รายงานวิจัยฉบับสมบูรณ์

โครงการ: การศึกษาตัวรับฮอร์โมนเอสโตรเจนและโปรเจสเตอโรนในสุกรสาวที่มีปัญหาทางการสืบพันธุ์

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บทคัดย่อ

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ชื่อโครงการ: การศึกษาตัวรับฮอร์โมนเอสโตรเจนและโปรเจสเตอโรนในสุกรสาวที่มีปัญหาทางการสืบพันธุ์

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การศึกษาครั้งนี้มีวัตถุประสงค์เพื่อศึกษาการแสดงออกของตัวรับฮอร์โมนเอสโตรเจนชนิดแอลฟ่าและโปรเจสเตอโรนในอวัยวะสืบพันธุ์ของสุกรสาวทดแทนที่ถูกคัดทิ้งเนื่องจากปัญหาความล้มเหลวทางระบบสืบพันธุ์ โดยทำการเก็บตัวอย่างอวัยวะสืบพันธุ์ซึ่งประกอบด้วย รังไข่ มดลูกและ คอมดลูกจากสุกรสาวที่ถูกคัดทิ้งจากฟาร์มสุกรจำนวนทั้งหมด 30 ตัว ทำการบันทึกข้อมูลทางระบบสืบพันธุ์ สาเหตุของการคัดทิ้ง และพยาธิสภาพที่ตรวจพบ ใช้วิธีอิมมูนโนฮิสโตเคมีในการตรวจหาการแสดงออกของตัวรับฮอร์โมนเอสโตรเจนชนิดแอลฟ่า และตัวรับฮอร์โมนโปรเจสเตอโรน พบว่าสาเหตุหลักของการถูกคัดทิ้งคือไม่แสดงอาการเป็นสัด ส่วนการตรวจทางพยาธิสภาพพบว่าสุกรสาวส่วนใหญ่ไม่แสดงความผิดปกติทางกายวิภาค (40%) ในขณะที่ลักษณะทางพยาธิวิทยาที่พบมากที่สุดคือ ถุงน้ำรังไข่ (33.3%) นอกจากนั้นพบการบวมน้ำและมีเลือดคั่งที่อวัยวะสืบพันธุ์ (26.7%) ผลจากขบวนการอิมมูนโนฮิสโตเคมีไม่พบความแตกต่างระหว่างสุกรสาวที่ถูกคัดทิ้งก่อนวัยเจริญพันธุ์และสุกรสาวที่ถูกคัดทิ้งในระยะลูเตียลยกเว้นการแสดงออกของตัวรับฮอร์โมนเอสโตรเจนชนิดแอลฟ่าในชั้นกล้ามเนื้อของมดลูก แสดงให้เห็นว่าสภาวะของระบบสืบพันธุ์ไม่มีผลต่อการแสดงออกของตัวรับฮอร์โมนในเนื้อเยื่อส่วนใหญ่ของคอมดลูกและมดลูกสุกรสาวที่ถูกคัดทิ้ง เมื่อพิจารณาถึงลักษณะทางพยาธิสภาพที่พบในสุกรเหล่านี้พบว่าการแสดงออกของตัวรับฮอร์โมนโปรเจสเตอโรนมีปริมาณน้อยกว่าอย่างมีนัยสำคัญในคอมดลูกของสุกรสาวที่ถูกคัดทิ้งก่อนวัยเจริญพันธุ์ที่พบการบวมน้ำและมีเลือดคั่งของอวัยวะสืบพันธุ์ ในส่วนของมดลูกสุกรพบว่ามีความแตกต่างของการแสดงออกของตัวรับฮอร์โมนเอสโตรเจนชนิดแอลฟ่าในชั้นเนื้อเยื่อเกี่ยวพันและกล้ามเนื้อมดลูกระหว่างสุกรสาวที่ถูกคัดทิ้งก่อนวัยเจริญพันธุ์และสุกรที่พบพยาธิสภาพในระยะลูเตียล สำหรับการแสดงของตัวรับฮอร์โมนโปรเจสเตอโรนพบว่ามีความแตกต่างเฉพาะในชั้นกล้ามเนื้อของมดลูกเท่านั้น เมื่อพิจารณาความแตกต่างระหว่างส่วนต่าง ๆ ของอวัยวะสืบพันธุ์ที่ศึกษาในครั้งนี้พบว่าส่วนกล้ามเนื้อของคอมดลูกและมดลูกเป็นเนื้อเยื่อที่มีการเปลี่ยนแปลงของการแสดงออกของตัวรับฮอร์โมนมากที่สุด จากผลการทดลองในครั้งแสดงให้เห็นถึงความแตกต่างของการแสดงออกของตัวรับฮอร์โมนในส่วนต่าง ๆ ของอวัยวะในระบบสืบพันธุ์

ของสุกรสาวที่ถูกคัดทิ้งซึ่งอาจจะมีความเกี่ยวข้องกับพยาธิสภาพที่พบในอวัยวะสืบพันธ์เหล่านี้ เนื่องจากการทำงานของฮอร์โมนผ่านตัวรับฮอร์โมนนั้นมีความแตกต่างและจำเพาะในเซลล์สืบพันธุ์แต่ละชนิดดังนั้นการแสดงออกของตัวรับฮอร์โมนเหล่านี้ย่อมมีความแตกต่างกันทั้งในสุกรสาวที่ปกติและสุกรสาวที่มีปัญหาทางระบบสืบพันธุ์ ทั้งนี้การที่พบการแสดงออกของตัวรับฮอร์โมนเอสโตรเจนชนิดแอลฟ่าและตัวรับฮอร์โมนโปรเจสเตอโรนในครั้งนี้ แสดงให้เห็นว่ามีการตอบสนองต่ออิทธิพลของฮอร์โมนที่มีส่วนเกี่ยวข้องกับสรีรวิทยาและพยาธิวิทยาของอวัยวะในระบบสืบพันธุ์ของสุกรสาวที่ถูกคัดทิ้ง

คำสำคัญ ตัวรับฮอร์โมนเอสโตรเจนชนิดแอลฟ่า ตัวรับฮอร์โมนโปรเจนเตอโรน สุกรสาว ปัญหาระบบสืบพันธุ์

Abstract

Project code: MRG5080007
Project title: Studies of oestrogen and progesterone receptors in reproductive organs of infertile gilt
Investigator: Assistant Professor Dr. Sayamon Srisuwatnasagul, Faculty of Veterinary Science, Chulalongkorn University
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Project period: 1 December 2006-30 November 2008

The present study aims to study the localization of steroid receptor, oestrogen receptor alpha and progesterone receptor in the reproductive organs of infertile gilts. The genital organs from 30 culled gilts which were ovary, uterus and cervix were collected from the slaughter houses. Historical data, reasons of culling and the macroscopic examination of the abnormalities of the ovaries and reproductive tracts were recorded. The immunohistochemistry was applied to investigate the expression of oestrogen receptor alpha (ERα) and progesterone receptor (PR) in formalin fixed paraffin-embedded tissues. The common culling reason of 30 gilts was anoestrus. Among these culled gilts, 40% of culling gilts showed no abnormality of the reproductive organs while the common macroscopic findings was cystic ovaries (33.3%) and the rest were found edema and/or congestion of the reproductive organs (26.7%). The results of immunohistochemistry between different reproductive statuses and regardless of the macroscopic finding were similar in the cervix and in the uterus except the expression of ERα in the myometrium. This indicated that reproductive status among these culled gilts may not have the significant effect on the expression of ER and PR in most compartments of the cervix and uterus. Regarding different macroscopic findings, there was the difference in the expression of PR in the surface epithelium and muscular layer of the cervix with lowest expression in the edema and/or congestion group before puberty. In the uterus, significant differences were observed for ERα score in the stroma and the myometrium between prepubertal gilts with no macroscopic abnormality and abnormal gilts culled during luteal phase. For PR the significant difference was seen only in the myometrium of edema and/or congestion gilts compared with no pathological prepubertal gilts. Comparing between different compartments of the reproductive tissue, it was found that the muscular layers both in the cervix and the uterus were the most dynamic tissue for the changes of steroid receptors. The results from the present study showed the difference in the expression of ERα and PR in different reproductive organs of culled gilts. The changes in the expression of these steroid receptors in some compartments of the cervix and uterus may involve with pathological status found in these culled gilts. As the regulation of steroid hormones through the expression of their receptors differed according to specific cell types of reproductive organs, therefore, the expression of steroid receptors in both normal and reproductive failure gilts should also vary. Moreover, the expression of ERα and PR in the reproductive organs of culled gilts indicated that there are responses to the influence of these steroid hormones and therefore, may have significant roles in reproductive physiology as well as pathology.

Keywords: oestrogen receptor alpha, progesterone receptor, gilt, infertile

Studies of oestrogen and progesterone receptors in reproductive organs of infertile gilt

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Abstract

The present study aims to study the localization of steroid receptor, oestrogen receptor alpha and progesterone receptor in the reproductive organs of infertile gilts. The genital organs from 30 culled gilts which were ovary, uterus and cervix were collected from the slaughter houses. Historical data, reasons of culling and the macroscopic examination of the abnormalities of the ovaries and reproductive tracts were recorded. The immunohistochemistry was applied to investigate the expression of oestrogen receptor alpha (ERα) and progesterone receptor (PR) in formalin fixed paraffin-embedded tissues. The common culling reason of 30 gilts was anoestrus. Among these culled gilts, 40% of culling gilts showed no abnormality of the reproductive organs while the common macroscopic findings was cystic ovaries (33.3%) and the rest were found edema and/or congestion of the reproductive organs (26.7%). The results of immunohistochemistry between different reproductive statuses and regardless of the macroscopic finding were similar in the cervix and in the uterus except the expression of ERα in the myometrium. This indicated that reproductive status among these culled gilts may not have the significant effect on the expression of ER and PR in most compartments of the cervix and uterus. Regarding different macroscopic findings, there was the difference in the expression of PR in the surface epithelium and muscular layer of the cervix with lowest expression in the edema and/or congestion group before puberty. In the uterus, significant differences were observed for ERα score in the stroma and the myometrium between prepubertal gilts with no macroscopic abnormality and abnormal gilts culled during luteal phase. For PR the significant difference was seen only in the myometrium of edema and/or congestion gilts compared with no pathological prepubertal gilts. Comparing between different compartments of the reproductive tissue, it was found that the muscular layers both in the cervix and the uterus were the most dynamic tissue for the changes of steroid receptors. The results from the present study showed the difference in the expression of ERα and PR in different reproductive organs of culled gilts. The changes in the expression of these steroid receptors in some compartments of the cervix and uterus may involve with pathological status found in these culled gilts. As the regulation of steroid hormones through the expression of their receptors differed according to specific cell types of reproductive organs, therefore, the expression of steroid receptors in both normal and reproductive failure gilts should also vary. Moreover, the expression of ERα and PR in the reproductive organs of culled gilts indicated that there are responses to the influence of these steroid hormones and therefore, may have significant roles in reproductive physiology as well as pathology

Keywords: Pig, Reproduction, Pathology, Ovary, Uterus

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1. Introduction

In general, culling and replacement rate of sows with gilts was approximately 35-55% each year (D'Allaire and Drolet, 1999) and therefore the numbers of replacement gilts in herd has a great effect on the overall output of the farm. The replacement gilts were introduced to herd at about 20-22 week of age when they have reached puberty. The factors which influence age at puberty varied such as breed, season, feeding including the influence from internal hormones which have the important roles in controlling the function of female reproductive organs.

The reproductive problems which lead to culling were varied such as anoestrus, repeat mating, not pregnant, vaginal discharge, abortion and dystocia. There are several earlier studies on pathological investigation of gilt reproductive organs from slaughter house (Ehnvall et al., 1981; Dalin et al., 1997; Heinonen et al., 1998) and the results showed the high number of culling pigs with no pathological lesions of reproductive organs. Kunavongkrit et al. (1988) studied the morphological of ovaries and uteri from the slaughter house in Thailand and revealed that 16.4% of culling gilts had reproductive lesions while 83.6% showed no pathological lesions of these reproductive organs. Moreover, 59.7% of normal culling gilts had ovulated. This is confirmed by the recent study in Thailand that about half of the gilts culled had shown normal reproductive organ (Tummaruk et al., 2009). Therefore, in addition to the macroscopic examination, there should be further studies of steroid hormones and their influences through specific steroid receptors in the reproductive organ of culling gilts. These may lead to better understanding of the mechanism of reproductive problems involved with the mechanisms under the influences of steroid hormones and their receptors.

The ovarian steroid hormones mainly oestrogen and progesterone, interplay the roles of controlling the morphological and functions of female reproductive organs of all mammals e.g. control of reproductive cycle, ovulation as well as pregnancy (Cooke et al., 1998; Spencer and Bazer, 2002; Lessey, 2003; Drummond, 2006). These steroid hormones elicit their functions by binding through specific receptor proteins in target tissues (Jensen and DeSombre, 1973; Jensen, 1991; Yamashita, 1998), therefore the presence of steroid receptors is as important as the levels of steroid hormones as they involve with the effective functions of reproductive control. There are several studies reported about the different localization of steroid receptor proteins in various reproductive organs and it was shown that steroid receptors such as oestrogen receptors and progesterone receptors could be found mainly in the uterus, cervix and ovary (Mowa and Iwanaga, 2000; Pelletier and El-Alfy, 2000; Pelletier et al., 2000; Wang et al., 2000). However, the study of steroid receptors in newly wean anoestrous sow demonstrated the high presence of steroid receptors in the uteri though the level of steroid hormones, oestradiol 17-β and progesterone were low (Sukjumlong et al., 2004a). Moreover, the presence of ER in the gene level was involved with the reproductive performance of the pigs (van Rens et al., 2000; Isler et al., 2002). Though the studies of steroid receptors in normal reproductive tracts are widely documented, the data of these receptors presence in reproductive disturbance gilts is still lacking. Therefore, the present study aims to investigate the presence of steroid receptor, oestrogen alpha and progesterone receptor in reproductive organs of infertile gilts.

2. Material and Methods

2.1 Animal and sample collection

Thirty crossbred Landrace x Yorkshire (LY) gilts were used. Historical data of all gilts was recorded. The data included herd, gilt's identity, breed, birth date, and date that the gilts enter the herd, first observed oestrus date, culling date, mating date, the BW at culling and the reason of culling. The genital organs of the slaughter gilts including ovary, uterus, and cervix were collected from the slaughterhouse within the herd or near the herd within 24 h after culling. The samples were placed in an ice box and sent to the laboratory within 24 h after slaughter.

They were dehydrated, embedded in paraffin and 4 μm thick sections were cut from each block and mounted on Polysine™ slides (Menzel-Glazer, Germany). These sections were used for immunohistochemistry.

2.3 Post-mortem examination

Post-mortem examination was performed on each part of the reproductive organs within 48 h after culling. The investigation focused on the abnormality of the ovaries, uterine horns and cervix.

Ovary

The ovaries were weighted. The number and diameter of copora lutea (CL) and number follicles with diameter ≥0.5 cm were counted. The ovaries were defined as being active when the ovaries contained CL or copora albicans (old CL) and follicles. In addition, the active ovaries were classified as luteal phase when CL were being active and it's diameter was larger than follicles and follicular phase when follicle's diameter was larger than CL. The ovaries were defined as pre-pubertal phase when ovaries contained follicles but had no CL or copora albicans. The gilts were defined as cystic ovaries when the formation of single or multiple cysts with diameter ≥1.5 cm were found.

Uterus

The uterine horn and the uterine body were dissected from the broad ligament and were weighted. The length of the left and right uterine horn and the uterine body were measured. The uterine horns were opened longitudinally and the endometrium was investigated. The macroscopic appearance of the endometrium was classified as normal, edema/congestion.

Cervix

The cervix was measured for length and the number of cervical fold. The vagina and vestibule were measured. The organs were dissected longitudinally and the epithelium was observed for abnormality. The appearance of the epithelium of the cervix, vagina and vestibule were classified as normal, edema/congestion and pyometra.

Reproductive status of the gilts

The reproductive status of the gilt was classified according to the appearance of the ovaries and the uterus. The gilts were defined as pre-puberty when the ovaries had no CL and contained follicles. The cyclic gilt was defined as follicular phase when follicles were ≥0.5 cm in diameter and small CL (≤0.4 cm or CL that smaller than follicle) were presented. The gilt was defined as luteal phase when large CL (diameter ≥0.5 cm or CL that larger than follicle) was presented. Of 30 gilts used in this study, 10 were culled during luteal phase and 20 gilts were culled before puberty.

2.3 immunohistochemistry

Before immunohistochemistry, sections were deparaffinized in xylene and rehydrated in graded alcohol. The immunohistochemical protocol was described previously by Sukjumlong et al., (2003). Briefly, antigen unmasking technique by mean of heating in the microwave (in 0.01M citrate buffer, pH 6.0) was performed in order to increase the antigen-antibody reaction. A standard avidin-biotin immunoperoxidase technique (Vectastain® ABC kit, Vector Laboratories, Inc., USA) was applied to detect ERα and PR. The primary antibodies used were mouse monoclonal antibody to oestrogen receptor alpha, ERα, (C-311: sc-787, Santa Cruz Biotechnology Inc., USA, dilution of 1:25) and mouse monoclonal antibody to PR (Immunotech, clone 10A9, dilution of 1:200). The incubation time for both primary antibodies was 1 h at room temperature.

Negative controls were obtained by replacing the primary antibodies to ERα or PR with normal mouse IgG (sc-2025, Santa Cruz Biotechnology Inc., USA) in a dilution of 1:200.

In the final step, a chromogen which was 3,3′-diaminobenzidine (DAB, Dakopatts AB, Älvsjö, Sweden) was added to visualize the bound enzyme (brown color). All sections were counterstained with Mayer's hematoxylin followed by mounting in glycerine-gelatin before investigation.

2.4Classification of positively stained cells

The classification of positively stained cells was done separately in each compartment of the reproductive organs. The ovary was classified to 4 compartments: surface epithelium, granulosa cells, luteal cells (if luteal tissue was presented) and thecal cells. However, as the immunostaining for ERα and PR was rare in the ovary in the present study, therefore the immunostaining score of the ovary was not studied in detail. The cervix consisted of 3 compartments: surface epithelium (SE), subepithelial layer of the stroma (STR) and muscular layer (M). The uterus was classified into 4 compartments: surface epithelium (SE), glandular epithelium (GE), subepithelial layer of the stroma (STR) and myometrium (Myo). The results of the immunostaining were evaluated semi-quantitatively by a manual scoring method. The scoring of ERα and PR positive cells was done by classification into three different levels of intensity: weak, 1; moderate, 2 and strong, 3. Since not all cells stained positively in some compartments of the tissue, the proportion of positive to negative cells was also included for these tissues. The proportions were estimated into four different levels (marked 1-4): low proportion (<30% of positive cells, 1); moderate proportion (30-60% of positive cells, 2); high proportion (>60-90% of positive cells, 3) and almost all cells positive (more than 90%, 4) (Sukjumlong et al., 2005). The total scores were calculated by the summary of intensity and proportional scores of each compartment of the reproductive tissues.

2.5 Evaluation of the results and statistical analysis

The result of immunohistochemical staining was evaluated semiquantitatively by using a staining score. Data were analysed using SAS (Statistical Analysis System, SAS Inst. V. 9.1, Cary, NC., USA.). Descriptive statistics including the mean and the standard deviations (SD) of all parameters were calculated. The total score of each compartment of the reproductive organs were compared between groups using Kruskal-Wallis's test and Wilcoxon rank sum test (NPAR1WAY procedure of SAS), and $P < 0.1$ were regarded to have statistical significance.

3. Results

3.1 Reproductive data

3.2 Culling reason and the reproductive status of the gilts

The present study revealed that of 30 culled gilts examined, the common culling reason was anoestrus. The macroscopic examination of the ovaries and the uterine horns revealed that 20 gilts were culled before puberty (66.7%), and 10 gilts were culled during the luteal phase (dioestrus, 33.3%). The pathological changes found from necropsy revealed that 10 gilts had cystic problem (33.3%), 8gilts showed edema and/or congestion of the uterine and/or cervical epithelium (26.7%) while 12 culled gilts (40%) showed no pathological lesion from macroscopic examination at all.

Immunohistochemistry

In general, positive immunostaining of both ERα and PR were observed in the nuclei of different cell types in all reproductive organs examined. In the cervix and uterus, the positive cells could be found in the surface epithelium, the glandular epithelium (only in the uterus), the stroma and the muscular layer (myometrium for the uterus). The immunostaining results were

summarized in Table 1-3 according to different reproductive status and the abnormalities found in the reproductive organs.

Regarding the different reproductive status, the results of immunohistochemistry for both ERα and PR in the cervix (Table 1.1, 1.2) were similar between prepuberty and luteal phase. In the uterus (Table 1.3, 1.4), difference was observed between prepuberty and luteal phase for ERα immunostaining in the stroma and myometrium while PR immunostaining score was similar between different stages of reproductive status.

When comparing among normal gilts and gilts with reproductive pathology (Table 2.1, 2.2), higher PR score was significantly observed in the SE and muscular layer of the cervix of normal prepubertal gilts than gilts with reproductive pathology; while ERα scores showed no significant difference though ERα score seemed to be higher in normal prepubertal gilts.

In the uterus (Table 3.1, 3.2), higher ERα scores were found in the surface epithelium, stroma, and the myometrium of normal prepubertal gilts while the lowest scores were found in gilts with pathology during luteal phase. On the other hand, higher PR scores were significantly lower in the myometrium of gilts with edema/congestion in prepuberty groups compared to the others.

For the results of immunostaining in the ovary, both ER and PR showed no difference as only a minority of positive cells was observed. The most prominent staining of positive cells was found in the germinal epithelium of the ovary and the granulosa cells and theca cells of different growing follicles, while there was no positive cell observed in the oocytes (germ cells) or in the luteal cells.

4. Discussion

From the present study, the immunohistochemical results showed that the localization of steroid receptors ERα and PR varied among different genital organs (cervix, uterus and ovary). This indicated the difference in physiological status among these different reproductive organs as described by other studies (Couse et al., 1997; Wang et al., 2000; Pfaffl et al., 2001; Okada et al., 2005). In the ovary, both ERα and PR were rarely found in all investigated culling gilts which may implied that these steroid receptors play a minor role in reproductive mechanism during these stages of the reproductive status (during prepuberty and luteal phase). However, it was shown that ER subtype beta (ERβ) were the major subtypes in the ovary which mediated the function of steroid hormones oestrogen rather than ERα which was the classical subtype (Hiroi et al., 1999; Mowa and Iwanaga, 2000; Pelletier and El-Alfy, 2000; Slomczynska and Wozniak, 2001). Moreover, it was shown that the mechanism of PR in regulating reproductive physiology involved with the presence of ERα in most of the target tissues (Sar and Welsch, 1999; Katzenellenbogen, 2000). Therefore, this may results in the low level of both ERα and PR found in the ovary of culling gilts in the present study.

Regarding the different reproductive status (prepuberty and luteal phase) of culling gilts, the present result showed no effect of reproductive status on the expression of both ERα and PR in the cervix. This demonstrates that the cervix may not be the main target of changes by the expression of steroid receptors, during cyclic and non cyclic periods as suggested by Cano et al., (1990) that the capacity of response to the steroid hormones of cervical cells is limited compared with other target tissue, such as endometrium. However, in the muscular layer of the cervix, which serve as the major compartment for cervical constriction or dilation under the influence of hormones (van Engelen et al., 2009), it was shown that higher score was observed in the prepubertal culling gilts though not significantly different. This may because the cervix needs more level of the steroid receptors before puberty in order to prepare for the incoming event which may occur during the cyclic period such as coitus or insemination. During luteal phase, the level of progesterone was high and it may result in the decrease of both ER and PR in the cervix as it was shown in other studies in human (Gorodeski et al., 1987; Kupryjanczyk, 1991) and in the ewes (Rodriguez-Pinon et al., 2008). Unfortunately that there is a limited data on the

expression of steroid receptors in the porcine cervix and thus, the normal value of steroid receptor expression during different reproductive status should be documented.

Also in the uterus, during the different reproductive status and regardless of the abnormality of the reproductive organs, the significant difference of ERα immunostaining was found only in the myometrium with the similar pattern that low expression of ERα score was found in gilts culled during luteal phase. These also supported the downregulation effect of progesterone on the presence of ERα in the myometrium as suggested in the cervix. As uterine contraction was needed in order to transport semen and sperm (Bulletti et al., 2000; de Ziegler et al., 2001; Kunz and Leyendecker, 2002; Fanchin, 2009), but later on after oestrus or during luteal phase, the uterus should undergo quiescent by lower the contractility of the myometrium and this may be mediated by the lower level of ER in the myometrium as it was demonstrated in other earlier studies (Laudanski et al., 2004).

According to the pathological/abnormality investigation in the reproductive tracts of culling gilt, it was shown that most of the gilts used in the present study showed no pathological lesions after post mortem examination (12 gilts) and the most common pathological lesions found was cystic problems (10 gilts) and the rest of culling gilts demonstrated uterine secretion and/or edema (8 gilts). In the cervix of culling gilts, the ERα scores showed no difference among gilts demonstrated reproductive pathological lesion either among normal gilts. However, significant difference was observed for PR immunostaining in the muscular layer of the cervix with higher scores in normal prepubertal gilts compared to prepubertal gilts with uterine edema/congestion problem. During the period of pregnancy, uterine quiescence is maintained by elevated progesterone acting through progesterone receptor (PR) (Brown et al., 2004; Boos et al., 2006; Mesiano and Welsh, 2007). Later on, uterine contraction at term and preterm was induced by the increased inflammatory response which involved with the impairment of PR to mediate cervical and uterine quiescence (Mendelson, 2009). Our results on the decrease of PR in the cervical muscle of the culling gilts with reproductive problems compared to normal gilts agreed with this concept that the lower expression of PR may relate to the inhibition of cervical and uterine quiescence in order to react to the inflammatory response which occurred in the gilts with reproductive problems. For the presence of PR in the surface epithelium of the cervix, similar pattern was observed that lower PR was found in the gilts with pathological lesions. This may involve with the inflammatory response within the cervical epithelium and cause the lower presence of PR as suggested for the muscular layer of the cervix.

In the uterus, the most prominent staining of ERα and PR was always observed in the myometrium compared to other compartments. For ERα, lower presence of ERα was found in culling gilts with pathological lesions during luteal phase. This finding was supported the influence of high level of progesterone during luteal phase which caused the lower presence of ERα in most compartments of the uterus. In general, the myometrium has a crucial function as it is the compartment which undergoes contraction in order to transport sperms (Kunz et al., 1997; Bulletti et al., 2000) and/or embryo if fertilization occurs (Nathanielsz et al., 1995; Bulletti and de Ziegler, 2006). However, in normal gilts, the expression of ERα was high during those periods in order to maintain the function of the myometrium (Sukjumlong et al., 2003; Sukjumlong et al., 2004b). In contrast, in abnormality gilts during luteal phase from the present study, lower presence of ERα was significantly found and this may be the cause of the impairment of reproductive activities in these gilts. In the study in human, uterine contractility participated in the emptying of the uterine content (de Ziegler et al., 2001). The changes of ERα expression may cause the inability of the uterus to clear the secretion, other waste substances or infectious agents and finally was the cause of the impairment of the uterus. On the other hand, the presence of PR in the myometrium of normal prepuberty gilts differed from infertile gilts culled before puberty but not to infertile gilts culled during luteal phase. The lower PR presence in pathological culled gilts before puberty compared to the others may due to the excessive imbalance levels of progesterone from pathological lesions and cause a lower presence of PR.

From the study in human about the inflammatory response in the uterus, it was shown that progesterone receptor has a major role in anti-inflammatory in the myometrium (Hardy et al., 2006). Therefore, the lower PR in the myometrium may involve with the inflammatory response to pathological lesions as it was shown in the culling gilts with secretion and/or edema problem. However, according to different isoforms of PR in reproductive organs, it was shown that progesterone mediated myometrial quiescence was suppressed by an increased expression of the type A PR (PR-A) (Mesiano et al., 2002). Unfortunately that the antibody to PR used in the present study could not differentiate the expression of PR-A and PR-B, so the result showed was the total score of both isoforms of PR. Hence, the high level of PR in the myometium of reproductive disturbance gilts during luteal phase may be the high level of PR-A only. Therefore, further investigation on the difference between both subtypes of PR in the myometrium is needed.

In the study of endometrial pathology in human, it was shown that there was a decrease of the percent of positive cells and of the staining intensity of both ERα and PR such as in poorly differentiated endometrial carcinomas (Ma et al., 2006; Amalinei et al., 2008), endometrial hyperplasia (Nunobiki et al., 2003) and in luteal phase failure syndrome (Savchenko et al., 1990; Abd-el-Maeboud et al., 1997). In addition, there are studies showed considerable changes in steroid receptors, ER and PR in infertile women (Thornburgh and Anderson, 1997; Bessmertnaia et al., 2008) and in polycystic ovarian syndrome (Li et al., 1998). Therefore, changes in hormonal status as well as the presence of specific receptors may attribute to the pathological status which occurred in the target organs of these steroid hormones as shown by our present results.

In the endometrial stroma, there was similar pattern of expression to the myometrium that significant lower expression of ERα in the stroma was observed in culled gilts during luteal phase. The explanation of these lower levels of ERα may due to the possible high level of progesterone during luteal phase. Moreover, as ERα-positive stroma was essential for several reproductive phenomenon occurred in the oestrogen target tissues in a paracrine manner such as proliferation of uterine epithelium, uterine secretory function (Buchanan et al., 1999). Negative expression or lower presence of ERα may cause the impairment in these regulatory mechanisms of oestrogen and should be the cause of infertility or pathological lesions found in these culled gilts. In pigs, the mechanism of dihydrotestosterone (DHT) on the antagonism of estrogenic effects in the pig uterus was demonstrated by downregulation of the ERα mainly in the endometrial stroma and the myometrium (Cardenas and Pope, 2004). In the present study, the significant lower of ERα was observed mainly in uterine stroma and the myometrium as well. Therefore, it could be explained that uterine stromal and myometrial cells were more sensitive to the changes of circulating hormones and/or other factors which may consequently cause the uterine dysfunction from alteration of these hormone receptors

For PR expression in the endometrium, the present study failed to detect the difference between normal and gilts culled during different reproductive status. As mentioned before that there are two isoforms of PR; PR-A and PR-B which were arisen from single gene. It was well documented that the levels of PR-A and PR-B are differentially regulated during the reproductive cycle and therefore, may mediate different physiological responses to progesterone. In the ovary and uterus, the studies in mice revealed that ablation of PR-A results in severe abnormalities in ovarian and uterine function leading to female infertility but not for PR-B (Conneely et al., 2003). Furthermore, there is a recent study showed that PR-A has been absent in all compartment of the uterus in anoestrous sows (Karveliene et al., 2007) .As it was shown in our results that the PR scores in this present study was the accumulation of PR-A and PR-B and therefore the difference in PR-A expression in culled gilts could not be demonstrated. On the other hand, there may be some difference in the expression of PR-A among these culled gilts, but it may also be balanced by the level of PR-B in the tissue compartments and therefore, cause the similar expression of PR in several compartments of the uterus among these culled gilts.

However, the different localization of PR-A in reproductive disturbance gilts should be further studied as it may reveal or explain the cause of pathological lesions found from this present study.

In conclusion, these results show the expression of steroid receptors, ERα and PR in culled gilts with reproductive problem. The data obtained indicate that the changes of steroid receptors in some compartments of the cervix and uterus may involve with pathological status found in these culled gilts. As the regulation of steroid hormones through the expression of their receptors differed according to specific cell types of reproductive organs, therefore, the expression of steroid receptors in both normal and reproductive failure gilts should also vary. Moreover, the expression of ERα and PR in the reproductive organs of culled gilts indicated that there are responses to the influence of these steroid hormones and therefore, playing significant roles in reproductive physiology as well as pathology.

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Table 1.1 The ERα scores of cervix in culling gilts with reproductive problem before puberty and during luteal phase

Stage	SE	Stroma	Muscular layer
Prepuberty	4.05 ± 1.76	3.25 ± 1.37	4.38 ± 1.75
Luteal	4.20 ± 1.79	3.20 ± 0.61	3.70 ± 1.70

Table 1.2 The PR scores of cervix in culling gilts with reproductive problem before puberty and during luteal phase

Stage	SE	Stroma	Muscular layer
Prepuberty	3.57 ± 2.17	2.6 ±1.56	3.72 ±2.09
Luteal	2.30 ± 1.94	2.0 ± 1.63	2.65 ± 2.20

Table 1.3 The ERα scores of uterus in culling gilts with reproductive problem before puberty and during luteal phase

Stage	SE	Stroma	GE	Myometrium
Prepuberty	2.72 ± 2.08	2.50±1.81a	3.32 ±2.43	4.35±1.24a
Luteal	1.38 ± 2.21	0.31±0.87b	1.84±1.82	1.37±1.89b

Table 1.4 The PR scores of the uterus in culling gilts with reproductive problem before puberty and during luteal phase

Stage	SE	Stroma	GE	Myometrium
Prepuberty	2.07 ± 2.13	1.97±1.82	2.07 ±2.51	3.92±1.59
Luteal	1.53 ± 1.81	2.56±2.12	2.00±2.22	4.50±0.83

Different letters within the same comlumn represent significantly different (table 1.3) ($P<0.05$). SE= surface epithelium, Stroma =cervical stroma (table 1.1, 1.2) or uterine stroma (table 1.3, 1.4); GE= glandular epithelium (table 1.3, 1.4)

Table 2.1
he immunohistochemical score of ERα in each compartment of the cervix of culling gilts with different pathological finding

Groups of gilts	ERα-SE	ERα-STR	ERα-M
Prepuberty-no abnormality	4.54±1.58	3.50+1.38	4.83±1.57
Prepuberty-cystic problem	2.87±2.25	2.75±1.89	3.50±2.61
Prepuberty-edema/congestion	3.75±1.55	3.00±0.81	3.88±1.81
Luteal-cystic problem	4.42±1.28	3.16±1.16	4.16±0.98
Luteal-edema/congestrion	3.87±2.59	3.25±2.36	3.0±2.44
Overall significant	NS	NS	NS

Different letters within the same column represent significant differences. NS= not significant; SE= surface epithelium, STR= cervical stroma, M=muscular layer of the cervix

Table 2.2
The immunohistochemical score of PR in each compartment of the cervix of culling gilts with different pathological finding

Groups of gilts	PR-SE	PR-STR	PR-M
Prepuberty-normal	4.5±1.29 a	3.00±1.47	4.50±1.66 a
Prepuberty-cystic problem	2.62±3.03 ab	2.50±2.08	3.63±2.75 ab
Prepuberty-edema/congestion	1.75±2.30 b	1.5±1.0	1.5±1.00 b
Luteal-cystic problem	2.33±1.36 ab	2.17±1.16	2.83±1.72 ab
Luteal-edema/congestion	2.25±2.87 ab	1.75±2.36	2.38±3.09 ab
Overall significant	P<0.1	NS	P <0.05

Different letters within the same column represent significant differences. NS= not significant; SE= surface epithelium, STR= cervical stroma, M=muscular layer of the cervix

Table 3.1
The immunohistochemical score of ERα in each compartment of the uterus of culling gilts with different pathological finding

Groups of gilts	ERα-SE	ERα-STR	ERα-GE	ERα-Myo
Prepuberty-normal	3.0±1.89 a	2.90+1.80 a	4.09±2.38	4.55±1.19 a
Prepuberty-cystic problem	2.60±2.79 ab	2.20±2.28 a	2.60±2.77	4.70±0.67 a
Prepuberty-edema/congestion	2.13±2.09ab	1.75±1.25a	2.13±1.84	3.38±1.70 ab
Luteal-cystic problem	0.87±1.80 b	0±0 b	1.81±2.10	1.37±1.92 b
Luteal-edema/congestion	1.87±2.59ab	0.62±1.18b	1.88±1.64	1.37±1.99 b
Overall significant	P<0.05	P<0.01	NS	P<0.01

Different letters within the same column represents significant differences. NS= not significant; SE= surface epithelium, STR= uterine stroma, GE= glandular epithelium, Myo=myometrium

Table 3.2
The immunohistochemical score of PR in each compartment of the uterus of culling gilts with different pathological finding

Groups of gilts	PR-SE	PR-STR	PR-GE	PR-Myo
Prepuberty-normal	2.59±2.05	2.45+1.75 a	2.27±2.70	4.36±1.16 a
Prepuberty-cystic problem	2.30±2.63	2.10±2.13ab	2.20±3.01	3.70±2.58 ab
Prepuberty-edema/congestion	0.37±0.75	0.50±1.00 b	1.38±1.70	3.00±0.81 b
Luteal-cystic problem	1.94±1.69	2.81±1.99	2.13±2.47	4.50±0.96 a
Luteal-edema/congestion	1.13±1.94	2.31±2.34	1.88±2.10	4.50±0.75 a
Overall significant	NS	NS	NS	P<0.1

Different letters within the same column represents significant differences. NS= not significant; SE= surface epithelium, STR= uterine stroma, GE= glandular epithelium, Myo=myometrium

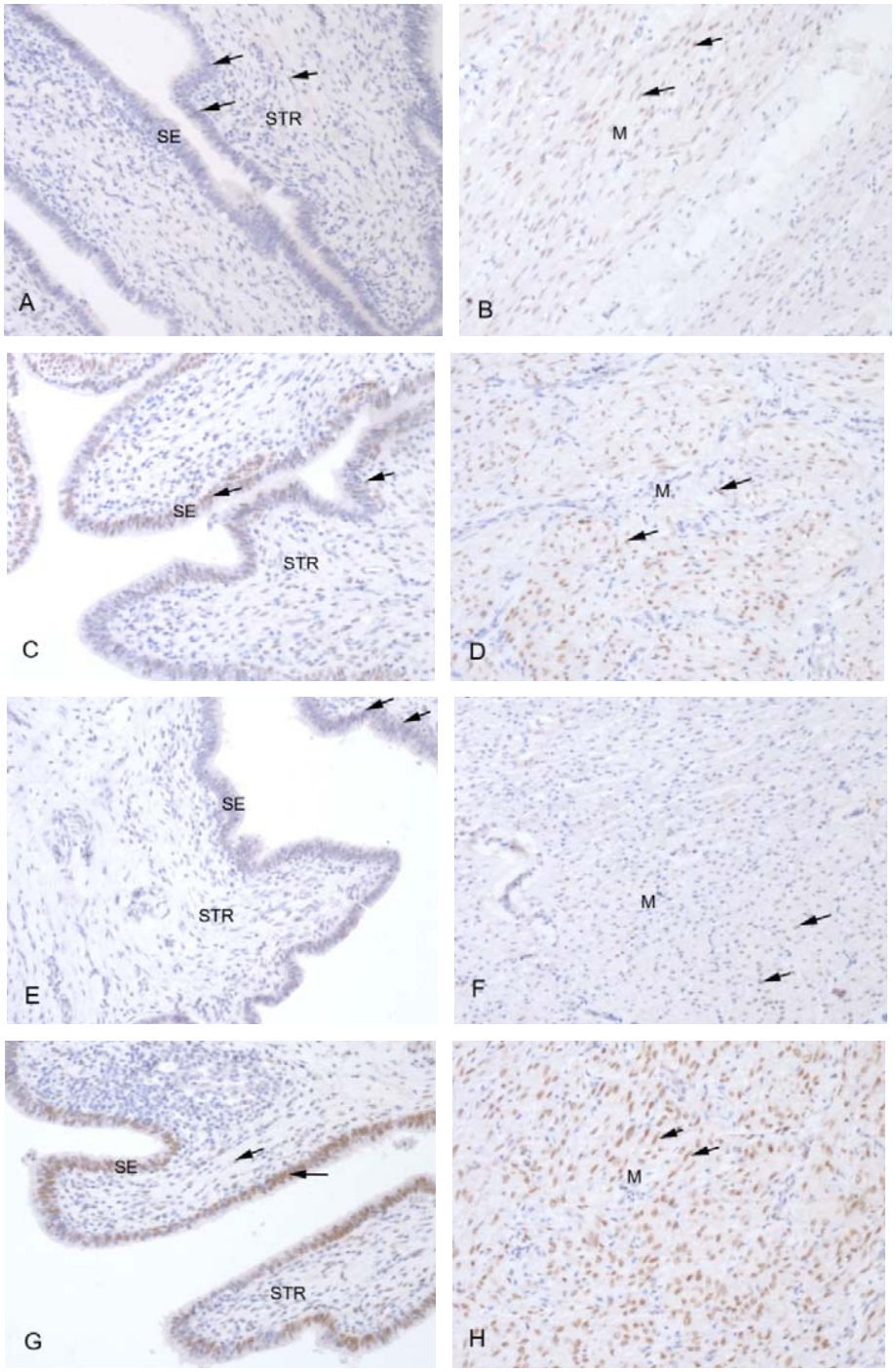

Figure 1 Immunohistochemical staining of ERα (A-D) and PR (E-H) in the cervix of culling gilts. A-B and E-F demonstrated low score of ERα and PR immunostaining respectively, C-D and G-H demonstrated high score of ERα and PR immunostaining respectively. SE = surface epithelium, STR = stroma, M = muscular layer of cervix, arrows represent positive cells.

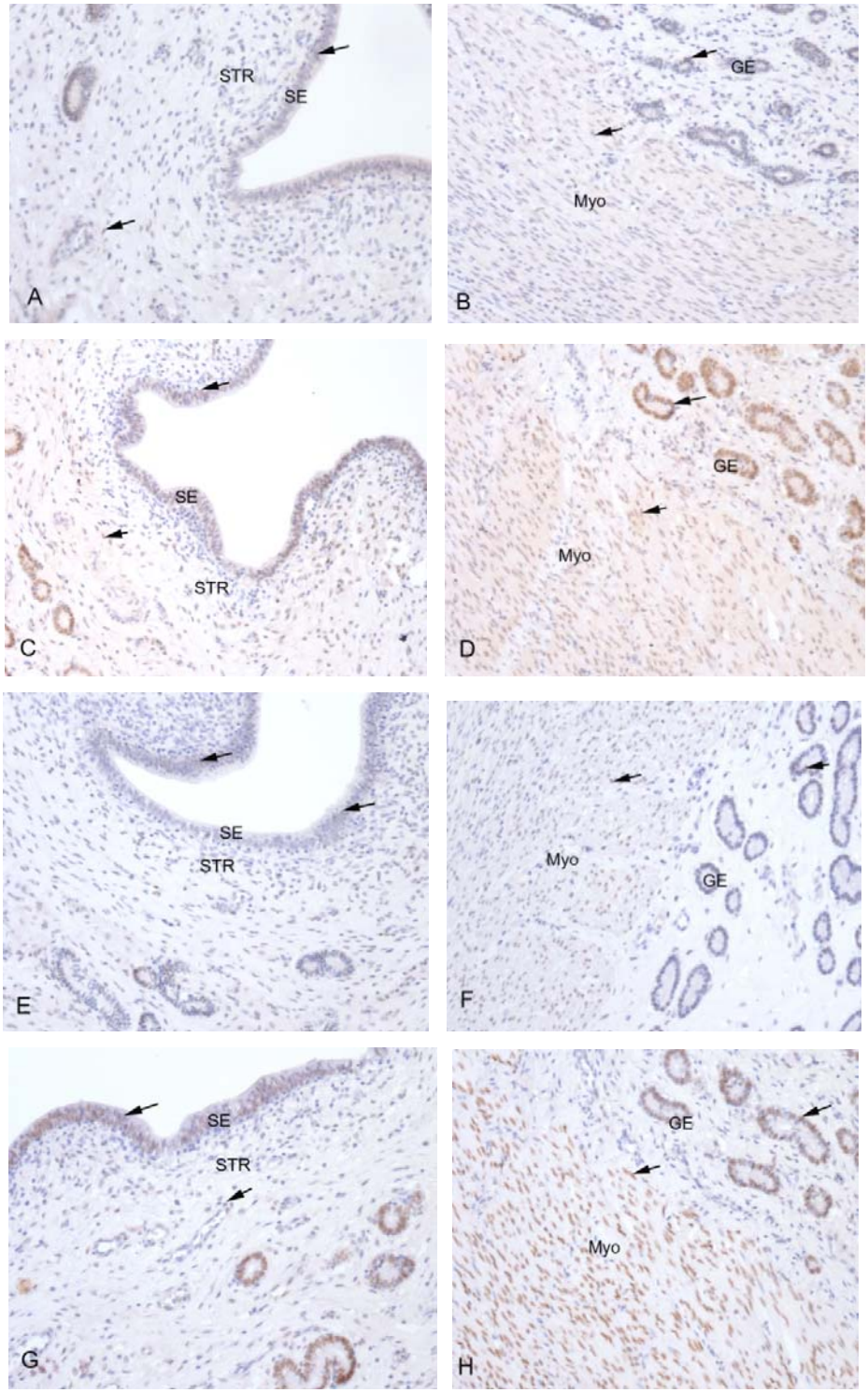

Figure 2 Immunohistochemical staining of ERα (A-D) and PR (E-H) in the uterus of culling gilts. A-B and E-F demonstrated low score of ERα and PR immunostaining respectively, C-D and G-H demonstrated high score of ERα and PR immunostaining respectively. SE = surface epithelium, STR = stroma, GE= glandular epithelium, Myo = myometrium, arrows represent positive cells.

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Output ที่ได้จากโครงการวิจัยที่ได้รับทุนจาก สกว.

เรื่องสั้นเพื่อส่งตีพิมพ์ในการประชุมวิชาการระดับนานาชาติ 2 เรื่อง และ ผลงานวิจัยเพื่อส่งตีพิมพ์ในวารสารวิชาการระดับนานาชาติ 1 เรื่อง

1. Sayamon Srisuwatanasagul, Padet Tummaruk, Annop Kunavongkrit. 2009. The study of oestrogen receptor alpha and progesterone receptor in the cervix of culling gilts with reproductive disturbance. Proc. The 2nd Federation of Asian Small Animal Veterinary Associations Congress 2009, In conjunction with The 35th Veterinary Medicine and Livestock Development Annual Conference.
2. Sayamon Srisuwatanasagul, Padet Tummaruk, Annop Kunavongkrit. The expression of steroid receptor ERα and PR in the uterus of prepubertal culling gilts Proc. The 2nd Federation of Asian Small Animal Veterinary Associations Congress 2009, In conjunction with The 35th Veterinary Medicine and Livestock Development Annual Conference.
3. Sayamon Srisuwatanasagul, Padet Tummaruk, Annop Kunavongkrit. The study of oestrogen receptor alpha and progesterone receptor in the reproductive organs of culling gilts with reproductive disturbance. (Manuscript)

การนำผลงานวิจัยไปใช้ประโยชน์

เชิงพาณิชย์ การตรวจหาตัวรับฮอร์โมนในสุกรที่มีความผิดปกติเหล่านี้สามารถใช้เป็นข้อมูลสำหรับสัตวแพทย์และเกษตรกร ในการวิเคราะห์ปัญหาทางระบบสืบพันธุ์ของสุกรสาวทดแทนในฟาร์มสุกรเพื่อหามาตราการในการแก้ไข เช่นการทดลองใช้ฮอร์โมนทดแทน เป็นต้น

เชิงนโยบาย เพื่อเป็นแนวทางในการจัดการสุกรสาวที่เหมาะสม โดยการเพิ่มมาตรการการกระตุ้นการเป็นสัดหรือความแม่นยำในการตรวจการเป็นสัดในสุกรสาวก่อนใช้งาน รวมทั้งเพื่อเป็นแนวทางในการเตรียมสภาวะของระบบสืบพันธุ์ให้เหมาะสมต่อการผสม เป็นต้น

เชิงสาธารณะ การนำผลการวิจัยไปขยายผลในศาสตร์ที่มีความใกล้เคียงกัน เช่น พยาธิ-วิทยา หรือวิทยาการสืบพันธุ์สุกร เป็นต้น

เชิงวิชาการ ทำให้มีแนวทางการทำวิจัยในแนวลึกต่อเนื่อง และเพื่อเกิดความร่วมมือในด้านการวิจัยในหลาย ๆ ด้าน เช่นความร่วมมือด้านพยาธิวิทยาและเพื่อประโยชน์แก่วิชาชีพสัตวแพทย์

3. อื่น ๆ--