Bisphenol S Administered to Pregnant Ewes with Contrasted Metabolic Status Impaired the Ovarian Follicular Development of Fetuses and Lambs

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ABSTRACTS WORKSHOPS

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TOPIC Female reproductive physiology

W29 GENE EXPRESSION PATTERNS IN UTERUS AND OVIDUCT DURING THE PREOVULATORY PERIOD IN EWES
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BACKGROUND-AIM
The uterine and oviduct environment soon after ovulation plays a major role in fertilization and embryo development. However, the knowledge about the uterine/oviductal environment during the preovulatory period is not fully understood. This study investigated the gene expression oviduct (ampulla and isthmus) and uterus (horns) during the preovulatory period in multiparous ewes.

METHODS
Multiparous Corriedale ewes (n=10) were synchronized during breeding season with intravaginal sponges and equine chorionic gonadotrophin (Menchaca and Rubianes 2004). Estrus was detected after sponge removal by using an aromatase inhibitor and the reproductive tract was dissected and the oviducts (ampulla and isthmus) and a portion of the upper third of the uterine horns were sampled to be stored at -80°C. Gene expression of PGR (nuclear progesterone receptor), ESR1 (estradiol receptor alpha), IGF2 (Insulin-like Growth Factor 2), PTGS2 (Prostaglandin-Endoperoxide Synthase 2), Serpina14 (Uterine milk protein), SOD1 and SOD2 (Superoxide Dismutases 1 and 2) as housekeeping genes BACT (Beta-Actin), and HPRT (Hypoxanthine-Guanine Phosphoribosyltransferase) were determined by real time PCR. All variables were analyzed using a mixed model analysis.

RESULTS
The expression of most of the genes (PGR, ESR1, IGF2, PTGS2, SOD1, SOD2) was greater (P<0.001) in the oviduct compared to the uterine tissue. Only the Serpina14 gene had greater (P<0.001) expression in the uterus (2.1±0.2; 8.6±1.4; 2.0±0.2; 2.1±0.1) compared to the oviduct (0.1±0.2; 1.3±1.2; 0.9±0.2; 0.7±0.1), respectively.

CONCLUSIONS
During the preovulatory period, the isthmus segment seems to have a greater gene expression activity of certain genes compared to the other regions, likely improving the environment for the cumulus oocyte complex transport, sperm migration for fertilization, and early embryo passage to the uterus.
W31 BISPHENOL S IMPAIRED HUMAN AND OVINE GRANULOSA CELL STEROIDOGENESIS

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BACKGROUND-AIM

Human being is exposed to bisphenol A (BPA), a plasticizer used in food containers, through diet. Granulosa cell (GC) play a fundamental role for oocyte growth and maturation. BPA impaired GC steroidoogenesis and has been classified as an endocrine disruptor (ED). BPA was banned from the food industry and replaced by structural analogues, particularly bisphenol S (BPS). Given that the ewe has similar follicle kinetics compared to women, the evaluation of the effects of BPS on the ovine would allow to investigate whether the ewe is a suitable model to study ED effects in human. Our objective was to assess BPS effects, and its mechanisms of action, on both human and ovine GC.

METHODS

After puncture of follicles from women undergoing in vitro fertilization or ewe ovaries (slaughterhouses), GC were collected, purified and treated in complemented serum-free McCoy Medium, with BPS (1 µM, 10 µM or 50 µM) for 48-h. We analysed GC viability (adenylate kinase activity assay) and proliferation (incorporation of BrDU), secretion of oestradiol and progesterone (ELISA essay), expression of steroidogenic enzymes, PR gene expression (quantitative RT-PCR) and MAPK3/1 phosphorylation. Results were analysed using non parametric permutational ANOVA and Tuckey post-hoc test.

RESULTS

BPS 10 µM significantly decreased progesterone secretion of both human (16 %; p = 0.0059) and ovine (22 %; p = 0.0402) GC compared to control. BPS 50 µM significantly decreased oestradiol secretion in human GC (46%; p < 0.0001), while it was significantly increased with BPS 10 µM in ovine GC (198%; p = 0.0082). In both ovine and human GC, BPS did not affect cell viability, proliferation, protein expression of steroidogenic enzymes, PR gene expression, or MAPK3/1 phosphorylation.

CONCLUSIONS

Thus, the effects of BPS on GC appear harmful and similar between human and ovine; except for oestriadiol secretion, likely due to the ovulatory stage of GC collection for women. Our data confirmed that the ewe is a relevant model, in term of sensitivity, for the human female reproduction. Ewe GC will allow us to further study the detailed mechanisms of action of BPS on female reproduction.

W33 INTERRELATIONSHIP BETWEEN INTRAFOLLICULAR DOPAMINE AND ESTRADIOL CONCENTRATIONS IN CYCLING MARES

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BACKGROUND-AIM

Follicular hormonal changes associated with oocyte maturation can be modulated locally by monoamines (Bódis et al., 1993). Animal models, ovariectomy decreases the release of dopamine (DA), that can be reversed with subsequent treatment of oestriadiol (E2) (Thompson and Moss, 1994). This is consistent with the elevation of DA presumably in relation to high levels of E2 during the estrous cycle. This response suggests that DA in FF could play an important role in the mechanism of ovulation in humans (Bódis et al., 1992). In mares, the effects of DA on follicular growth may be mediated by FSH secretion (King et al., 2008). However, the relationship between DA and E2 concentrations has not yet been investigated. The objective of this study was to investigate the interrelationship between DA and E2 in follicular fluid (FF) in different categories of follicular sizes in cycling mares.

METHODS

A total of 60 samples of FF by aspiration of slaughterhouse ovarian follicles of 30 clinically healthy mares aged 6.6 ± 1.3 years are evaluated. FF samples were classified according to diameter as: small (20-30 mm), medium (31-40 mm) and large (≥ 41 mm) size. Intrafollicular DA (pg/mL) concentrations were measured with a radioimmunoassay technique (Labor Diagnostika Nord GmbH, Nordhorn, Germany). The concentrations of E2 (ng/mL) in FF are determined by a competitive enzyme-linked immunosorbent assay (Estradiol sensitive ELISA Demeditec DE4399) validated specifically for FF in the equine species. The intrafollicular concentrations of E2 in small follicle were significantly lower (652.9 ± 241.3 pg/mL) than in medium (1,498.9±205.8 pg/mL) and large (1,692.7±146.8 pg/mL) follicle sizes (P < 0.05). In FF, the concentrations of DA in medium size follicles were significantly higher (707.9±360.7 pg/mL) and large follicles (1,692.7±146.8 pg/mL) follicle sizes (P < 0.05). DA and E2 concentrations are positively correlated (r=0.66; P < 0.05).

CONCLUSIONS

These results suggest that, as is the case at the circulating level, ovarian estrogens are involved in dopaminergic activity in follicular development, or that catecholamines modulate the stimulating effect of oestradiol in follicular fluid.