



HAL
open science

Dataset of zootechnical, biological, oocyte and embryo production indicators, from ewes with contrasted metabolic status and submitted to chronic bisphenol S exposure

A Desmarchais, Sandrine Freret, Svetlana Uzbekova, Virginie Maillard, Sébastien Elis

► **To cite this version:**

A Desmarchais, Sandrine Freret, Svetlana Uzbekova, Virginie Maillard, Sébastien Elis. Dataset of zootechnical, biological, oocyte and embryo production indicators, from ewes with contrasted metabolic status and submitted to chronic bisphenol S exposure. *Animal - Open Space*, 2025, 4, pp.100088. <10.1016/j.anopes.2024.100088>. <hal-04926090>

HAL Id: hal-04926090

<https://hal.inrae.fr/hal-04926090v1>

Submitted on 3 Feb 2025

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons CC BY-NC-ND 4.0 - Attribution - Non-commercial use - No Derivative Works - International License



Contents lists available at ScienceDirect

animal - open space

journal homepage: www.elsevier.com/locate/anopes



Data paper

Dataset of zootechnical, biological, oocyte and embryo production indicators, from ewes with contrasted metabolic status and submitted to chronic bisphenol S exposure

A. Desmarchais ^{a,1,*}, S. Fréret ^a, S. Uzbekova ^a, V. Maillard ^a, S. Elis ^a

^a INRAE, CNRS, Université de Tours, PRC, 37380, Nouzilly, France

ARTICLE INFO

Article history:

Received 2 September 2024

Revised 11 December 2024

Accepted 12 December 2024

Handling editor: Charlotte Gaillard

Keywords:

Endocrine disruptor
Energy metabolism
Hormones
Ovary
Sheep

ABSTRACT

This dataset consists of raw data from an experimental design in the ovine model: zootechnical traits, biological indicators, oocyte collection and embryo production from ewes with contrasted metabolic status and submitted to chronic Bisphenol S (BPS) exposure. For 2 consecutive years, 120 primiparous ewes (60 different ewes for each year) were managed in a loose sheepfold, assigned to two diet groups – Restricted (R, n = 30) and Well-fed (WF, n = 30) – and supplemented with or without BPS (0, 4 or 50 µg/kg/day), generating six experimental groups named R-0, R-4, R-50, WF-0, WF-4, and WF-50 (n = 10 ewes/group). Oocyte collection was carried out during the breeding season of sheep between September and December. Diet management started in May and BPS treatment started in June, that is to say at least 3 months before oocyte collection. The dataset presents zootechnical traits (age, live weight, body condition score), metabolic indicators and hormones concentrations measured in plasma (non-esterified fatty acids, glucose, thyroid hormones, Anti-Mullerian hormone, progesterone, oestradiol and other steroids) as well as in follicular fluid for some of them (progesterone, oestradiol and others steroids), BPS and BPS-glucuronide concentrations in plasma and urine, data from follicular puncture and oocyte collection by ovum pick-up followed by *in vitro* embryo production (during the 2nd year of experiment). This large dataset can be re-used on the one hand by researchers working on reproductive physiology in interaction with energy metabolism in ruminants, to further investigate the effects of a restricted diet; on the other hand, by toxicologists and endocrinologists to investigate the effects of BPS (potential endocrine disruptor) on several hormones (i.e. steroids) and metabolites.

© 2025 The Authors. Published by Elsevier B.V. on behalf of The animal Consortium. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Reader comments

We invite you to comment on the article on the PubPeer platform by clicking on this link [discuss this article](#).

Specification table

Subject	Physiology and Functional Biology
Specific subject area	Reproductive physiology in sheep with contrasted metabolic status and

	submitted to chronic Bisphenol S exposure.
Type of data	Table
How data were acquired	Zootechnical data were recorded: ewes were weighed and their body condition was determined (visual examination and palpation, scale of 0–5) monthly. Plasma samples were analysed using biochemical assays performed for metabolic and hormonal indicators, using published methods. Ovarian follicles were punctured, and oocytes were collected using Ovum Pick Up by endoscopy under general anaesthesia (published procedure).

(continued on next page)

* Corresponding author.

E-mail address: alice.desmarchais@inrae.fr (A. Desmarchais).

¹ Present address: INRAE Centre Val de Loire, Physiologie de la Reproduction et des Comportements, 37380 Nouzilly, France.

	<p>Oocyte quality and embryo development after <i>in vitro</i> fertilisation were evaluated by microscopy observation.</p> <p>Preovulatory follicular fluid (diameter ≥ 6 mm) samples were analysed for hormonal indicators using ELISA assays or by TSQ 8000 tandem mass spectrometer MS/MS and Excalibur 3.0 software analysis for steroidome measurements.</p> <p>Urine samples were analysed by Acquity U-HPLC device coupled to a Xevo-TQ triple quadrupole mass spectrometer.</p> <p>All experimental procedures were conducted in accordance with the European Directive 2010/63/EU on the protection of animals used for scientific purposes and approved by the French Ministry of National Education, Higher Education, Research and Innovation after ethical assessment by the local ethics committee "Comité d'Ethique en Expérimentation Animale Val de Loire (CEEA VdL)" (protocols registered under APAFIS numbers 13965–201804 2008519239v2 and 14014–201803071 7477406v2).</p>
Data format	Raw data (https://doi.org/10.57745/EGUA5H)
Parameters for data collection	During 2 consecutive years, 120 primiparous ewes were managed in a loose sheepfold. Each year (n = 60 different ewes/year), ewes were assigned to two diet groups, Restricted (n = 30) and Well-fed (n = 30), and were supplemented with or without BPS (0, 4 or 50 $\mu\text{g}/\text{kg}/\text{day}$), generating six experimental groups named R-0, R-4, R-50, WF-0, WF-4, and WF-50 (n = 10 ewes/group). Oocyte collection was carried out during the breeding season of sheep between September and December. Diet management started in May and BPS treatment started in June, at least 3 months before oocyte collection.
Description of data collection	<p>Ewes were weighed and their body condition was determined (visual examination and palpation, scale of 0–5) monthly.</p> <p>Blood samples were collected before morning feeding, after jugular venipuncture in heparinised tubes and blood centrifugation, and stored at -20 °C until analysis.</p> <p>Ovarian follicles were punctured and oocytes were collected using Ovum Pick Up by endoscopy under general anaesthesia.</p>

	<p>Follicular fluid samples from preovulatory follicles were obtained at the time of sacrifice, isolated from granulosa cells by centrifugation and stored at -80 °C until analysis.</p> <p>Urine samples were obtained at the time of sacrifice by bladder puncture and stored at -80 °C until analysis.</p>
Data source location	INRAE Val de Loire, Unité mixte de recherche Physiologie de la reproduction et des comportements (UMR PRC), 37380, Nouzilly, France. GPS coordinates Latitude N 47° 32' 41.046" Longitude E 0° 46' 54.595"
Data accessibility	Data are hosted in Recherche Data Gouv repository. https://recherche.data.gouv.fr/ https://doi.org/10.57745/EGUA5H Licenses of use: etalab 2.0 (https://spdx.org/licenses/etalab-2.0.html).
Related research article	Desmarchais, A., Têteau, O., Kasal-Hoc, N., Cognié, J., Lasserre, O., Papillier, P., Lacroix, M., Vignault, C., Jarrier-Gaillard, P., Maillard, V., Binet, A., Pellicer-Rubio, M-T., Fréret, S., Elis, S. 2022. Chronic low BPS exposure through diet impairs <i>in vitro</i> embryo production parameters according to metabolic status in the ewe. <i>Ecotoxicology and Environmental Safety</i> , Volume 229, 113096. https://doi.org/10.1016/j.ecoenv.2021.113096 . https://www.sciencedirect.com/science/article/pii/S0147651321012082

Value of the data

- This large dataset, obtained in experimental conditions in the sheep model during 2 consecutive years, consists of three zootechnical traits, eight plasmatic biological indicators and hormones measured in plasma, 36 steroids measured in plasma and follicular fluid, two indicators of Bisphenol S (BPS) exposure measured in plasma and urine, and 13 indicators concerning oocyte collection by Ovum-Pick Up (OPU) and *in vitro* embryo development (IVD).
- This dataset can benefit to researchers working on reproductive physiology in interaction with energy metabolism in ruminants, to further investigate the effects of a restricted diet.
- This dataset provides an overall view of ovine folliculogenesis and embryo development through *in vitro* maturation and fertilisation of oocytes, collected *in vivo* by ovum pick-up.
- This dataset provides rare biological information in sheep experimentally exposed to BPS and can be useful for toxicologists and endocrinologists to study the effects of BPS on several hormones (i.e. steroids) and metabolites, in order to help to establish recommendations.

Data Description

The dataset (<https://doi.org/10.57745/EGUA5H>) is hosted in “Recherche Data Gouv repository” (<https://recherche.data.gouv.fr/>) and consists in 15 Excel® datafiles (each datafile being associated to a text file with the dictionary of variables) containing raw data from an experimental design in the ovine model: zootechnical traits, biological indicators, oocyte collection and embryo production from ewes with contrasted metabolic status and submitted to chronic BPS exposure.

For 2 consecutive years, 120 primiparous ewes (60 ewes/year: 60 ewes in the 1st year, and another 60 ewes in the 2nd year) were managed in a loose sheepfold, assigned to two diet groups, Restricted (R, n = 30) and Well-fed (WF, n = 30), and supplemented with or without BPS (0, 4 or 50 µg/kg/day), generating six experimental groups named R-0, R-4, R-50, WF-0, WF-4, and WF-50 (n = 10 ewes/group). The experimental design is illustrated and detailed in the datapaper in Figs. 1–3.

The 15 datafiles included in the dataset are listed and described below (all datafiles containing the individual identification of the 120 ewes, numbered from 1 to 60 in year 1 and from 61 to 120 in year 2):

- Datafiles n°1 and 2 present zootechnical traits (age, live weight, body condition score).
- Datafiles n°3, 4, 5, 6 and 7 present metabolic indicators and hormones concentrations measured in plasma: non-esterified fatty acids and glucose (n°3), thyroid hormones (n°4), Anti-Mullerian hormone (n°5), progesterone (n°6) and oestradiol (n°7).
- Datafile n°8 presents progesterone and oestradiol concentrations measured in follicular fluid.
- Datafiles n°9, 10 and 11 present steroidome analysis in plasma (n°9 and 10) and follicular fluid (n°9 and 11).
- Datafiles n°12 and 13 present BPS and BPS-glucuronide concentrations in plasma (n°12) and urine (n°13).
- Datafiles n°14 and 15 present data from follicular puncture and oocyte collection by OPU (n°14 and 15), followed by *in vitro* embryo production during the 2nd year of experiment (n°15).

In this article, all zootechnical traits, biological indicators and oocyte/embryo data are summarised in 3 tables: Table 1 presents the list of zootechnical traits, biological indicators and hormones, indicators of BPS exposure; Table 2 presents the list of steroids analysed by gas chromatography coupled to tandem mass spectrometry; and Table 3 presents the list of indicators concerning oocyte collection by OPU and IVD.

Experimental design, material and methods

The experimental design of the study, conducted for two consecutive years (2018–2019), is described in Fig. 1. A total of 120 primiparous Ile-de-France ewes (60 different ewes for each year, numbered from 1 to 60 in year 1 and from 61 to 120 in year 2), 2.5 years old on average, were managed in a loose sheepfold at the INRAE Experimental Unit (Unité Expérimentale de Physiologie Animale de l’Orfrasière, Nouzilly, France, <https://doi.org/10.15454/1.5573896321728955E12>). Because of the seasonality of reproduction in sheep, oocyte collection was carried out each year during the breeding season between September and December (from late summer to early winter).

The related research article by Desmarchais et al. (2022), focusing on the results concerning *in vitro* embryo production, only includes data obtained during the 2nd year of the experiment. Another related research article by Téteau et al. (2022) is dedicated

to the results concerning the steroidome analysis, obtained during the 2 years of the experiment.

Experimental groups

For each year (n = 60 different ewes/year), at the beginning of the experiment, the ewes were assigned (according to their age and live weight) to two diet groups named Restricted (R, n = 30) and Well-fed (WF, n = 30) groups (see following section). At the beginning of the experiment, the average age of the ewes was 2.1 ± 0.2 years for the R group and 2.2 ± 0.2 years for the WF group in year 1, 2.8 ± 0.2 years for the R group and 2.8 ± 0.2 years for the WF group in year 2; the average live weight was 60.4 ± 4.1 kg for the R group and 63.5 ± 3.9 kg for the WF group in year 1, 56.9 ± 3.7 kg for the R group and 62.3 ± 4.2 kg for the WF group in year 2; the average body condition score (BCS) was 2.7 ± 0.3 for the R group and 3.1 ± 0.4 for the WF group in year 1, 2.4 ± 0.1 for the R group and 2.7 ± 0.1 for the WF group in year 2.

Among the R and WF groups, ewes were supplemented (see section below) with or without BPS (0, 4 or 50 µg/kg/day), therefore generating six experimental groups named R-0, R-4, R-50, WF-0, WF-4, and WF-50 (n = 10 ewes/group) (Desmarchais et al., 2022).

Diet management and Bisphenol S exposure

Diet management started in May and BPS treatment started in June, that is to say at least three months before oocyte collection (Fig. 2), as detailed in the following sections. Feed was distributed once a day, in the morning, and ewes had free access to water and mineral licks to provide essential micronutrients. The diet, designed using the INRAE recommendations for the growth and maintenance needs of adult, non-pregnant ewes (Agabriel, 2013), consisted of straw *ad libitum* added with dietary supplement in the form of pellets (AXEREAL Elevage, Saint Germain de Salles, France) composed of wheat (60%), alfalfa, sugar cane treacle and vitamins with nutritional values of 6 276 MJ of net energy and 72 g of metabolisable protein per kilogram of DM.

The live weight and BCS (determined using a scale of 0 (emaciated) to 5 (grossly obese) (Russel et al., 1969)) of the ewes were recorded monthly. Ewes were fed in order to reach the goal of a median BCS of 2.0 in R groups and 4.0 in WF groups. The quantity of the diet offered was adjusted to the mean live weight of the animals in each treatment group (Desmarchais et al., 2022). Ewes from R groups (n = 60) received 50% of their daily energy maintenance requirements (0.15 kg of feed per animal, with an average daily intake of 0.941 MJ of net energy and 10.8 g of metabolisable protein) until the target BCS was reached and then 80% of their daily energy maintenance requirements until the end of the experiment. Ewes from WF groups (n = 60) received 165% of their daily energy maintenance requirements (0.50 kg of feed per animal, with an average daily intake of 3 184 MJ of net energy and 36 g of metabolisable protein) throughout the experiment.

For the R and WF groups, BPS was added (R-4, R-50, WF-4, and WF-50 groups) or not (R-0 and WF-0 (groups) in the diet (0, 4 or 50 µg/kg/day) according to the guidelines established for Bisphenol A tolerable daily intake in Europe in 2015 and in 2006), and BPS was added to the diet (in the pellets), and adjusted once a month according to the mean BW of the ewes.

Hormonal treatment for ovum pick-up: oestrus synchronisation and ovarian stimulation

All 120 ewes (Years 1 and 2) were submitted, after oestrus synchronisation and ovarian stimulation according to a protocol adapted from Lahoz et al. (2014), to oocyte collection by OPU with

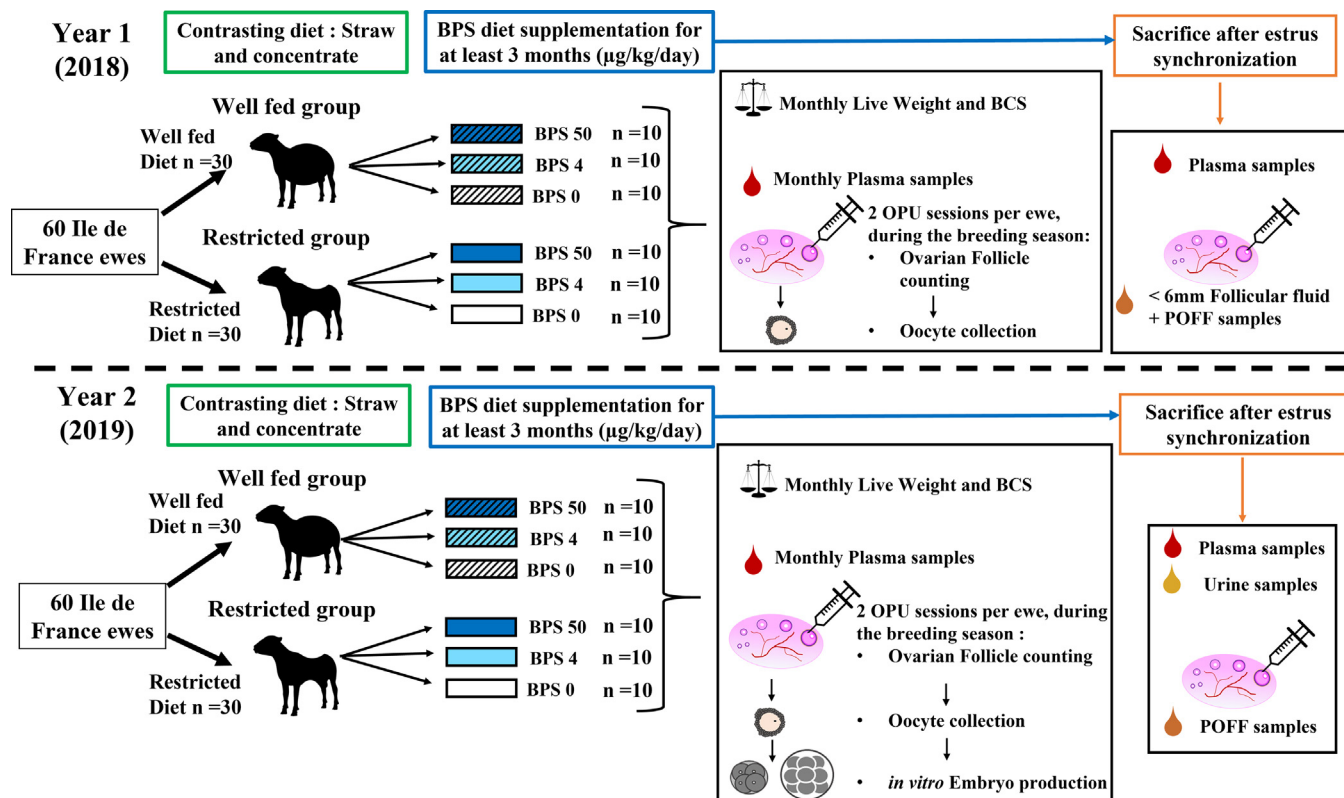


Fig. 1. Experimental design for nutritional management of ewes and Bisphenol S (BPS) exposure, recording of zootechnical traits and collection of biological samples during two consecutive years. During two consecutive years, 120 primiparous ewes were managed in a loose sheepfold. Each year ($n = 60$ different ewes/year), the ewes were assigned to two diet groups, Restricted (R, $n = 30$) and Well-fed (WF, $n = 30$), and were supplemented with or without BPS (0, 4 or 50 $\mu\text{g}/\text{kg}/\text{day}$), generating six experimental groups named R-0, R-4, R-50, WF-0, WF-4, and WF-50 ($n = 10$ ewes/group). Abbreviations: BCS = body condition score, POFF = Preovulatory follicular fluid, OPU = Ovum Pick Up.

two sessions conducted at a 7-day interval (Fig. 2). For technical feasibility, the number of ewes submitted to OPU per day was limited to 10, therefore, each year, the 60 ewes were distributed into six OPU batches (named B1 to B6 in year 1, and B7 to B12 in year 2) of 10 ewes, in order to the six experimental groups (R-0, R-4, R-50, WF-0, WF-4, and WF-50) were distributed into each OPU batch (Fig. 2): one to two ewes from each experimental group in each OPU batch (except for B1 with two or three ewes from R0, WF0, R50 and WF50 groups only). During ovarian stimulation, prior to the first OPU session, ewes received two intramuscular injections of 125 $\mu\text{g}/\text{ewe}$ cloprostenol (Estrumate[®], MSD Santé Animale, Beaucouzé, France) eight days apart, and five days after the second injection, a 30-mg fluorogestone acetate (FGA) vaginal sponge (SYNCHRO-PART[®] 30 mg, CEVA Santé Animale, Libourne, France) was inserted for 12 days, until the first OPU session; this FGA sponge was renewed on the day of the first OPU session to prepare the ewes to the second one, seven days later (Desmarchais et al., 2022). During the three days before each OPU session, each ewe was treated with a protocol of ovarian stimulation (Lahoz et al., 2014) consisting in a total of 32 mg FSH (Stimufol[®], Reprobiol, Liège, Belgium) in five intramuscular injections in decreasing doses: 8 mg (60 h before OPU session), 8 mg (48 h before), 6 mg (36 h before), 6 mg (24 h before), and 4 mg (12 h before). Clo-

prostenol (Estrumate[®], MSD Santé Animale, Beaucouzé, France) was systematically administered (125 $\mu\text{g}/\text{ewe}$) with the first injection of FSH.

Recovery of oocytes by OPU

After undergoing a 12-h fasting period, ewes were anaesthetised intravenously (i.v.) with 0.05 mg/kg xylazine (ROMPUN[®] 2%, Bayer Division Animal Health, La Garenne-Colombes, France) and 2–4 mg/kg ketamine (IMALGENE[®], Merial, Lyon, France), then, anaesthesia was maintained with inhalation of 2.5% isoflurane. Follicular puncture procedure was performed using endoscopy. All visible ovarian follicles with a diameter ≥ 2 mm were punctured and aspirated with an Ova-Stiff[™] EchoTip[®] needle (Cook medical, Ireland), linked to a vacuum pump. Cumulus oocyte complexes (COCs) from both ovaries were individually collected for each ewe into a 15 mL Falcon tube containing phosphate buffer saline at 38.5 °C. Throughout the surgery, the number and diameter of punctured follicles were measured for each ewe and OPU session, using a 2 mm scale positioned on the needle. Following the procedure, warm physiological saline (0.9% NaCl) was administered into the abdominal cavity before closing trocar holes with surgical staples (Desmarchais et al., 2022). At the conclusion of the intervention, a long-acting non-

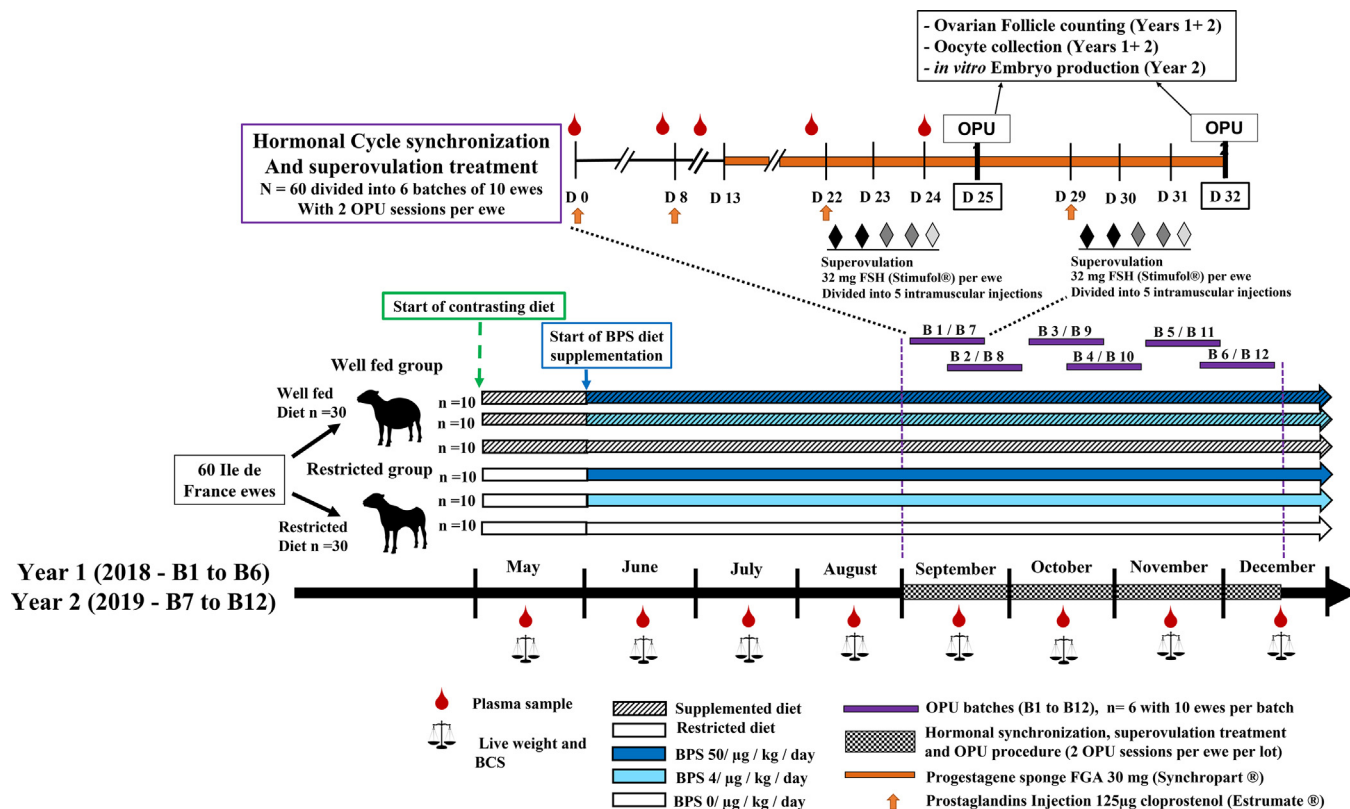


Fig. 2. Experimental design for hormonal treatment (oestrus synchronisation and ovarian stimulation) and recovery of oocytes by ovum pick-up in sheep. During two consecutive years, the ewes (n = 60 different ewes/year) were assigned to two diet groups, Restricted (R, n = 30) and Well-fed (WF, n = 30), and were supplemented with or without Bisphenol S (BPS) (0, 4 or 50 µg/kg/day), generating six experimental groups named R-0, R-4, R-50, WF-0, WF-4, and WF-50 (n = 10 ewes/group). Diet management started in May and BPS exposure started in June, that is to say at least three months before oocyte collection by ovum pick up performed during the breeding season of sheep (between September and December), after sequential oestrus synchronisation and superovulation treatment (12 batches of 10 ewes -purple rectangle- over the two years, with six batches/year). During sessions (years 1 and 2), ovarian follicles were counted and cumulus oocyte complexes were collected; *in vitro* embryo production was performed only during year 2. Abbreviations: B = batch, BCS = body condition score, D = day, OPU = ovum pick up, FGA = fluorogestone acetate.

steroidal anti-inflammatory drug (2 mg/kg live weight of flunixin meglum) was administered intramuscularly.

In vitro maturation, *in vitro* fertilisation and *in vitro* embryo development

In vitro maturation (IVM), *in vitro* fertilisation (IVF) and IVD were performed only in year 2, the results being presented in the related research article focusing on embryo production (Desmarchais et al., 2022). In year 2, cumulus–oocyte complexes recovered from each ewe, were then subjected to *in vitro* maturation, fertilisation and embryo development procedures (Fig. 3).

First, COCs were placed in 4-well dishes up to 13 COC per 100 µL of modified TCM199 maturation medium (as described in Desmarchais et al., 2020) and then covered with mineral oil. *In vitro* maturation lasted 24 h at 38.8 °C in a humidified atmosphere with 5% CO₂. Then, COC were subjected to *in vitro* fertilisation consisting in a 24–h co-incubation with spermatozoa from frozen ram semen, in IVM medium drops covered with mineral oil, at 38.8 °C under a humidified atmosphere with 5% CO₂ in air (as described in Desmarchais et al., 2020). Subsequently, after removing cumulus cells, presumptive zygotes of each ewe were bathed and underwent IVD in a modified Synthetic Oviductal Fluid medium covered with mineral oil. On day two of IVD, foetal calf serum was added to each medium drop to enable embryo development as blastocyst. Cleavage rates and embryo development were evaluated on days two and six of IVD using a Zeiss inverted microscope (Zeiss,

Germany) and SPOT 4.5 software. On day two of development, the total number of cleaved embryos and > 4 cell cleaved embryo were recorded. On the day seven of development, the total number of early unexpanded blastocysts and expanded blastocysts were recorded (Fig. 3). These data enabled us to evaluate cleavage rates (total number of cleaved embryos divided by the total number of fertilised COC) and blastocyst rates (total number of blastocysts divided by the number of cleaved embryos).

Oestrus synchronisation before sacrifice

After the two consecutive OPU sessions, ewes underwent oestrus synchronisation (in batches of six to eight ewes including one to two ewes per experimental group) using a 20-mg FGA vaginal sponge (Chronogest® CR Eponge, MSD Santé Animale, Beaucauzé, France) during 11 days. Then, an intramuscular injection of eCG (equine chorionic gonadotrophin, Synchro-Part® PMSG Bovins-Ovins-Caprins, CEVA Santé Animale, Libourne, France) was administered at the dose of 400 UI/ewe to stimulate final follicular growth. Two days after eCG administration and oestrus synchronisation, ewes were sacrificed at the stage of the presumptive pre-ovulation, three weeks after the second OPU session. Ewes were sacrificed after electronarcosis in the experimental slaughterhouse of the INRAE Experimental Unit PAO (Nouzilly, France, <https://doi.org/10.15454/1.5573896321728955E12>). Within 15 min after death, for each ewe, blood, urine, and pre-ovulatory follicle fluid (POFF) were then collected.

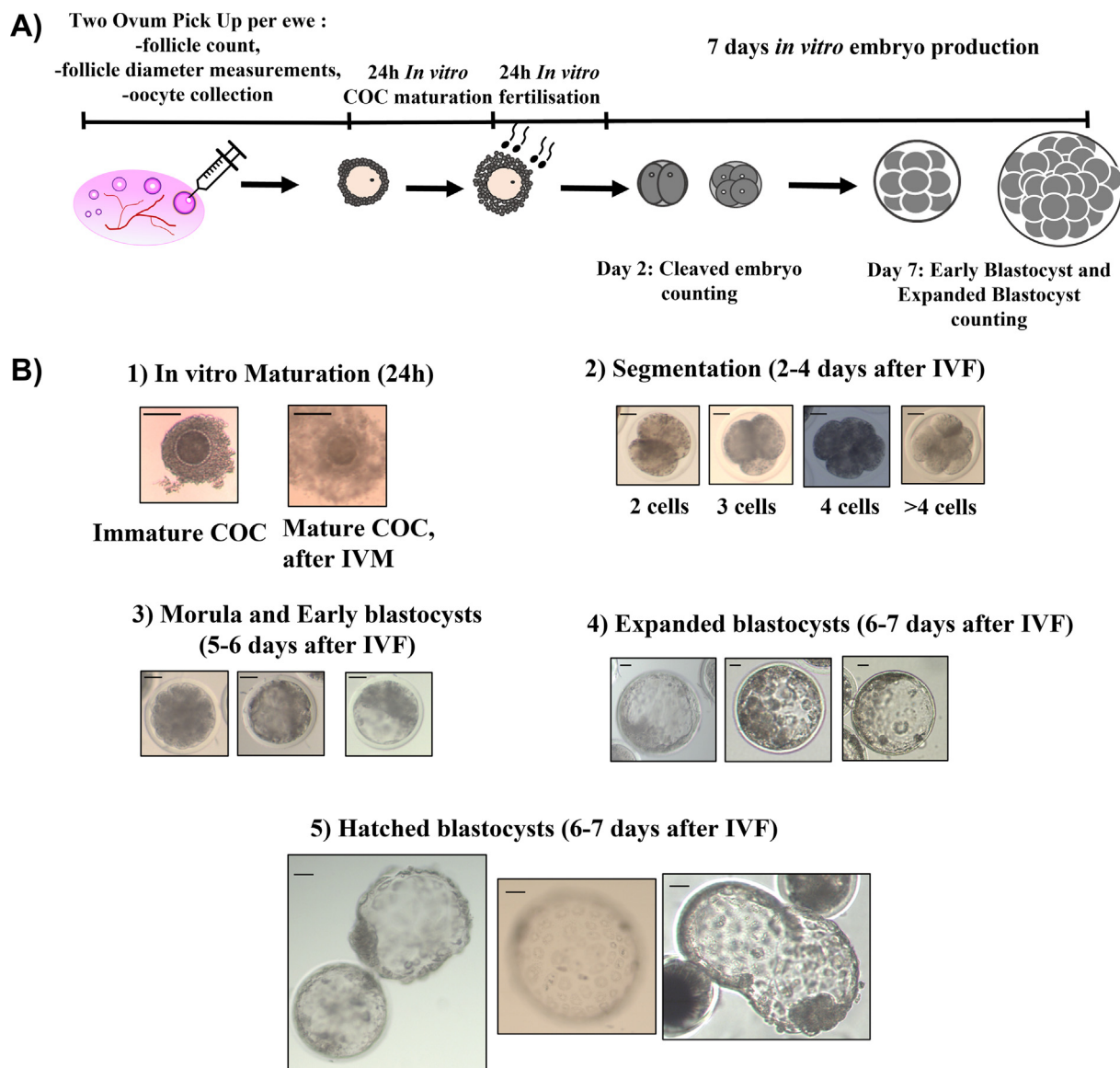


Fig. 3. (A) Experimental design for recovery of cumulus-oocyte complexes by Ovum Pick Up in sheep, followed by *in vitro* maturation, *in vitro* fertilisation and *in vitro* embryo development; (B) Illustrations for evaluation of ovine cumulus-oocyte complexes and embryos according to their developmental stage. Images were acquired using a Zeiss inverted microscope (Zeiss, Germany) and SPOT 4.5 software. Scale bar represents 100 μ m. Abbreviations: COC = Cumulus oocyte complexes, IVM = *in vitro* maturation, IVF = *in vitro* fertilisation.

Biological samples and assays

Biological samples and methods for assays are described in [Table 1](#).

Plasma

Blood samples were collected after jugular venipuncture in 5 mL heparinised tubes (17 IU/mL sodium heparin, Vacutainer®; Becton Dickinson and Company, Le Pont de Claix, France). After centrifugation (3 700 g for 30 min at 4 °C), plasma aliquots were stored at -20 °C until analysis.

During years 1 and 2, plasma samples were collected ([Figs. 1 and 2](#)):

- monthly and at OPU1 for non-esterified fatty acids and glucose assays,
- 1 and 5 days before OPU1 for progesterone assay,

- at the time of sacrifice for steroidome assays,
- before exposure to BPS and the day before OPU1 for BPS and BPS-g assays.
- Only in year 2, plasma samples were collected:
- at OPU1 for total thyroxine, free thyroxine and free triiodothyronine assays,
- the day before OPU1 for oestradiol assay,
- 12 days before OPU1 for Anti-Mullerian hormone assay.

Follicular fluid and urine

After sacrifice and ovarian punctures, follicular fluid samples from pre-ovulatory follicles (follicles with diameter \geq 6 mm) were isolated from granulosa cells by centrifugation (9 600 g for 3 min, 4 °C) and then stored at -80 °C until analysis.

Pre-ovulatory follicular fluid samples were collected at the time of sacrifice and used for steroidome assays. Among these samples, those of the 2nd year were used for progesterone and oestradiol assays.

Table 1

List of zootechnical traits, biological indicators and hormones, and indicators of Bisphenol S (BPS) exposure in ewes with contrasted metabolic status and submitted to chronic BPS exposure [for each indicator, the number of corresponding datafile in the dataset is indicated in the 1st column].

Datafile number	Indicator	Biological samples	Abbreviation (Unit)	Year	Number of ewes	day and/or frequency of sampling	Methods –Technologies	Equipment
Zootechnical traits								
1	Age	Animal	Age (year)	Y1 + Y2	120	At the beginning of the experiment		
2	Live weight	Animal	BW (kg)	Y1 + Y2	120	once a month during the experiment	Weighing	
2	Body condition score	Animal	BCS	Y1 + Y2	120	once a month during the experiment	visual assessment and palpation	BCS was determined using a scale from 0 (emaciated) to 5 (grossly obese) ¹
Biological indicators and hormones								
3	Non-esterified fatty acids	Plasma	NEFA (µM)	Y1 + Y2	120	once a month during the experiment, and at the time of first OPU session	Colorimetric enzymatic method	Konelab 20 analyzer (Thermo Scientific, Gometz le Châtel, France) and kits provided by Sobioda (Montbonnot, France) ²
3	Glucose	Plasma	Gluc (mg/L)	Y1 + Y2	120	once a month during the experiment, and at the time of first OPU session	Colorimetric enzymatic method	Konelab 20 analyzer (Thermo Scientific, Gometz le Châtel, France) and kits provided by Thermo Scientific (Villebon sur Yvette, France) ²
4	Total thyroxine	Plasma	T4T (ng/mL)	Y2	60	Day before the 1st OPU	T4 Elisa Assay Spectrophotometry	DIAsource ImmunoAssays® S.A., Louvain La Neuve, Belgium
4	Free thyroxine	Plasma	FT4 (pg/mL)	Y2	60	Day before the 1st OPU	FT4 Elisa Assay Spectrophotometry	Absorbance was measured at 450 nm with the Sunrise-basic plate reader spectrophotometer (TECAN Life Sciences, Switzerland) and the Magellan software.
4	Free triiodothyronine	Plasma	FT3 (pg/mL)	Y2	60	Day before the 1st OPU	FT3 Elisa Assay Spectrophotometry	
5	Anti-Mullerian hormone	Plasma	AMH (pg/mL)	Y2	60	12 days before the 1st OPU session (5 days after second cloprostenol injection on FGA sponge insertion day).	Elisa assay Spectrophotometry	AMH Gen II ELISA assay (Beckman Coulter, Villepinte, France) ³ .
6	Progesterone	Plasma	P4_P (ng/mL)	Y1 + Y2	120	5 and 1 day(s) before the 1st OPU	Elisa Assay Spectrophotometry	Absorbance was measured at 405 nm with the Sunrise-basic plate reader spectrophotometer (TECAN Life Sciences, Switzerland) and the Magellan software ⁴ .
8	Progesterone	Preovulatory Follicular Fluid	P4-FF (ng/mL)	Y1 + Y2	120	Postmortem, at presumptive oestrus after oestrus synchronisation	Elisa Assay Spectrophotometry	Cf. P4 in plasma.
7	Estradiol	Plasma	E2_P (pg/mL)	Y2	60	Day before the 1st OPU	Elisa Assay Spectrophotometry	E2-EASIA-kit DIAsource ImmunoAssays® S.A., Louvain La Neuve, Belgium, adapted for oestradiol detection in ovine plasma
8	Estradiol	Preovulatory Follicular Fluid	E2-FF (ng/mL)	Y1 + Y2	120	Postmortem, at presumptive oestrus after oestrus synchronisation	Elisa Assay Spectrophotometry	Absorbance was measured at 450 nm with the Sunrise-basic plate reader spectrophotometer (TECAN Life Sciences, Switzerland) and the Magellan software ⁵ .
9 & 10	Steroidome (see Table 2 for complete list of steroids)	Plasma (pools)	See Table 2 for details	Y1 + Y2	68 (4–6 biological replicates containing 3–4 ewes)	Postmortem, at presumptive oestrus after oestrus synchronisation	Gas chromatography coupled with tandem mass spectrometry	AI1310 Autosampler Trace 1310 GC TSQ 8000 tandem mass spectrometer MS/MS (Thermo Scientific)
9 & 11	Steroidome (see Table 2 for complete list of steroids)	Pre ovulatory Follicular fluid (pools)	See Table 2 for details	Y1 + Y2	69 (4–6 biological replicates containing 3–4 ewes)	Postmortem, at presumptive oestrus after oestrus synchronisation		Signal analysis with Excalibur 3.0 software (Thermo Scientific)

(continued on next page)

Table 1 (continued)

Datafile number	Indicator	Biological samples	Abbreviation (Unit)	Year	Number of ewes	day and/or frequency of sampling	Methods –Technologies	Equipment
12	Indicators of Bisphenol S exposure Bisphenol S	Plasma	BPS_P (nM)	Y2	60	before the beginning of the exposure and on the day before 1st OPU.	Liquid chromatography-mass spectrometry operated with positive electrospray ionisation and Multiple Reaction Monitoring mode.	Acquity U-HPLC device coupled to a Xevo-TQ triple quadrupole mass spectrometer ⁶
12	Bisphenol S glucuronide	Plasma	BPS_g_P (nM)	Y2	60	before the beginning of the exposure and on the day before 1st OPU.	Chromatographic separation Waters Phenyl-Hexyl U-HPLC column (2.1 × 100 mm; 1.6 µm) with an acidified H ₂ O/AcN gradient elution (0.3 mL/min, 40 °C) ⁶ .	Chromatographic separation Waters Phenyl-Hexyl U-HPLC column (2.1 × 100 mm; 1.6 µm) with an acidified H ₂ O/AcN gradient elution (0.3 mL/min, 40 °C) ⁶ .
13	Bisphenol S	Urine	BPS_U (nM)	Y2	6	Postmortem after synchronised estrus	Chromatographic data were monitored using Targetlynx [®] software (Waters Corporation).	
13	Bisphenol S glucuronide	Urine	BPS_g_U (nM)	Y2	6	Postmortem after synchronised estrus		

Abbreviations: Y = Year, FCA = fluorogestone acetate, OPU = Ovum Pick Up.

- ¹ Russel et al., 1969.
- ² Baéza et al., 2015.
- ³ Rico et al., 2009.
- ⁴ Canepa et al., 2008.
- ⁵ Fabre-Nys et al., 2015.
- ⁶ Desmarchais et al., 2022.

Table 2

List of steroids analysed by gas chromatography coupled to tandem mass spectrometry in plasma and pre-ovulatory follicular fluid of ewes with contrasted metabolic status and submitted to chronic Bisphenol S (BPS) exposure [corresponding datafiles: 9, 10 & 11].

Steroids*	Abbreviation
Pregnenolone	PREG
20α-dihydropregnenolone	20α-DHPREG
17α-hydroxypregnenolone	17α-OHPREG
Progestagens	
Progesterone	PROG
5α-dihydroprogesterone	5α-DHP
5β-dihydroprogesterone	5β-DHP
20α-dihydroprogesterone	20α-DHP
3α5α-tetrahydroprogesterone	3α5α-THP
3α5β-tetrahydroprogesterone	3α5β-THP
3b5α-tetrahydroprogesterone	3b5α-THP
3α5α20α-hexahydroprogesterone	3α5α20α-HHP
3α5β20α-hexahydroprogesterone	3α5β20α-HHP
3b5α20b-hexahydroprogesterone	3b5α20b-HHP
3b5α20α-hexahydroprogesterone	3b5α20α-HHP
17α-hydroxyprogesterone	17α-OHP
16α-hydroxyprogesterone	16α-OHP
Androgens	
Testosterone	T
5α-dihydrotestosterone	5α-DHT
5β-dihydrotestosterone	5β-DHT
Dehydroepiandrosterone	DHEA
Androstenediol	ADIOL
Androstenedione	ADIONE
5α-dihydroandrostenedione	5α-DHADIONE
Estrogens	
Estradiol	E2
Estrone	E1
Estriol	E3
2-methoxyestradiol	2-ME2
2-hydroxyestradiol	2-OH-E2
Glucocorticoids	
Cortisol	F
Cortisone	E
11-deoxycortisol	11-deoxyF
3α5β-tetrahydrocortisol	3α5β-THF
Mineralocorticoids	
Corticosterone	B
11-dehydrocorticosterone	11-dehydroB
3α5α-tetrahydrodeoxycorticosterone	3α5α-THDOC

* Units: ng/mL for all steroids analysed in follicular fluid, ng/mL for steroids analysed in plasma except for Progesterone, 5α-dihydroprogesterone, Testosterone

Only in year 2, urine samples were collected at the time of sacrifice for BPS and BPS-g assays, by bladder puncture and stored at –80 °C until analysis.

Peer Review Summary

Peer Review Summary for this article (<https://doi.org/10.1016/j.anopes.2024.100088>) can be found at the foot of the online page, in Appendix A.

Ethics approval

All experimental procedures were conducted in accordance with the European Directive 2010/63/EU on the protection of animals used for scientific purposes and approved by the French Ministry of National Education, Higher Education, Research and Innovation after ethical assessment by the local ethics committee “Comité d’Ethique en Expérimentation Animale Val de Loire (CEEA

Table 3

List of indicators concerning oocyte collection by Ovum-Pick Up and *in vitro* embryo development in ewes with contrasted metabolic status and submitted to chronic Bisphenol S (BPS) exposure [corresponding datafiles: 14 & 15].

Indicators	Day of data collection	Year (number of ewes)
Nb punctured follicles	OPU day	Year 1 and 2 (n = 120)
Nb 2 mm follicles	OPU day	Year 1 and 2 (n = 120)
% 2 mm follicles/punctured follicles	OPU day	Year 1 and 2 (n = 120)
Nb 3–5 mm follicles	OPU day	Year 1 and 2 (n = 120)
% 3–5 mm follicles/punctured follicles	OPU day	Year 1 and 2 (n = 120)
Nb ≥ 6 mm follicles	OPU day	Year 1 and 2 (n = 120)
% ≥ 6 mm follicles/punctured follicles	OPU day	Year 1 and 2 (n = 120)
Nb recovered COC	OPU day	Year 1 and 2 (n = 120)
% recovered COC/punctured follicles	OPU day	Year 1 and 2 (n = 120)
Nb oocytes in IVM	OPU day	Year 2 (n = 60)
% oocytes in IVM/recovered COC	OPU day	Year 2 (n = 60)
Nb cleaved embryos	Day 2 of IVD	Year 2 (n = 60)
% cleaved embryos/oocytes in IVM	Day 2 of IVD	Year 2 (n = 60)
Nb Embryos 2–4 cells	Day 2 of IVD	Year 2 (n = 60)
Nb Embryos > 4 cells	Day 2 of IVD	Year 2 (n = 60)
% Embryos > 4 cells/cleaved embryos	Day 2 of IVD	Year 2 (n = 60)
Nb blastocysts	Day 7 of IVD	Year 2 (n = 60)
% blastocysts/cleaved embryos	Day 7 of IVD	Year 2 (n = 60)
Nb early blastocysts (non-expanded)	Day 7 of IVD	Year 2 (n = 60)
% early blastocysts /cleaved embryos	Day 7 of IVD	Year 2 (n = 60)
Nb expanded blastocysts	Day 7 of IVD	Year 2 (n = 60)
% expanded blastocysts/cleaved embryos	Day 7 of IVD	Year 2 (n = 60)
Nb hatched blastocysts	Day 7 of IVD	Year 2 (n = 60)

Abbreviations: Nb = Number of, COC = Cumulus oocyte complexes, IVM = *in vitro* maturation, IVD = *in vitro* development, OPU = Ovum Pick Up.

VdL)" (protocols registered under APAFIS numbers 13965–20180 42008519239v2 and 14014–2018030717477406v2).

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) did not use any AI and AI-assisted technologies.

Author ORCIDs

Alice Desmarchais: <https://orcid.org/0000-0002-6937-214X>.

Sandrine Fréret: <https://orcid.org/0000-0002-3179-0506>.

Svetlana Uzbekova: <https://orcid.org/0000-0002-6989-6904>.

Virginie Maillard: <https://orcid.org/0000-0001-9306-4545>.

Sebastien Elis: <https://orcid.org/0000-0002-0376-0641>.

Author contributions

Alice Desmarchais: Methodology, Validation, Formal analysis, Investigation, Visualisation, Writing – original draft, Reviewing and Editing.

Sandrine Fréret: Validation, Formal analysis, Visualisation, Data curation, Writing – reviewing & editing.

Svetlana Uzbekova: Reviewing and Editing.

Virginie Maillard: Reviewing and Editing.

Sebastien Elis: Methodology, Validation, Formal analysis, Investigation, Visualisation, Writing – reviewing & editing, Supervision, Project administration, Funding acquisition.

Declaration of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors would like to thank Olivier Lasserre and the “ruminants” team in the Experimental Unit “PAO” (INRAE, Nouzilly) for taking care of animals, Nathalie Kasal Hoc, Juliette Cognié, and the Pixanim platform (INRAE, Nouzilly) for OPU sessions, Jean-Philippe Dubois and Sylvain Bourgeois (experimental slaughterhouse, Experimental Unit PAO, INRAE, Nouzilly) and the Endocrinology and Phenotyping Laboratory (“Physiology of reproduction and behavior” Unit, INRAE Nouzilly) for progesterone assay.

Financial support statement

This work was financially supported by INRAE, “Centre-Val de Loire” Region (Bemol project, APR IR 2017-00117108), the French National Research Agency (project ANR-18-CE34-0011-01 MAMBO) and the BioMedecine Agency (Project 18AMP006 FertiPhenol).

References

- Agabriel, J., 2013. INRA Alimentation des Bovines, Ovins et Caprins: Besoins des Animaux – Valeurs des Aliments. Tables INRA 2007, mise à jour 2010. Editions Quae, Versailles, France.
- Baéza, E., Jégou, M., Gondret, F., Lalande-Martin, J., Tea, I., Le Bihan-Duval, E., Berri, C., Collin, A., Métayer-Coustard, S., Louveau, I., Lagarrigue, S., Duclos, M.J., 2015. Pertinent plasma indicators of the ability of chickens to synthesize and store lipids1. *Journal of Animal Science* 93, 107. <https://doi.org/10.2527/jas.2014-8482>.
- Canepa, S., Laine, A.-L., Fagu, A., Flon, C., Monniaux, D., 2008. Validation d'une méthode immunoenzymatique pour le dosage de la progesterone dans le plasma des ovins et des bovins. *Les Cahiers Techniques De L'INRA* 64, 19.
- Desmarchais, A., Têteau, O., Papillier, P., Jaubert, M., Druart, X., Binet, A., Maillard, V., Elis, S., 2020. Bisphenol S impaired *in vitro* ovine early developmental oocyte competence. *International Journal of Molecular Science* 21, 1238. <https://doi.org/10.3390/ijms21041238>. PMID: 32059612; PMCID: PMC7072985.
- Desmarchais, A., Têteau, O., Kasal-Hoc, N., Cognié, J., Lasserre, O., Papillier, P., Lacroix, M., Vignault, C., Jarrier-Gaillard, P., Maillard, V., Binet, A., Pellicer-Rubio, M.-T., Fréret, S., Elis, S., 2022. Chronic low BPS exposure through diet impairs *in vitro* embryo production parameters according to metabolic status in the ewe. *Ecotoxicology and Environmental Safety* 229, 113096. <https://doi.org/10.1016/j.ecoenv.2021.113096>.
- Fabre-Nys, C., Chanvallon, A., Debus, N., François, D., Bouvier, F., Dupont, J., Lardic, L., Lomet, D., Ramé, C., Scaramuzzi, R.J., 2015. Plasma and ovarian oestradiol and the variability in the LH surge induced in ewes by the ram effect. *Reproduction* 149, 511. <https://doi.org/10.1530/REP-14-0587>.
- Lahoz, B., Alabart, J.L., Cocero, M.J., Monniaux, D., Echegoyen, E., Sanchez, P., Folch, J., 2014. Anti-Müllerian hormone concentration in sheep and its dependence of age and independence of BMP15 genotype: an endocrine predictor to select the best donors for embryo biotechnologies. *Theriogenology* 81, 347–357. <https://doi.org/10.1016/j.theriogenology.2013.09.033>.
- Rico, C., Fabre, S., Medigue, C., Clemente, N.D., Clement, F., Bontoux, M., Touze, J.-L., Dupont, M., Briant, E., Remy, B., Beckers, J.-F., Monniaux, D., 2009. Anti-müllerian hormone is an endocrine marker of ovarian gonadotropin-responsive follicles and can help to predict superovulatory responses in the cow. *Biology of Reproduction* 80, 50. <https://doi.org/10.1095/biolreprod.108.072157>.
- Russel, A.J.F., Doney, J.M., Gunn, R.G., 1969. Subjective assessment of body fat in live sheep. *Journal of Agricultural Science* 72, 451–454. <https://doi.org/10.1017/S0021859600024874>.
- Têteau, O., Lière, P., Pianos, A., Desmarchais, A., Lasserre, O., Papillier, P., Vignault, C., Lebachelier de la Rivière, M.-E., Maillard, V., Binet, A., Uzbekova, S., Saint-Dizier, M., Elis, S., 2022. Bisphenol S alters the steroidome in the preovulatory follicle, oviduct fluid and plasma in ewes with contrasted metabolic status. *Frontiers in Endocrinology (lausanne)* 13, 892213. <https://doi.org/10.3389/fendo.2022.892213>. PMID: 35685208; PMCID: PMC9172638.