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

Ophélie Têteau, Peggy Jarrier-Gaillard, Olivier Lasserre, Pascal Papillier, Alice Desmarchais, Claire Vignault, Virginie Maillard, Aurélien Binet, Marie-Emilie Lebachelier de la Riviere, Nicole Picard-Hagen, et al.

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# ABSTRACT BOOK



ICAR2020+2

Bologna, Italy

19<sup>th</sup> International Congress  
on Animal Reproduction  
BOLOGNA (ITALY), 26<sup>th</sup>-30<sup>th</sup> JUNE 2022

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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 on 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 androgenized ram. The ewes were euthanized (thiopental sodium Tiobarbital and T-61) from 36 to 48 h after sponge removal (i.e., before ovulation), 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 procedure in SAS.

## 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 ( $P < 0.001$ ) RNA expression in the uterus ( $62.3 \pm 9.0$ ) compared to both ampulla ( $1.9 \pm 9.7$ ) and isthmus ( $18.1 \pm 9.0$ ). Regarding the oviduct tissue, the genes IGF2, PTGS2, SOD1 and SOD2 had greater ( $P < 0.001$ ) RNA expression in the isthmus ( $2.1 \pm 0.2$ ;  $8.6 \pm 1.4$ ;  $2.0 \pm 0.2$ ;  $2.1 \pm 0.1$ ) compared to the ampulla ( $0.1 \pm 0.2$ ;  $1.3 \pm 1.2$ ;  $0.9 \pm 0.2$ ;  $0.7 \pm 0.1$ ), 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.

**W30****BISPHENOL S ADMINISTERED TO PREGNANT EWES WITH CONTRASTED METABOLIC STATUS IMPAIRED THE OVARIAN FOLLICULAR DEVELOPMENT OF FETUSES AND LAMBS**

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## BACKGROUND-AIM

The size of the ovarian reserve, which is established by the number of primordial follicles, is defined before birth in ovine. The prenatal period is a critical window of sensitivity to environmental factors. Maternal metabolic status during gestation can influence fetal programming, and affect the ovarian reserve. Bisphenol A (BPA), a plasticizer used in food packaging, has deleterious effects on fetal folliculogenesis. It has been banned from the food industry and mainly replaced by bisphenol S (BPS). The objective of this study was to determine the effects of BPS 50 µg/kg/d in utero exposure on female fetuses and lambs from ewes with contrasted metabolic status on follicular population by ovarian histology.

## METHODS

This study was divided into two experiments performed on pregnant ewes exhibiting a contrasted metabolic status (lean versus well-fed). First, pregnant ewes were exposed to BPS by daily subcutaneous injection for 3 months, to analyze the ovarian development of the fetuses at 130 days of gestation. Second, pregnant ewes were exposed daily to BPS through food for 3 months and the lambs were then monitored up to 4 month-old (pre-puberty). Follicular population was characterized through follicle classification and counting on ovarian sections.

## RESULTS

In utero BPS exposure led to an increase in the number of pre-antral ( $p = 0.005$ ) and antral follicles ( $p < 0.001$ ) in fetuses and conversely to a decrease in 4 month-old lambs ( $p = 0.007$ ). Besides, a significant interaction between maternal metabolic status and BPS exposure during gestation was reported for the number of primordial ( $p = 0.019$ ), pre-antral ( $p = 0.003$ ) and antral ( $p < 0.001$ ) follicles of fetuses. In offspring of fat mothers, the plasma anti-Müllerian hormone decreased in 1 and 2 month-old lambs ( $p < 0.001$ ). In offspring of well-fed mothers, the body weight of the female fetuses increased ( $p < 0.001$ ). Moreover, the body weight of female lambs that were in utero exposed to BPS increased compared to control ( $p = 0.040$ ).

## CONCLUSIONS

In conclusion, bisphenol exposure during gestation had deleterious consequences on the body and folliculogenesis of the offspring. BPS effects also vary according to the maternal metabolic status. Further research will be needed to determine if these alterations will have deleterious effects on the ovarian reserve and on the reproductive function in adulthood.

## 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 steroidogenesis 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 kinetic 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 therefore 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 Mc Coy Medium, with BPS (1  $\mu$ M, 10  $\mu$ M or 50  $\mu$ M) for 48-h. We analysed GC viability (adenylate kinase activity assay) and proliferation (incorporation of BrDU), secretion of oestradiol and progesterone (ELISA assay), expression of steroidogenic enzymes (Western Blot), hormonal receptor gene expression (quantitative RT-PCR) and MAPK3/1 signalling pathway, as it is involved in both survival and proliferation cellular process (Western Blot). Results were analysed using non parametric permutational ANOVA and Tuckey post-hoc test.

## RESULTS

BPS 10  $\mu$ M significantly decreased progesterone secretion of both human (16 %;  $p = 0.0059$ ) and ovine (22 %;  $p = 0.0402$ ) GC compared to control. BPS 50  $\mu$ M significantly decreased oestradiol secretion in human GC (46%;  $p < 0.0001$ ), while it was significantly increased with BPS 10  $\mu$ M 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 oestradiol 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 estradiol (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  $6.6 \pm 1.3$  years are evaluated. FF samples were classified according to diameter as: small (20-30 mm), medium (31-40 mm) and large ( $\geq 41$  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.

## RESULTS

The intrafollicular concentrations of E2 in small follicle were significantly lower ( $652.9 \pm 241.3$  pg/mL) than in medium ( $1.498.9 \pm 205.8$  pg/mL) and in large ( $1.692.7 \pm 146.8$  pg/mL) follicle sizes ( $P < 0.05$ ). In FF, the concentrations of DA in medium size follicles were significantly higher ( $707.9 \pm 360.7$  ng/mL) than in small ( $306.2 \pm 113.8$  ng/mL) and large ( $351.2 \pm 132.5$  ng/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 estradiol in follicular fluid.