An investigation into the short- and long-term effects of prenatal maternal stress in the domesticated dog *(accompanied by a research study proposal).*

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Prenatal Maternal Stress

Prenatal maternal stress (often shortened to prenatal stress) describes the exposure of an expectant mother to physical and/or emotional stress that can directly compromise the social behaviours, sensory processing, and levels of anxiety in the offspring (Abbot et al., 2018).

INTRODUCTION:

The prenatal period of development is the gestation period, or time between conception and birth (Hill, 2021). For the domesticated dog this is an average of 63-64 days/9 weeks, ranging from between 56 and 66 days (Hill, 2021). This makes it by far the longest developmental stage for puppies, rivalled only by the postnatal socialisation period which also equals a total of circa sixty days. Despite the many physical changes happening in the neonatal and transitional periods when the puppy has been born, it is the prenatal period that is responsible for the creation of the puppies themselves. From as early as twelve hours into the prenatal period there is a fertilized cell in the mother, which starts to split until there are a total of sixty-four cells implanted in the uterine wall (Haffenden, Brown and Nicholas, 2018). By week four of the prenatal period puppies are about 1.5cm long and are most susceptible to birth defects. Up until day 33 all puppies are female; after day 33 some become male (Haffenden, Brown and Nicholas, 2018). From week eight puppies can be born safely (Hill 2021).

It is well documented that prenatal stress has some severe short term and long-term impacts on the growth, development and mental and physical health of humans (McLaughlin et al., 2021; Humphreys et al., 2020; Saeki et al., 2021). Equally multiple studies have been done to assess the biological changes as a result of prenatal maternal stress in other mammalian species (Thompson, 1957; Braastad, 1998; Creutzberg et al., 2021). There have been some small studies on the impacts of prenatal stress in dogs, but they have been isolated to individual breeds such as Leroy's experiment on five beagles (Leroy et al., 2009). Tønnessen highlights that prenatal maternal stress can be directly linked to high perinatal puppy mortality rates (Tønnessen et al., 2012). A larger study encompassing a

greater number of dogs, from a greater spectrum of breeds, across a longer time frame has not yet been explored as a way of uncovering the impact of prenatal stress on the modern, domestic dog, and the potential links to the rise in behavioural issues that we see in the twenty first century.

LITERARY SURVEY:

In 1957 the first work emerged on the damaging effects of prenatal maternal stress and anxiety on offspring (Thompson, 1957). The subject of these experiments were rats, and it was Thompson et al who opened the door to understanding a whole new influential factor in the development of mammalian species. However, it was Braastad in 1998 who found and compiled research on the types of stressors that could affect development of offspring from laboratory and farmed mammals (Braastad, 1998). This experiment was then followed up by his blue fox experiments in the early twenty-first century, where he delved deeper into the impacts of prenatal stress on the foetal gonadal and the foetal pituitary-adrenal systems (Braastad, 2001; 2003).

Braastad used daily handling of the pregnant mother foxes to cause significance amounts of stress in these unhandled animals (Braastad, 2001). They were then raised to day 48 of their 52 days gestation, and then humanely killed (Braastad, 2001). The data collection consisted of weighing of the adrenal glands from the foetuses, as well as measuring their steroid and cortisol content (Braastad, 2001). The ethics of this study must be called into question as a significant limitation of the study. Unfortunately, in much scientific research, animals are raised and killed to provide the best scientific results. Ideally, this experiment could have sought to find a better way to understand the impacts of prenatal stress even if it meant less accurate data. However, the results of this experiment proved enlightening. Braastad discovered that those foetuses from mothers who had been handled daily had smaller adrenal glands and higher cortisol and plasma levels in comparison to those that had not undergone any prenatal stress. Prenatal maternal stress was demonstrated to have a significant influence on the development of the Hypothalamic–Pituitary–Adrenal (HPA) Axis in the offspring (Braastad, 2001). The HPA Axis is a complex feedback system of neurohormones which regulate stress, immunity and fertility (Knapp, 2020). The HPA Axis is responsible for cortisol production and as any changes to the Axis will have a significant effect on organisms ability to handle stress (Knapp, 2020). Braastad also noted that it was the female offspring in his experiment that were more vulnerable to the changes on the HPA Axis then their male siblings (Braastad, 2001).

Braastad's study, despite some compromised ethics, demonstrated how mammalian species were susceptible to prenatal stress on a biological and ethological level – there were physical and chemical changes happening as a result of stress that would stay with the foetus for the remainder of their life.

In 2021 Creutzberg et al. set out to dive deeper into the same research with a focus on the HPA Axis and inflammation markers in adult rodents' brains (Creutzberg et al., 2021). Their method, instead of experimenting directly on rodents, was to reanalysis over thirty research papers of prenatal stress on rodent species and use the data collected in them for their own study (Creutzberg et al., 2021). This bypassed some of the ethical present in Braastad's experiment as rather than subjugate more animals to stress (and potential death) the researchers used data that was already available to them. However, this presents an additional limitation to the results as there are more likely to be errors or misinterpretations of other researchers' data.

Creutzberg's meta-analysis study concluded that there was evidence of changes to the HPA Axis, as Braastad had found earlier with his blue foxes (Creutzberg et al, 2021). What they did not find was any evidence for change in the inflammatory markers, but they noted that this could demonstrate that any case-by-case inflammatory differences might not be an 'immunological phenotype' but instead a 'vulnerability' that could still be connected to prenatal stress even if this experiment had not yet proved a connection (Creutzberg et al., 2021). Creutzberg's analysis took into account a vast range of experiments over many decades of research which makes this a unique study that compiled all the data seeking to find one outcome: whether prenatal stress do indeed cause these ethological and biological changes, particularly to the HPA Axis (Creutzberg et al., 2021). Just like Braastad they certified the influence of prenatal maternal stress and how it should not be ignored.

In over sixty years since Thompson first published his work there has been an increase in enthusiasm in the number of studies into understanding prenatal stress on offspring development. Another study in 2021 by Crombie et al. focused on the impacts of prenatal stress on both behavioural and neurodevelopment, as well as seeing whether there was a potential way to reverse these changes (Crombie et al., 2021). Their study used guinea pigs, exposing them to strobe lighting for two hours a day as a form of stress (Crombie et al., 2021). Similarly to Braastad, this experiment involved euthanising the participants on day 30 of gestation and then collecting samples of the plasma and testing cortisol, allopregnanolone and progesterone levels (Crombie et al., 2021). It would seem that twenty years later science is still on the backfoot when it comes to ethical considerations in its experiments. Crombie et al. discovered that there was a difference in how the offspring behaved based on their sex, confirming what Braastad had noticed twenty years earlier (Crombie et al., 2021; Braastad, 2001). Of the offspring exposed to prenatal stress: the males were more hyperactive, whilst the females were more anxious (Crombie et al., 2021). Perhaps most interesting in their research was that these negative effects on offspring behaviour as a result of prenatal stress could be improved through additional postnatal stress (stress experienced from birth to adolescence) – including separation from their dam (Crombie et al., 2021). It appeared that subjecting a young animal to more stress postnatally reduced the behaviours of hyperactivity and anxiety which had been caused by prenatal maternal stress. But at what cost?

Although Crombie's study seems to demonstrate that additional stress in the postnatal period could be used as a treatment option for offspring who underwent stress in the prenatal period, I would argue that this is too simplistic (Crombie et al., 2021). Behavioural improvements might be seen, but there is not yet enough research to demonstrate the long-term impact of postnatal stress. By causing stress in the postnatal period what trauma could we be forcing them to deal with throughout their life? Might we be forcing offspring into states of suppression? There is not yet enough data to safely use postnatal stress as a treatment option for prenatal stress – more research on the impacts of postnatal stress is required.

In 2009 the first canine-specific prenatal maternal stress study was released. The full study was never published, but a shortened summary was included in the *Journal of Veterinary Behaviour.* Leroy et al. exposed five pregnant beagle bitches to one of three environments: acute stress experiences, enrichment experiences and ordinary housing (Leroy et al., 2009). The sixty-six puppies born from the five beagles were tested for their problem-solving skills through the use of a maze and results were compared against each other (Leroy et al., 2009). The results of this experiment were fascinating. The puppies that had undergone prenatal stress were found to be much faster when escaping the maze: they made fewer changes of direction, mistakes, and U-turns in comparison to the puppies that did not undergone any prenatal stress (Leroy et al., 2009). It is likely that these puppies fear responses were so well conditioned as a result of prenatal stress that they were motivated and better equipped to escape faster due to the anxiety of the experience. These same puppies however did not maintain attentional focus or as good eye-contact with the experiments compared with the non-stressed puppies. Seventeen of the puppies, from across all three study groups, were later tested with an obstacle course in adulthood (Leroy et al., 2009). They demonstrated no difference between those that had been prenatally stressed and those who had not. All dogs had spent their adult lives in similar stable home environments, suggesting perhaps that positive life-experiences and adaption to their high levels of cortisol meant there were no long-lasting effects of prenatal stress for the dogs in this study (Leroy et al., 2009).

Outside of canine or other test species the world of human psychology caught onto the possible implication of prenatal stress in humans. A plethora of research studies have been done surrounding the topic examining how prenatal maternal stress is impacting the children and adults of today. Despite these studies being human-centric it is becoming increasingly likely that all mammalian species respond to prenatal stress in a similar way and thus the literature can be applied to our dogs just as it can be for ourselves. Humphreys et al. in 2020 conducted an experiment looking at how prenatal stress affected the amygdala-medial prefrontal cortex in infants. The amygdala found in the medial temporal lobe, and the medial prefrontal cortex (mPFC), a neocortical structure, work together in a partnership in the establishment and extinction of fear and fear memory (Marek et al., 2013). They are vital structures that are involved in any kind of fear conditioning but are also connected to

the development of many anxiety disorders (Marek et al, 2013). If prenatal stress was to alter the way the amygdala-mPFC functioned, then it would certainly have an impact on fear learning and anxiety levels in mammalian species.

This is exactly what Humphreys discovered in 2020. Her experiment, focusing on humans, was ethically acceptable. Humphreys recruited a string of expectant mothers and recorded what stressors had happened to them over the last six months to gage an understanding of how much prenatal maternal stress they had experienced (Humphrey et al., 2020). Their infants, once born, were subject to a series of Magnetic Resonance Imaging (MRI) scans to assess function and structure of the amygdala-mPFC. The results proved that infants who had experienced prenatal stress had an increased 'structural integrity between the amygdala and mPFC' (Humphreys et al., 2020). A greater connection between the amygdala and the mPFC could establish stronger fear pathways and lead to an increase in anxiety disorders, demonstrating the very real physiological changes as a result of prenatal maternal stress.

McLaughlin et al. also sought to examine the implication of prenatal stress in 2021. Her study focused on understanding the differences in responses to prenatal stress between the sexes. Similar to the work of Crombie who discovered differences in responses between his male and female guinea pigs, McLaughlin wanted to address the same in humans (Crombie et al., 2021; McLaughlin et al., 2021). McLaughlin's participants were mothers who had experienced ordinary life stress events during pregnancy and then enrolled on the study: like Humphreys had done a year earlier. Ethically, the human-centric studies keep the participants interests at heart and model how and effective, ethical canine study should be performed. McLaughlin's experiment spanned decades and followed the participants through their childhood and into adulthood, with the final tests taking place at aged eighteen (McLaughlin et al., 2021).

What she discovered was that there was indeed a sex-specific response to prenatal stress (McLaughlin et al., 2021). Initially, male children showed a more significant stress response measured by elevated cortisol levels to stressors compared to females (McLaughlin et al., 2021). This sex differences reversed in adolescence when females showed a more significant stress response (McLaughlin et al., 2021). Finally, at the adolescent (measured here as eighteen years), the sex difference had reverted back to the original results with males having more elevated cortisol levels in response to stressors. There are many additional factors that must be taken into account when analysing this data, such as the individual development of the participants, the understanding that females typically go through adolescence earlier than males, the individual lives, homes and experiences the participants had between tests, and the fact that no participant was exposed to significant prenatal trauma (they focused on common stress events instead) (McLaughlin et al., 2021).

Much more research is needed into the possibilities of a sex-specific response to the gravity of what has been discovered by McLaughlin et al.

What these studies highlight is that there is a significant amount of research on prenatal stress and its impacts across many mammalian species, including humans. It is fine time that a study, specifically on canines, with all the ethical concerns conducted in the human-centred studies, was undergone to establish exactly how prenatal stress has affected the domestic dogs today. Understanding this will hopefully put on the path to being able to help mitigate, manage and treat this in the future.

RESEARCH QUESTIONS:

This research study proposal seeks to answer a few of the questions plaguing canine trainers and behaviourists, as well as gaining more information on the impact of the experiences of the bitch during pregnancy.

1) Does prenatal maternal stress affect canines just as it does in other species? Are there possible variations across the types and breeds of domestic dog?
2) What consequences are there of prenatal maternal stress in dogs? Is it something we should be concerned about?
3) Are domesticated dogs more at risk of complications as a result of prenatal maternal stress in comparison to other species? Is this down to the lifestyle and management of the pregnant bitches?
4) Is prenatal maternal stress and its consequences on the rise for the canine species? Is this also a result of bad management of pregnancies of bitches? Does it also reflect the more recent increase in puppy-demand?
5) Are puppy farms and unethical commercial establishments responsible for the creation of a generation of dogs suffering from the impacts of prenatal maternal stress?
6) How responsible is prenatal maternal stress for the rise of behavioural issues we see today?

The hope of this research proposal is to shed light on the impact of prenatal stress and explore the extent to which the current management practices of pregnant bitches may be contributing to creating a generation of dogs with fewer social skills and an inability to cope with stress.

HYPOTHESIS:

My hypothesis is: prenatal maternal stress plays a highly influential role in biological changes within newly born domesticated puppies, just as it does in humans. These changes result in birth defects,

physical changes and higher levels of cortisol which lead to behavioural imbalances. Dogs that experienced prenatal stress are less socially capable, have impaired sensory processing and a higher baseline of anxiety leading them more likely to display typical behavioural problems faced by modern behaviourists: including but not limited to, reactivity, fear-aggression, resource guarding and separation anxiety.

A COMMENT ON ETHICS:

For the proposed research study a controlled experiment that might obtain the best scientific/biological results would follow the protocols set out by Braastad et al. on their study in blue foxes, or by Crombie et al and their study with guinea pigs (Braastad et al., 2001; Crombie et al., 2021). This would include having a wide-range of dams in a controlled environment and subjecting them to stress each day: either through handling, food-deprivation, strobe lighting, traumatic events etc (Braastad et al., 2001; Crombie et al., 2021). However, in the modern age of animal rights it is important that any study does not compromise the participants. The study proposed is therefore qualitative, based on dogs that have found themselves in stressful circumstances (for example dogs in rescue, or from traumatic backgrounds), not dogs that are exposed to stress as part of an experiment. Although the study will not use controlled condition and therefore many more variables are potentially introduced, it will be physically and emotionally safer for the dogs at the centre of the study - something that should not be compromised in the name of science. The improvements that could be made to amend some of these flaws in approaching the study in this way are outlined at the end of the proposal.

PROPOSED METHODOLOGY:

Terminology

“Experimental group” refers to the bitches who underwent prenatal maternal stress, and their offspring. “Control group” refers to the bitches who did not undergo any prenatal maternal stress, and their offspring. “Test group” refers to all dogs used in the study.

Animals, Sourcing and Timeframe

The Experimental Group in this study will use female bitches of three of the most common breed types in the UK and most readily found in rescue centres as highlighted by the data from Battersea Dogs and Cats Home. These breeds are a Staffordshire Bull Terrier, German Shepherd and a Border Collie (Pets4Homes, 2013). Of these breeds, the Staffordshire Bull Terrier and German Shepherd also

coincide with *UK Pets* fifteen most popular breeds of 2021 (UK Pets, 2021); whilst the Border Collie falls in the top fifty (Petpedia, 2021). There will be five bitches of each breed, taking the total dogs in the experimental group to fifteen. A second group of a further fifteen female bitches, all matching the same breeds as the experimental group will form the control group.

This experiment does not need to take place in a designated window of time. To ensure the ability to collect a wide range of results over a long period of time this experiment can be ongoing. Pregnant females do not need to be identified at the same time; instead the data can be recorded as and when pregnant bitches meeting the criteria become available.

The pregnant bitches that make up the experimental group must all have been identified from somewhere where they have been exposed to high states of stress whilst pregnant. This includes, but is not limited to: long-distance travel, caged or confined living, starvation, injury, extreme temperatures, isolated incidents where the bitch feared for her life, pre-existing mental health conditions such as anxiety or depression. Once the experimental group of bitches have been identified they would be removed from the high-stress environment as soon as possible in favour of a more comfortable home for the remainder of the pregnancy.

The control group of bitches must be from stable households. These dogs must have no pre-existing mental health issues, and not be exposed to high levels of stress through pregnancy. They must come from households where there are secure attachments with their caregivers, they must not be trained with punitive methods, undergo any invasive procedures, or have had a significant injury through the gestation period. Any bitches in the control group that undergoes a high-stress experience unintentionally during gestation must be removed from the control group.

All bitches when identified will then be kept in stable, secure environments with all their needs met for the remainder of the pregnancy and birth of the puppies. This includes plentiful access to food, water and space, such as a whelping box, to retreat to and give birth to the litter.

Initial Data Collection

Once the litters have been born, immediate data collection can begin. This will include physical weight and length measurements of the puppies at birth. The experimental group bitches result can be pitched against the control group bitch of the same breed to decipher whether prenatal stress contributed to impairing the growth of the prenatally stressed pups (Braastad et al, 2001).

A Canine Cortisol ELISA Kit should then be used on each of the puppies produced in every litter, in the entire test group (Cusabio, 2020). This Salimetrics cortisol assay kit will be able to detect the baseline level of cortisol in the puppies. Healthy dogs typically have cortisol saliva tests readings

below 5nmol/L, whilst dogs struggling with excessive cortisol or hypercortisolism can range from anywhere as low as 5nmol/L to 25nmol/L (Wenger-Riggenbach et al, 2010). The results of the experimental group puppies will be pitched against the control group to see if there is indeed an increased cortisol concentration in the prenatally stressed puppies.

Example Results Table*:

*Data in table above is fictious and designed purely to demonstrate what a final results table may look like.

Ongoing Handling & Treatment of Puppies in the Test Group

Once these initial observations have been recorded all puppies in the test group need to follow the same protocol over the coming weeks and months as they grow.

Between the ages of 3 and 16 days the puppies should undergo a protocol of *Early Neurological Stimulation* (ENS) as outlined by Battaglia – exposing the puppies to various different types of stressors in mild forms like cold towels, being separated from their mother, being handled and rotated

Test Subject	**Group (Experimental or Control)**	**Breed**	**Sex**	**Length (cm)**	**Weight (g)**	**Cortisol (ngmol/L)**	**Birth Defects**	**Stillborn (yes/no)**
Puppy #1	Experimental	Border Collie	*M*	*15.2*	*198*	*10.1*	*n/a*	*No*
Puppy #2	Experimental	German Shep.	*F*	*21.7*	*380*	*7.9*	*Twisted RH Leg*	*No*
Puppy #3	Control	Border Collie	*M*	*19.3*	*360*	*3.2*	*n/a*	*No*
Puppy #4	Experimental	Staffie.	*M*	*14.9*	*180*	*18.7*	*n/a*	*No*
Puppy #5	Experimental	Staffie.	*F*	*12.1*	*120*	*n/a*	*n/a*	*Yes*

upwards, downwards, left and right, etc (Battaglia, 2009). This can lead to dogs whom in adulthood have stronger adrenal glands, heartbeats, greater disease resistance and had better problem-solving abilities (Battaglia, 2009).

The puppies will then be observed on Day 3 and Day 16 of the ENS protocol with qualitative data recorded. Qualitative data will include observations on the puppies' reaction to separation from the mother, whether distress sounds were made, whether the puppy responded well (or indifferently) to novel scenarios, or whether they expressed signs of stress and fear illustrated by crying, trying to escape the experiment area and return to the mother. A second record of data will be taken at Day 16 after the protocol has been enacted for thirteen days to see if there has been any improvement in the puppies ability to handle the stress, or if the observations remained the same, or worsened.

Example Table for ENS Protocol:

*Border Collie Litter #3, Day 3 (Experimental Group)**

Test Subject	**Separated from Mother**	**Placed on Cold Towel**	**Invasively Handled**
Puppy A	*Crying, whelping, wriggling away.*	*Crying whilst wriggling off the towel.*	*Crying and attempt to get out of hands.*
Puppy B	*No crying, frozen.*	*Frozen still.*	*Hardly wriggled, except when lifted upside down then tried to wriggle.*
Puppy C	*Crying and wriggling.*	*Short sharp whelps, but remained on the towel.*	*Crying whilst trying to get out from hands.*
Puppy D	*Whimpering whilst remaining quite still.*	*Less crying, slowly movement too.*	*Crying, but remained still and manoeuvrable.*
Puppy E	*Crying.*	*Crying whilst wriggling off towel.*	*Crying and attempt to get out of hands.*

*Data in table above is fictitious for the purpose of outlining this proposal.

After day 16 the puppies will be cared for using an ordinary socialisation and habituation programme, whilst also seeking to find appropriate homes for the pups. Repeat observations checks will be made at 3 weeks, 5 weeks and 8 weeks before the transition to the new homes to explore the puppies response to the introduction of a novel object. The observer will record whether a novel object is greeted with enthusiasm and confidence, or whether promotes a fear-response whereby the puppy is scared, stressed or unsettled by the experience.

Example Table for Novel Object:

*Border Collie Litter #3, Week 5 (Experimental Group)**

Test Subject	**Response to Novel Object**
Puppy A	*Unwilling to approach, but not leaving training area.*
Puppy B	*Frozen still, unwilling to approach, backing away, stiff movements.*
Puppy C	*Crying for mum and little mates, did eventually approach object.*
Puppy D	*Observing object from a distance, no attempt to engage, but not scared.*
Puppy E	*Enthusiastic engagement, attempted play with object.*

*Data in all tables above is fictitious, and only as a means to outline the data-gathering method for this proposed experiment.

Continuing Development & Data Collection of Test Group

New homes for the Test pups from 8 weeks of age will have to meet a standard to both enable appropriate living conditions for the dogs, but also to aid in reducing variation between the lives of the test dogs. These standards include stable homes, not with novice caregivers, outdoor space, no young children, and no current dog with mental health or stress-induced behaviour issues. These restrictions are in place to prevent additional stress being placed on the puppies in the control and experimental groups in their early developmental periods. Any differences in the results between the puppies in the control and experimental groups can be linked more clearly to their prenatal experiences.

Once the puppies have been transferred into their new homes at aged 8 weeks, a second observational visit will be arranged. This visit will observe how the pup is coping in a new environment, what their response is in their new surroundings. The observation will record whether the puppies behaved confidently and curiously, or whether they were timid and scared, whether there was distress vocalisation, whether they were able to engage and play with their caregivers, whether they were comfortable eating, sleeping and drinking.

Repeat visits will then take place at 12 weeks, 6 months, 1 year, 2 years, 5 years and 10 years. On each observational visit a set of activities will be presented to the dog, and their reactions recorded. These include:

- Response to a novel object (balloon, beach ball, bunting, toy dog, etc).
- Interactions with a group of other dogs.
- Interactions with a group of people.

Note: if the dog has previously demonstrated to the caregiver that it is unable to cope in close interactions with novel objects, people and/or dogs, then the subject must not be put in a compromising position. Instead work with the dog at a distance it is comfortable, and record that it is unable to get within however many metres of the triggers.

At each activity the observer should be looking for and recording any signs of aggression, stress, fear or unwillingness to engage. The observer will record Calming Signals and behaviours associated with the Ladder of Aggression as a means of keeping recorded data comparable to the results of other dogs in the study. Rugaas's *On Talking Terms with Dogs: Calming Signals* outlines many of the commonly observed behaviours including head turns, diverted gaze, lip and nose licking, and yawning (Rugaas, 1997). The Ladder of Aggression was devised by Kendal Shepherd and illustrated in *The Canine Commandments* (Kendal, 2007). The ladder begins with typical calming signals, but escalates to avoidance behaviours and aggression, including growling, snapping and biting (Kendal, 2007).

Example Tables:

*Test Subject #7 (Experimental Group)**

	Engagement	**Repetitions of Vocalisation**	**Calming Signals**	**Ladder of Aggression**
Novel Object	*Engaged*	*2*	*Yawning, lip-licking*	*n/a*
Dog Interactions	*Engaged*	*5*	*Lip-licking, shake.*	*Stiffening, growling, snapping.*
People Interactions	*Engaged*	*0*	*n/a*	*n/a*

*Test Subject #12 (Control Group)**

	Engagement	**Repetitions of Vocalisation**	**Calming Signals**	**Ladder of Aggression**
Novel Object	*Engaged*	*0*	*Shake off.*	*n/a*
Dog Interactions	*Engaged*	*0*	*n/a*	*n/a*
People Interactions	*Engaged*	*0*	*n/a*	*n/a*

*Test Subject #19 (Experimental Group)**

	Engagement	**Repetitions of Vocalisation**	**Calming Signals**	**Ladder of Aggression**
Novel Object	*Engaged*	*7*	*Turning away, crouching, exited test area.*	*Stiffening.*
Dog Interactions	*Engaged*	*n/a*	*Lip licking.*	*Stiffening.*
People Interactions	*Did not engage.*	*0*	*n/a*	*n/a*

*Data in all tables above is fictitious, and only as a means to outline the data-gathering method for this proposed experiment.

Application of the Data

The data recorded from each interaction of the years of all test subjects can be compared and contrasted to see if the social ability, problem solving skills and stress responses of the prenatally stressed pups (experimental group) is impaired.

EXPERIMENTAL IMPROVEMENTS:

As outlined above on the comment on ethics this study is not fool proof. It will not harness the most scientifically accurate results due to the vast number of uncontrolled factors. It is my opinion that the welfare of the participants of the experiment is a higher priority than the value of the results. This section lists, and explains, the types of flaws in this experiment and how to be mindful of them when learning from the results, and how to improve the experiment going forward.

Range of Breeds

The initial proposal focuses on a narrow study across very few breed types. This however does not provide accurate data for the whole of the canine species. This study should ideally involve a wide range of domesticated female dogs. Ideally pregnant females from every breed, including some mixed breeds would be used to provide a more accurate widespread result that can be termed under 'dog' rather than just a specific breed.

Litter Size

Borge et al. demonstrates that there are a multitude of factors that affect litter sizes in dogs, including: breed, size and age of bitch, whether there have been any previous litters, the time of year (winter versus summer etc), the number of matings and whether these were AI or natural (Borge et al., 2011). It is recognised that large litters in other species create additional stressors and problems for the prenatal and neonatal pups, including reduce birth weights directly affecting survival rates (Peltoniemi, Yun, Björkman, and Han, 2021). In dogs there is an increase in the chance of stillbirth, runts, birth defects and perinatal mortality – particularly in letters of over 12 puppies (Tønnessan et al., 2012). This has to be allowed for in the interpretation of results as birth defects and smaller puppies may not be a result of prenatal maternal stress, and instead due to a larger than average litter size for any of the above listed reasonings (Borge et al., 2011).

Neonatal Stress

Another important limitation to this study was that the calculations, particularly of cortisol, at birth could be influenced by other stress in that neonatal period. The birthing process itself can be particularly stressful and additional trauma like starvation of oxygen or mismanagement of the pups by the mother could easily affect cortisol levels in the puppy. It would be important therefore that the study takes notes of any puppy that undergoes an additionally stressful experience before the first set of quantitative data is taken. Additional neonatal stress should be factored in the analysis of the results.

Breeders & Homes

One of the most influential factors that could hinder results is the impact of external stressors that individual dogs go through in their normal day-to-day lives. This could also be impacted by the types of homes they are raised in, and by their caregivers. A potential improvement to this experiment would be to set up a system of adopters who all meet a more intense criteria than outlined in the experiment above. This would not be time and cost effective but would allow the dogs to be raised in mirroring lifestyles so that there are as few variables as possible coming from differences in their management, training, and life experience.

Difference in Trimester

It is worth being aware that by selecting pregnant bitches from all walks of life there could be a range in at which trimester the prenatal stress occurred. Some of the bitches may have experienced trauma and stress for all three trimesters, being recovered just before birth only. Whilst other bitches may have only been introduced to a short amount of stress in the last trimester only. Although there is not enough research out there yet to note if there would be a difference in the impacts of prenatal stress, it is reasonable to assume that a dog under stress for their entire pregnancy is more likely to have significant changes to the offspring than a dog who experiences stress in the very last days before birth once the puppies are already grown. A method to mitigate this variable would be to accept dogs that fit a certain profile only: i.e. those that underwent stress in trimester one only. This however narrows down the dogs available for the study and may not be possible.

DISCUSSION & SIGNIFICANCE:

The main aim of this study is to investigate the short and long-term impacts of prenatal maternal stress on the domestic dog, with the hope of communicating a wider understanding of the gravity of subjecting pregnant mothers to high stress loads. It would hope to emphasise the importance of good management, not only of the puppies, but of the mother through her pregnancy. This study might also potentially highlight how the rise of typical behavioural issues today might be connected to mismanagement of their mothers while pregnant.

The results of this study would provide both quantitative and qualitative data that could be compared and contrasted to draw a conclusion about the likely negative impacts of prenatal maternal stress. The sizes, weights, cortisol levels, birth defects and potential stillborns recorded across the litters at birth could indicate the immediate results of prenatal stress and its consequence on the dogs that are brought into the world – particularly when these findings can be contrasted against the control group of the same breeds of dog. This study aims to be long-term, covering many years, collecting qualitative data. The dogs in the test group will be monitored from puppyhood, to adolescence, to young dogs into adulthood and finally old age in the hope of giving an accurate picture of the potential very long-lasting impact of prenatal stress.

I theorise that prenatal maternal stress is the sole biggest factor in the upward trend of domestic dogs with behavioural issues requiring assistance from behavioural practitioners. For many years we have been breeding a generation of dogs from prenatally stressed mothers as a consequence of the existence of puppy mills, the rise in monetary value of puppies, inexperienced caregivers, imports from other countries, and the increase of dogs in rescue. Therefore, the thousands of puppies born in these circumstances, or from these mothers, are already on a backfoot when it comes to their health,

development, and ability to function in a modern human world. Humans have bred a generation of highly stressed dogs, and if we combat prenatal maternal stress we may be able to reverse that trend.

This is why findings of this study would be highly valuable. It has long been understood that prenatal stress has had negative impacts, but a complex, long-term, broad ranging study of just the domesticated dog will give a unique understanding of how prenatal stress is affecting man's best friend. This in turn will help people understand, and potentially even combat, the reoccurring and rising behavioural issues found among modern day canines. It is hoped that this will also enable behaviour practitioners to better help and support the dogs they are working with, as well as providing knowledge to help prevent a vicious cycle of breeding prenatally stressed dogs.

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