscularis indicating an intimate relation between the receptor expression and the sexual activity; it is known that, after ligand binding the AhR is translocated into the nucleus ***(Hoffman et al, 1991; Reyes et al, 1992; Whitelaw et al, 1993)*** so the nuclear staining

points to the activation of the receptor in the chicken oviduct.The AhR was found to be activated in the absence of added exogenous ligands suggesting the presence of an endogenous.

AhR ligand (s) ***(Sadek and Allen-Hoffman, 1994; Ma and Whitlock, 1996; Weiss et al, 1996).***

On the other hand, our findings were concomitant with results obtained in the human endometrium where a highest expression level of AhR was observed in the periovulatory period***(Kuechenhoff et al,1999b)*** suggesting a possible role of the ovarian steroids and in the rabbit uterus, the staining was restricted to the epithelium in the uterine glands and occured for the first time in the stromal cells in the pregnancy, decidual cells, descendants of stromal cells, are AhR positive ***(Tscheudschilsuren et al, 1999b)***. In addition, the receptor was expressed in the uterus and vagina showing high regulation during the preimplantation period ***(Hasan and Fischer, 2003).***

We conclude that AhR expression in the chicken genital tract shows specific developmental changes. It seems to be involved in the process of egg production and its expression is controlled by the ovarian steroids (estrogen and progesterone). Further studies are necessary to clarify the relation between AhR and steroids activities.

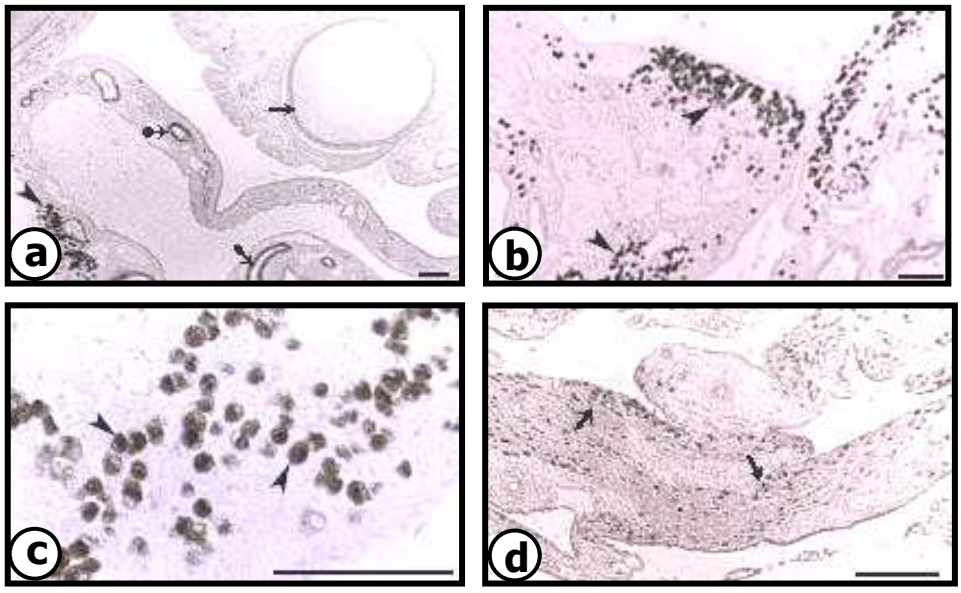

Fig (2): The ovary of the laying hen, AhR protein was expressed in the interstitial cells (a, b, c, arrowhead ™) but not in the follicles (a, arrow). Stromal cells were immunopositive (d ∧). AhR was expressed in the cytoplasm of the interstitial cels(c™)and nuclei of the stromal cells (d). Walls of the ovarian bood vessels were immunopositive (a, arrow ⌋). Bars 50 µm.

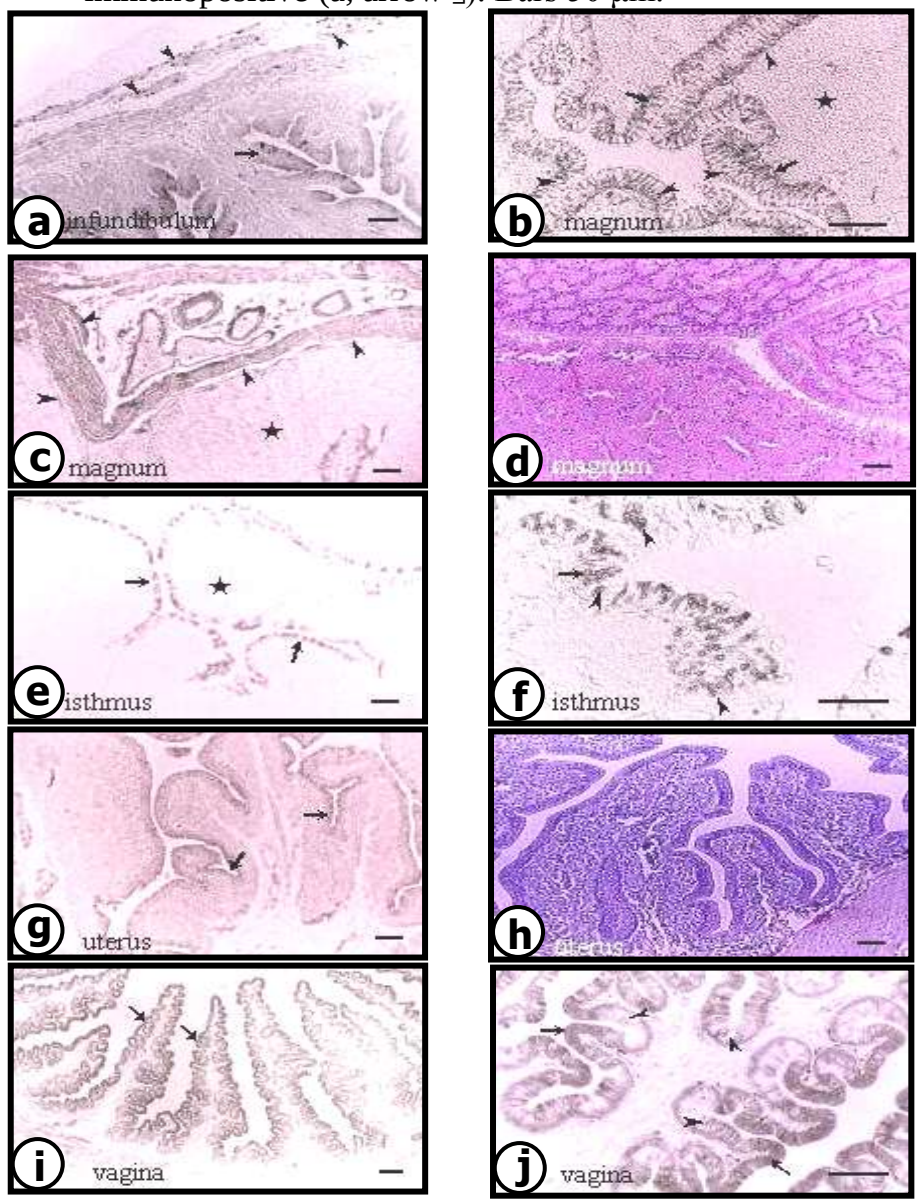

Fig (3): Legend on the next page.The oviduct of the laying hens,AhR was expressed mainly in the luminal epithelium of infundibulum, magnum, isthmus, uterus and vagina respectively (a, b, e, f, g, I arrows ∧). The glands were immunonegative (b,c,e,H).Smooth muscle cells were immunopositve along the oviduct (a, c, ™). Nuclear staining was evident in the positive luminal epithelial cells (b, f, arrow head ™). Bars 50 μm.

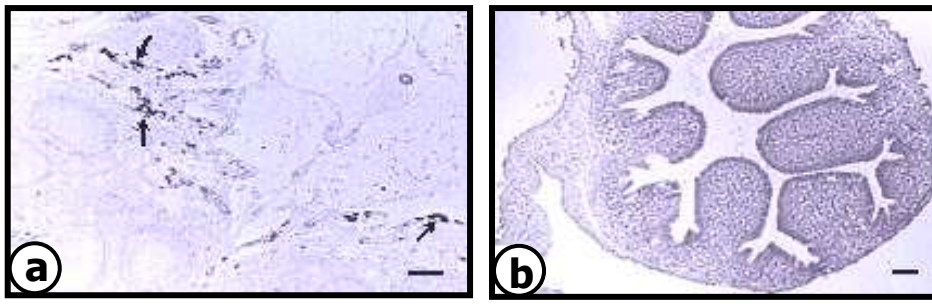

Fig (4): The ovary of immature chickens, AhR was expressed only in the interstitial cells in the ovary (a, arrow). The oviduct of the immature chickens was immunonegative (b). Bars 50 μm.

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