Original Article

Expression of Oestrogen Receptor Alpha and Progesterone Receptor in Sow Oviducts after Different Artificial Insemination

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Abstract

In pig production, different insemination methods which allow deposition of sperm in the uterine body (intrauterine insemination, IUI) and uterine horn (deep intrauterine insemination) have been applied in order to reduce number of spermatozoa. Earlier studies reported that steroid hormones influenced the transportation of spermatozoa, ovum and embryos in the sow reproductive tract which was related to the presence of their specific receptors; oestrogen receptor alpha (ERα) and progesterone receptor (PR). Therefore, the present study aims to evaluate the immunolocalization of ERα and PR in different parts of sow oviducts, after different artificial insemination methods. Twelve sows were divided into 3 groups according to the different insemination methods, which were AI (n = 3), IUI (n = 4) and DIUI (n = 4). Percentage of ERα and PR immunostaining was evaluated by manual scoring and image analysis system. Results showed significantly higher percentage of positive staining in AI group compared with IUI and DIUI groups in the oviduct for ERα and PR. It has been demonstrated that oestrogen (E_2) in boar semen can up-regulate steroid receptors in the pig reproductive tract. The small volume of semen used for IUI and DIUI groups might also influence the lower expression of these steroid receptors due to the lower amount of E2. In conclusion, the different insemination methods regarding the volume of semen can have the effects on the expression of steroid receptors in the sow oviduct.

Keywords: artificial insemination, deep intrauterine insemination, intrauterine insemination, oestrogen receptor alpha, progesterone receptor

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บทคัดย่อ

การแสดงออกของตัวรับฮอร์โมนเอสโตรเจนชนิดแอลฟ่าและตัวรับฮอร์โมนโปรเจสเตอโรนในท่อนำไข่ของสุกรนางหลังจากการผสมเทียมด้วยวิธีต่างกัน

ศยามณ ศรีสุวัฒนาสกุล [1] เผด็จ ธรรมรักษ์ [2] อรรณพ คุณาวงษ์กฤต [2]*

ในอุตสาหกรรมการผลิตสุกรได้มีการนำเทคนิคต่าง ๆ ของการผสมเทียมมาประยุกต์ใช้ โดยทำการปล่อยน้ำเชื้อที่ตำแหน่งมดลูก (การผสมเทียมแบบปล่อยน้ำเชื้อที่มดลูก, IUI) และที่ปีกมดลูก (การผสมเทียมแบบปล่อยน้ำเชื้อที่ปีกมดลูก, DIUI) เพื่อลดความเข้มข้นของจำนวนอสุจิในน้ำเชื้อลง จากการศึกษาก่อนหน้านี้พบว่าสเตียรอยด์ฮอร์โมนมีความเกี่ยวข้องกับการขนส่งตัวอสุจิ เซลล์ไข่ และตัวอ่อนในท่อทางเดินสืบพันธุ์ของสุกรเพศเมียโดยมีความสัมพันธ์กับการแสดงออกของตัวรับสเตียรอยด์ฮอร์โมนที่จำเพาะคือ ตัวรับฮอร์โมนเอสโตรเจนชนิดแอลฟ่า และตัวรับฮอร์โมนโปรเจสเตอโรน การทดลองในครั้งนี้มีจุดประสงค์เพื่อศึกษาการแสดงออกของตัวรับฮอร์โมนเอสโตรเจนชนิดแอลฟ่าและตัวรับฮอร์โมนโปรเจสเตอโรนในส่วนต่าง ๆ ของท่อนำไข่ของสุกรนาง โดยใช้สุกรนางจำนวนทั้งสิ้น 12 ตัวแบ่งเป็น 3 กลุ่มตามวิธีการผสมเทียมได้แก่ การผสมเทียมแบบดั้งเดิม การผสมเทียมแบบปล่อยน้ำเชื้อที่มดลูก และการผสมเทียมแบบปล่อยน้ำเชื้อที่ปีกมดลูก โดยผลการศึกษาจะถูกวิเคราะห์สองวิธี คือ วิธีการให้คะแนนจากภาพ และการใช้โปรแกรมวิเคราะห์ภาพ ผลการศึกษาพบว่าการแสดงออกของตัวรับฮอร์โมนเอสโตรเจนชนิดแอลฟ่าและตัวรับฮอร์โมนโปรเจสเตอโรนในท่อนำไข่สุกรนางมีระดับสูงในกลุ่มที่ผสมเทียมแบบดั้งเดิมเปรียบเทียบกับกลุ่มที่ผสมเทียมแบบปล่อยน้ำเชื้อที่มดลูกและปีกมดลูก จากการศึกษาก่อนหน้านี้พบว่าในน้ำเชื้อสุกรนั้นมีระดับของฮอร์โมนเอสโตรเจนในระดับสูงทำให้สามารถกระตุ้นให้เกิดการแสดงออกของตัวรับฮอร์โมนที่เพิ่มขึ้นได้ในอวัยวะสืบพันธุ์ของสุกร ดังนั้นการผสมเทียมด้วยวิธีปล่อยน้ำเชื้อที่ตำแหน่งมดลูกและปีกมดลูกซึ่งใช้ปริมาณของน้ำเชื้อและตัวอสุจิลดลงอย่างมากอาจส่งผลให้การแสดงออกของตัวรับฮอร์โมนน้อยกว่าในกลุ่มที่ผสมเทียมแบบดั้งเดิม จากผลการศึกษาครั้งนี้สรุปได้ว่าการผสมเทียมด้วยวิธีต่าง ๆ มีอิทธิพลต่อการแสดงออกของตัวรับสเตียรอยด์ฮอร์โมนในระบบทางเดินสืบพันธุ์ของสุกรนาง

คำสำคัญ: การผสมเทียมแบบดั้งเดิม การผสมเทียมแบบปล่อยน้ำเชื้อที่ปีกมดลูก การผสมเทียมแบบปล่อยน้ำเชื้อที่มดลูก ตัวรับฮอร์โมนเอสโตรเจนชนิดแอลฟ่า ตัวรับฮอร์โมนโปรเจสเตอโรน

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Introduction

In Thailand, artificial insemination (AI) has an important role in swine industry in order to increase the efficiency in swine production. Regarding conventional artificial insemination, diluted fresh semen was used and released at the cervix of female pigs. This can reduce the numbers of spermatozoa to 5-10 folds compared to natural mating. However, Sumransap et al. (2007) found that this technique caused a loss of more than 90% of spermatozoa before they could reach the fertilization site which was caused by a drawback of the semen (Steverink et al., 1998). In order to overcome this problem, a newer technique of artificial insemination was introduced. It is the insertion of insemination cohet into the female reproductive tracts and the release of semen in the uterus, which is called "intrauterine insemination" (IUI), and deeper into the uterine horn, which is called "deep intrauterine insemination" (DIUI) without any surgical treatment. An early study on the IUI technique by Watson and Behan (2002) showed that the number of spermatozoa used was only $1x10^9$ per dose which did not affect the number of fertilization rate. In addition, a study by Martinez et al. (2001; 2002) revealed that the DIUI technique, which released the semen at the uterine horn, could reduce the concentration of the semen to 60 folds compared to convention AI. In Thailand, the number of 150 million spermatozoa was used for DIUI technique which resulted in the number of fertilization rate in 5 from 8 gilts. Moreover, the embryos were also found in both sides of uterine horn with the average of 11.4 embryos per gilts (Tummaruk et al., 2007). This certifies that these new methods of artificial insemination may be able to use with freezing semen, sex determination semen and may also be able to develop to use for embryonic transfer in the future.

The mechanism of sperm transportation in the female reproductive tracts is complicated and regulated by several biological factors both from the female as well as the quality and concentration of semen (Rodriguez-Martinez et al., 2005). After insemination, spermatozoa will be transported through the reproductive tract to sperm reservoir. Studies revealed that ovulation could affect sperm distribution and transportation, i.e. sperm distribution from sperm reservoir, by the changes in hormonal levels during ovulation (Hunter, 2008). In addition, the contraction of female reproductive tract was also needed in order to transport sperm to sperm reservoir (Langendijk et al., 2002[a]). Moreover, the contraction of the oviduct took part in the transportation of ovum to the fertilization site (Orihuela et al., 2001).

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, 1991; O'Malley et al., 1991; Yamashita, 1998), therefore the presence of steroid receptors is as important as the levels of steroid hormones as they involve in the effective functions of reproductive control. Several studies reported 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, oviduct 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 sows demonstrated the high presence of steroid receptors in the uteri, although the level of steroid hormones, oestradiol 17-β and progesterone, were low (Sukjumlong et al., 2004). Moreover, the presence of ER in the gene level was involved in 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, there are no data of these receptors localization in the different parts of the sow oviduct after different artificial insemination methods. Therefore, the present study aims to investigate the presence of steroid receptors, oestrogen receptor subtype alpha (ERα) and progesterone receptor (PR) in the sow oviduct after different artificial insemination methods.

Materials and Methods

Animals: Twelve crossbred Landrace x Yorkshire multiparous sows were purchased from a commercial swine herd and were brought to the department of Obstetrics, Gynaecology and Reproduction, Nakhon Pathom province, Thailand on the day of weaning. The sows were kept in individual pen and were fed twice a day (approximately 4.0-5.0 kg per day) on a commercial feed (Starfeed176® BP Feed Co Ltd, Saraburi, Thailand) containing 15.0% protein, 2.0% fat and 10.0% fiber. Water was provided ad libitum via water nipples. The sows were carefully detected for the onset of standing estrus twice a day (am/pm) after weaning. The proposal of the present study was approved by the Institutional Animal Care and Use Committee (IACUC) (Approval No. 1031018), Chulalongkorn University.

Detection of estrus and ovulation: Estrus detection was initially performed on the day after weaning (Day 1), by allowing the sows to have nose-to-nose contact with a mature boar and applying the back pressure test. Sows with a standing reflex were considered in estrus. The onset of estrus was defined as the first time the sow showed standing reflex minus 6 hours. At standing estrus, hCG (Chorulon®, Intervet Ltd, Boxmeer, The Netherlands) 750 IU was administrated intramuscularly to the sows in order to induce ovulation (Wongkaweewit et al., 2012). The time of ovulation was determined by monitoring an appearance of the follicles every 8 h using transrectal realtime B-mode ultrasonography adjusted to a 5-MHz linear transducer (Honda Electronics Co, Ltd, Tokyo, Japan). The ovulation time was defined as 4 hours before the first time when no follicle was visible.

Artificial insemination: All sows were divided into 3 groups according to the insemination methods, which were conventional artificial insemination (AI, n = 4), Intrauterine insemination (IUI, n = 4) and Deep intrauterine insemination (DIUI, n = 4). The sows were inseminated with a single dose of diluted semen during the second oestrus after weaning. The time of ovulation during the first oestrus was used to determine the timing of insemination, which was carried out at 6-8 hours prior to the expected time of ovulation. Semen with a motility of > 70%, a concentration of > $150x10^6$ spermatozoa/ml and with normal sperm > 85% was extended with Beltsville thawing solution (Pursel and Johnson, 1976). The sperm dose contained 3000x106 spermatozoa in 100 ml for AI, $1000x10^6$ spermatozoa in 50 ml for IUI and $150x10^6$ spermatozoa in 5 ml for DIUI. Both the IUI and the DIUI methods were adapted from Sumransap et al. (2007). Briefly, after cleaning the perineal area of the sows, a commercial AI catheter (Goldenpig®, Minitube, Tiefenbach, Germany) was inserted through the vagina into the cervix, where the diluted semen was deposited in AI group. In IUI group, the IUI device (Magaplus®, Magapor, Ejea de los Caballeros, Spain) was inserted through the vagina into the cervix. Thereafter, the inner tube extended about 20 cm beyond the tip of the outer catheter and resided in the uterine body or the posterior uterine horn in order to deposit the diluted semen. In DIUI group, the long flexible catheter (1.8 m) was inserted through the conventional AI catheter. This long catheter was moved forward and deposited diluted semen in the uterine body along one uterine horn (unknown side) for its full length. The diluted fresh semen with $150x10^6$ motile sperm in 5.0 ml was deposited in the proximal third of one side of the uterine horn. Subsequently, a warm BTS, 2.5 ml in volume, was used to flush the semen into the uterine

horn after insemination.

Tissue collection: Approximately 12 hours after insemination, all sows were slaughtered and the reproductive tracts were removed. Post-mortem examination was performed on each part of the reproductive organs. Three different parts of the oviduct, which were isthmus, ampulla and infundibulum, were collected and preserved separately in 4% paraformaldehyde for 24-36 hours. Thereafter, they were routinely histological processed and 4 µm thick sections were cut from each block and mounted on Polysine™ slides (Menzel-Glazer, Germany). One section from each block was stained with Hematoxylin-Eosin for histological investigation and the other sections were used for immunohistochemistry.

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 at 750 watt (in 0.01M citrate buffer, pH 6.0) was performed, followed by endogenous peroxidase blocking in 3% hydrogen peroxide in methanol as well as a background blocking with normal horse serum. 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 (clone 1D5, Dako Denmark, dilution of 1: 25) and mouse monoclonal antibody to PR (clone 10A9, Immunotech, dilution of 1: 200). The PR primary antibody can recognize both PR-A and PR-B, so the results shown in the present study was the accumulation of PR-A and PR-B. The incubation time for both primary antibodies was 1 hour at room temperature. The sow uterus at oestrus which was known to contain both ERα and PR was served as positive controls, while the negative controls were obtained by replacing the primary antibodies to ER 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.

Evaluation of the results and statistical analysis: The oviduct was divided into 3 parts which were isthmus, ampulla and infundibulum. The results of the immunostaining were evaluated semi-quantitatively by a manual scoring method as well as image analysis by computer software (Image-pro plus version 6.0, Media Cybernetics, Inc, MD, USA).

The manual 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 tissues, 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 oviductal tissues which were surface epithelium (SE), stromal layer (STR) and muscular layer (M). Since the muscular layer of the infundibulum was not accessible, therefore the manual scoring was done only in the SE and STR in this part of the oviduct.

Quantification of the immunostaining was performed on five randomly selected fields which comprised all compartments of the oviduct. The results from image analysis are presented as mean percentage of total area of positive staining per total area of cell nuclei.

The data obtained from both manual scoring and image analysis 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 or percentage of positive staining from each compartment of the oviduct were compared between groups using Kruskal-Wallis's test and Wilcoxon rank sum test (NPAR1WAY procedure of SAS), and $p < 0.05$ were regarded to have statistical significance.

Results

Immunohistochemistry

In general, positive immunostaining of both ERα and PR were observed in the nuclei of oviductal epithelium, stroma and muscular layer, but with different proportions and intensities (Figs 1 and 2). Furthermore, cytoplasmic staining for ERα was observed periodically whereas it was not found for PR immunostaining. The immunostaining results were summarized according to the different artificial insemination methods in Tables 1.1 and 2.1 for image analysis and in Tables 1.2 and 2.2 for manual scoring.

By using image analysis, higher percentage of both ERα and PR was found in the AI groups compared to the other insemination methods. Regarding different oviductal parts, significantly higher percentage of ERα was observed in the AI group for the ampulla and the infundibulum part

Table 1.1 Percentage of ERα positive staining from image analysis in different parts of the oviduct (mean±SD)

Insemination	AI	IUI	DIUI
Isthmus	37.78 ± 9.4	31.08 ± 16.54	33.89 ± 18.5
Ampulla	31.95 ± 13.82 [a]	37.66 ± 23.58 [a]	15.36 ± 10.63 [b]
Infundibulum	45.33 ± 26.01[a]	36.15 ± 21.89 [a]	25.19 ± 19.26 [b]

while higher percentage of PR was found in the isthmus and the infundibulum part of AI group ($p < 0.05$) (Tables 1.1 and 2.1.). For the manual scoring results, similar pattern was observed that high immunostaining score was found in the AI groups compared to the other insemination groups for both ER and PR (Tables 1.2 and 2.2). In addition, significant difference was observed mainly in the SE of the oviductal tissue from the manual scoring method. Moreover, for PR immunostaining, significant difference was found only in the SE of the infundibulum part by manual scoring (Tables 2.2).

Table 1.2 ERα positive staining score by manual scoring in different parts of the oviduct (mean±SD)

ERα immunostaining	AI	IUI	DIUI
Isthmus-SE	4.0 ± 2.0	2.16 ± 0.28	2.83 ± 0.76
Isthmus-STR	3.16 ± 1.04	2.16 ± 0.28	2.6 ± 1.15
Isthmus-M	5.5 ± 0.4	3.83 ± 1.04	4.16 ± 0.28
Ampulla-SE	4.16 ± 2.0[a]	2.16 ± 0.28 [b]	2.16 ± 0.28 [b]
Ampulla-STR	3.33 ± 0.76	1.83 ± 0.28	2.67 ± 1.15
Ampulla-M	3.33 ± 0.5	3.5 ± 1.3	4.67 ± 0.5
Infundibulum-SE	3.6 ± 2.08[a]	1.34 ±1.15 [b]	0.67 ± 1.15 [b]
Infundibulum-STR	3.00 ± 1.0	1.83 ± 1.15	2.33 ± 0.5
Infundibulum-M	NA	NA	NA

AI: conventional artificial insemination, IUI: intrauterine insemination, DIUI: deep intrauterine insemination, SE: surface epithelium of the oviduct, STR: stromal layer of the oviduct, M: muscular layer of the oviduct
Different letters within the same row represent significant difference, NA: not accessible.

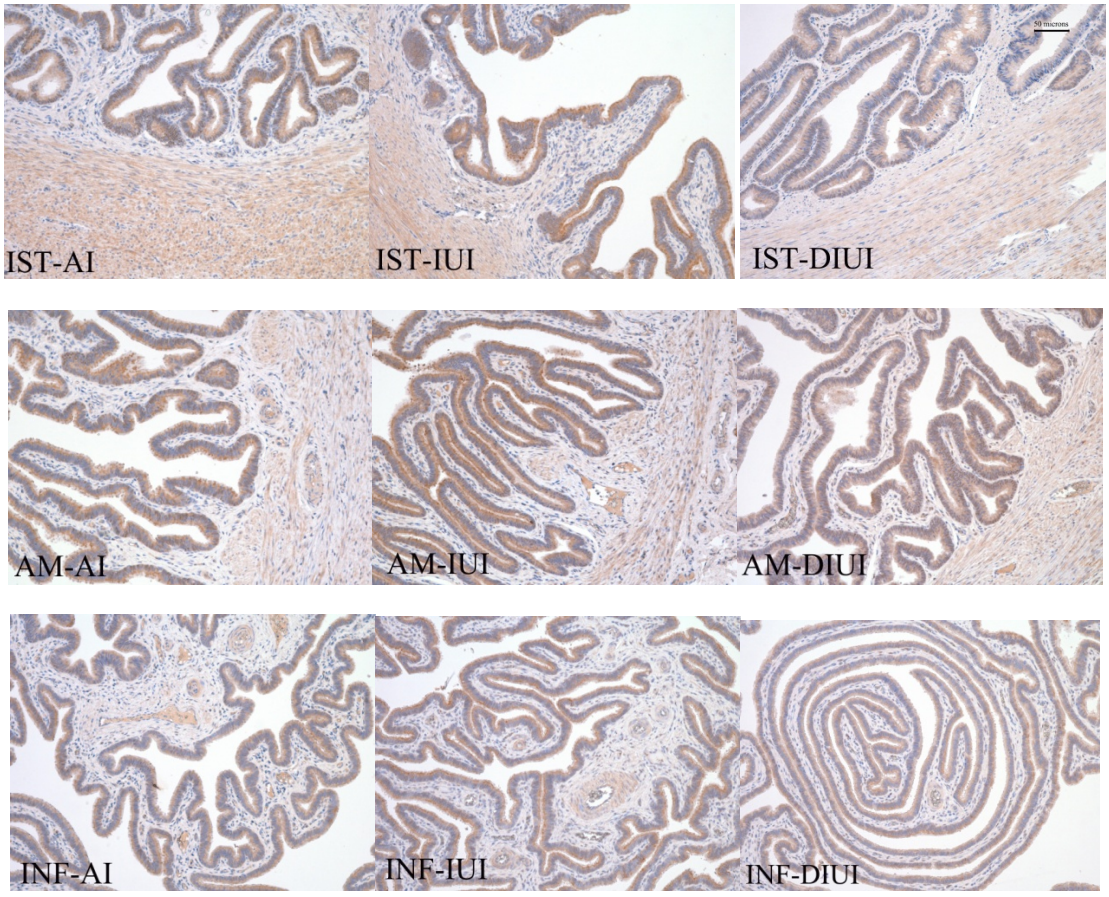

Figure 1 ERα immunostaining in different parts of the sow oviduct after different insemination methods. IST-AI: isthmus after conventional artificial insemination, IST-IUI: isthmus after intrauterine insemination, IST-DIUI: isthmus after deep intrauterine insemination, AM-AI: ampulla after conventional artificial insemination, AM-IUI: ampulla after intrauterine insemination, AM-DIUI: ampulla after deep intrauterine insemination, INF-AI: infundibulum after conventional artificial insemination, INF- IUI: infundibulum after intrauterine insemination, INF-DIUI: infundibulum after deep intrauterine insemination

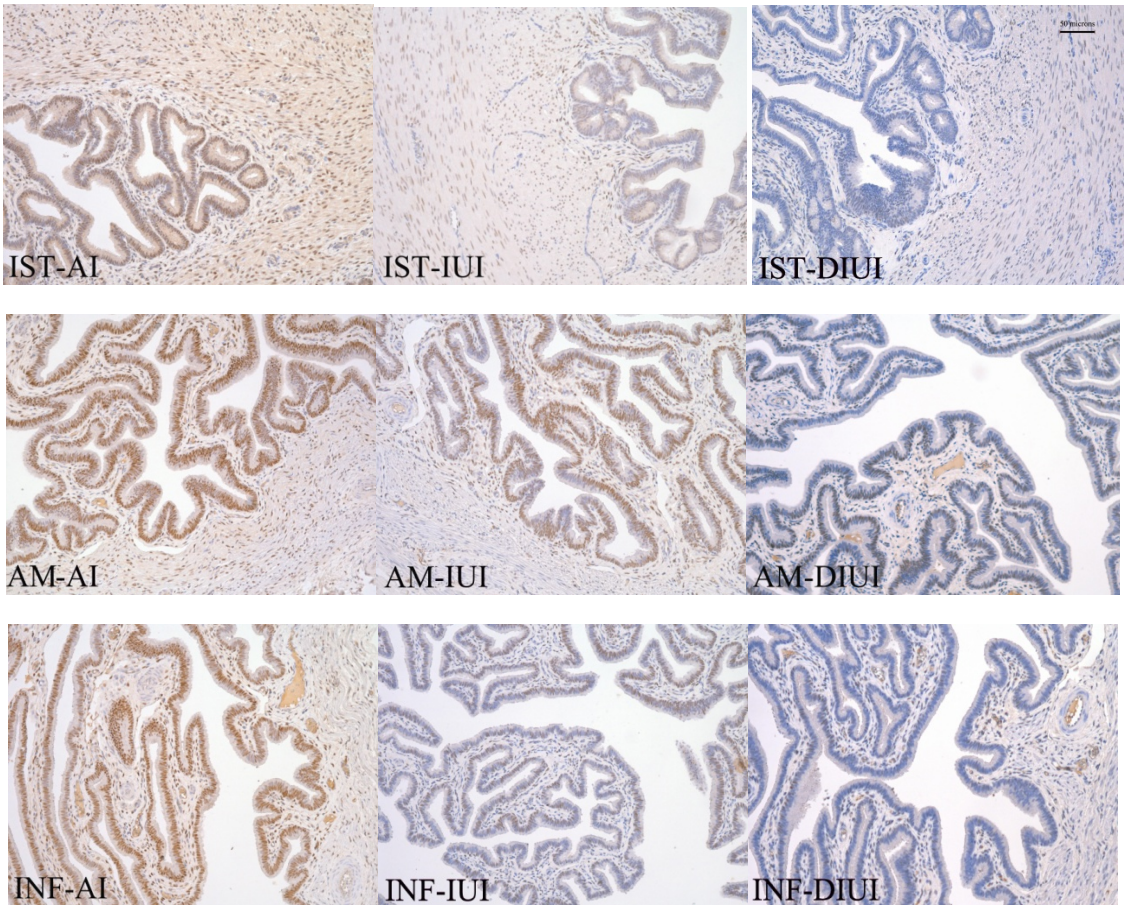

Figure 2 PR immunostaining in different parts of the sow oviduct after different insemination methods. IST-AI: isthmus after conventional artificial insemination, IST-IUI: isthmus after intrauterine insemination, IST-DIUI: isthmus after deep intrauterine insemination, AM-AI: ampulla after conventional artificial insemination, AM-IUI: ampulla after intrauterine insemination, AM-DIUI: ampulla after deep intrauterine insemination, INF-AI: infundibulum after conventional artificial insemination, INF- IUI: infundibulum after intrauterine insemination and INF-DIUI: infundibulum after deep intrauterine insemination

Table 2.1 Percentage of PR positive staining from image analysis software in different parts of the oviduct (mean±SD)

Insemination	AI	IUI	DIUI
Isthmus	51.97 ± 11.95[a]	27.89 ± 26.07[b]	30.27 ±16.42[b]
Ampulla	41.72 ± 8.6	33.07 ± 25.01	46.63 ± 15.23
Infundibulum	43.59 ± 24.12[a]	23.45 ± 18.65[b]	26.61 ± 24.08[b]

Table 2.2 PR positive staining score by manual scoring in different parts of the oviduct (mean±SD)

PR immunostaining	AI	IUI	DIUI
Isthmus-SE	6.67 ± 0.28	4.5 ± 0.86	6.3 ± 0.29
Isthmus-STR	5.5 ± 0.5	3.83 ± 0.76	5.83 ± 0.58
Isthmus-M	5.8 ± 0.75	3.1 ± 1.32	4.16 ± 0.2
Ampulla-SE	5.33 ± 0.28	4.67 ± 0.76	6.16 ± 0.28
Ampulla-STR	4.33 ±0.29	3.5 ± 0.86	5.16 ± 0.28
Ampulla-M	5.3 ± 0.57	3.5 ± 1.29	4.6 ± 0.50
Infundibulum-SE	4.83 ± 0.57[a]	2.0 ±0[b]	2.5 ±1.0[b]
Infundibulum-STR	3.83 ± 0.76	2.33 ± 0.57	4.66 ± 0.28
Infundibulum-M	NA	NA	NA

AI: conventional artificial insemination, IUI: intrauterine insemination, DIUI: deep intrauterine insemination, SE: surface epithelium of the oviduct, STR: stromal layer of the oviduct, M: muscular layer of the oviduct. Different letters within the same row represent significant difference, NA: not accessible.

Discussion

Regarding the different insemination methods, the present results showed higher immunostaining in the AI group compared to the others. This demonstrates that different insemination methods may have the influence on the expression of these steroid receptors. It has been demonstrated that oestrogen (E_2) in boar semen can up-regulate steroid receptors in the pig reproductive organs (Langendijk et al., 2002[a]; Langendijk et al., 2002[b]). The small volume of semen used for IUI and DIUI groups might also influence the lower expression of these steroid receptors due to the lower amount of E_2.

In the oviducts, the present study demonstrated the higher PR staining in the oviductal part of uterotubule junction (UTJ) of the AI sows comparing to the IUI and DIUI sows (Tummaruk et al., 2010). DIUI may influence the lower expression of PR in the UTJ from the lower number of spermatozoa in the UTJ which serves as sperm reservoir (Tummaruk and Tienthai, 2008). Similar to our present results, the lower PR was found in the isthmus part of the oviduct, which is located near the UTJ, as well as in the infundibulum part. As progesterone can influence the transportation of spermatozoa both before and after fertilization

(Mburu et al., 1996), this mechanism may mediate through the expression of PR which showed lower expression when the number of sperm was lower by IUI and DIUI techniques.

In addition to sperm transportation, oviductal cilia are believed to have a critical role in ovum transport from the oviduct to the uterus in cyclic and pregnant rats (Halbert et al., 1989). Oestrogen may have roles in ovum transport by regulating oviductal ciliogenesis in rats indirectly via ERα in the epithelium (Okada et al., 2003). Moreover, a single injection of 17β-estradiol (E_2) on day 1 of the reproductive cycle or pregnancy can shorten oviductal transportation of eggs (Croxatto, 2002) and this mechanism was believed to mediate via ERα in the oviductal epithelium as well (Croxatto, 2002; Orihuela et al., 2003). From the results of the present study, we found positive ERα in all parts of the oviducts (isthmus, ampulla and infundibulum) which may involve in the mechanism of ciliogenesis regulation via ERα. However, the oviductal epithelium consisted of two cell types which were ciliated cells and secretory cells, thus further study should be considered regarding the differentiation between these two cell types. Comparing different insemination methods in regards to gamete transportation, the higher ERα staining found in the AI group may indicate that more successful fertilization after conventional artificial insemination could result from the better transportation of ovum which may be regulated by ERα in the oviductal epithelial cells as well.

In general, there are two isoforms of PR; PR-A and PR-B which arise from single gene. It was well documented that the levels of PR-A and PR-B are differently 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 resulted in severe abnormalities in ovarian and uterine function leading to female infertility but not for PR-B (Conneely et al., 2003). Furthermore, a recent study showed that PR-A was absent in all compartments of the uterus in anoestrous sows (Karveliene et al., 2007). As the results of the present study were the accumulation of PR-A and PR-B, therefore the difference between these two isoforms of PR could not be demonstrated. On the other hand, there may be some differences in the expression of PR-A in these inseminated sows, but it may also be balanced by the level of PR-B in the tissue compartments and, therefore, causes the similar expression of PR in some of the oviductal tissues. However, the different localization of PR-A should be further studied as it may reveal or explain the possible effects on the localization of PR in different inseminated sows.

From the results of the present study, it was also demonstrated that ERα immunostaining was found in the cytoplasm of the oviductal epithelium. This is in agreement with previous studies reporting localization of ERα in non-nuclear sites of reproductive cells (Welshons et al., 1984; Marquez and Pietras, 2001; Monje and Boland, 2001; Monje et al., 2001). Moreover, the study in rats demonstrated that mating could increase the number of ERα in non-nuclear compartments. The increase in ERα in non-nuclear compartment involved the changes from non-genomic pathway in cyclic rat to genomic pathway in mating rat (Okada et al., 2003). The changes in these pathways have been designated "intracellular path shifting, or IPS" (Parada-Bustamante et al., 2007). Therefore, the marked ERα cytoplasmic staining could be involved in the changes of IPS induced by mating or insemination in the present study since no cytoplasmic staining was observed in cyclic sows (Sukjumlong et al., 2005) nor in the epithelial cells for PR immunostaining.

In the present study, immunostaining results of both ERα and PR were evaluated by two different methods, the manual scoring and the image analysis. The results from these two methods were in agreement with each other, although minor differences could be observed in some parts of the oviduct. The explanation of the difference was that the manual scoring showed variations in patterns of immunostaining with regard to both proportion and intensity while the image analysis quantified the total amount of positive staining in randomly selected area. Moreover, cytoplasmic staining could be excluded from manual scoring method but still be detected by image analysis system.

In conclusion, the present study showed the differences in steroid receptor expression among different insemination methods and in varied parts of the sow oviduct. The methods of insemination regarding the volume of semen can have the effects on the expression of steroid receptors in the sow oviduct and this may also influence the successful fertilization in sows.

Acknowledgements

The present study was granted by Thailand Research Fund (MRG5380173). We would like to thank Dr. Panida Chananpiwat and Dr. Sukanya Manee-in for assistance with sample collection, Mr. Sinchai Pianchop for his assistance with histological technique and Miss Jongkol Sangvirun for assistance with immunohistochemical procedure.

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