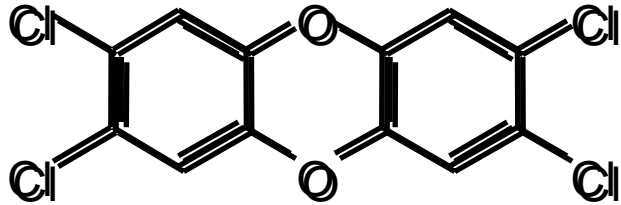


# ***Functions of AhR and its Ligands in the Ovary***

***Ken-ichirou Morohashi***  
***Division for Sex Differentiation,***  
***National Institute for Basic Biology***

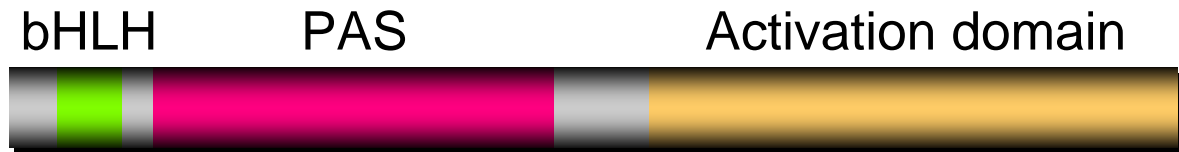
# TCDD causes a Broad Spectrum of Toxicological Effects via the Function of AhR (Arylhydrocarbon Receptor)

TCDD  
(2,3,7,8-tetrachlorodibenzo-*p*-dioxin)



TCDD causes Teratogenesis  
Immunosuppression  
Tumor promotion  
Estrogenic effect  
etc...

Arnt  
AhR nuclear  
translocator



Heterodimer Formation

DNA Binding

AhR  
Arylhydrocarbon  
Receptor



Heterodimer Formation

Ligand  
Binding

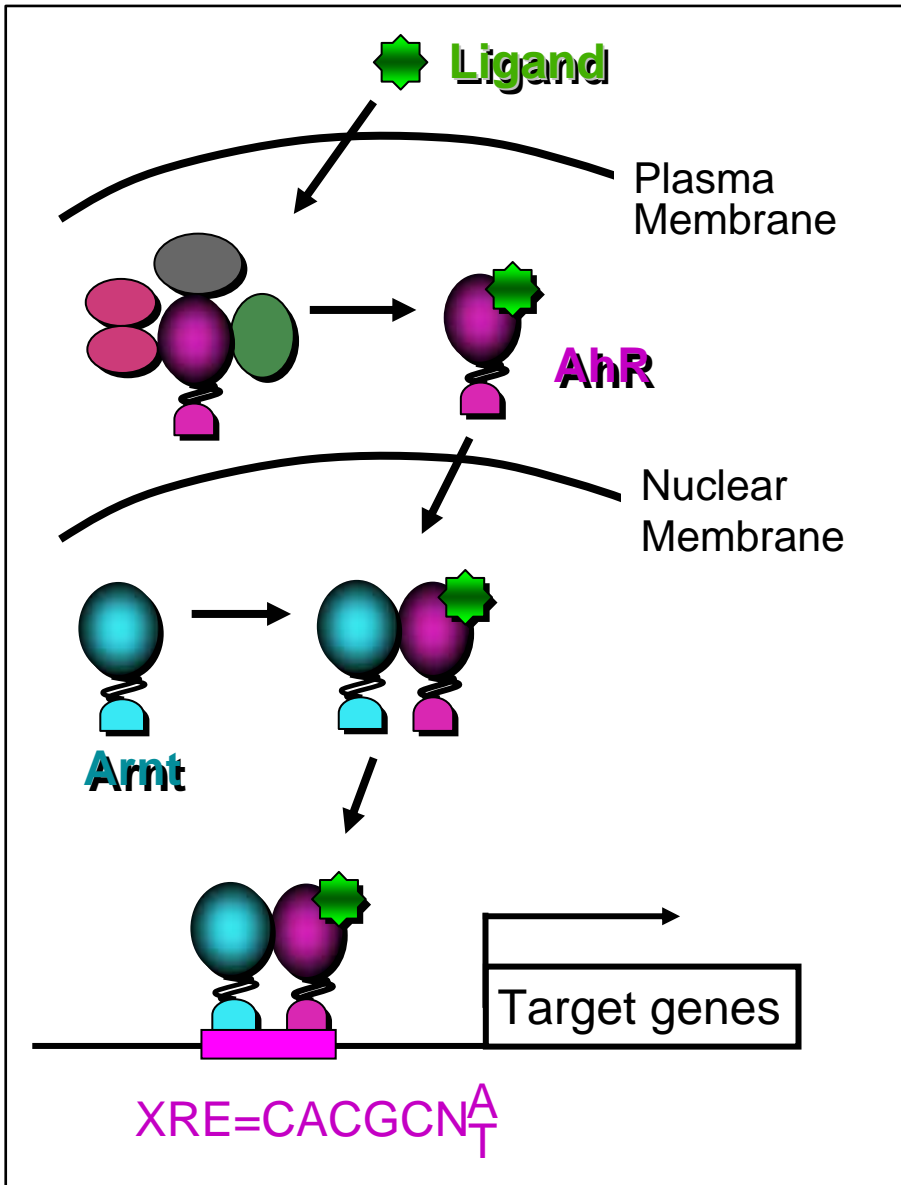
AhRR  
AhR Repressor



bHLH PAS

Repression domain

# Ligand-dependent Transcriptional Activation of Target Genes by AhR and Arnt Heterodimer

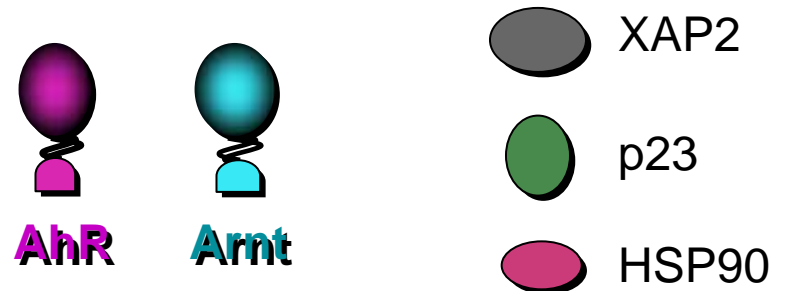


## Exogenous Ligands for AhR

2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)  
 3-methylcholanthrene (3MC)  
 9,10-dimethylbenzanthracene (DMBA)  
 Benz[a]pyrene (B[a]P)  
 etc...

## Target Genes of AhR

*Cyp1A1*  
*Cyp1A2*  
*Glutathione S transferase*  
*UDP-glucuronosyl transferase*  
*AhRR*



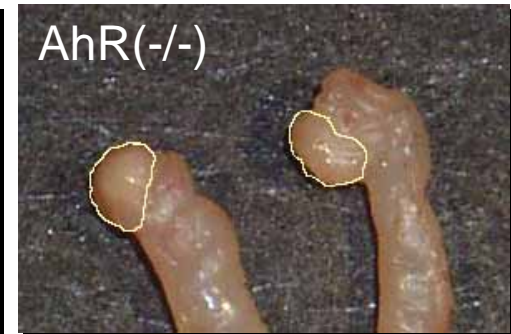
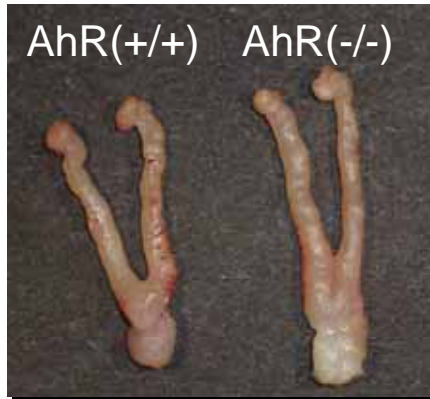
# Phenotypes of AhR Knockout Mice related to Reproduction

(1) Subfertility - Litter size and frequency of pregnancy were decreased.

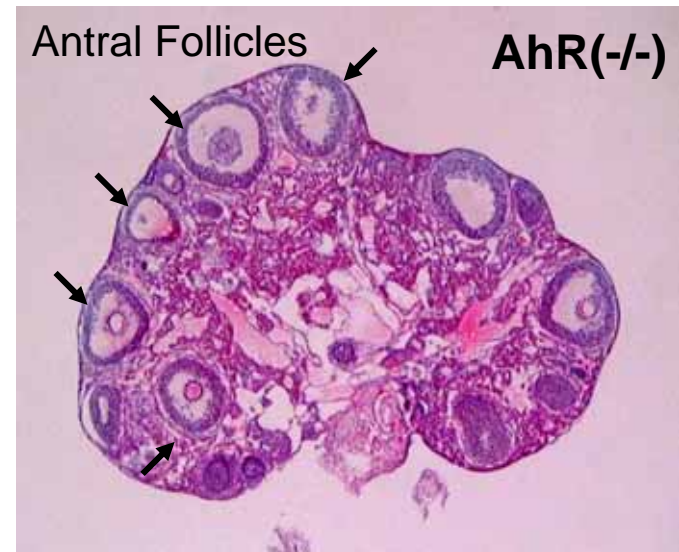
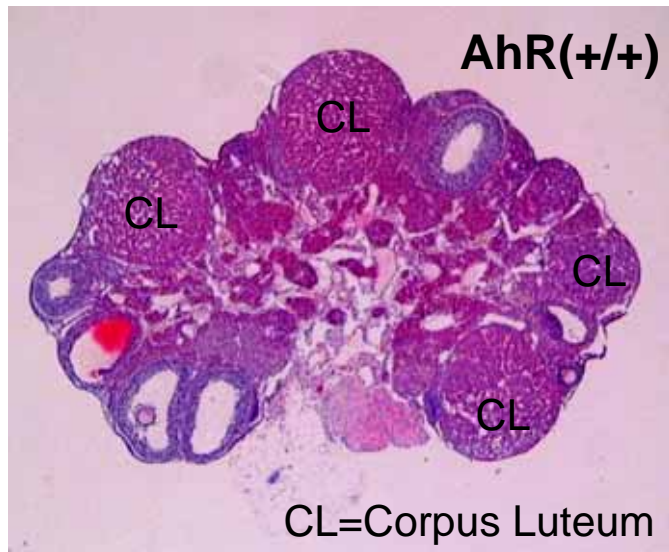
Genotype		Litter Size			Average	Total pups			
Female	Male	1st	2nd	3rd					
<b>+/+</b>	+/-		8	11	10	9.7		29	
			6	11	-	9.5	9.7	19	
			10	10	-	10.0		20	
			9	11	9	9.7	29		
							10.1	213	
		11	13	11	11.7	35			
		+/+		9	10	11	10.0	10.5	30
				9	10	10	9.7		29
				11	11	-	11.0		22
	<b>-/-</b>	+/-		4	5	-	4.5		9
			8	-	-	8.0	5.0	8	
			3	5	-	4.0		8	
			-	-	-	0.0	0		
							4.4	57	
		2	1	-	1.5	3			
		+/+		2	3	-	2.5	4.0	5
				7	3	-	5.0		10
				10	4	-	7.0		14

# Phenotypes of AhR Knockout Mice related to Reproduction

(2) Small ovary- Ovaries were diminished to about 65% in wet weight.

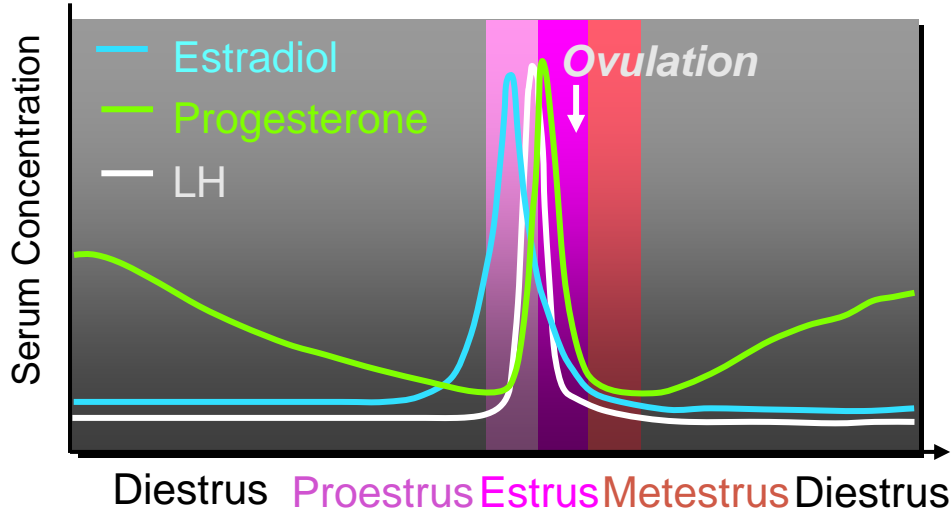


(3) Incomplete follicular maturation - Many antral follicles but few preovulatory follicles were observed, while CL was rear.

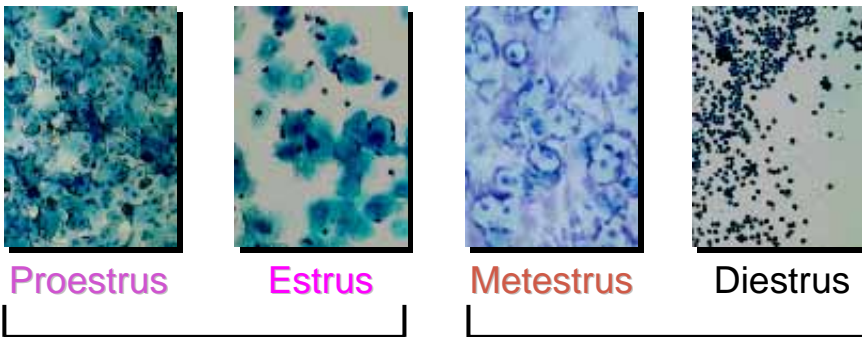


# Phenotypes of AhR Knockout Mice related to Reproduction

## (4) Estrus cycle was disordered

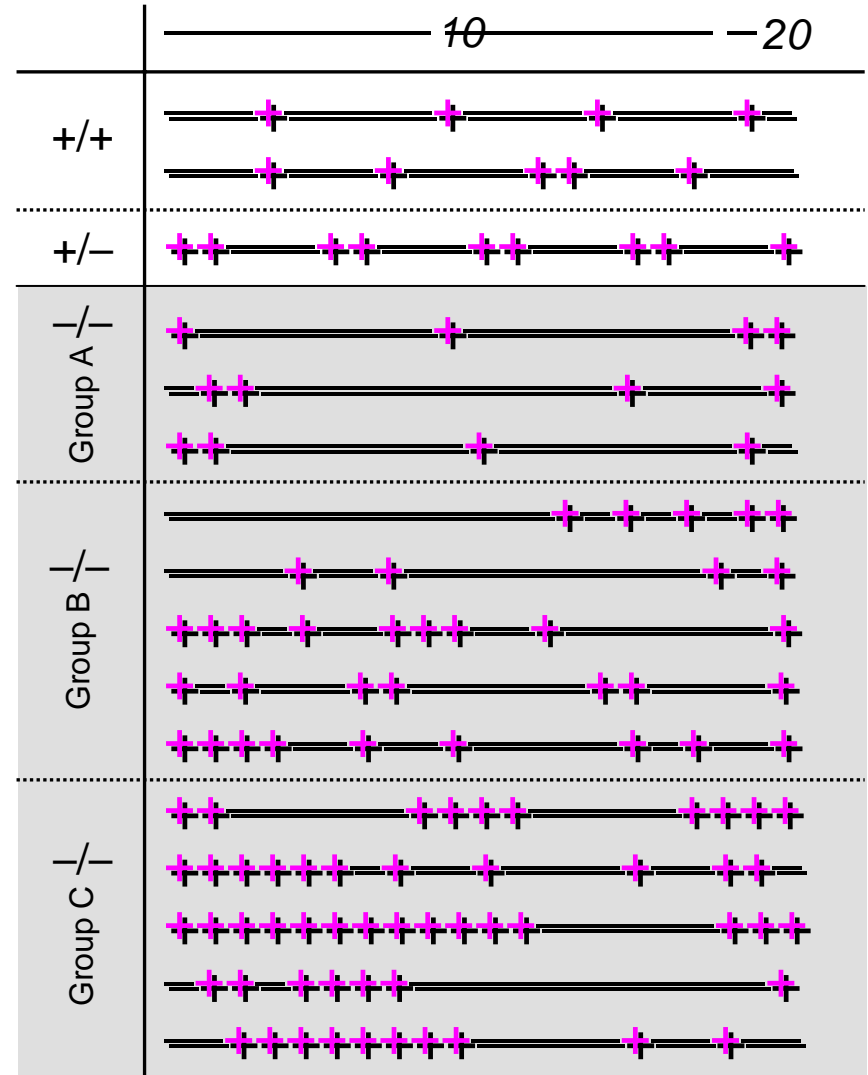


< Vaginal smears prepared at each estrus period >



Represented by “+”

Represented by “-”

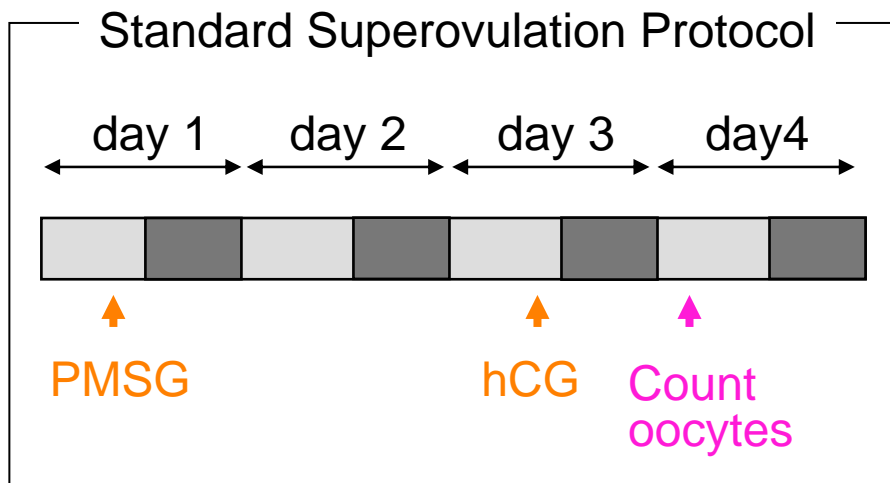


Group A: prolonged cycle.

Group B: shortened or prolonged cycle.

Group C: prolonged estrus period and no cyclicity.

# Phenotypes of AhR Knockout Mice related to Reproduction

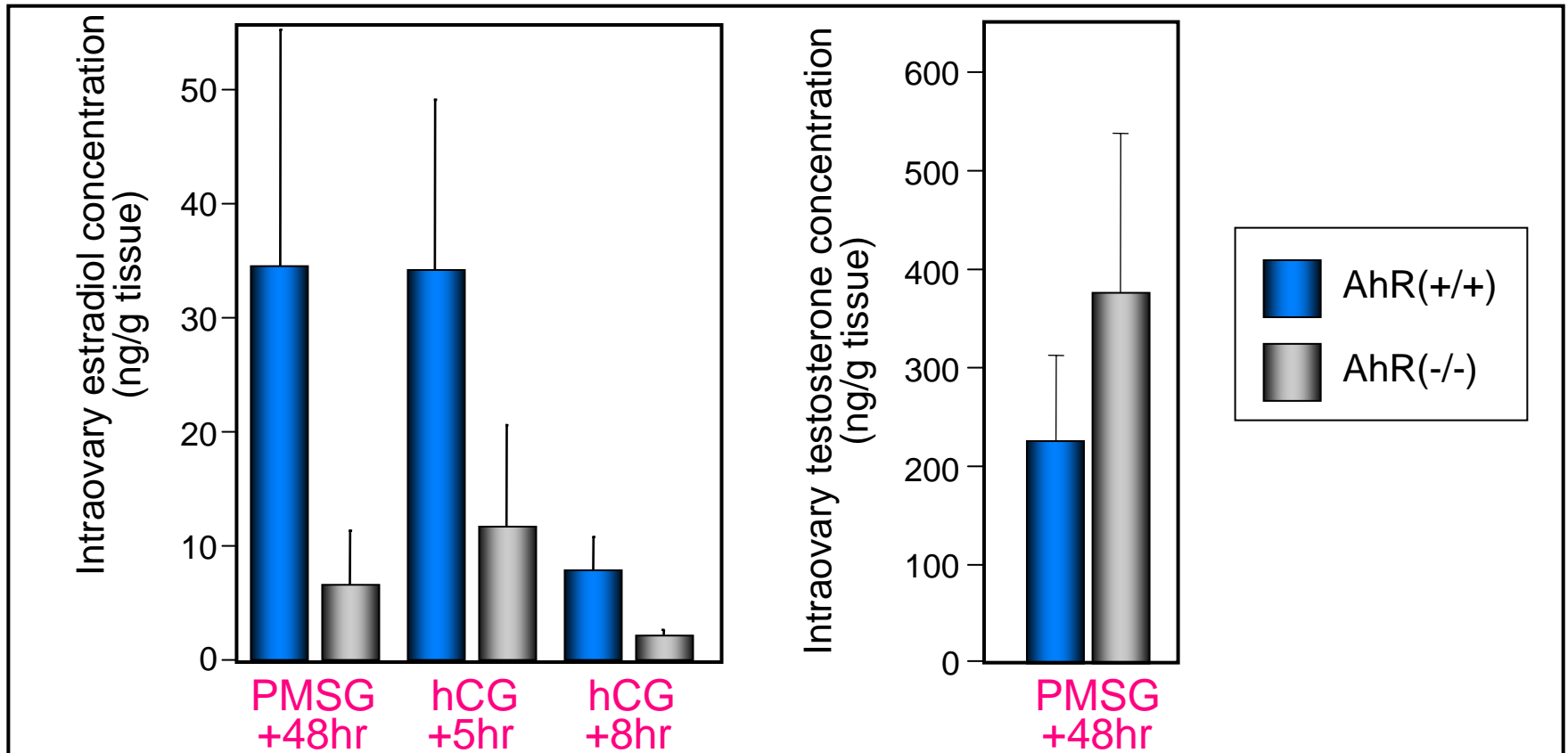
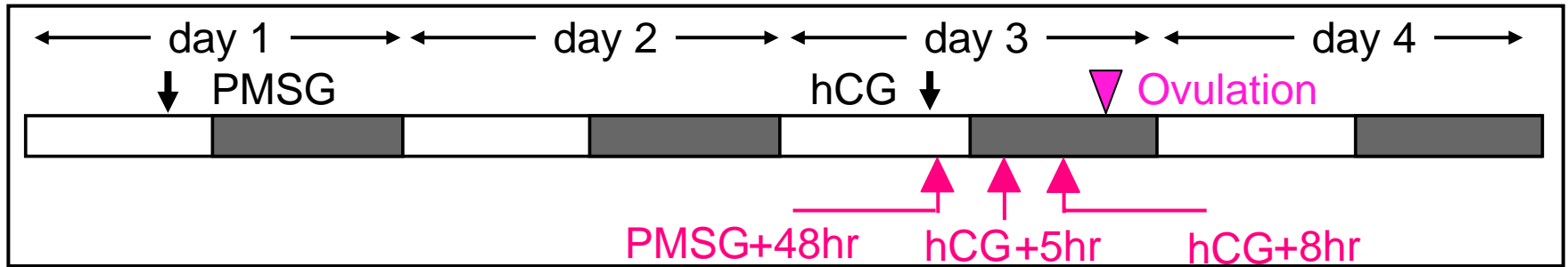


genotype		No. of ovulated Oocytes (mean $\pm$ S.D.)	
+/+	n=4	51.3 $\pm$ 2.2	(3 week-old)
	n=3	25.0 $\pm$ 1.0	(3 month-old)
-/-	n=3	8.7 $\pm$ 8.5	(3 week-old)
	n=3	4.0 $\pm$ 4.6	(3-month-old)

(5) Reduced ovulation - Ovulation capacity was decreased to approximately 1/6.

# Phenotypes of AhR Knockout Mice related to Reproduction

(6) Insufficient ovarian estradiol - Intraovarian estradiol concentration was decreased to approximately 1/3.





# **Summary of the Phenotypes in Reproduction of AhR KO Mice**

## **(1) Subfertility**

Decreased litter size and efficiency of fertility in AhR KO mouse.

## **(2) Small Ovary**

The ovary of AhR KO mouse is approx 65% of wild type.

## **(3) Maturation Defect of Ovarian Follicle**

Many immature follicles and few corpus luteum are in the KO ovary.

## **(4) Disordered Estrus Cycle**

All KO mice show abnormal patterns of estrus cycle.

## **(5) Decreased Number of Ovulated Eggs**

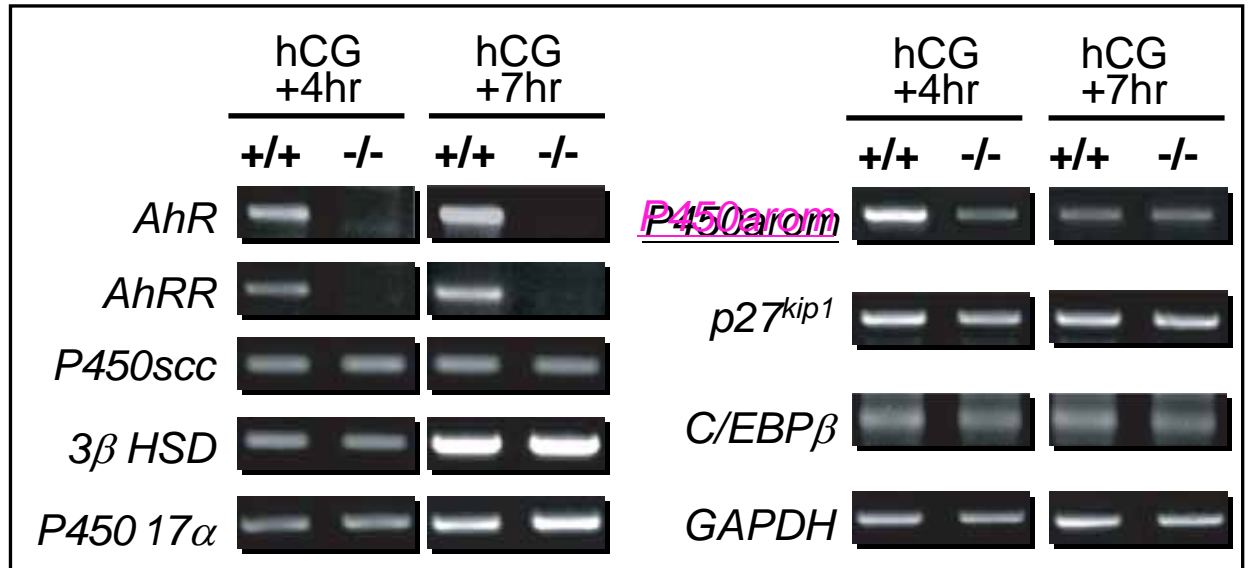
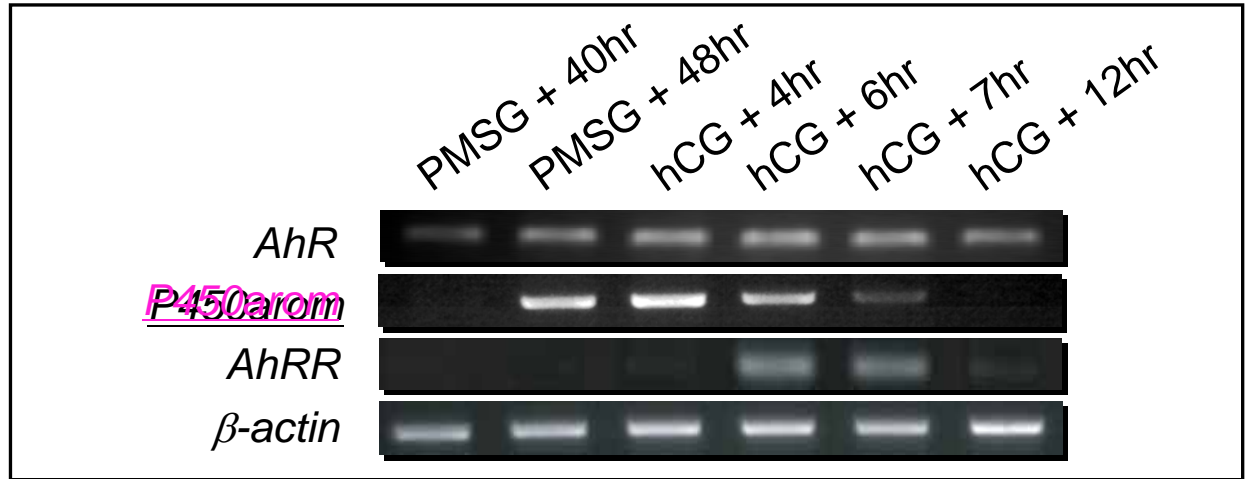
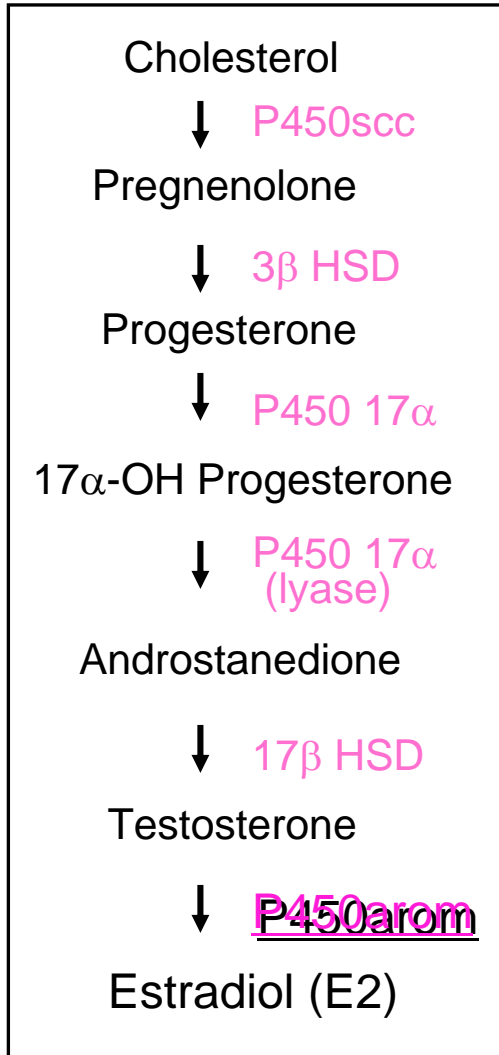
The number of the ovulated eggs from KO is 1/6 of wild type.

## **(6) Decreased Estradiol**

Serum concentration of estradiol is 1/3 of wild type.

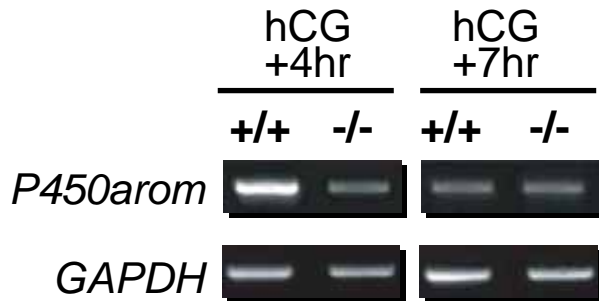
**Question: why estrogen production is decreased?**

# Upregulation of P450 Aromatase Gene Expression from Proestrus to Estrus disappeared from the AhR(-/-) Ovary

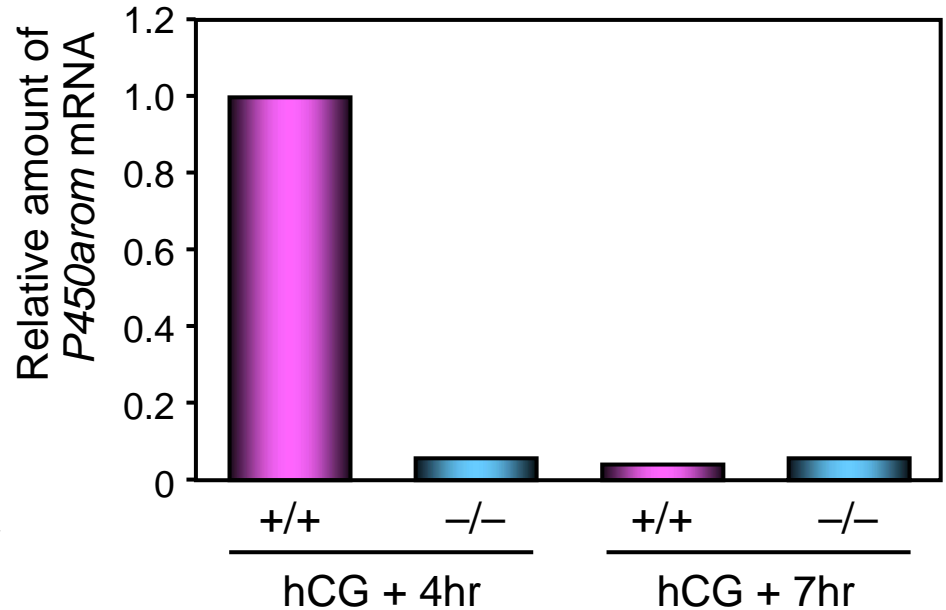


# Defect in Enhancement of Cyp19 Expression in AhR(-/-) Females confirmed both at mRNA and Protein Levels

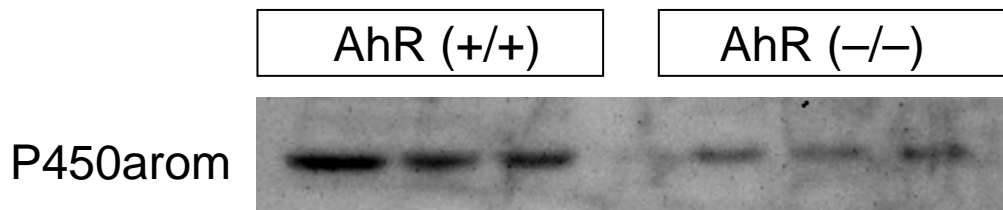
## RT-PCR



Quantification of Cyp19 mRNA by using Real-time RT-PCR.



## Western blotting



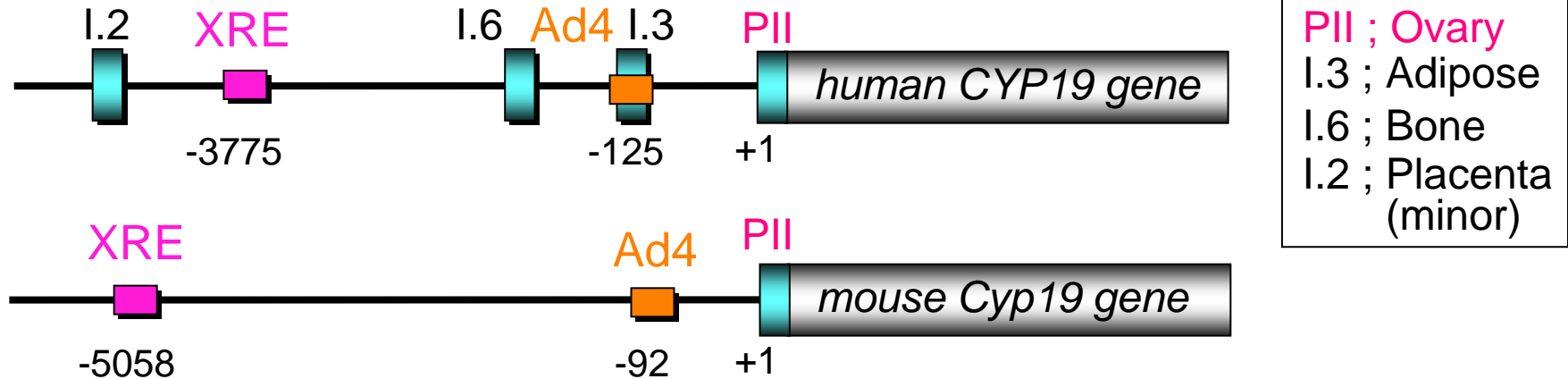
Whole cell extract was prepared from PMSG and hCG treated (**hCG + 5hr**) ovaries.

AhR regulates P450arom expression in the ovary?



Localization of AhR and P450arom in the ovary?

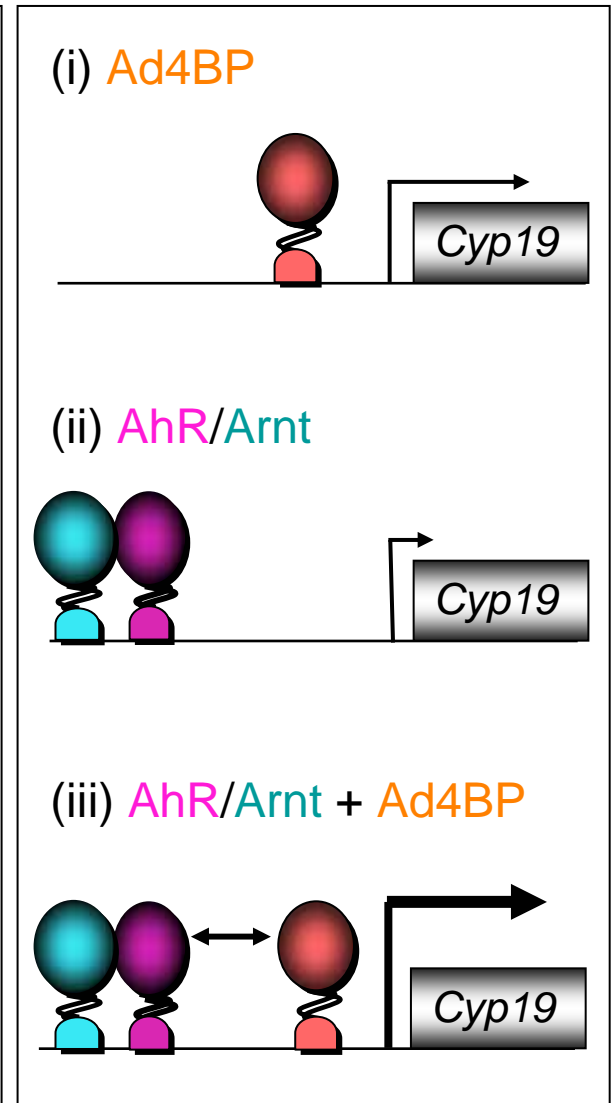
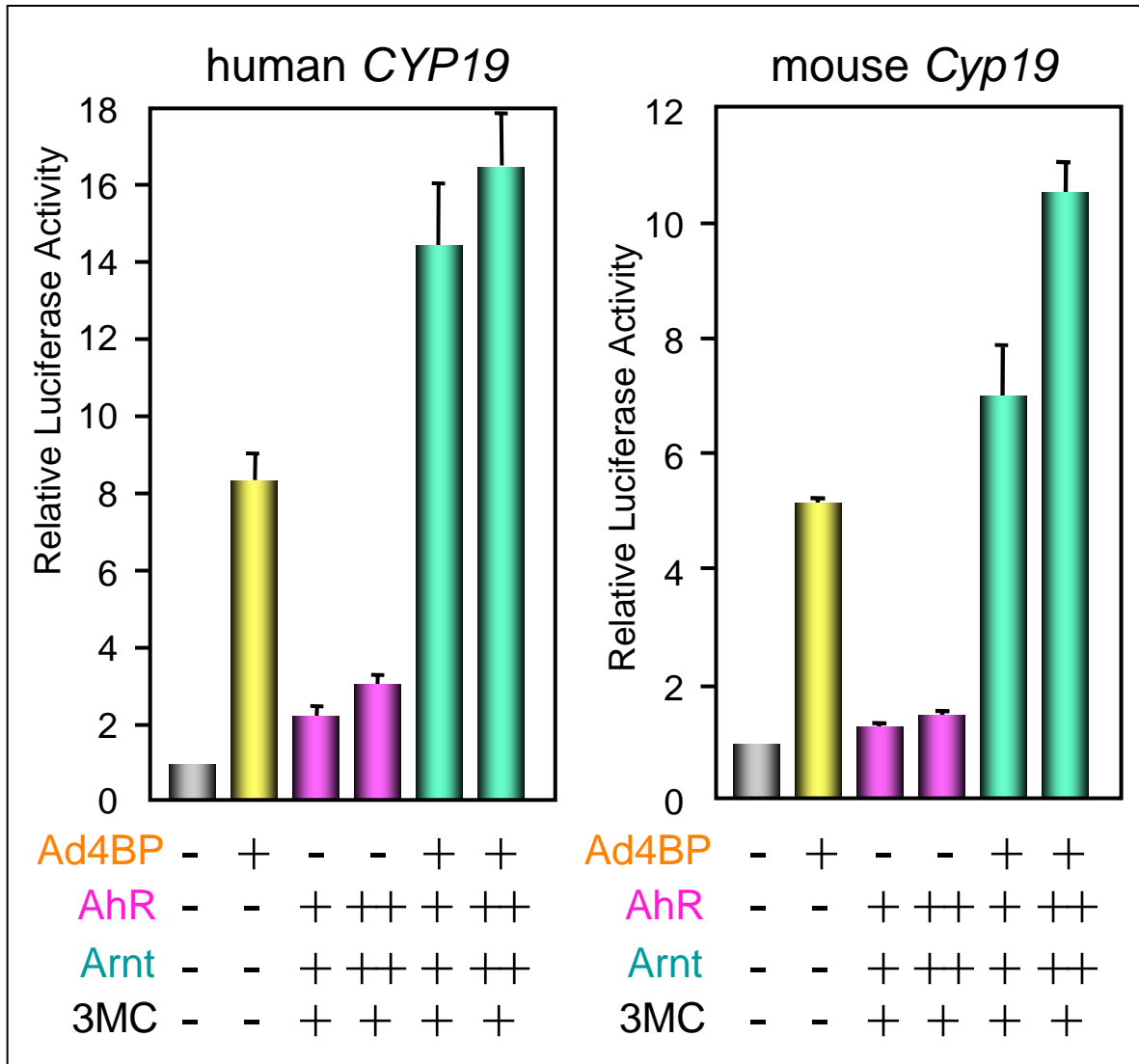
# Schematic Representation of Upstream Region of Human and Mouse Cyp19 (Aromatase) Gene



## Ad4BP/SF-1

is a member of nuclear receptor superfamily.  
 activates transcription of genes encoding steroidogenic enzymes.  
 is expressed at granulosa and theca cells in the ovary.

# Synergistic Transcriptional Activation of Aromatase (Cyp19) Gene by AhR and Ad4BP/SF-1



# Interaction between AhR and Ad4BP/SF-1 on Cyp19 Gene Promoter Revealed by ChIP Assays with Preovulatory Granulosa Cells

