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14. ABSTRACT

This study aims to identify if exposure to estrogen during the neonatal period increases the sensitivity of the prostate to hormonal induction of malignant and pre-malignant lesions in the adult. Significant progress had been made and it has been demonstrated that co-administration of high concentration of testosterone and estradiol can induce carcinogenesis in the prostate of mice including pathologies ranging from hyperplasia to dysplasia and carcinoma in situ. Comparison of the prostates of wild-type and estrogen deficient ArKO mice has shown that although both develop focal dysplastic pathologies in discrete sites the estrogen-deficient ArKO prostate shows a significantly reduced incidence of aberrant pathology compared to that of normal animals. This strongly suggests that exposure to increased estrogen increases the risk of developing prostate cancer.

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INTRODUCTION

The causes of prostate cancer are unclear and it is not understood what factors may predispose a man to development of prostate cancer, although fluctuation in steroid hormones levels has been suggested. The ultimate aim of this study is to determine if exposure to elevated estrogen levels results in altered susceptibility to the development of prostate cancer, with the ultimate hypothesis being that exposure to estrogen during neonatal life increases the susceptibility to hormonal induction of prostate cancer in adult life. If this can be proven it will demonstrate that the risk of developing prostate cancer in adulthood can be adversely influenced by exposure to estrogen during periods of prostate development far removed from adulthood.

BODY

Task 1: Establish aromatase knockout heterozygote breeding pairs for specific production of experimental mice (Months 1-3).

Establishment of the ArKO breeding pairs was accomplished by the end of the first 3 months of this project. Due to space constraints, this was initially begun as 6 pairs and increased over subsequent months to a total of 10. As per institutional protocols for the maintenance of breeding pairs animals breeding pairs were turned over after production of 6 litters or after not achieving any pregnancy for 6 weeks.

Task 2: ~~Induction of prostate malignancy by combined T+E treatment of adult ArKO mice~~ (Months 3-22)

This study addressed the hypothesis that "**Preexisting prostate hyperplasia and high androgens and prolactin increases the susceptibility to hormonal induction of prostate cancer" which was generated by a previous pilot study.**

To induce carcinogenesis mice were treated with silastic implants of testosterone and estradiol, a protocol that has been described to induce carcinogenesis in rats and canines and transformation of human prostate cells (Noble, 1977; Leav et al., 1988; Yu et al., 1993; Wang et al., 2001). Normally ArKO and wild-type (WT) mice show significant different serum androgen profiles (Figure 1) however administration of T+E implants in both ArKO and WT mice displaying similar androgen profiles. Initially levels of both serum testosterone and DHT were highly elevated but declined over the course of treatment. Thus both ArKO and were exposed to similar hormonal influences.

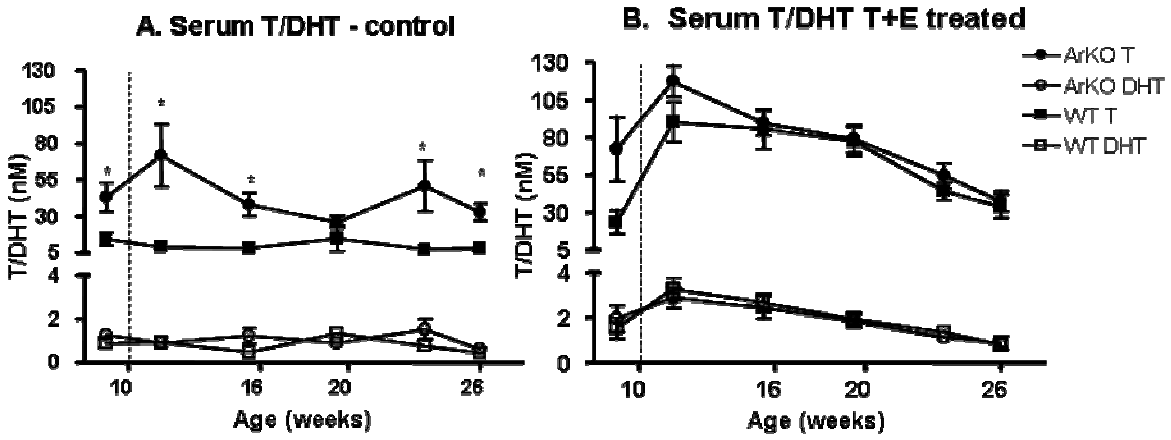

Figure 1. Serum androgen concentration following T+E treatment. A Serum T, but not DHT is significantly elevated in Ar Komice compare to WT. B. Serum T and DHT levels for both ArKO and WT mice following T+E treatment are not significantly different. Dashed line indicaes time of treatment with T+E or placebo. * $p>0.05$, $n>10$

Analysis of tissue demonstrated significant weight increases in ventral, and lateral prostate lobes (VP, LP respectively) of WT mice although no significant changes were detected in dorsal and anterior prostate lobes (DP, AP). No significant change was observed in any lobe of the ArKO prostate following treatment (Figure 2). This was supported by volumetric analysis which showed significant increases in the volume of the epithelial cell compartment of WT VP and LP with no significant effect on other tissue compartments tissue or in DP or AP. Corresponding to the organ weights ArKO prostate lobes showed no effect on tissue compartmental volumes.

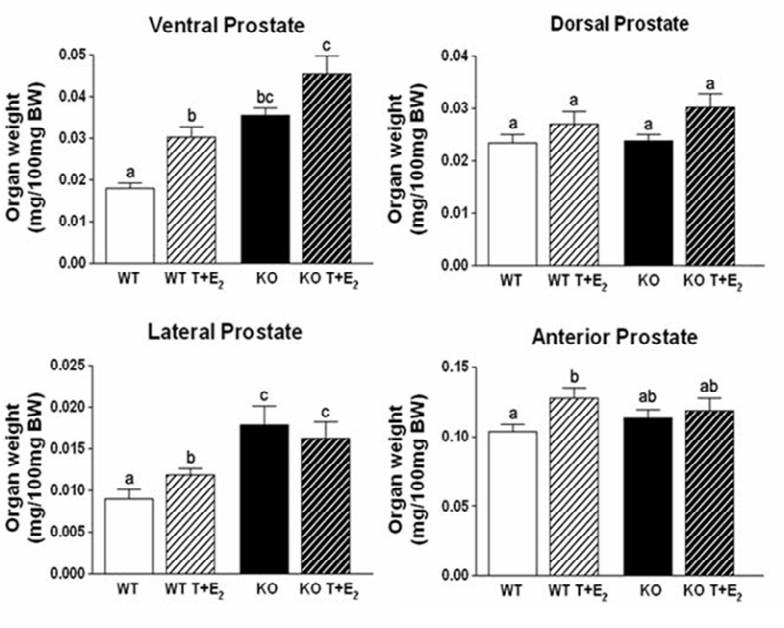

Figure 2: Effect of T+E treatment on prostate weight Treatment of adult mice with T+E2 resulted in significant increases in WT ventral, lateral and dorsal prostate weight but had no effect on ArKO organ weight. Data expressed as mean ± SEM ; same superscripts indicate groups that are not significantly different, $p<0.05$, $n \geq 16$.

Morphological and immunohistological analysis of tissues clearly identified the appearance of epithelial hyperplasia in WT tissues and pre-malignant lesions consistent with the development of prostatic intraepithelial neoplasia and dysplasia within T+E treated tissues of both ArKO and WT animals. Immunohistological studies identified apparent increase in proliferative activity within these lesions as well as up-regulation of androgen receptor, estrogen receptor α and β. It was also apparent that some lesions demonstrated the loss of the tumor suppressor E-cadherin (Appendix 1a).

It was initially proposed that stereological analysis would be conducted to determine alteration in immunohistological markers within the prostate, however the random distribution of prostatic lesions following T+E treatment presents major problems in defining parameters and boundaries for analysis. Therefore although extensive immunohistochemistry has been conducted stereological analysis has not been conducted (Appendix 1b).

The incidence of these premalignant lesions was assessed throughout each prostate lobe with highest incidence lesions observed in the LP followed by VP, DP and AP (Table 1). However the incidence of these pathologies was reduced by up to 50% in ArKO VP and LP.

	VP		LP		DP		AP	
Pathology	WT	ArKO	WT	ArKO	WT	ArKO	WT	ArKO
Hyperplasia	100	100	100	100	100	100	67	44
Atypical hyperplasia	83	38*	100	60*	55	30	17	17
PIN/CIS	50	25*	90	50*	45	20	17	17

Table 1. Incidence of hormone induced prostate pathologies is reduced in ArKO mice, * $p < 0.05$, $n \geq 15$.

These results suggest that the ArKO mouse prostate is not predisposed to the hormonal induction of malignancy compared to WT mice which appear more susceptible to hormonal induction of carcinogenesis.

Outcome:

Although both ArKO and WT mice displayed evidence of hormonal induction of carcinogenesis, it is obvious that in the absence of endogenous estrogen production the ArKO mouse shows a reduced susceptibility to prostatic carcinogenesis arising for altered adult androgen and estrogen levels. Thus the initial hypothesis that suggested that the presence of preexisting pathology would increase the risk of carcinogenesis has been disproved.

Significance

These data demonstrate that the presence of estrogen in WT mice results in significantly increased incidence of premalignant lesions within the prostate compared to animals that do not have estrogen. Thus it is apparent that exposure to estrogen has the potential to predispose the prostate to carcinogenesis.

However it remains to be determined if the apparent resistance of ArKO mice to hormonal carcinogenesis results from the neonatal absence of estrogen/ elevation of androgen, or may be the direct result of the absence of aromatase activity in adult tissues. Identifying the cause of this response will provide new insight into the role of aromatase in prostate development and disease

Task 3: Adult exposure to high does of testosterone and estrogen, following neonatal estrogenisation (Months 8-36).

Exposure to estrogen during early life ahs been reported to have significant effects on prostate development ranging from hypertrophy, hyperplasia and dysplasia to inflammation. Given the outcome of the previous task the hypothesis that "**Exposure to estrogen during neonatal life increases the susceptibility to hormonal induction of prostate cancer in adult life"** has been proposed.

Therefore this study involves treating neonatal ArKO and WT mice with the synthetic estrogen diethylstilbestrol (DES). Animal are subsequently allowed to age to 90 or 180 days before receiving the same T+E treatment as applied previously. Animals receive the T+E implants for a further 4 months before tissue is collected for analysis.

Major renovations of animal holding facilities was undertaken during 2007 that significantly reduced animal holding capacity and breeding. This has significantly delayed the breeding and treatment of experimental animals therefore there is no data yet available for this project given the time frames of experimentation although tissues are due to be collected shortly.

KEY RESEARCH ACCOMPLISHMENTS

List of key research accomplishments emanating from this research

- Immunohistological characterization of pre-malignant lesions
- Demonstration of hormonal profiles following T+E treatment of ArKO and WT mice
- Stereological quantiation of prostate pathology in T+E treated animals identifying epithelium as site of aberrant growth
- Demonstration of reduced susceptibility to hormonal carcinogenesis in absence of estrogen synthesis
- Utility of T+E treatment as a model of carcinogenesis in mice

REPORTABLE OUTCOMES:

	Reportable outcomes that have resulted from this research
Manuscripts	William A. Ricke, Stephen J. McPherson, Joseph J Bianco, Gerald R. Cunha, Yuzhuo Wang, Gail P. Risbridger. Prostatic hormonal carcinogenesis is mediated by *in situ* estrogen production and estrogen receptor alpha-signaling. FASEB J 2007 (in press).
Abstracts/Presentations	SJ McPherson, JS Pedersen, GP Risbridger. Absence of Endogenous Aromatase Activity and Estrogen Results in Reduced Susceptibility to Hormonal Induction of Prostate Malignancy in Adulthood. *Department of Defense, Prostate Cancer Research Program, Innovative Minds in Prostate Cancer Today (IMPaCT) meeting.*, Atlanta, USA, 5-8

	September 2007. (See Appendix 2a) McPherson SJ, Bianco JJ, Wang H, Pedersen JS, Wang YZ, Cunha GR, Risbridger GP. Estrogen deficient mice are less susceptible to hormone dependent induction of prostate malignancy. *12th International Congress on Steroid Hormones and Cancer*, Athens, Greece, 2006. (See Appendix 2b)
Patents & licenses	nil
Degrees obtained	nil
Development of cell lines	nil
Tissue or serum repositories	nil
Informatics such as databases and animal models	nil
Funding applied for based on this award	nil
Employment or research opportunities applied for and/or received based on experience/training supported by this award	nil

CONCLUSION:

In summary this project has provided significant data regarding the influence of estrogen on the susceptibility to prostatic carcinogenesis. Specifically this study has shown that the presence of increased estrogen in a male can cause an increase in the incidence of prostatic lesions caused by alteration in adult hormone levels that can lead to carcinogenesis. Currently it is unclear if this is due to the long term effect originating from estrogen exposure during early life or is specifically due to aberration in adult hormonal balances. A recent publication in rats has suggested that elevated exposure to estrogen during neonatal life may be a predisposing factor for adult disease

(Yuen et al., 2005) and the outcomes of this study, when completed, will provide further insight and understanding
to the potential role of estrogen, and androgens, in predisposing the prostate to cancer.

Therefore at the conclusion of this investigation this study is sure to provide significant new understanding and insight into prostatic carcinogenesis, specifically into the role of both estrogen and androgens, in influencing the susceptibility of an individual to this disease.

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Yu, M., Leav, B.A., Leav, I., Merk, F.B., Wolfe, H.J., and Ho, S.M. (1993) Early alterations in ras protooncogene mRNA expression in testosterone and estradiol-17 beta induced prostatic dysplasia of noble rats. Lab Invest 68:33-44.

Yuen, M.T., Leung, L.K., Wang, J., Wong, Y.C., and Chan, F.L. (2005) Enhanced induction of prostatic dysplasia and carcinoma in Noble rat model by combination of neonatal estrogen exposure and hormonal treatments at adulthood. International journal of oncology 27:1685-1695.

APPENDIX 1: ADDITIONAL FIGURES.

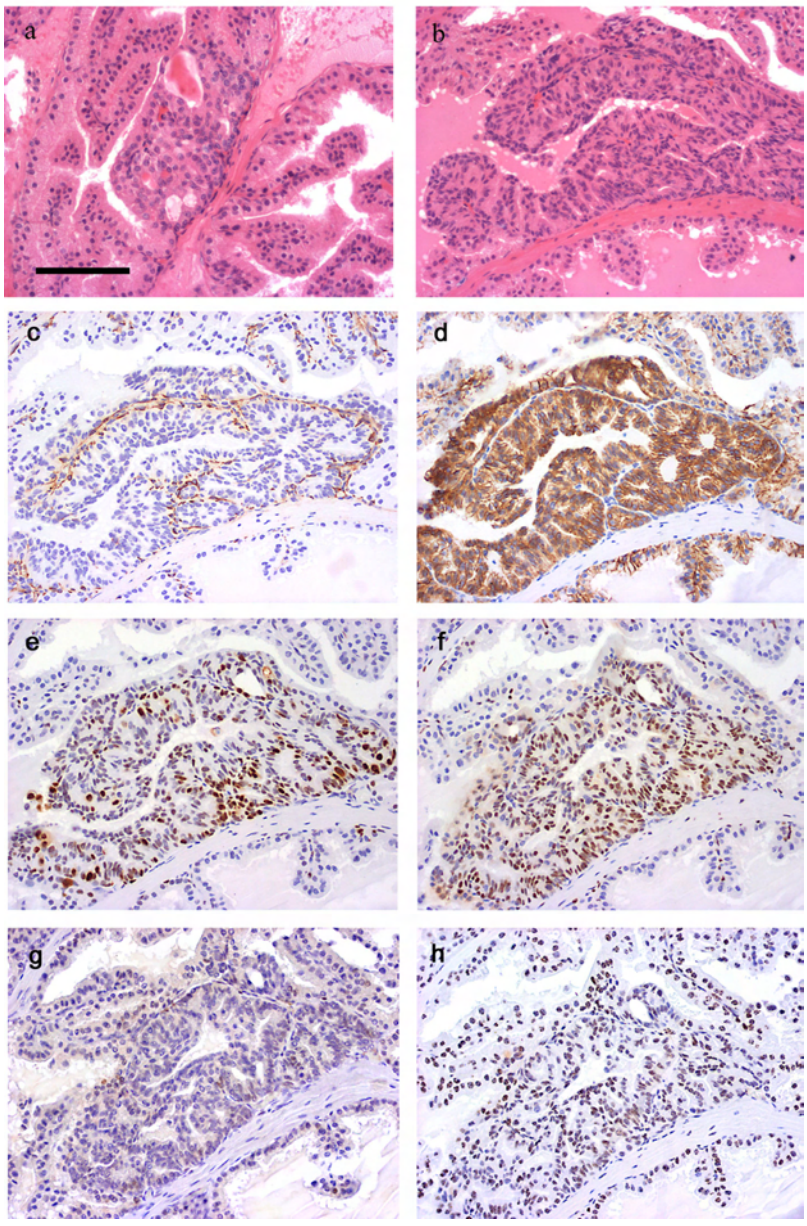

Figure 3: Identification of dysplasia in mouse prostate lobes following T+E2 hormonally-treated mice. Focal dysplastic lesions could be identified by histological parameters in prostates from both WT and ArKO mice following T+E2 treatment. Both low (b) and high (b) grade lesions were identifiable.Immunohistochemistry was also used to identify lesions. c-h: High grade dysplastic lesion showing high molecular weight cytokeratin (CKH; c), E-cadherin (d), PCNA (e), AR (f), ERα (g), ERβ (h). Scale bar = 25μm.

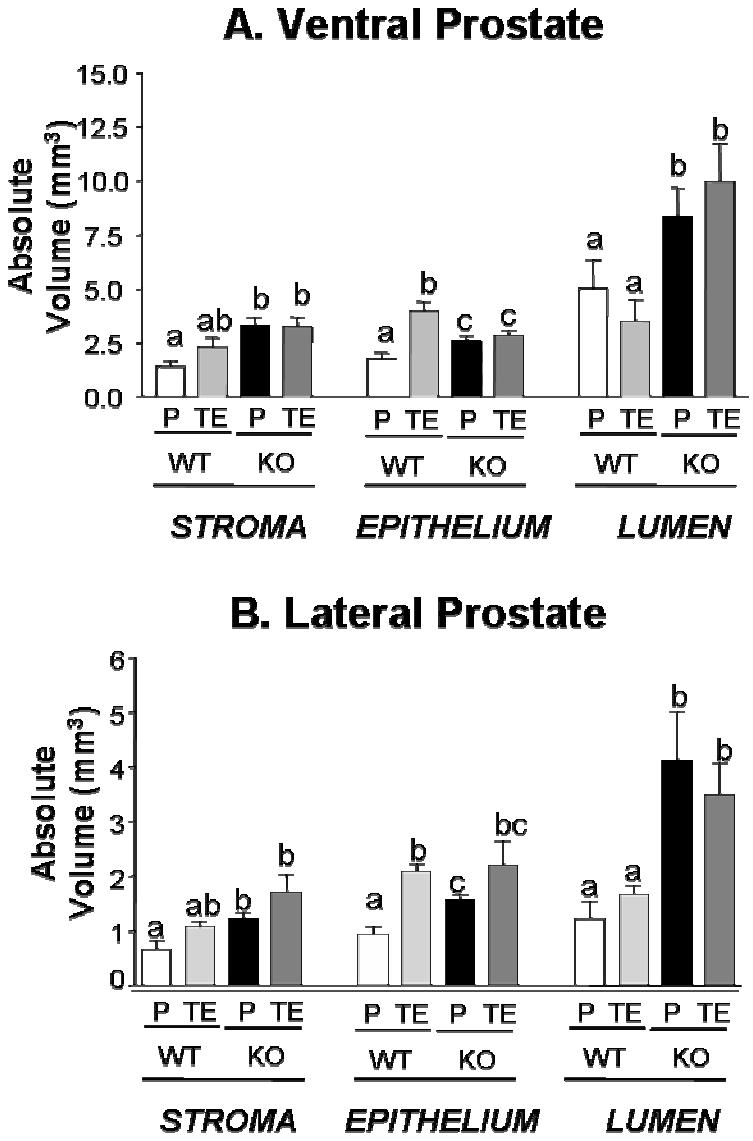

Figure 4. Stereological analysis of prostate lobes following T+E2 treatment. Consistent with histological and weight data, stereological analysis of (A)Ventral and (B) Lateral prostate lobes demonstrated significant increases in absolute volumes of epithelial compartment of WT VP and LP ($p<0.05$). Same superscript indicates no significant difference ($p < 0.05$, $n >10$) in same tissue compartment.

APPENDIX 3. ABSTRACT PRESENTATIONS

1. Poster presentation give nby Stephen McPherson at the inaugural *Department of Defense, Prostate Cancer Research Program, Innovative Minds in Prostate Cancer Today (IMPaCT) meeting.*, Atlanta, USA, 5-8 September 2007.

Absence of endogenous aromatase activity and estrogen results in reduced susceptibility to hormonal induction of prostate malignancy in adulthood.

Stephen J McPherson; John S Pedersen (Tissupath, Melbourne, Victoria, Australia); Gail P Risbridger

The incidence of prostate cancer in men increases with age and although dependent on the presence of androgens, commonly occurs at an age where the levels of serum testosterone are declining and the ratio of estrogens: androgens is increasing. The contradictory nature of this observation has focused attention on estrogens and particularly on how the balance between androgens and estrogens might alter the incidence of prostate cancer that develops upon aging. Evidence exists that exposure to high levels of estrogen during early neonatal life (imprinting) can have permanent and irreversible effects leading to aberrant prostate development and the development of premalignant and inflammatory pathologies in adult life. It has also been shown that altering the hormonal milieu in adult animals, using combined androgen and estrogen treatment, can induce prostatic malignancy. In both cases it is the presence of estrogen that determines the resultant pathology.

Given the involvement of estrogen in the process of prostatic carcinogenesis the purpose of this project is to determine if suppression of adult and/or neonatal estrogen levels results in a reduced sensitivity to the development of hormonally induced prostate cancer.

In the initial phase of this investigation we have administered silastic implants containing Testosterone + Estradiol ($T+E_2$) implants into estrogen-deficient aromatase knockout (ArKO) and WT mice for 4 months to determine if the absence of estrogens results in altered sensitivity to hormonally induced carcinogenesis. The results of this study have demonstrated the induction of malignant and premalignant pathologies in prostates of ArKO mice, is reduced by up to 50% compared to WT animals. Interestingly it has also shown that the most androgen sensitive prostate lobes (ventral and lateral) are demonstrating the greatest reduction in incidence of pathological lesions. Thus it is apparent that the absence of endogenous aromatase activity and thus systemic estrogen results in a reduced incidence of hormonally-induced malignant and non-malignant prostatic pathologies.

It is unclear if reduced sensitivity of the ArKO prostate to hormonal induction of carcinogenesis is due to reduced adult estrogen levels or an effect of altered neonatal imprinting occurring between the estrogen-deficient ArKO mice and WT mice. This issue is currently being addressed

in studies combining neonatal estrogenisation of both ArKO and WT mice with exposure to $T+E_2$ treatment in adulthood.

IMPACT: Given the sensitivity of the neonatal prostate to abnormal hormone levels and the fact that the balance of hormones alters in aging men, this project may have significant impact on the ability to identify individuals with increased risk of developing prostate disease/cancer. Further this project will determine if susceptibility to prostate disease/cancer may be influenced by hormonal exposure during early life.

2. Poster presentation give nby Gail Risbridger on behalf of Stephen McPherson at *12th International Congress on Steroid Hormones and Cancer*, Athens, Greece, 2006.

ESTROGEN DEFICIENT MICE ARE LESS SUSCEPTIBLE TO HORMONE DEPENDENT INDUCTION OF PROSTATE MALIGNANCY.

McPherson SJ (1), Bianco JJ (1), Wang H (1), Pedersen JS (2), Risbridger GP (1)
(1) Monash Institute of Medical Research, Monash University Melbourne, Australia, 3168;
(2)TissuPath, Melbourne Australia

Androgens and estrogens are critical in regulating early and late life development of the prostate gland. Yet estrogens are regarded as potentially carcinogenic and neonatal estrogenisation is linked to the potential development of prostate malignancy in late life. Estrogen deficiency occurs in aromatase knockout (ArKO) mice that spontaneously develop prostatic hypertrophy and hyperplasia, but do not develop prostatic malignancy. As ArKO mice show inherent prostate growth abnormalities and a lifelong hormone imbalance, we predicted that they would show increased susceptibility to the induction of prostate cancer in adulthood. To test this hypothesis, the rate of hormonal induction of malignancy was compared in adult ArKO and wild-type (WT) mice, using combined testosterone + estradiol (T+E) treatment for 4 months.

Following T+E treatment both ArKO and WT serum androgen levels were elevated to similar levels. T+E-treated WT prostates demonstrated increased localisation of androgen receptor (AR) and significant increases in prostate lobe weights and epithelial volume. Hyperplasia and dysplasia, was identifiable in all prostate lobes in WT mice, although the rate of incidence varied between lobes; lateral - 90% incidence of dysplasia, ventral - 50%, dorsal - 45%, anterior - 17%. T+E treated ArKO tissues showed no significant changes to weight, volume or AR localisation, however dysplastic lesions were detectable, but at an incidence approximately half that seen in WT (lateral 50%; ventral 23%; dorsal 20%) although anterior prostate was no different.

The results demonstrate that in the absence of endogenous estrogen, hormonal induction of premalignant dysplastic lesions of the prostate is significantly reduced. This suggests that exposure to estrogen may increase the predisposition of the prostate to malignant change resulting from hormonal imbalances in adulthood. It remains to be determined if the apparent resistance of ArKO prostate to induction of premalignant lesions is the result of hormonal imprinting during neonatal life or of altered hormonal influences in adulthood.