Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

The Toulouse GOLD Cohort: a prospective canine birth cohort investigating early-life determinants of health and disease into adulthood

Aurelien Grellet

aurelien.grellet@envt.fr

NeoCare, ENVT, Université de Toulouse

Ilyas Bendahmane

NeoCare, ENVT, Université de Toulouse

Chloe Chauvel

NeoCare, ENVT, Université de Toulouse

Laurie Milesi

NeoCare, ENVT, Université de Toulouse

Jessie Brun

ENVT, Université de Toulouse

Petra Rouch-Buck

ENVT, Université de Toulouse

Amelie Marchand

ENVT, Université de Toulouse

Christelle Baur

NeoCare, ENVT, Université de Toulouse

Ines Khemri

NeoCare, ENVT, Université de Toulouse

Ugo Beaujardin

NeoCare, ENVT, Université de Toulouse

Jullia Landi Sato

NeoCare, ENVT, Université de Toulouse

Patricia Ronsin

NeoCare, ENVT, Université de Toulouse

Jean-Sébastien Desmet

ENVT, Université de Toulouse

Océane Richard

Estelle Legendre

ENVT, Université de Toulouse

Laurie Michaud
ENVT, Université de Toulouse
Marie Nguyen
ENVT, Université de Toulouse
Muhammad Zahid Tahir
NeoCare, ENVT, Université de Toulouse
Marie Christine Cadiergues
Toulouse Institute for Infectious and Inflammatory Diseases (INFINITy), Université de Toulouse, INSERM, CNRS
Hanna Mila
NeoCare, ENVT, Université de Toulouse

Article

Keywords: Dog, Cohort, Biobank, DOHaD, Neonatalogy, Chronic non-communicable diseases

Posted Date: November 23rd, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11284516/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: No competing interests reported.

The Toulouse GOLD Cohort: a prospective canine birth cohort investigating early-life determinants of health and disease into adulthood

Aurelien Grellet[1*], Ilyas Bendahmane[1,2], Chloe Chauvel[1], Laurie Milesi[1], Jessie Brun[3], Petra Rouch-Buck[1,3], Amelie Marchand[3], Christelle Baur[1], Betty Duc[1], Ines Khemri[1], Ugo Beaujardin[1], Jullia Landi Sato[1], Patricia Ronsin[1], Jean-Sébastien Desmet[1], Océane Richard[1], Estelle Legendre[1], Laurie Michaud[1], Marie Nguyen[1], Muhammad Zahid Tahir[1], Marie Christine Cadiergues[3,4], Hanna Mila[1]

1. NeoCare, ENVT, Université de Toulouse, Toulouse, France
2. GenPhySE, Université de Toulouse, INRAE, ENVT, Castanet Tolosan, France
3. Small Animal Clinics, ENVT, Université de Toulouse, Toulouse, France
4. Toulouse Institute for Infectious and Inflammatory Diseases (INFINITy), Université de Toulouse, INSERM, CNRS, Toulouse, France.

*Corresponding author. E-mail: Aurelien.grellet@envt.fr

Abstract

Early-life exposures can induce lasting biological changes that shape health trajectories, yet their contribution to disease development remains poorly characterized in dogs. The Toulouse GOLD Cohort is a prospective longitudinal birth cohort designed to investigate how maternal, paternal, environmental, nutritional, microbial, and management-related factors interact during early life to influence health from conception to adulthood. Golden Retrievers undergo standardized longitudinal phenotyping, clinical monitoring, owner-reported assessments, and repeated biological sampling. The initial Early Cohort comprised 123 puppies from 18 dams. Stillbirth and neonatal mortality rates were 5.7% and 1.7%, respectively, resulting in 7.3% mortality by two months. Early growth trajectories and reference centile curves were generated from 6,479 body-weight measurements collected in 120 puppies during the first 56 days of life. All 113 puppies remaining in the programme underwent clinical examination and biological sampling at 18 months. The continuously expanding biobank currently contains over 10,000 samples collected across developmental stages. By integrating early-life exposures with lifelong phenotyping and repeated biosampling, this cohort provides a unique framework for identifying developmental determinants, biomarkers, and critical windows associated with subsequent health and disease in dogs.

Keywords
Dog, Cohort, Biobank, DOHaD, Neonatalogy, Chronic non-communicable diseases

1. Introduction

In the early 1950s, Dr David Barker demonstrated that children born to food-restricted mothers were more likely to exhibit low birth weight and to develop cardiomyopathy in adulthood [1,2]. These initial observations led to the emergence of a revolutionary theory of the Developmental Origins of Health and Disease (DOHaD). According to the DOHaD concept, events occurring during the perinatal period may induce physiological changes that ensure survival but persist throughout an individual's life and may have consequences for postnatal development and long-term health [3].

Longitudinal studies monitoring health status are one of the most powerful approaches for determining the effects of environmental and lifestyle factors on disease development [4]. In humans, long-term birth cohorts that follow individuals from early life through childhood, adolescence, and adulthood are invaluable tools for understanding the developmental origins of health and disease. By capturing environmental, nutritional, microbial, genetic, and socioeconomic exposures during critical windows of development, these cohorts provide unique insights into the pathways leading to chronic diseases later in life [5]. Such longitudinal approaches have substantially improved our understanding of immune-mediated disorders, such as atopic dermatitis, by identifying early-life determinants of disease onset, persistence, and progression [6,7]. Similarly, birth cohorts help elucidate the origins of chronic gastrointestinal disorders and recurrent diarrheal diseases through the characterization of host–microbiome interactions and environmental exposures during infancy [8]. Overall, these studies are instrumental in investigating the developmental origins of many non-communicable diseases, including obesity, metabolic syndrome, allergic conditions, cardiovascular disease, and type 2 diabetes in addition to those already cited [9]. The integration of multi-omics technologies, environmental monitoring, and long-term clinical follow-up now offers new opportunities to identify early biomarkers, uncover disease mechanisms, and develop preventive strategies across the life course.

Despite major advances in veterinary medicine, chronic disorders such as obesity, chronic enteropathies, and atopic dermatitis remain among the most common reasons for veterinary consultations in dogs, representing a substantial burden for animal welfare and healthcare. The prevalence of overweight and obesity is estimated to affect 6-44 % of the canine

population worldwide [10,11]. Gastrointestinal disorders are among the most frequent problems and account for approximately 10–30% of consultations in primary veterinary practice, and canine atopic dermatitis is currently estimated to affect over 10 per cent of the general canine population in industrialised countries [12–14]. Although these chronic diseases are highly prevalent, their etiologies remain only partially understood. This highlights the need to investigate critical developmental periods in dogs (i.e., gestation, neonatal period and early pediatric period), during which key life events may influence long-term health trajectories and ultimately affect the risk of chronic disease in adulthood.

To date, six major canine longitudinal studies are ongoing: the Dogslife cohort, the Golden Retriever Lifetime Study, the Generation Pup, the Dog Ageing Project, Mars Petcare Biobank, VetCompass Australia and VetCompass UK [15]. The objectives of these cohorts are to identify the environmental, genetic, and lifestyle factors that influence ageing in dogs and the risk of common disorders, mainly cancers, and behavioural problems. Nevertheless, none of these cohorts has evaluated the influence of gestation and early life on short, medium, and long-term health.

This work aims to describe the Toulouse GOLD Cohort (TGC), a prospective longitudinal birth cohort established to collect comprehensive data on Golden Retriever dogs from the fetal stage through adulthood. The overall objective of TGC is to investigate how maternal, paternal, environmental, nutritional, microbial, and management-related factors interact during early life to shape health trajectories across the lifespan. More specifically, the cohort has been designed to address several major research questions, including: (i) the course of pregnancy and parturition and their influence on neonatal health; (ii) the immunological, hormonal, nutritional, and microbial composition of maternal milk and their associations with neonatal development; (iii) the physiology of neonatal adaptation during the first weeks of life; (iv) growth trajectories from birth to adulthood; (v) the consequences of the weaning transition particularly weaning-associated diarrhea and behavioral disorder; (vi) the developmental origins of major chronic non-communicable diseases, including chronic diarrhea, obesity, and atopic dermatitis; and (vii) the contribution of paternal programming to offspring development and long-term health. Through the integration of detailed longitudinal phenotyping, biological sampling, and environmental data, the cohort aims to identify critical developmental windows and actionable determinants that may improve canine health throughout life (Figure 1).

2. Methods/design

2.1. Setting up the project

The TGC was created by the NeoCare research unit of the National Veterinary School of Toulouse (ENVT, France). In June 2021, the Visio Foundation officially funded the establishment of the Early Cohort (EC), a prospective longitudinal cohort designed to enroll and follow 100 dogs from birth to 18 months of age. In order to make this project possible, a steering committee was assembled, consisting of two senior research leaders and veterinarians (H.M., A.G.), a veterinary specialist in behavioural medicine (O.R.), two veterinary clinicians in nutrition (P.B.R) and preventive medicine (P.B.R., P.R) at the vet teaching hospital, a PhD student (Q.G.), an administrator (B.D.), and three veterinary students (L.M., E.L., M.N.). The steering committee was responsible for developing the project's infrastructure. Its responsibilities included establishing the maternity unit, developing a selection programme for breeding females, identifying partner breeders to provide bitches, prioritising data collection topics, submitting the project to the ethics committee, developing consent forms and participant contracts, and establishing the project's communication strategy, including the creation of the cohort name, website, and logo. Moreover, the project was submitted to the decentralized state services (Directions Départementales de la Protection des Populations, DDPP) in order to define the legal status of the cohort. Given that no invasive procedures were planned to be performed beyond routine high-quality clinical monitoring, the cohort was registered as a breeding kennel with the French competent authorities (registration no.2022-0017721).
The study protocol was reviewed by the Ethics Committee of the École Nationale Vétérinaire de Toulouse (Science et Santé Animales Ethics Committee, Committee No. 115). The protocol underwent full ethical review and received a favourable opinion from the Committee (reference SSA_2023_007). All procedures were carried out in accordance with applicable French legislation, institutional animal-welfare requirements, and relevant veterinary guidelines.

In February 2022, a dedicated maternity unit was opened, comprising three whelping pens that could accommodate up to three bitches from late gestation until the end of lactation, as well as their puppies (Supplementary file 1). The pens were separated by plastic sandwich panels, perforated along part of their length to allow the female dog privacy during whelping and to provide visibility of the surrounding area when needed. Each $8m^2$ pen was equipped with a whelping box, a sleeping area raised off the ground, wooden bedding, bowls, toys, a heating lamp, and a surveillance camera to continuously monitor the mothers' behavior remotely as

their whelping date approached, while minimizing disturbance. The whelping unit was also equipped with a hygro-thermometer for continuous monitoring of the room temperature and humidity, with the aim of maintaining a temperature of approximately 24°C at the unit level, and 26-30°C at the level of the puppies during the first three weeks of life. Additionally, access to an outdoor area (a relaxation zone) of 80 m^2 was provided for the brood bitch and its puppies.

2.2. Design of the Early Cohort

Eighteen Golden Retriever females from a French partner breeding kennel were enrolled in the EC over two consecutive years. The health status of the selected females was assessed by clinical examination, body weight and body condition, screening for hip and elbow dysplasia, and behavioural evaluation. Additionally, the following genetic diseases of Golden Retrievers were screened: congenital eye malformations, congenital myasthenic syndrome, degenerative myelopathy, dystrophic epidermolysis bullosa, ichthyosis Type 2, muscular dystrophy, neuronal ceroid lipofuscinosis 5, progressive Retinal Atrophy (GR-PRA1 and GR-PRA 2 variants), and sensory ataxic neuropathy.

Bitches were progressively transitioned (between 28 and 40 days of gestation) from their maintenance diet to a commercial complete and balanced diet adapted to the requirements of the final third of pregnancy with appropriate levels of energy density, high-quality protein, essential fatty acids, vitamins, and minerals (Supplementary file 2).

Pregnant bitches were housed in the maternity unit in groups of three females simultaneously from approximately two weeks before whelping until weaning of their puppies at two months of age. After weaning, the adult bitches returned to their breeding kennel, while the puppies had three possible outcomes. Some were destined to become assistance or mediation dogs and were placed into “foster families” before being handed over to a beneficiary at approximately 18 to 24 months of age; some females were retained for future breeding within the cohort and placed with “breeding families”, and others were placed as companion dogs. Thus, after weaning, all the dogs were maintained in household environments, where they were exposed to numerous uncontrolled and potentially interacting environmental factors. So, no animals were euthanized or sacrificed for this study, and no anaesthesia or sedation was performed as part of the study procedures reported here.

Future dog owners were informed about the study through a dedicated website (https://toulousegoldcohort.envt.fr/). Interested individuals could complete a contact

questionnaire on the website and were subsequently contacted by a member of the Neocare unit, who explained the study in detail, including its objectives, follow-up procedures, and the rights and responsibilities associated with the adoption of a cohort puppy. Each owner signed a contract for the acquisition of the dogs, including consent for its continued participation in the study. During the consent process, dog owners were informed of the study's data sharing policy. Clinically relevant findings arising from routine diagnostic investigations performed as part of the dog's clinical care were communicated to the owners, whereas research-use-only data and results without established clinical significance were not routinely returned. After their adoption at two months, puppies were followed at the GOLD Clinic, a preventive medicine service dedicated to dogs enrolled in the TGC. They were systematically monitored at 2, 3, 4, 6, 12 and 18 months of age and then annually starting at 24 months. They received a standardized preventive care protocol based on the World Small Animal Veterinary Association (WSAVA) recommendations for vaccination (Supplementary file 3) and European Scientific Council for Companion Animal Parasites (ESCCAP) recommendations for deworming [16].

The TGC aims to follow the dogs throughout their lives, some of whom may remain healthy, while others may develop illnesses or chronic conditions. Enrolled dogs that subsequently develop health conditions continue to participate at the discretion of their owners. Participation in the TGC does not interfere with the routine veterinary care of enrolled dogs. Throughout the follow-up period, all preventive, diagnostic, and therapeutic measures considered necessary to preserve the health and welfare of each dog are implemented according to standard veterinary practice, irrespective of the study protocol. When a health condition is identified, appropriate veterinary care is provided without delay, and the animal’s health and welfare always take precedence over research procedures. Depending on their health problem, dogs may be examined by their regular veterinarian or by one of the specialized services at the Veterinary Teaching Hospital of Toulouse Veterinary School. In all cases, subsequent examinations, diagnosis, treatments, and the animal's health follow-up were recorded in the cohort database. No single sample size was defined a priori for the cohort, given its longitudinal design and multiple exploratory objectives. Sample size calculations will be performed for individual studies conducted within the cohort, where appropriate.

Currently, the project aims to continue to recruit between 40 and 60 puppies each year, all of whom will be systematically born and followed in the maternity and at the Gold Clinic. This cohort therefore has no predefined end date. If the population is insufficient for a specific study, recruitment will continue until the required number of individuals has been reached.

2.3. Breeding program following the Early Cohort

To establish a breeding stock for continuation of the TGC, a subset of female dogs born within the EC was pre-selected at two months of age based on health and behavioural criteria for inclusion in the breeding programme. The future reproductive females are housed with their breeding families throughout the project.

During each oestrus phase, at least one examination is performed by practitioners in the reproductive medicine department of the veterinary school to monitor reproductive function and characterize the progression of the cycle. At 18 months of age, the bitches undergo the same selection process as described above. Females meeting the selection criteria are enrolled in the breeding programme. Sires are selected from partner breeding kennels in order to limit inbreeding within the study population. All sires undergo the same health and behavioural selection procedures as those applied to the females.

When a litter is planned, each confirmed reproductive female undergoes a complete oestrus monitoring using a multimodal approach combining physical examination, vaginal cytology, progesterone assays and abdominal ultrasound to determine the day of ovulation. Vaginal smears are collected and stained using standard cytological techniques (RAL 555® and Diag-Oestro® kit, RAL Diagnostics, Martillac, France). Serum progesterone concentrations are measured at repeated time points using a validated immunoassay (Dri-Chem Immuno AU10V, Fujifilm, Tokyo, Japan). Transabdominal ovarian ultrasonography is performed concurrently to assess follicular dynamics (SonoScape E11 ultrasound system - SonoScape Medical Corp., Shenzhen, China). For each examination, at least three ovarian follicles are identified and measured in two perpendicular planes, and the mean follicular diameter is calculated. Ovulation day (designated as day 0 of gestational dating) is determined when the vaginal smear is consistent with oestrus, the serum progesterone concentration reaches 4–10 ng/mL and doubles on the following day, and ovarian follicles are no longer visible on ultrasonographic examination. Subsequently, two artificial inseminations are performed within 72 hours after ovulation using fresh semen from the same previously assigned stud dog.

A pregnancy follow-up is conducted on days 21, 28, 42, 56 post-ovulation in all inseminated bitches, and then daily until whelping. At each time point, serum progesterone concentrations are measured using the same validated immunoassay as described for oestrus monitoring, allowing assessment of luteal function. Abdominal ultrasonography is performed at day 28 post-ovulation to record the presence and number of embryonic vesicles. At days 42 and 56

post-ovulation, and between days 56 and 62, ultrasonographic examination focuses on fetal viability and development, with measurements collected to further characterize the developmental progression, as previously described [17]. From approximately 42 days post-ovulation onward, females are typically transitioned to a diet designed for late gestation and lactation. As specific nested studies may investigate the effects of targeted nutritional interventions, such as supplementation with selected fibres, prebiotics, probiotics, or yeasts, specific dietary components may be modified or supplemented according to the study protocol. In all cases, the overall diet remains complete and balanced and meets the nutritional requirements and current recommendations for canine reproduction, gestation, and lactation. Each bitch is limited to a maximum of three litters between two and five years of age. Following completion of the breeding programme, bitches are retired from reproduction, sterilised, and remain with their breeding families, who become their permanent owners for the remainder of their lives.

2.4. Data collection

Handwritten and digital data were collected in accordance with standardized procedures and recorded on standardized clinical follow-up sheets (Supplementary file 4).

The study timeline was structured according to key reproductive and developmental stages based on previously published data and key physiological transitions in canine growth (Figure 2) [18]. The maternal reproductive period comprised heats and mating (from the onset of heats to the last insemination), gestation (from the last insemination to 57 days post-ovulation), the pre-whelping period (from 57 days post-ovulation to the day before parturition), parturition, and lactation (from the end of parturition to puppy weaning). From the offspring perspective, the growing period extended from birth to 2 years of age and encompassed the neonatal (D0–D21), puppy (D21–6 months), juvenile (6–12 months), and young-adult (12 months–2 years) stages. The neonatal period was further subdivided into an early neonatal period (D0–D2) and a late neonatal period (D3–D21). Within the puppy stage, weaning constituted a progressive nutritional and maternal transition occurring at approximately 8–9 weeks of age (D56–D63), separating the pre-weaning and post-weaning phases. From 2 years of age onwards, dogs entered the adult period, which was further divided into adult (2–6 years), mature (6–9 years), senior (9–13 years), and geriatric (≥13 years) stages. This classification was designed to facilitate the interpretation of longitudinal data in relation to biologically relevant developmental milestones.

The type and frequency of measurements are summarized in Table 1. Briefly, clinical and management variables, including treatments administered, fecal score (scale from 1-liquide feces - to 5-hard feces in adults, and scale from 1-liquide feces -to-13-hard feces in puppies), body weight, body condition score (scale from 1-very thin- to 9 - obesity), muscular condition score (1 – normal muscle mass- to 4 – severe muscle loss), food intake, and general clinical examinations, are recorded at regular intervals before and after whelping in mothers and their puppies [19–21].

Puppies enrolled in the cohort were followed longitudinally from birth to early adulthood according to a standardized data collection schedule (Table 2, Figure 2). Data were collected during their stay in the maternity, during clinical follow-ups at the Veterinary Teaching Hospital, and via regular data submissions by host families. At birth (D0), an Apgar score (a standardized clinical assessment of neonatal vitality) and an initial clinical examination were performed [22]. During the neonatal and early pediatric periods (D1 to D56), puppies underwent standardized longitudinal assessments including blood glucose concentration, body temperature, body weight, morphometric measurements, and comprehensive clinical examinations. All medical treatments and interventions were prospectively recorded throughout this period. Following adoption, participants continued to undergo scheduled follow-up evaluations, including clinical examinations, growth monitoring, body composition assessment (body weight, body condition score, and muscle condition score), and laboratory investigations, such as complete blood counts. Additional information on health, nutrition, behavior, and lifestyle was collected through standardized owner-completed questionnaires. At selected time points, fecal consistency was also evaluated using photographs submitted by owners, allowing standardized remote assessment of fecal scores [23]. Whenever possible, the follow-up protocol relies on previously validated assessment tools to ensure standardized and reproducible data collection. For example, the Dog Obesity Risk and Appetite (DORA) questionnaire was used to evaluate owner-reported feeding behavior and obesity-related traits [24]. Questionnaires have been, and continue to be, developed by the study team with input from subject specialists, as appropriate.

For data management, an Interoperable Study and Research Data Sharing System (SPIDER) was developed by ENVT. It is a web application designed to optimize research data management, such as data obtained from the TGC. This adaptable and customizable application brings together interconnected data points and facilitates their analysis in an Open Science approach. SPIDER is used to structure and secure cohort data by guaranteeing their traceability,

enabling the development of data management plans, and facilitating the sharing and valorization of research data in accordance with FAIR (Findable, Accessible, Interoperable, and Reusable) and Open Science principles. A total of 587 variables are recorded and could be shared with future collaborators. The complete list of variables with their description is available in Supplementary file 5.

2.5. Samples collection

In parallel with data recording, biospecimens (blood, urine, saliva, hair, feces, and swabs of different mucosal membranes or body surfaces) are collected from bitches and their puppies (Tables 3 and 4). For each pregnant bitch core samples are collected for biorepository storage one week before whelping and 1, 7, 21 days post partum. For each puppy, the same procedure is performed at birth (D0, within the first 5 minutes of life and before any colostrum ingestion), 1, 7, 21, and 56 days of age in the maternity and at 3, 6, 9, and 12 months of age, and annually thereafter.

To accommodate routine management while preserving the longitudinal design of the cohort, all scheduled sample collections and clinical assessments were performed within a ± one day window of the planned time point. The only exceptions were the D0 and D1 (between 24 and 36h after the end of parturition) assessments in puppies and the first assessment in dams after whelping, which were conducted on the designated day because of their importance for characterizing the immediate postnatal and early lactation periods.

To support the long-term objectives of the Toulouse GOLD cohort, a dedicated biobank was established at the inception of the project. It enables the standardized processing, traceable storage, and long-term preservation of all biological samples collected throughout the longitudinal follow-up, thereby providing a sustainable resource for future translational and multi-omics research. This biobank is supervised by the NeoCare unit, with specific measures in place to ensure appropriate resources and adequate safety procedures, including approved equipment for storage at -70°C (FROILABO BM17586, Genas, France), and sample traceability through a dedicated software (ESIToul Barcodes, Get-Plage INRAE, Toulouse, France). To ensure sample integrity, all storage freezers are equipped with continuous temperature monitoring devices linked to an ENVT-developed monitoring platform. Temperature measurements are recorded continuously, and automated alarm notifications are issued whenever temperatures deviate from predefined acceptable ranges. This system enables prompt corrective actions in the event of equipment malfunction or failure of storage conditions.

3. Preliminary results on the Early Cohort population

A total of 123 puppies born at ENVT between 7 June 2022 and 1 March 2024 from 18 dams were enrolled and followed up, constituting the EC. The median age of the bitches at mating was 3.8 years (range: 1.9–5.8). All dams had favourable hip dysplasia screening results, with both hips graded A or B according to the Fédération Cynologique Internationale (FCI) classification: 10/18 (55.6%) were graded A/A, 3/18 (16.7%) A/B, and 5/18 (27.8%) B/B. The median parity of the dams before enrolment in the cohort was 2 previous litters (range: 0–6), with 2/17 (11.8%) dams being nulliparous. Most oestrous cycles were spontaneous (15/18), while three were induced using GnRH agonist implants (Suprelorin® 4,7 mg, Virbac, Carros, France). A total of five different sires were used for breeding, with a median of two matings per male (range: 1–4). All breedings were performed by natural mating, with no artificial insemination used. The median gestation length was 64 days (range: 63–66). Prepartum body condition score (BCS) was available for 17 of the 18 dams. The median BCS was 4 (range: 3–7), assessed at a median of 12 days before parturition (range: 3–24 days). No caesarean sections were performed. The median litter size was 7.5 puppies (range: 2-11).

Among the 123 puppies born, the stillbirth rate was 5.7% (7/123), and neonatal mortality reached 1.7% (2/116), resulting in an overall mortality rate of 7.3% (9/123) within the first two months of life. The sex ratio was 0.98. Median birth weight was 409 g (range: 168-573), with 0.8% (1/120) of puppies classified as very low birth weight (<188 g) and 50% (60/120) classified as low birth weight (188-417g) [25]. The median Apgar score at birth was 8 (range: 5–10) with 10.4% (12/115) having an Apgar score <7 considered as neonatal distress syndrome [26].

Median growth rate over the first two days of life, reflecting the overall colostrum intake, was 6.3 % (range: -7.5%; +20.4%), with 6.9% of puppies presenting retarded early growth and thus a deficit in colostrum intake (growth rate <-4%) [27]. A total of 6,479 body-weight measurements from 120 puppies, collected from birth to 56 days of age, were used to model early growth trajectories and construct smoothed weight centile curves. The resulting smoothed P10, P25, P50, P75, and P90 growth curves are presented in Figure 3, together with individual puppy growth trajectories. Morphometric measurements increased progressively throughout the first 8 weeks of life (Table 5). Mean head circumference increased from 15.3 ± 0.9 cm at day 1 to 31.4 ± 1.8 cm at day 56, thoracic circumference from 17.4 ± 1.3 to 41.7 ± 3.5 cm, and crown-rump length from 18.4 ± 1.6 to 46.1 ± 4.1 cm.

Among the 114 puppies alive at 56 days of age, one was subsequently withdrawn from the program due to congenital abnormality (a bilateral ectopic ureter associated with a pelvic bladder). Among the remaining 113 puppies that underwent a follow-up visit at 18 months of age, including a clinical examination and biological sample collection, 85 were placed as companion dogs, 8 entered an assistance-dog program, and 6 were assigned to animal-assisted intervention activities. In addition, 15 female puppies were selected at 2 months of age as potential future breeding dogs; among these, 8 were subsequently excluded from the breeding program before their first mating because they did not meet the program's selection criteria. Among the seven females ultimately retained for breeding, six produced at least one litter during the follow-up period, with a total of 10 new litters included in the cohort.

4. Discussion

The Toulouse GOLD Cohort is an original prospective canine cohort, designed to follow dogs' development and health from conception to adulthood. This project demonstrates the feasibility of collecting detailed and standardized clinical and physiological data in canine neonates from the very first minutes of life within a breeding facility. Such early data acquisition is rarely achievable under field conditions, where monitoring is often inconsistent and limited to later time points. Indeed, most often births take place at breeders' or private homes, where access for research teams is impossible or at best limited by factors such as the size of the kennel (too small), concerns regarding biosecurity, or the practical difficulty of staying on site for extended periods. Previous studies in canine neonatology have typically relied on retrospective data or information collected several hours to days after birth, thereby limiting the understanding of immediate postnatal adaptation [28]. In addition, many investigations have focused on puppies delivered by cesarean section, a mode of delivery that bypasses several physiological processes associated with natural parturition. Growing evidence indicates that birth mode influences early neonatal adaptation and the establishment of the gut microbiota, potentially affecting subsequent immune and metabolic development [29]. Consequently, data obtained from cesarean-delivered puppies may not fully reflect the physiological events occurring during spontaneous birth. The Toulouse GOLD cohort, therefore, provides a unique framework for characterizing the perinatal period in dogs and investigating its associations with health outcomes throughout life.

This cohort was designed to reflect real-world canine developmental conditions rather than highly controlled laboratory settings. Puppies are born and raised in a breeding

environment representative of the majority of French dog breeding facilities, comprising a limited number of whelping areas (three maternity units), access to outdoor environments, and routine husbandry practices [30]. Whenever possible, parturition occurs naturally, allowing the study of physiological neonatal adaptation under these conditions. Puppies remain with their dam throughout the legally recommended maternity period of at least two months, receiving maternal care and milk as the primary source of nutrition, with milk supplementation provided only when clinically indicated. Preventive healthcare, nutrition, and husbandry protocols are based on current veterinary recommendations and standard breeding practices [31]. Following weaning, puppies transition from the maternity environment to their adoptive households, where they are exposed to the diverse environmental, nutritional, and social conditions encountered by pet dogs in everyday life. Although dogs are subject to intensive longitudinal monitoring, their living conditions closely resemble those of the general canine population, thereby enhancing the external validity and translational relevance of the cohort.

Health is a complex and multifactorial process that cannot be explained by a single parameter alone. Instead, it results from the dynamic interaction of multiple factors, including early-life conditions, parental influences, nutrition, environment, and management practices, which together shape health outcomes over time [32,33]. The longitudinal design of the Toulouse GOLD Cohort since birth provides a unique opportunity to follow individuals over time and to characterize the occurrence of chronic conditions such as chronic enteropathies, obesity, or atopic dermatitis. These multifactorial disorders are highly prevalent in companion animals and constitute major challenges for veterinary medicine due to their chronic nature, impact on welfare, and associated healthcare costs [34,35]. By continuously monitoring animals from birth onwards, this study will generate longitudinal data that may allow the detection of early and subtle variations in health status before the onset of clinical disease. Such an approach may help to identify early-life determinants of chronic conditions and to define potential windows for intervention. The cohort will also enable the identification of distinct health trajectories, distinguishing animals with favorable outcomes from those developing chronic disorders.

In addition, understanding the complex mechanisms underlying health and disease requires integrative and multidisciplinary approaches capable of capturing the interactions between environmental exposures, nutrition, microbial colonization, physiological adaptation, and clinical outcomes. By combining these complementary sources of information within a single longitudinal framework, the cohort provides a unique opportunity to investigate how

multiple factors interact across critical developmental periods and are associated with health trajectories throughout life. Such an approach moves beyond the study of isolated risk factors and enables a more comprehensive understanding of the biological and environmental processes driving resilience or susceptibility to disease. In the medium term, these data could support the design of pragmatic interventional studies aimed at improving health trajectories through targeted modifications of management and care practices. These approaches may facilitate the identification of early biomarkers or patterns associated with later disease, thereby opening new perspectives for preventive veterinary medicine. Ultimately, this cohort-based strategy could contribute to the development of optimized, evidence-based care pathways in canine reproduction, neonatology, and pediatrics, with potential applications in routine clinical practice.

As with any real-world longitudinal cohort, the Toulouse GOLD Cohort presents both important strengths and inherent limitations. A major strength of this study lies in its field-based design, which aims to reproduce conditions representative of conventional dog breeding while allowing close, standardized, and prospective monitoring of bitches and puppies. This design may support the external validity of the findings while providing clinically relevant data under practical breeding conditions. However, the same optimized management and intensive follow-up implemented within the cohort may also reduce the occurrence of adverse outcomes, thereby limiting the number of cases available for studying specific neonatal risk factors. Notably, the mortality rate among puppies in this cohort was 7.3% from birth to two months of age, which is lower than the 15–20% mortality rates commonly reported in the literature [36–39]. This suggests that intensive monitoring and optimized management of breeding bitches and their litters may substantially reduce neonatal mortality. These findings challenge the perception that high early-life mortality in dogs is unavoidable and instead support the idea that it may be mitigated through improved clinical follow-up, early detection of complications, and appropriate supportive care. The prevalence of very low birth weight puppies in our cohort was also low (0.8%), whereas previous studies have reported substantially higher proportions, often ranging from 5% to over 20%, depending on breed and study design [40]. Very low birth weight has been consistently identified as a major risk factor for neonatal mortality, with early mortality reported to be approximately 13 times higher in affected puppies than those with normal birth weight [40]. While this is a positive outcome from an animal health perspective, which supports the effectiveness of the management practices implemented, it also constitutes a limitation, as the small number of affected individuals precludes robust investigation of risk factors associated

with very low birth weight and neonatal mortality in the short term. This lack of extreme variability reduces statistical power and highlights the need for larger cohorts or multicentric studies to address these questions. Despite this limitation, our findings suggest that appropriate management of the dam throughout gestation—particularly nutritional management—may play a key role in reducing the occurrence of low birth weight.

A major asset of the Toulouse GOLD cohort is the establishment of a large and continuously expanding longitudinal biobank. To date, more than 10,000 biological samples have been collected and stored from participating dogs across more than ten distinct developmental time points, spanning the gestational, neonatal, pediatric, and adult periods. The biobank includes nine complementary sample types (blood, urine, feces, and rectal, oral, skin, ear, vaginal and preputial swabs). Importantly, sample collection is ongoing both in dogs enrolled in the EC as they continue to age and in newly born puppies entering the cohort. This dual strategy enables simultaneous extension of longitudinal follow-up and continuous recruitment of new individuals, thereby increasing the depth and breadth of the biobank over time. By integrating repeated biological sampling with comprehensive phenotypic, clinical, environmental, nutritional, and longitudinal data, this biobank provides a unique platform for integrative and multi-omics research. It will enable the investigation of the complex molecular, physiological, microbial, and developmental mechanisms governing early-life adaptation, as well as the interactions between early-life exposures and the subsequent emergence of acute and chronic diseases. Moreover, this dynamic design ensures the continuous generation of both longitudinal and generational data, creating a sustainable resource for future research.

Importantly, the Toulouse GOLD cohort is designed as an open and collaborative research infrastructure. It aims to make available to the scientific community not only selected datasets but also its broader research platform, thereby facilitating the exploration of new hypotheses and the development of innovative tools and technologies in the field of canine developmental biology and neonatology. Such an open-science approach is expected to accelerate knowledge generation and foster multidisciplinary collaboration. Similar strategies have proven highly successful in large human cohort studies such as ALSPAC, Generation R, and the UK Biobank, where broad access to data and biological resources has substantially amplified scientific output and enabled discoveries extending far beyond the original objectives of the cohorts [41–43]. Comparable initiatives are emerging in veterinary medicine, notably through the Dog Aging Project and the Golden Retriever Lifetime Study, which have

demonstrated the value of shared longitudinal resources for addressing complex questions related to health, disease, and lifespan [44,45]. By adopting a comparable framework, the Toulouse GOLD cohort aims to become a sustainable research platform supporting collaborative, interdisciplinary, and translational investigations into canine health and development.

Finally, this initiative aligns with the broader One Health framework, as improving the understanding of early-life determinants of health in dogs may also provide valuable comparative insights into human pediatrics. Indeed, companion dogs represent a highly relevant translational model for studying the DOHaD. The relatively short lifespan in dogs, compared to humans, enables longitudinal and interventional studies across the entire life course (from early development to ageing) within a limited timeframe. In children, this critical period has been defined as the first 1000 days of life (from conception until 2 years of age). Given the shorter lifespan of dogs and taking into account the key stages of physiological development, the first 120 days of life—from conception to approximately 2 months of age—may be considered a critical developmental window broadly analogous to the concept of the first 1000 days in humans. By 8 weeks of age, puppies have undergone most of the major early-life physiological transitions: they have reached a functional level of immunocompetence, are generally fully weaned and capable of efficiently digesting solid food, exhibit a substantially more mature gut microbiota, and have acquired the fundamental motor, sensory, and behavioral abilities required for independent adaptation to their environment [46–48]. These characteristics make this period a particularly relevant target for investigating the developmental origins of health and disease in dogs [49]. Importantly, dogs share the same physical, chemical, and social environments as humans, which are key determinants of early-life programming and long-term health trajectories, and which are difficult to replicate in controlled laboratory settings [50]. This close environmental and biological overlap strengthens the relevance of canine models for investigating how early-life exposures influence later-life outcomes, a central concept of the DOHaD framework. Furthermore, as companion dogs are integrated into human households and considered family members by a large proportion of the population, their follow-up allows for the collection of detailed, real-world data on nutrition, environmental exposures, and life experiences from the earliest stages of life. Altogether, this unique combination of shared environment, accelerated life course, and close human–animal relationship makes the companion dog a powerful and complementary model to traditional approaches for exploring the early-life determinants of health and disease, with strong translational potential for human medicine.

5. Conclusions

The Toulouse GOLD cohort represents, to our knowledge, one of the first comprehensive longitudinal canine birth cohorts designed to investigate the developmental origins of health and disease from the earliest stages of life. By combining prospective phenotyping, extensive biological sampling, long-term follow-up, and real-world environmental exposures, this initiative provides a unique framework for studying how early-life factors shape health trajectories throughout the canine lifespan. As the cohort continues to expand and mature, it is expected to generate new insights into the mechanisms underlying resilience, disease susceptibility, and healthy ageing in dogs. Through its open and collaborative framework, the cohort aims not only to advance veterinary knowledge but also to contribute to broader comparative and translational research on the developmental origins of health and disease. In the long term, these efforts may contribute to a paradigm shift in canine medicine, moving from the management of established diseases toward a more predictive, preventive, and individualized approach to healt.

References

1. Barker, D. J. P. Adult consequences of fetal growth restriction. *Clin. Obstet. Gynecol.* **49**, 270–283 (2006).
2. Roseboom, T. J. *et al.* Coronary heart disease after prenatal exposure to the Dutch famine, 1944-45. *Heart* **84**, 595–598 (2000).
3. Gretchev, A. *et al.* Unpacking developmental programming: a conceptual and historical analysis in the context of DOHaD. *J. Dev. Orig. Health Dis.* **17**, e17 (2026).
4. Oken, E. *et al.* When a birth cohort grows up: challenges and opportunities in longitudinal developmental origins of health and disease (DOHaD) research. *J. Dev. Orig. Health Dis.* **14**, 175–181 (2023).
5. Boyd, A. *et al.* Cohort Profile: the 'children of the 90s'--the index offspring of the Avon Longitudinal Study of Parents and Children. *Int. J. Epidemiol.* **42**, 111–127 (2013).
6. Kooijman, M. N. *et al.* The Generation R Study: design and cohort update 2017. *Eur. J. Epidemiol.* **31**, 1243–1264 (2016).
7. Kummeling, I. *et al.* Etiology of atopy in infancy: the KOALA Birth Cohort Study. *Pediatr. Allergy Immunol. Off. Publ. Eur. Soc. Pediatr. Allergy Immunol.* **16**, 679–684 (2005).
8. Lenover Moyer, M. B., Jasani, K., Waldman, A. B., Chinchilli, V. M. & Shenk, M. K. The Developmental Origins of Irritable Bowel Syndrome: A Systematic Review and Meta-Analysis. *Am. J. Hum. Biol.* **37**, e24209 (2025).
9. TEDDY Study Group. The Environmental Determinants of Diabetes in the Young (TEDDY) Study. *Ann. N. Y. Acad. Sci.* **1150**, 1–13 (2008).
10. Mao, J., Xia, Z., Chen, J. & Yu, J. Prevalence and risk factors for canine obesity surveyed in veterinary practices in Beijing, China. *Prev. Vet. Med.* **112**, 438–442 (2013).
11. McGreevy, P. D. *et al.* Prevalence of obesity in dogs examined by Australian veterinary practices and the risk factors involved. *Vet. Rec.* **156**, 695–702 (2005).
12. Robinson, N. J., Dean, R. S., Cobb, M. & Brennan, M. L. Investigating common clinical presentations in first opinion small animal consultations using direct observation. *Vet. Rec.* **176**, 463–463 (2015).
13. O'Neill, D. G., James, H., Brodbelt, D. C., Church, D. B. & Pegram, C. Prevalence of commonly diagnosed disorders in UK dogs under primary veterinary care: results and applications. *BMC Vet. Res.* **17**, 69 (2021).
14. Gentry, C. M. Updates on the Pathogenesis of Canine Atopic Dermatitis and Feline Atopic Skin Syndrome: Part 2, the Skin Barrier, the Microbiome, and Immune System Dysfunction. *Vet. Clin. North Am. Small Anim. Pract.* **55**, 173–187 (2025).
15. Landolt, J. I., O'Neill, D. G., Unterer, S., Hartnack, S. & Kowalska, M. E. A mapping review of worldwide current and previous cohort research programmes in cats and dogs. *PLOS ONE* **20**, e0321007 (2025).
16. Squires, R. A., Crawford, C., Marcondes, M. & Whitley, N. 2024 guidelines for the vaccination of dogs and cats – compiled by the Vaccination Guidelines Group (VGG) of the World Small Animal Veterinary Association (WSAVA). *J. Small Anim. Pract.* **65**, 277–316 (2024).
17. Kutzler, M. A., Yeager, A. E., Mohammed, H. O. & Meyers-Wallen, V. N. Accuracy of canine parturition date prediction using fetal measurements obtained by ultrasonography. *Theriogenology* **60**, 1309–1317 (2003).
18. Harvey, N. D. How Old Is My Dog? Identification of Rational Age Groupings in Pet Dogs Based Upon Normative Age-Linked Processes. *Front. Vet. Sci.* **8**, 643085 (2021).
19. Burkholder, W. J. Use of body condition scores in clinical assessment of the provision of optimal nutrition. *J. Am. Vet. Med. Assoc.* **217**, 650–654 (2000).

20. Laflamme, D. Development and validation of a body condition score system for dogs. **22**, 10–15 (1997).
21. Grellet, A. *et al.* Validation of a fecal scoring scale in puppies during the weaning period. *Prev. Vet. Med.* **106**, 315–323 (2012).
22. Veronesi, M. C., Panzani, S., Faustini, M. & Rota, A. An Apgar scoring system for routine assessment of newborn puppy viability and short-term survival prognosis. *Theriogenology* **72**, 401–407 (2009).
23. Chauvel, C. *et al.* Validation of photographic fecal scoring in puppies. *Prev. Vet. Med.* **246**, 106729 (2026).
24. Raffan, E., Smith, S. P., O'Rahilly, S. & Wardle, J. Development, factor structure and application of the Dog Obesity Risk and Appetite (DORA) questionnaire. *PeerJ* **3**, e1278 (2015).
25. Mugnier, A. *et al.* Low and very low birth weight in puppies: definitions, risk factors and survival in a large-scale population. *BMC Vet. Res.* **16**, 354 (2020).
26. Mila, H. *et al.* Monitoring of the newborn dog and prediction of neonatal mortality. *Prev. Vet. Med.* **143**, 11–20 (2017).
27. Mila, H., Grellet, A., Feugier, A. & Chastant-Maillard, S. Differential impact of birth weight and early growth on neonatal mortality in puppies,. *J. Anim. Sci.* **93**, 4436–4442 (2015).
28. Chastant-Maillard, S. *et al.* Reproductive performance and pre-weaning mortality: Preliminary analysis of 27,221 purebred female dogs and 204,537 puppies in France. *Reprod. Domest. Anim.* **52**, 158–162 (2017).
29. Garrigues, Q., Apper, E., Chastant, S. & Mila, H. Gut microbiota development in the growing dog: A dynamic process influenced by maternal, environmental and host factors. *Front. Vet. Sci.* **9**, (2022).
30. Leroy, G., Verrier, E., Wisner-Bourgeois, C. & Rognon, X. Breeding goals and breeding practices of French dog breeders: results from a large survey. *Rev. Méd Vét* (2007).
31. Squires, R. A., Crawford, C., Marcondes, M. & Whitley, N. 2024 guidelines for the vaccination of dogs and cats – compiled by the Vaccination Guidelines Group (VGG) of the World Small Animal Veterinary Association (WSAVA). *J. Small Anim. Pract.* **65**, 277–316 (2024).
32. Rutter, H. *et al.* The need for a complex systems model of evidence for public health. *The Lancet* **390**, 2602–2604 (2017).
33. Lerner, R. M., Brindis, C. D., Batanova, M. & Blum, R. Wm. Adolescent Health Development: A Relational Developmental Systems Perspective. in *Handbook of Life Course Health Development* (eds. Halfon, N., Forrest, C. B., Lerner, R. M. & Faustman, E. M.) 109–121 (Springer International Publishing, Cham, 2018). doi:10.1007/978-3-319-47143-3_6.
34. O'Neill, D. G., James, H., Brodbelt, D. C., Church, D. B. & Pegram, C. Prevalence of commonly diagnosed disorders in UK dogs under primary veterinary care: results and applications. *BMC Vet. Res.* **17**, 69 (2021).
35. Drechsler, Y., Dong, C., Clark, D. E. & Kaur, G. Canine Atopic Dermatitis: Prevalence, Impact, and Management Strategies. *Vet. Med. Res. Rep.* **15**, 15–29 (2024).
36. Gill, M. A. Perinatal and late neonatal mortality in the dog. https://ses.library.usyd.edu.au/handle/2123/4137 (2001).
37. Mila, H. *et al.* Monitoring of the newborn dog and prediction of neonatal mortality. *Prev. Vet. Med.* **143**, 11–20 (2017).
38. Tønnessen, R., Borge, K. S., Nødtvedt, A. & Indrebø, A. Canine perinatal mortality: A cohort study of 224 breeds. *Theriogenology* **77**, 1788–1801 (2012).

39. Chastant-Maillard, S. *et al.* Reproductive performance and pre-weaning mortality: Preliminary analysis of 27,221 purebred female dogs and 204,537 puppies in France. *Reprod. Domest. Anim.* **52**, 158–162 (2017).
40. Mugnier, A. *et al.* Low and very low birth weight in puppies: definitions, risk factors and survival in a large-scale population. *BMC Vet. Res.* **16**, 354 (2020).
41. Boyd, A. *et al.* Cohort Profile: the 'children of the 90s'--the index offspring of the Avon Longitudinal Study of Parents and Children. *Int. J. Epidemiol.* **42**, 111–127 (2013).
42. Kooijman, M. N. *et al.* The Generation R Study: design and cohort update 2017. *Eur. J. Epidemiol.* **31**, 1243–1264 (2016).
43. Sudlow, C. *et al.* UK Biobank: An Open Access Resource for Identifying the Causes of a Wide Range of Complex Diseases of Middle and Old Age. *PLOS Med.* **12**, e1001779 (2015).
44. Creevy, K. E., Akey, J. M., Kaeberlein, M. & Promislow, D. E. L. An open science study of ageing in companion dogs. *Nature* **602**, 51–57 (2022).
45. Guy, M. K. *et al.* The Golden Retriever Lifetime Study: establishing an observational cohort study with translational relevance for human health. *Philos. Trans. R. Soc. B Biol. Sci.* **370**, 20140230 (2015).
46. Schäfer-Somi, S., Bär-Schadler, S. & Aurich, J. E. Immunoglobulins in nasal secretions of dog puppies from birth to six weeks of age. *Res. Vet. Sci.* **78**, 143–150 (2005).
47. Malm, K. & Jensen, P. Weaning in dogs: within- and between-litter variation in milk and solid food intake. *Appl. Anim. Behav. Sci.* **49**, 223–235 (1996).
48. Guard, B. C. *et al.* Characterization of the fecal microbiome during neonatal and early pediatric development in puppies. *PloS One* **12**, e0175718 (2017).
49. Garrigues, Q., Apper, E., Chastant, S. & Mila, H. Gut microbiota development in the growing dog: A dynamic process influenced by maternal, environmental and host factors. *Front. Vet. Sci.* **9**, (2022).
50. Sexton, C. & Ruple, A. Canine sentinels and our shared exposome. *Science* **384**, 1170–1172 (2024).

Acknowledgements

The authors gratefully acknowledge all staff members, students, veterinarians, veterinary nurses, animal care personnel, and research assistants who contributed to the establishment and longitudinal follow-up of the Toulouse GOLD Cohort, including animal care, clinical examinations, biological sample collection, sample processing, and data collection. The authors also thank the families and partner organizations involved in the cohort for their continued participation and commitment to the long-term follow-up of the dogs.

The authors sincerely thank the Fondation VISIO for its early support of the project and for the financial support that made the establishment of the Early Cohort possible. The authors are also grateful for the constructive and valuable exchanges with the Fondation VISIO throughout the development of the project.

Finally, the authors are particularly grateful to all the dogs enrolled in the Toulouse GOLD Cohort and to the families caring for them, whose long-term participation makes this research possible.

Author contributions

A.G. and H.M. conceived the Toulouse GOLD Cohort, developed its overall methodology, and have jointly coordinated and supervised the cohort since its inception. **A.G.** contributed to conceptualization, methodology, project administration, supervision, funding acquisition, formal analysis, data interpretation, visualization, and writing of the original draft. **H.M.** contributed to conceptualization, methodology, project administration, supervision, funding acquisition, clinical investigation, biological sample collection, and writing – review and editing.

I.B. and C.C. contributed to the development and implementation of the cohort. **I.B.** contributed to investigation, including monitoring of parturition and biological sample collection. **C.C.** contributed to data curation and database structuring and to the longitudinal clinical follow-up of dogs from 12 months of age onwards.

L.Mi., J.B., and A.M. contributed to investigation and project administration through assistance with biological sample collection and management of study documentation. **C.B.** contributed to animal care and to the operational management of the maternity facility.

P.R.-B., I.K., U.B., M.Z.T., J.L.S., and P.R. contributed to investigation through clinical monitoring and examinations of the dogs during the maternity and subsequent follow-up periods. **M.C.C.** contributed to clinical investigation and follow-up, particularly dermatological assessments. **O.R.** contributed to

behavioural assessment of the founder dogs, puppy socialization, and assessment and selection of puppies for placement with families and partner organizations.

E.L., L.Ma., and M.N. contributed to methodology and project development through their participation in the cohort steering committee. **B.D.** contributed to the initial development of the cohort and its organizational management. **J.-S.D.** contributed to data curation, database structuring, and development of the SPIDER tool used for data management within the cohort.

All authors contributed to the critical review of the manuscript, read and approved the final version.

A.G. and H.M. jointly supervised this work.

Data Availability

The datasets generated and/or analysed within the Toulouse GOLD Cohort are not publicly available, as the cohort is ongoing and longitudinal data collection is continuing. Data supporting the findings of the present study are available from the corresponding author upon reasonable request, subject to applicable ethical, legal, and institutional requirements.

Additional Information

Funding

The establishment of the Early Cohort of the Toulouse GOLD Cohort was supported by the Fondation VISIO. The funder had no role in the study design, data collection, data analysis or interpretation, preparation of the manuscript, or decision to submit the manuscript for publication.

Competing interests

The authors declare no competing interests.

Figure legends

Figure 1. Main health domains evaluated in the Toulouse GOLD Cohort, a prospective longitudinal canine cohort.

Figure 2. Study timeline showing the main periods investigated in the Toulouse GOLD Cohort.

Figure 3. Individual growth trajectories and smoothed body-weight centile curves from birth to 56 days of age. Individual puppy growth trajectories are shown in grey. Smoothed centile curves represent the 10th (P10), 25th (P25), 50th (P50), 75th (P75), and 90th (P90) percentiles and were estimated using a generalized additive model for location, scale and shape (GAMLSS) with a Box-Cox Power Exponential (BCPE) distribution.

Table legends

Table 1. Main data collected from breeding bitches followed in the Toulouse GOLD Cohort. G, gestational day; L, day of lactation. *Available only for breeding bitches born within the Toulouse GOLD Cohort. #Repeated daily thereafter until whelping. °Repeated daily thereafter until L(X) + 7.

Table 2. Main data collected from puppies followed in the Toulouse GOLD Cohort. D, day of life; M, month of life; AA, after adoption. *Available only for the Early Cohort. °Repeated daily thereafter until D(X) + 7.

Table 3. Biospecimens collected from pregnant and lactating bitches followed in the Toulouse GOLD Cohort.

G, gestational day; L, day of lactation. *Available only for breeding bitches born within the Toulouse GOLD Cohort.

Table 4. Biospecimens collected from puppies followed in the Toulouse GOLD Cohort. D, day of life; M, month of life. *Available only for the Early Cohort.

Figure 1: Main health domains evaluated in the Toulouse GOLD Cohort: a prospective longitudinal cohort

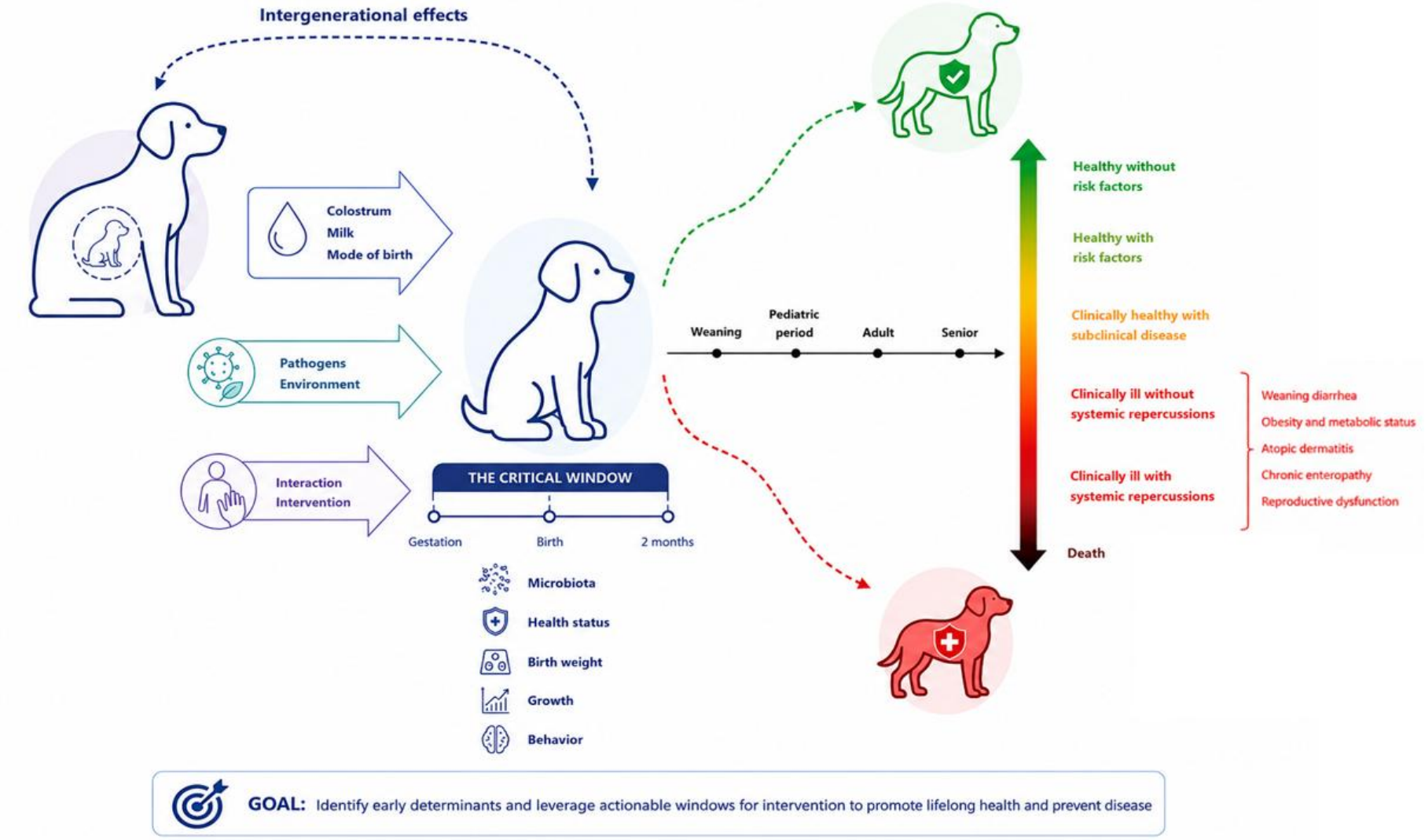

Figure 2: Study timeline showing the main periods investigated in the Toulouse GOLD Cohort.

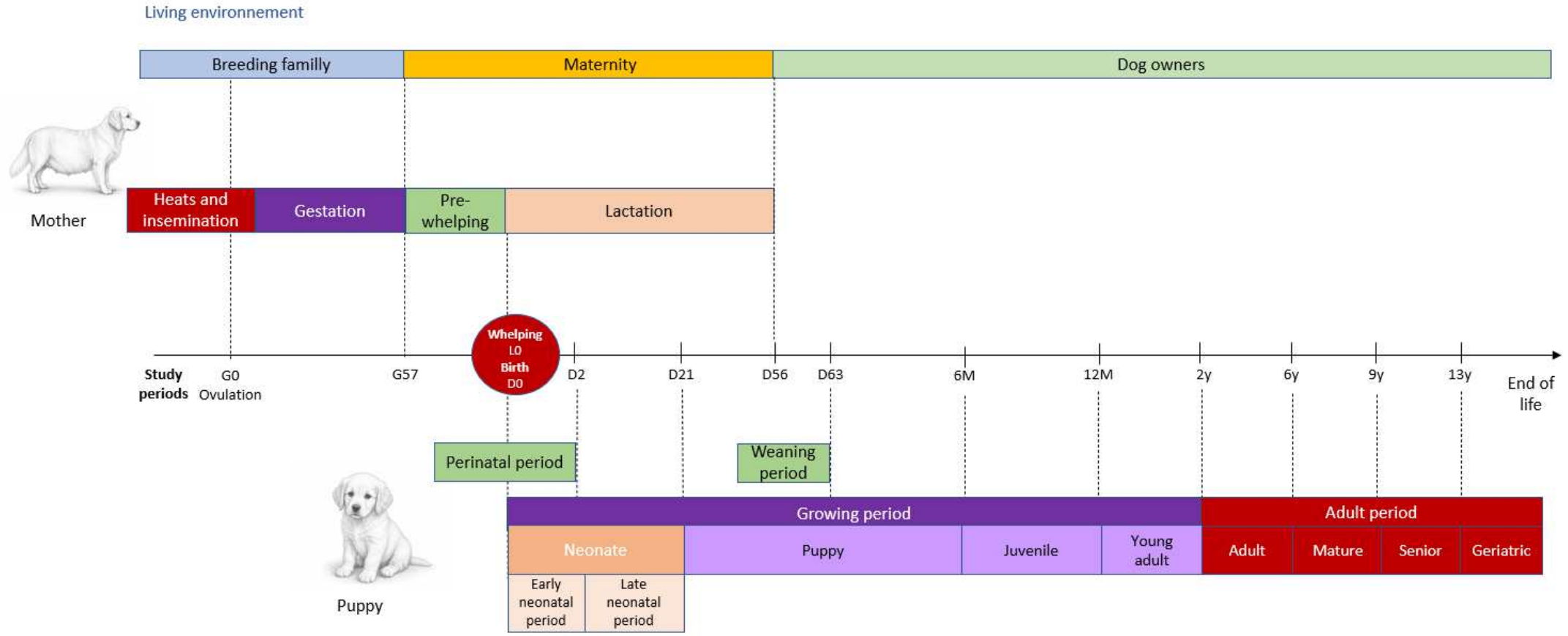

Figure 3. Individual growth trajectories and smoothed body-weight centile curves from birth to 56 days of age. Individual puppy growth trajectories are shown in light grey. Smoothed centile curves represent the 10th (P10), 25th (P25), 50th (P50), 75th (P75), and 90th (P90) percentiles and were estimated using a generalized additive model for location, scale and shape (GAMLSS) with a Box-Cox Power Exponential (BCPE) distribution.

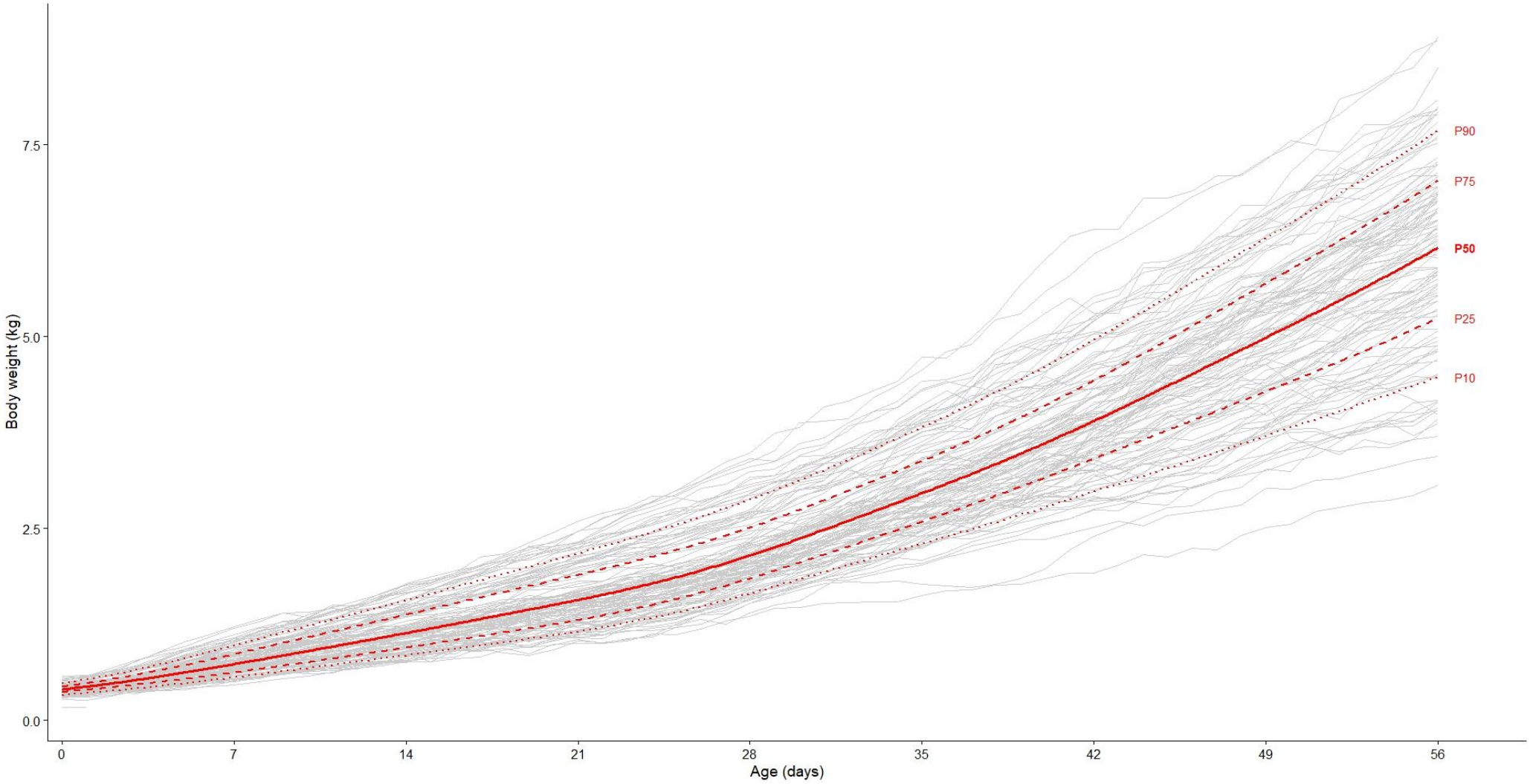

Table 1: Main data collected from reproductive bitches followed in the Toulouse GOLD Cohort

Type	**Collection time**											
	Heats and insemination	**Gestation**		**Pre-whelping**	**Whelping**	**Lactation**						
	Ovulation = G0*	G28*	G42*	G57	L0	L1	L7	L21	L28	L35	L42	L56
Progesterone	X	X	X	X#								
Fetal ultrasound measurements		X	X	X#	X							
Body temperature	X	X	X	X#	X	X°	X°	X°	X°	X°	X°	X
Treatments administered	X	X	X	X#	X	X°	X°	X°	X°	X°	X°	X
Body weight	X	X	X	X#		X°	X°	X°	X°	X°	X°	X
Food amount and ingestion	X	X	X	X#	X	X°	X°	X°	X°	X°	X°	X
Body Condition Score (BCS)	X	X	X	X		X	X	X	X	X	X	X
Muscular condition score (MCS)	X	X	X	X		X	X	X	X	X	X	X
General clinical exam	X	X	X	X		X	X	X	X	X	X	X
Dental plaque				X		X	X	X	X	X	X	X
Fecal score				X#		X°	X°	X°	X°	X°	X°	X

G – gestational day; L – day of lactation; *Available only on the reproductive bitches born in the TGC; # And than daily until whelping; °And then daily until L(X)+7

Table 2: Main data collected from puppies followed in the Toulouse GOLD Cohort

Type	**Collection time**																	
	Birth D0	D1	D7	D14	D21	D28	D35	D42	D49	D56	7 days AA*	3M	4M	6M	9M	12M	18M*	Every year from the age of 2
APGAR	X																	
Glycemia	X	X			X					X	X	X	X	X	X	X	X	X
Body temperature	X	X	X	X	X	X°	X°	X°	X°	X	X	X	X	X	X	X	X	X
Morphometric measurements		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
General clinical exam	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Body Weight	X	X	X	X	X	X°	X°	X°	X°	X	X	X	X	X	X	X	X	X
Body Condition Score (BCS)												X	X	X	X	X	X	X
Muscular condition score (MCS)												X	X	X	X	X	X	X
Treatments administered	X°	X°	X°	X°	X°	X°	X°	X°	X°	X°	X	X	X	X	X	X	X	
Complete blood count														X	X	X	X	X
Health events declared by owners											X	X	X	X	X	X	X	X
Fecal score										X	X	X	X	X	X	X	X	X

D – day of life; M – month of life; AA = After Adoption; *Available only for the Early Cohort; °And then daily until D(X)+7

Table 3: Biospecimens collected from pregnant and lactating bitches followed in the Toulouse GOLD Cohort

Type of sample	Collection time						
	Ovulation = Day 0*	G28*	G42*	G56	L1	L7	L21
Buccal swab	X	X	X	X	X	X	X
Skin swab	X	X	X	X	X	X	X
Vaginal swab	X	X	X	X	X	X	X
Rectal swab	X	X	X	X	X	X	X
Blood sample (serum, plasma)	X	X	X	X	X	X	X
Hair sample	X	X	X	X			
Feces	X	X	X	X	X	X	X
Vaginal swab	X	X	X	X	X	X	X
Colostrum / Milk					X	X	X

G – gestational day; L – day of lactation; *Available only from the reproductive bitches born in the Toulouse GOLD Cohort

Table 4: Biospecimens collected from puppies followed in the Toulouse GOLD Cohort

Type of sample	Detail about the sample	Collection time											
		Birth D0	D1	D7	D21	D56	D63	M3	M6	M9	M12	M18*	Every year from the age of 2
Urine sample		X	X	X									X
Rectal swab		X	X	X	X	X	X	X	X	X	X	X	X
Buccal swab			X	X	X	X	X	X*	X*	X*	X*	X	
Skin swab			X	X	X	X	X	X*	X*	X*	X*	X	
Blood sample	1 ml collected until 3 months (serum), 4 ml collected after 3 months (serum, plasma, whole blood)		X		X	X	X	X	X	X	X	X	X
Hair sample					X	X	X	X	X	X	X	X	X
Feces						X	X	X	X	X	X	X	X
Ear swab									X*	X*	X*	X	
Vaginal/preputial swab									X	X	X	X	X

D – day of life; M – month of life; *Available only for the Early Cohort

Table 5: Morphometric measurements of puppies from 1 to 56 days of age. Data are presented as mean ± standard deviation (SD).

Age (days)	n	Head circumference (cm)	Thoracic circumference (cm)	Crown-rump length (cm)
1	115	15.3 ± 0.9	17.4 ± 1.3	18.4 ± 1.6
7	114	18.6 ± 1.2	21.2 ± 1.7	22.1 ± 2.4
14	111	21.0 ± 1.5	23.6 ± 2.3	25.6 ± 2.3
21	114	22.9 ± 1.5	26.3 ± 2.4	28.2 ± 2.6
28	114	25.0 ± 1.7	28.8 ± 2.5	30.9 ± 2.7
35	114	26.7 ± 1.5	32.4 ± 2.7	35.2 ± 3.0
42	114	28.3 ± 1.7	35.8 ± 2.9	38.5 ± 3.2
49	114	29.7 ± 1.9	38.2 ± 3.0	41.7 ± 3.2
56	113	31.4 ± 1.8	41.7 ± 3.5	46.1 ± 4.1

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryFile1SchematicrepresentationofToulouseGOLDCohortmaternityunit.docx
- SupplementaryFile2Compositionofthedrydiet.docx
- SupplementaryFile3AntiparasiticandvaccinationprotocolfordogsofTGC.docx
- SupplementaryFile4ToulouseGoldCohortStandardizedAssessmentForms.docx
- SupplementaryFile5TGCDataDictionary.xlsx