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Technical Note

Precision-cut luteal slices: A promising approach for studying luteal function in pigs

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ABSTRACT

The study was aimed to validate the precision-cut luteal slices to investigate porcine luteal function. Corpora lutea (CLs) were cut into 180-μm thick slices using Krumdick Tissue Slicer. The viability, tissue structure and steroidogenic acute regulatory protein (STAR) expression in the luteal slices did not differ between the beginning and the end of the 24-h incubation period. The luteal progesterone secretion showed a time- and dose-dependent response to porcine luteinizing hormone. The effects of prostaglandin $F_{2\alpha}$ and 17β-estradiol on progesterone secretion by porcine luteal slices were comparable to the previously reported *in vivo* results of the CL microdialysis system in the pig.

1. Introduction

In most domestic animal species, the corpus luteum (CL) acquires the capacity to undergo luteolysis (luteolytic sensitivity) between days 6 and 9 of the estrous cycle. Porcine CL acquires luteolytic sensitivity relatively late in the cycle [1,2], *i.e.*, near day 13. Several hypotheses on the delayed acquisition of luteolytic sensitivity in the pig have been stated, including auto-amplification of luteal production of prostaglandin (PG) $F_{2\alpha}$ and differences in the activation of the $PGF_{2\alpha}$ signaling pathway. However, the mechanism responsible for this delay in the pig is not fully recognized.

Studies on the acquisition of luteolytic sensitivity usually include both *in vivo* and *in vitro* experiments [3] or use an intricate CL microdialysis *in vivo* system [4]. The current study aimed at examining whether precision-cut tissue slices of porcine CL can be used to investigate luteal function as an alternative to *in vivo* experiments in pigs and other species. To meet this aim two distinct experiments were performed. In the first experiment: (1) the optimal *in vitro* conditions for incubation of luteal slices, and (2) time- and dose- dependent effects of luteinizing hormone (LH) on progesterone (P4) secretion were established. In the second experiment, the effects of 17β-estradiol (E2) and $PGF_{2\alpha}$ on P4 secretion by luteal slices were determined under the conditions established in the first experiment.

2. Materials and methods

The experiments were conducted in accordance with the national guidelines for agricultural animal care and approved

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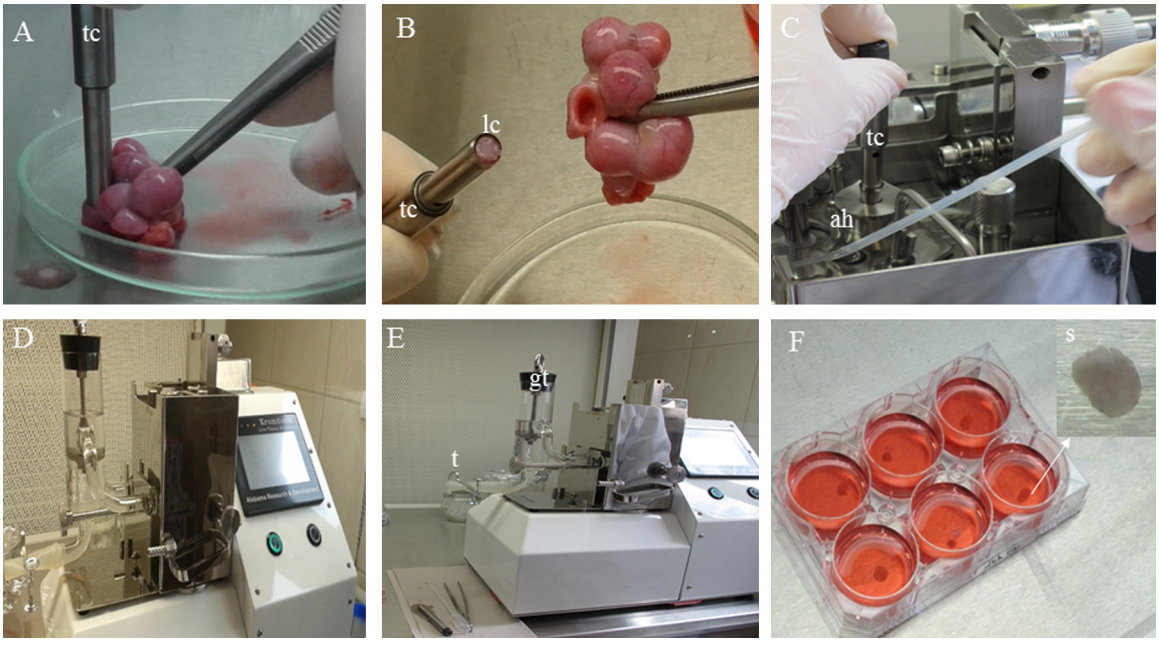

Fig. 1 – Preparation and incubation of porcine luteal slices. Luteal cores (lc) were prepared using a tissue coring (tc) tool (8 mm diameter) (A and B) and transferred to the cylindrical arm holder (ah) (C) of the Krumdick Tissue Slicer (D). High-quality slices (s) (*i.e.*, round with a smooth edge and uniform color) were generated using a rapidly reciprocating disposable blade and were removed from the glass trap (gt) by opening the tap (t) (E). The slices were collected in a Petri dish filled with medium and gently transferred (one per well) to 6-well culture plates filled with oxygenated medium (F). The slices were incubated under a highly oxygenated atmosphere (95% O_2 and 5% CO_2) with shaking. For more details please see Section 2.

by the Local Animal Ethics Committee. Crossbred gilts (7–8 months old) during their second estrous cycle were used in the study. Corpora lutea collected during the mid-luteal phase (days 10–12) or late-luteal phase (days 14–15) were cut into slices (180-μm thick, 8 mm diameter, 4–5 mg wet weight) using a Krumdick Tissue Slicer (K&F Research, Birmingham, AL, USA) (Fig. 1A–F; [5]). This procedure allows collection of 24 luteal slices within 2–3 min. To validate the model, the precision-cut luteal slices (PCLS) collected during the mid-luteal phase ($n = 4$) were used to examine: (1) P4 concentration in different medium volumes (2, 4 and 6 mL, medium-199; Sigma-Aldrich, St. Louis, MO, USA) after treatment with LH, a positive control (1, 10 or 100 ng/mL; pLH B-1, USDA Hormone Program, Bethesda, MD, USA); and (2) the time- and dose-dependent response of P4 secretion to LH (10 or 100 ng/mL) determined in the optimal medium volume. Since in preliminary experiments the lowest LH dose (1 ng/mL) was ineffective, this dose was not included in the subsequent experiments. The slices (one per well) were incubated in M-199 supplemented with 0.1% bovine serum albumin (BSA; ICN Biomedicals, Inc., Costa Mesa, CA, USA), antibiotics and anti-fungal drug (amphotericin B; Sigma–Aldrich). The post-incubation medium was collected to determine P4 concentration, using enzyme-immunoassay (EIA) described previously [6]. Since the greatest dose-dependent effect of LH on P4 secretion was found in 6 mL of medium, the viability of luteal slices, measured as lactate dehydrogenase (LDH) activity, was determined in this volume, before and after 24 h of incubation. The integrity of luteal slices (before and after the 24-h incubation period, in medium only) was assessed with hematoxylin and eosin (H&E) staining. RNA isolation from luteal tissue, reverse-transcription and expression of steroidogenic acute regulatory protein (STAR) mRNA using real-time PCR were performed as previously described [7].

In the second experiment, the effects of E2 (1 and 10 ng/mL) and $PGF_{2\alpha}$ (10^{-7} M) on P4 secretion by slices obtained from CLs harvested during the mid- ($n = 4$) and late-luteal phase ($n = 4$) were determined in 6 mL of medium and after 24 h of incubations, *i.e.*, in conditions established in the experiment 1. In these conditions, the viability of luteal slices was confirmed and the slices maintained an appropriate tissue structure. The medium was collected every 6 h during the 24-h incubation period to determine P4 concentration (EIA). The changes in medium P4 concentration, differences in STAR mRNA expression and LDH activity in luteal tissue were analyzed with Student's *t*-test (GraphPad Prism v.5.0; GraphPad Software, San Diego, CA, USA). $p < 0.05$ was considered statistically significant, and the numerical data were expressed as mean ± SEM.

3. Results and discussion

Although porcine CL is considered to be independent on the pituitary LH until day 12 of the estrous cycle [8], 10 and 100 ng/mL of LH increased ($p < 0.01$) P4 secretion by mid-luteal slices incubated for 24 h in 6 mL of medium (Fig. 2A). It should be emphasized that we have applied approximately 20 times less luteal tissue [9] and have included LH in doses 10–1000 times lower in comparison to previous reports [8,9]. When the 4-mL

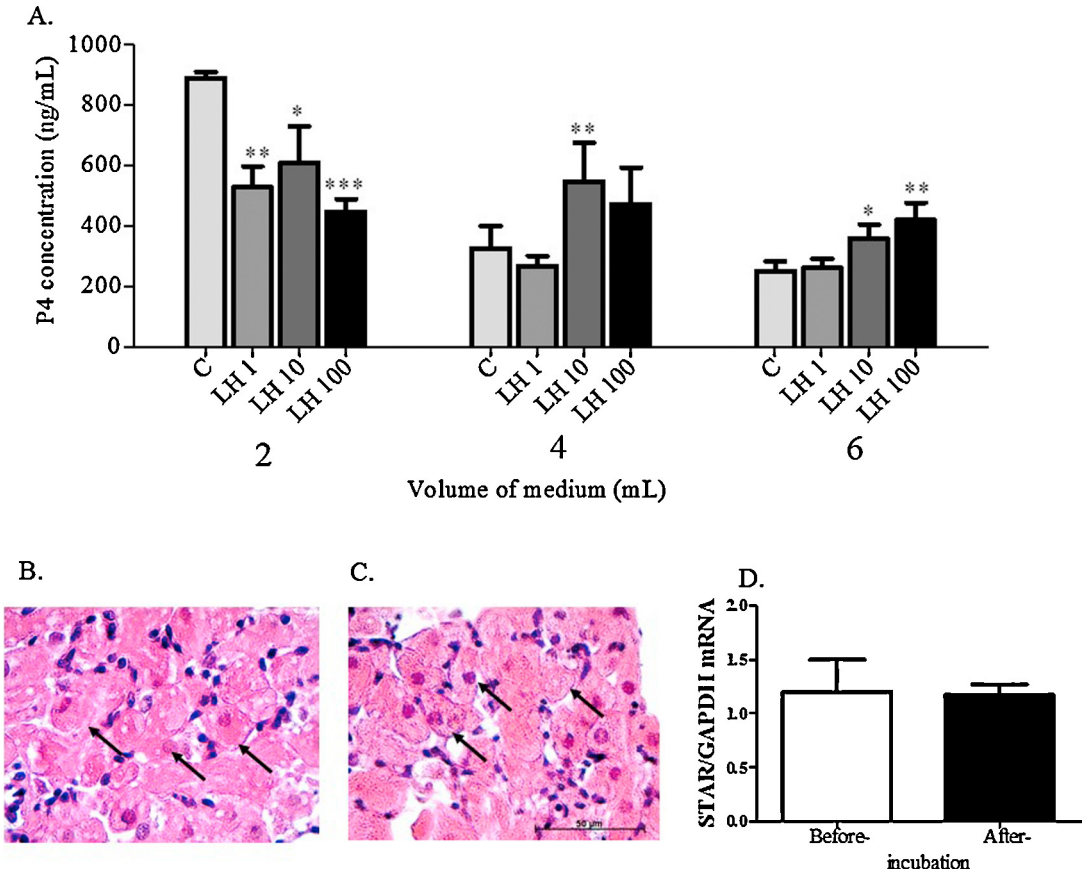

Figure 2 – Progesterone (P4) accumulation (A; mean ± SEM) in three different volumes of medium (2, 4 and 6 mL) collected after LH (1, 10 and 100 ng/mL) treatment (24-h incubation) of luteal slices obtained during the mid-luteal phase (days 10–12) of the porcine estrous cycle (n = 5 independent experiments). The images present the structural integrity of luteal tissue before (B; 0 h) and after the incubation (C; 24 h); arrows indicate luteal cells. (D) STAR mRNA expression in luteal slices before and after the 24-h incubation. Data were analyzed with Student's t-test. Asterisks denote significant differences between the treatment and corresponding control (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$); C, control; LH1, 1 ng/mL; LH10, 10 ng/mL; LH100, 100 ng/mL of LH; bar = 50 μm.

volume of incubation medium was examined, only 10 ng/mL of LH significantly ($p < 0.05$) increased P4 concentration. The most consistent dose-dependent LH influence was observed in the 6-mL volume of incubation medium (Fig. 2A). Interestingly, the most obvious time-dependent response was recorded in this volume after 18-h incubation period (Fig. 3). Because of it and since P4 concentration did not differ between the 18- and 24-h incubation periods, only data for 18-h period were presented (Fig. 4). Furthermore, considering 2–3 fold lower P4 concentration in control 4- and 6-mL incubation media, we assume that abundant concentration of P4 in 2-mL volume of incubation medium might inhibit its own production (Fig. 2A).

The LDH activity determined in the mid-luteal slices did not differ between 0 and 24 h of incubation (1.67 ± 0.04 *vs.* 1.77 ± 0.11 mU/mL/mg). Additionally, the H&E staining showed that the tissue structure was not altered after the 24-h incubation period (Fig. 2B and C). Similarly, STAR expression did not differ between the beginning and the end of the incubation (Fig. 2D). These data confirmed that the applied *in vitro* conditions ensured an adequate oxygen and nutrient supply and did not affect the viability of porcine luteal tissue during the examined incubation periods ($p < 0.05$).

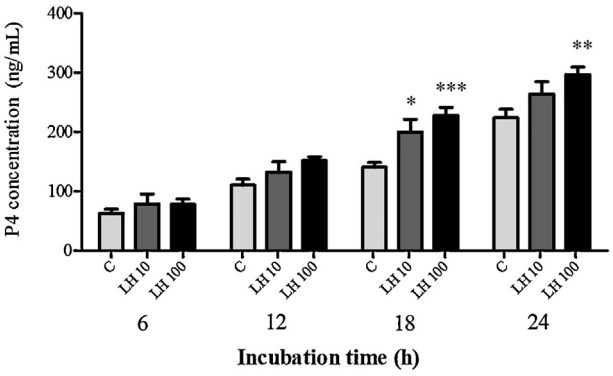

Fig. 3 – Time- and dose-dependent effects of LH (10 and 100 ng/mL) on progesterone (P4) medium (6 mL) accumulation (mean ± SEM) after the 24-h incubation period of the luteal slices collected during the mid-luteal phase (days 10–12) of the porcine estrous cycle (n = 4 independent experiments). Data were analyzed with Student's t-test. Asterisks denote significant differences between the treatment and corresponding control (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$); C, control; LH10, 10 ng/mL; LH100, 100 ng/mL of LH.

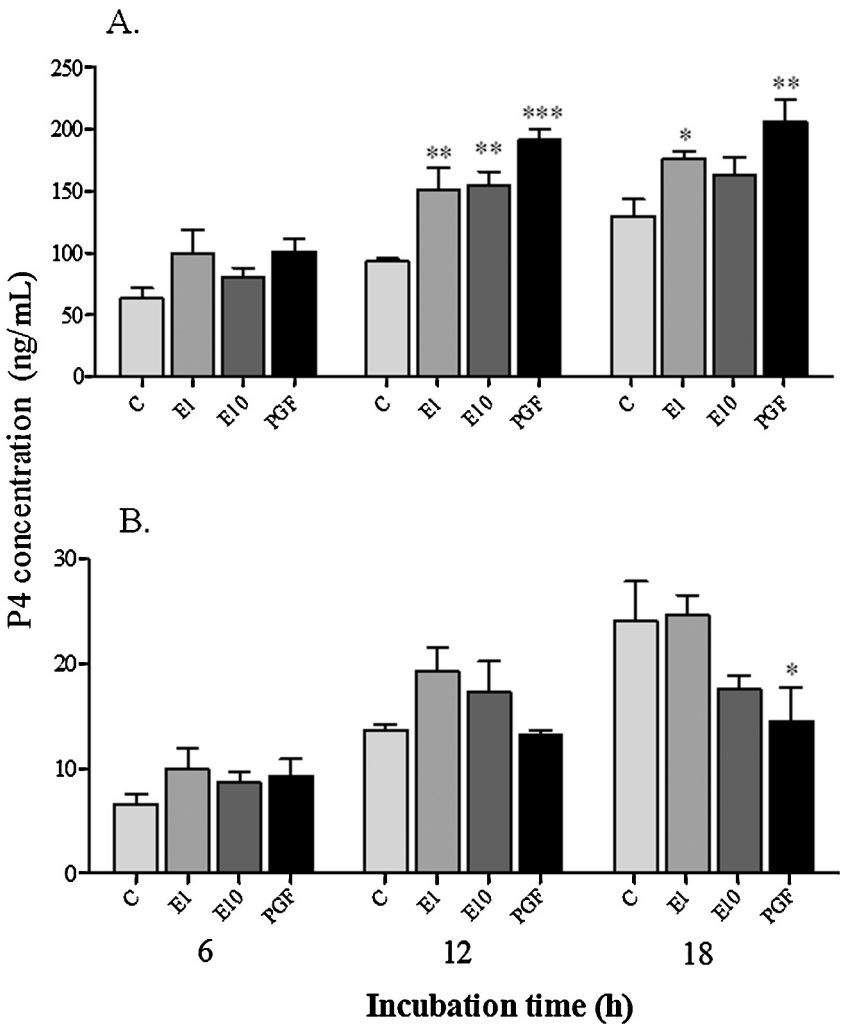

Fig. 4 – Progesterone (P4) accumulation (mean ± SEM) in medium (6 mL) collected after $PGF_{2\alpha}$ (10^{-7} M) or E_2 (1 and 10 ng/mL) treatment of luteal slices (6-, 12- or 18-h incubation) obtained during the mid-luteal (A) or late-luteal (B) phase of the porcine estrous cycle ($n = 4$ independent experiments). Data were analyzed with Student's t-test. Asterisks denote significant differences between the treatment and corresponding control ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$); C, control, E1, 1 ng/mL; E10, 10 ng/mL of E2; PGF, $PGF_{2\alpha}$ 10^{-7} M.

Despite the fact that luteolytic sensitivity is a complex and still not fully recognized phenomenon, the further research in this field has been limited due to a lack of an appropriate experimental model. Thus, we applied the PCLS model to examine the effects of $PGF_{2\alpha}$ and E2 on medium P4 concentration. $PGF_{2\alpha}$ (10^{-7} M) enhanced ($p < 0.001$) P4 secretion by slices obtained from CLs collected during the mid-luteal phase (Fig. 4A; 12- and 18-h incubation) and diminished ($p < 0.05$) P4 secretion by luteal slices collected during the late-luteal phase (Fig. 4B; 18-h incubation). The observed effects were consistent with the results of the *in vivo* experiments employing the microdialysis system [4]. In the latter study, $PGF_{2\alpha}$ increased P4 secretion on days 11 and 12, but decreased on day 14 (during luteolysis) of the porcine estrous cycle. Estradiol, an acknowledged luteotropin in pigs [10], increased P4 production by porcine luteal slices collected during the mid-luteal phase (Fig. 4A), but not during the late-luteal phase (Fig. 4B).

In summary, the *in vitro* PCLS model to examine the physiological functions of the CL in the pig was validated in the current study. The model may be also applied in other domestic animals or primates, as well as may provide a new alternative to laborious and expensive *in vivo* experiments and limit the number of animals used in the research.

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