PHYSIOLOGIC RESPONSES OF THE FETAL LAMB TO EIGHT DAY FLUOXETINE EXPOSURE DURING LATE GESTATION

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Abstract

Clinical depression is diagnosed in 10-15% of pregnancies frequently resulting in antidepressant therapy with selective serotonin reuptake inhibitors such as fluoxetine. Human studies have suggested that third trimester exposure to fluoxetine results in negative birth outcomes such as preterm delivery, low birth weight and increased admission to neonatal intensive care units. Based on these findings, we undertook an eight day maternal IV infusion of fluoxetine (FX, 98.5 μg/kg.d) in 14 chronically instrumented pregnant sheep during late gestation (125-140 d, term = 147 d) with a control group of 15 animals receiving sterile water. In the FX group maternal and fetal plasma FX and norfluoxetine (NFX) concentrations were within the therapeutic range reported in humans. Maternal fluoxetine infusion increased plasma serotonin levels within 15 min of infusion associated with a 20% decrease in uterine artery blood flow. Both plasma serotonin levels and uterine artery blood flow returned to control values by 1 h after infusion. The transient decrease in uterine artery blood flow resulted in fetal hypoxemia during the first 24 h of infusion. Minor alterations in fetal blood gases continued throughout the eight-day infusion period. The incidences of low voltage electrocortical activity (EcoG), eye movements and fetal breathing movements were significantly reduced in the FX group while the incidence of high voltage ECoG was significantly increased during the first 6 h of maternal fluoxetine infusion. These alterations continued throughout the eight days of infusion to a lesser degree. Fetal ACTH and cortisol plasma concentrations in the FX group increased on Infusion Days 7 and 8 more than in the control group. Late gestation infusion of fluoxetine in sheep did not alter birth weight

or gestational age at delivery. In conclusions, late gestation exposure to FX in pregnant sheep transiently altered fetal blood gas status with greater effects on fetal behavioural state and neuroendocrine function. The longer term consequences of these in utero perturbations remains to be determined.

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μm	micrometre
5HT	serotonin
5HTP	5-hydroxytryptophan
ACTH	adrenocorticotropin hormone
AgCl	silver chloride
$AUC_{0-\infty}$	area under the curve
AVP	vasopressin
cm	centimetres
C_{max}	maximum plasma concentration
CRF	corticotropin releasing factor
d	day
ECoG	electrocorticogram
EEG	electroencephalogram
EMG	electromyogram
EOG	electrooculogram
FBM	fetal breathing movements
FX	fluoxetine
g	gram
h	hour
HPA axis	hypothalamic-pituitary-adrenal axis
HPLC	high pressure liquid chromatography
ID	inner diameter

IP	intraperitoneal
IV	intravenous
MAOI	momoamine oxidase inhibitor
M	molar
mEq/l	milliequivalent per litre
min	minute
min	minutes
ml	millilitre
mm	millimetre
mmHg	millimetre Mercury
mV	millivolt
nA	nanoampere
NaCl	sodium chloride
NFX	norfluoxetine
nM	nanomolar
PO	per oral
REM	rapid eye movement sleep
SC	subcutaneous
SSRI	selective serotonin reuptake inhibitor
SWS	slow wave sleep
TCA	tricyclic antidepressant
t_{max}	time to maximum plasma concentration
V_{dapp}	apparent volume of distribution

wk	week

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CHAPTER 1

Background

1.1 What is Depression?

Individuals suffering from depression experience a pervasive dysphoric mood day-in and day-out with a natural time course of 4-12 months (Kandel, 1991). A clinical diagnosis of depression is made when an individual suffers from either a depressed mood or a marked decline in interest or pleasure in all, or most, activities for more than two weeks. The DSM-III-R criteria listed in Table 1 are used for the diagnosis of major depressive disorders. The estimated prevalence of this disorder is 13-20% in the United Sates and 5% world-wide, occurring two to three times more frequently in women than in men (Kandel, 1991; Wells & Hayes, 1993). Adults 25-44 years of age comprise the most susceptible age group (Kandel, 1991; Wells & Hayes, 1993).

1.1.1 Theories of Depression

In the 1960s, the catecholamine hypothesis of affective disorders was proposed suggesting that depression is associated with a deficiency of catecholamines, specifically noradrenaline, at functionally important aminergic receptor sites (Schildkraut, 1965). This was followed by the indoleamine theory, which suggests that the neurotransmitter deficiency does not involve noradrenaline but serotonin (Wells & Hayes, 1993). Serotonin plays a significant role in regulating

some of the basic behaviours disrupted during depression such as sleep, appetite, energy levels, sexual activity, cognitive function, psychomotor function and mood

Table 1.1. DSM-III-R Diagnostic Criteria for Major Depressive Disorder.

A. At least five of the following symptoms have occurred in the past two weeks, reflecting a change in previous functioning and at least one of which is 1 or 2.
    1. Depressed mood
    2. Markedly diminished interest or pleasure in all, or almost all, activities
    3. Significant weight loss/gain or an increase/decrease in appetite
    4. Insomnia or hypersomnia
    5. Psychomotor agitation or retardation
    6. Fatigue or loss of energy
    7. Feelings of worthlessness or excessive or inappropriate guilt
    8. Diminished ability to think or concentrate, or indecisiveness
    9. Recurrent thoughts of death, recurrent suicidal ideation, or a suicide attempt or specific suicide plan
B. The change in mood can not be linked to an organic factor or the normal reaction to the loss of a loved one
C. Delusions and hallucinations do not occur without significant mood symptoms
D. Schizophrenia, schizophreniform, or delusional disorder have been ruled out

Adapted from Pharmacotherapy: A pathophysiologic Approach, J.T. DiPrio, R.L. Talbert, P.E. Hayes, G.C. Yee, G.R. Matzke

(Wells & Hayes, 1993). Additionally, it has been suggested that the effects of antidepressant drugs require a functional serotonin system as a low tryptophan diet can lead to a depressive episode (Delgado et al., 1990). More recently, Siever & Davis (1985) proposed the dysregulation hypothesis of depression, suggesting that an inability to regulate the catecholaminergic neurotransmitter system results in depression without specifying the particular neurotransmitter involved. Kandel (1991), however, suggests that depression is likely a single disorder involving disturbances in any one of several neurotransmitters, which are known to interact. Antidepressant drugs may also have their therapeutic action through normalisation of hypothalamic-pituitary-adrenal axis function (Holsboer, 2000). This hypothesis is based on observations of altered corticotropin-releasing hormone, adrenocorticotropin hormone (ACTH) and cortisol levels in depressed patients.

1.1.2 Incidence of Depression during Pregnancy and the Antepartum Period

Depression is more common in women than men across all age groups and cultural backgrounds with a female to male ratio of 1.68 (Kessler et al., 1993). It has been suggested that this gender difference in prevalence of depression may be related to biologic factors such as endocrinologic or genetic determinants or that a universal social factor may play a role (Weissman & Olfson, 1995). Whatever the cause, depression is a disease affecting predominantly women during their childbearing and child-rearing years. In fact, 10-15% of women may be diagnosed with depression during pregnancy (Ledward, 1996; Weissman & Olfson, 1995;

O'Hara et al., 1984). Postpartum depression occurs in about 10 to 15% of women with symptoms beginning two weeks after delivery including despondency, feelings of guilt and inadequacy, loss of sexual interest and sleep and eating difficulties which can be treated with support, both practical and psychological, as well as with antidepressants (Ledward, 1996). It is possible that significant postpartum changes in hormone levels such as progesterone, estrogen, cortisol and β-endorphins may lead to the symptoms of depression in the postpartum period (Weissman & Olfson, 1995).

1.2 Treatment of Depression During Pregnancy

Treatment of depression during pregnancy is important to fetal outcome by preventing poor maternal self-care and nutrition, disturbed sleep, lack of prenatal care, increased exposure to alcohol and drugs and a higher risk of suicide by the mother (Weissman & Olfson, 1995). The prevalence of clinical depression was assessed at 28 weeks gestation in 389 women aged 18 to 29 years using the Beck Depression Inventory (Steer et al., 1992). Scores of ≥ 21 are diagnostic of clinical depression. The results of this study indicated that scores ≥ 21 were related to an increase in negative pregnancy outcome including an increased risk of low birth weight newborns (20% vs. 7.6%), preterm delivery (25% vs. 8.1%) and small for gestational age newborns (15% vs. 6.2%). A study of the five year old children of postnatally depressed women found that male offspring are at significantly greater risk of clinically significant behavioural disturbances than girls or children of women who did not experience depression postnatally (Sinclair & Murray, 1998).

One would like to suggest that psychotherapy is an effective and sufficient method of dealing with depression during pregnancy and thus negate the requirement of pharmaceutical intervention. Unfortunately few studies exist which assess the efficacy of psychotherapy during pregnancy. In addition, even if psychotherapy and pharmacological therapy were proven to have equivalent efficacy, antidepressants may be the preferred course of treatment as the onset of action is faster and more consistent (Weissman & Olfson, 1995).

1.3 Pharmacological Treatment of Depression

A brief description of neurotransmission in the noradrenergic and serotonergic systems, as illustrated in Figure 1.1, will be useful in understanding the mechanism of action of antidepressants. The propagation of an action potential along the axon to the nerve terminal causes an influx of calcium into the cell. The calcium influx causes a change in membrane potential, triggering the exocytosis of vesicles filled with either noradrenaline or serotonin (5HT) resulting in the release of these neurotransmitters into the synaptic cleft. The neurotransmitters diffuse into the extracellular fluid and act on postsynaptic receptors to cause excitatory endplate potentials and action potentials if the threshold potential is reached. Noradrenaline is inactivated by the enzyme catechol-O-methyl transferase in the synapse and by an active uptake process into the presynaptic cell, the surrounding glial cells and the postsynaptic cell while serotonin is inactivated by the latter process only (Kandel, 1991). Once in the cell, the neurotransmitters are either repackaged into vesicles or metabolized in the mitochondria by monoamine oxidase.

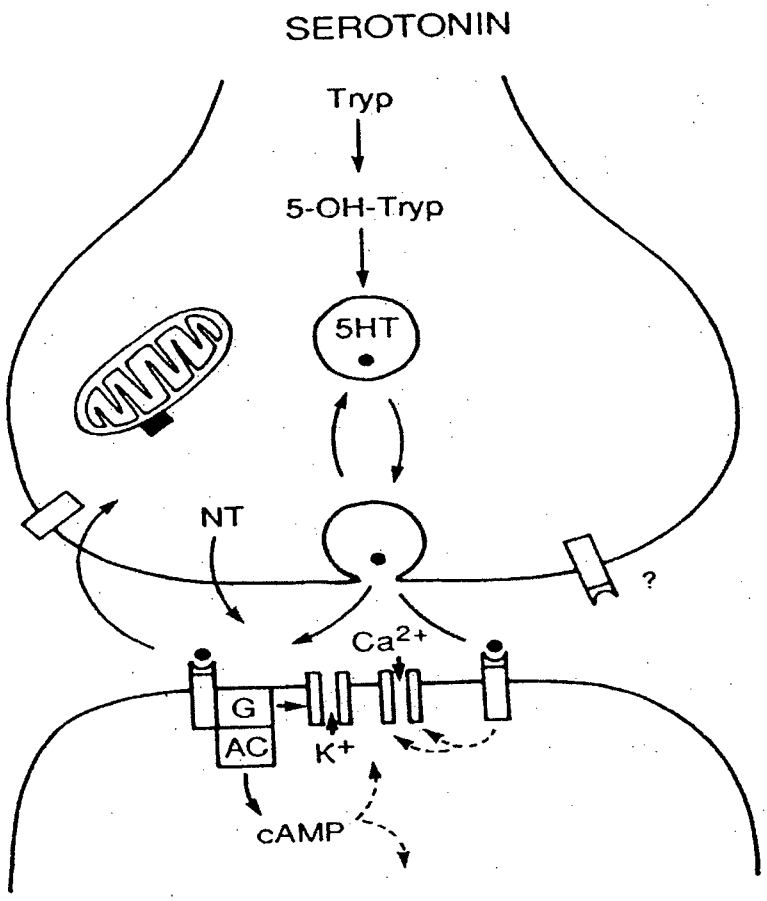


Figure 1.1. Diagrammatic depiction of neurotransmission at a serotonergic synapse. Tryp, tryptophan; 5-OH-Tryp, tryptophan hydroxylase; 5HT, serotonin; NT, neurotensin; Ca^{2+}, calcium; K^{+}, potassium, G, G protein; AC, adenylate cyclase; cAMP, cyclic adenosine monophosphate. G.M. Shepherd, Neurobiology, 2nd Ed., Oxford University Press, New York, 1988, p. 158.

The goal of drug therapy in the treatment of depression is to increase the amount of neurotransmitter produced for release or the duration of time the neurotransmitter spends in the synapse. There are seven major classes of antidepressants that have been designed to fulfil these goals. Monoamine oxidase inhibitors (MAOI) which prevent the degradation of noradrenaline and serotonin in the presynaptic cell, and tricyclic antidepressants (TCA) which block the reuptake of noradrenaline and serotonin to varying degrees into the presynaptic cell are considered the classical therapies (Kandel, 1991). Five newer antidepressant treatments have been developed including selective serotonin reuptake inhibitors (SSRIs), dual serotonin and noradrenaline reuptake inhibitors, serotonin-2 antagonist/reuptake inhibitors, norepinephrine and dopamine reuptake inhibitors and noradrenergic and specific serotonergic antidepressants (Stahl, 1998). Each of these classes of antidepressants act similarly by affecting the uptake or accumulation of noradrenaline and serotonin (Kandel, 1991). Remission or marked improvement in depressive symptomology is observed in 70% of patients treated with MAOIs or TCAs while 85% of patients show improvement with SSRIs or higher concentrations of TCAs (Kandel, 1991).

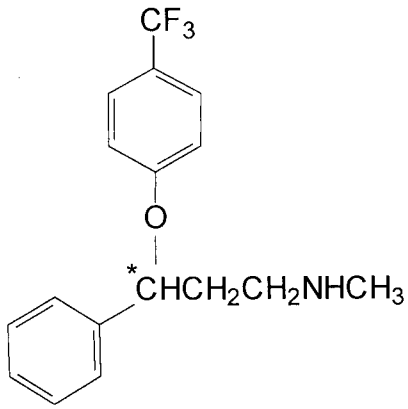
1.4 Physiological Changes in Pregnancy Affecting Drug Therapy

Pregnancy causes many changes in maternal physiology. As a result, changes in drug dosing may be necessary. An increase in dose may be required due to decreased protein binding, altered hepatic metabolism and progesterone induced decreased gastrointestinal motility which decreases drug absorption during

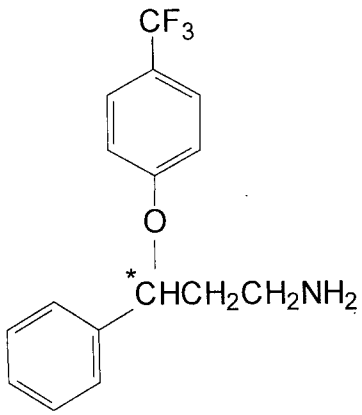
pregnancy (Weissman & Olfson, 1995; Wisner, et al., 1999). Maternal blood volume increases 40-50% during pregnancy, which contributes to the increase in the volume of distribution that may lead to a lower drug serum concentration (Gilstrap & Little, 1992). In addition, renal output is increased and this may increase the clearance of drugs. It is also interesting to note that progesterone has been shown to inhibit the activity of several cytochrome P450 isozymes including CYP2D, CYP11β, CYP1A1 and CYP1A2 which may lead to altered metabolism of drugs during pregnancy (Baum & Strobel, 1997; Delorme et al., 1995; Eugsterlt et al., 1993). It is also interesting to note that estrogen has been shown to desensitise hypothalamic $5HT_{1A}$ receptors in rats (Raap et al., 2000) leading one to speculate that this may result in an altered response to various antidepressant drugs.

1.5 Fluoxetine

Fluoxetine (FX), an example of an effective SSRI, was introduced to the market in 1987 with the trade name Prozac®. By 1989, Prozac® held nearly 20% of the market share for antidepressants in the United States (Brosen & Skelbo, 1991). In 1993 and 1994, 46% of 1000 patients treated with SSRIs for depression received FX (Hylan et al., 1999). A recent study in Great Britain found a 460% increase in prescriptions for SSRIs between 1991 and 1996 with 50% receiving FX (Lawrenson et al., 2000). Both FX and it's' pharmacologically active major metabolite, norfluoxetine (NFX), are chiral compounds (Stevens & Wrighton, 1993). Prozac® is marketed as a racemic mixture composed of both the R- and S- enantiomers. The chemical structure of these compounds is illustrated in Figure 1.2.



Fluoxetine



Norfluoxetine

Figure 1.2. Chemical structure of FX and its' demethylated metabolite, norfluoxetine. The asterisks indicate asymmetric carbon atoms.

1.5.1 Mechanism of Action

As mentioned previously, the SSRIs inhibit the reuptake of serotonin into the presynaptic cell and thus increase the availability of serotonin at postsynaptic receptor sites. The therapeutic action of SSRIs is due to delayed disinhibition of serotonin release, resulting in increased serotonin neurotransmission (Stahl, 1998). After release into the synapse, serotonin is cotransported with sodium back into the presynaptic cell by a transporter with a serotonin recognition site (Goodnick & Goldstein, 1998). FX binds to the serotonin recognition site or a closely overlapping site preventing the reuptake of serotonin (Owens & Nemeroff, 1994) with 80% inhibition of the pump (Preskorn, 1997). Acute administration of FX causes an increase in serotonin especially in the raphe nucleus. Somatodendritic $5HT_{1A}$ autoreceptors on serotonin cell bodies in the raphe nucleus inhibit cell firing, reducing serotonin release from the cell (de Montigny et al., 1990). The increase in serotonin caused by chronic FX exposure desensitises these receptors resulting in a disinhibition of neuronal firing, thus increasing serotonin release from the cell (Owens & Nemeroff, 1994; Kandel, 1991). The latency between increased extracellular serotonin levels due to immediate reuptake inhibition and increased neuronal impulse flow representing $5HT_{1A}$ receptor desensitisation may account for the delay in therapeutic action (Stahl, 1998). The delay in therapeutic action may be decreased by coadministration of a $5HT_{1A}$ receptor antagonist such as pindolol, to prevent the initial decrease in serotonin release (Hjorth & Auerbach, 1996; Goodwin, 1996). Increased serotonin levels in the synapse cause desensitisation of

presynaptic terminal $5HT_{1B}$ (rat) and $5HT_{1D}$ (human and guinea pig) receptors that normally inhibit serotonin release from the neuron. Disinhibition of these autoreceptors increases serotonin neurotransmission. It is unclear as to whether chronic SSRI treatment causes decreased sensitivity of postsynaptic $5HT_{1A}$ receptors (Cowen, 2000) or has no effect on these receptors (Lanfumey & Hamon, 2000; de Montigny et al., 1990). On the other hand, no change in postsynaptic $5HT_2$ receptors has been observed (Johnson, 1991). Receptor downregulation may require 2-3 weeks and thus may explain the 2-3 week delay in either therapeutic action of the drug or remission of acute side effects. Although serotonin neurons project from the raphe nucleus to many brain regions, the pathway from the midbrain raphe to the prefrontal cortex is thought to be involved in the antidepressant effects of SSRIs (Stahl, 1998).

Studies in rats show that a single dose of FX causes an increase in serotonin synthesis in the brain (Badaway et al., 1996; Tsuiki et al., 1995). Conversely, chronic FX treatment (fourteen days) in both mice and rats decreases cerebral serotonin synthesis due to presynaptic feedback inhibition and decreased tryptophan uptake into the presynaptic cell (Cacia et al., 1992; Hwang et al., 1980; Trouvin et al., 1993). The finding in animal models that chronic FX treatment decreases serotonin synthesis may not be clinically relevant as FX will allow the serotonin in the synapse to remain in close proximity to receptors longer and the doses used in the animal work may have been higher than most clinical doses. In addition, 5-hydroxyindole acetic acid levels are lower in the brain with FX treatment suggesting that the metabolism of serotonin is decreased (Johnson, 1991). Microdialysis studies in rats

show that acute FX increased serotonin and dopamine in the prefrontal cortex while chronic FX increased only serotonin (Tanda et al., 1996). Acute FX (10 mg/kg IP) caused an increase in extracellular serotonin levels in the striatum (Marsden et al., 1979). Studies with fluvoxamine, a potent SSRI, have found an increase in extracellular serotonin in the rat frontal cortex and dorsal raphe nucleus with acute dosing (Bel & Artigas, 1992), while chronic treatment (1 mg/kg for 14 days) increased extracellular serotonin in the frontal cortex but not the dorsal raphe nucleus (Bel & Artigas, 1993). It has also been noted that noradrenaline turnover is decreased after SSRI administration (Wells & Hayes, 1993).

Changes in other neurotransmitters are common with the TCAs, and this may be responsible for the broader side effect profile of these drugs compared to the SSRIs. The SSRIs do not alter histamine, dopamine, α1- or α2-adrenoceptor binding (Johnson, 1991; Preskorn, 1997), however, an increase in $GABA_B$ binding sites has been observed with chronic FX treatment (Johnson, 1991). FX has also been reported to upregulate β-adrenoceptors (Goodwin, 1996) whereas several TCAs have been shown to downregulate β-adrenoceptors (Fuller & Wong, 1987).

1.5.2 Side Effects

Common side effects of FX treatment include nausea, nervousness, headache, anxiety and insomnia (Lader, 1988; Rickels et al., 1985). More than 20% of subjects treated with 20 mg/d FX for eight weeks (n=61) complained of headache, dry mouth, nausea, somnolence, diarrhea and agitation (Rush et al., 1998). The incidence of side effects generally increases as drug dosage is increased [e.g. 60

mg/day] (Altamura et al., 1988; Beasley et al., 1990; Tyrer et al., 1990). The side effects of SSRI treatment are a result of the disinhibition of serotonin neurotransmission at other effector sites as illustrated in Table 1.2.

1.5.3 Efficacy

Comparisons of the efficacy of FX at 5, 20, 40 and 60 mg/day doses have been made over a period of six weeks. Subjects receiving doses of 20, 40 and 60 mg/day showed significant improvement over the placebo group on several scales of depression (Altamura et al., 1988). SSRIs are as effective as TCAs in treating depression with fewer side effects (Preskorn, 1997) although they may be less effective in severe depression (Goodwin, 1996). FX has a slower onset of action than sertraline, another SSRI (Newhouse, 1996). After 6 weeks of FX treatment, subjects were classified as responders if they scored less than 8 on the Hamilton Rating Scale for Depression, with 57% responding at 60 mg/d (Kelly et al., 1989) and 54% responding with a 20 mg/d dose (Norman et al., 1993).

1.5.4 Plasma Concentration and Therapeutic Response

No relationship has been found between plasma concentrations of FX or NFX and desirable therapeutic response (Preskorn, 1997; Baumann, 1996; Kelly et al., 1989; Norman et al., 1993). FX doses of 40 and 60 mg for three weeks result in a FX plasma concentration of 154.5 ng/ml and 309 ng/ml respectively with higher levels observed at 6 weeks of treatment (Asberg & Martenson, 1993). Measurement of FX and NFX at 2, 4 and 6 weeks of treatment with 20 mg/d found that the plasma

Table 1.2. Summary of serotonin receptor types, second messenger systems, target tissues and physiological effects.

Receptor	Second Messenger System	Target Tissue	Effect
5-HT_{1A}	Adenylate cyclase	Presynaptic -dorsal raphe nucleus Postsynaptic - Hippocampus, lateral septum, frontal cortex, thalamus, hypothalamus	Inhibits 5-HT neuronal firing Blunts growth hormone, cortisol and hypothermic response to serotonin
5-HT_{1B}	Adenylate cyclase	Terminal autoreceptor (rat)	Inhibits 5-HT release
5-HT_{1C}	Phosphoinositide Turnover	Choroid plexus and brain regions	Induction of specific behaviours, feeding and anxiety
5-HT_{1D}	Adenylate cyclase	Terminal autoreceptor (human)	Inhibits 5-HT release
5-HT_2	Phosphatidyl Inositol Turnover	Postsynaptic in hippocampus, frontal cortex, spinal cord	Sedation & insomnia ($5HT_{2C}$) Delayed ejaculation or orgasm ($_{2C}$) Decreased libido Decrease food intake ($5HT_{2C}$) Anxiety ($5HT_{2C}$)
5-HT_3	Ion Channel	Area postrema, limbic regions Nucleus tractus solitarius	Modulation of neurotransmitter release (dopamine, acetylcholine)
		Gut, brainstem, hypothalamus	Nausea & vomiting

Compiled from Goodwin, 1996; Goodnick & Goldstein, 1998; Lanfumey & Hamon, 2000; Cowen, 2000; Martin et al., 1998; Glennon, 1990; Marsden, 1991; Stahl, 1998.

concentration of FX increased (59, 63 & 82 ng/ml) while NFX levels stabilised (43, 65 and 65 ng/ml) (Norman et al., 1993). Fluorine magnetic resonance spectroscopy in 12 subjects showed that brain FX concentration is 10 times higher than plasma concentration (Bolo et al., 2000). It should be noted that these studies used high pressure liquid chromatography (HPLC) to determine the concentration of FX and NFX and were are not capable of analysing the concentrations of the S and R isomers of either compound. It is possible that a relationship may exist between one or more of these isomers and therapeutic response or side effect profile.

1.5.5 Platelet Function

Almost all of the serotonin produced by the enterochromaffin cells of the gut for peripheral use is stored in platelets. Platelets do not synthesise serotonin but do have serotonin uptake transporters and are rich in $5HT_2$ receptors (Marsden, 1991). As a result, platelets are a good model of neuronal serotonin uptake and receptor activity (Fuller & Wong, 1987). FX treatment in humans has been shown to decrease serotonin uptake as expected, and this inhibition is correlated with a decrease in mean score on the Hamilton Depression Scale, indicating clinical improvement in subjects (Bakish et al., 1997). Two human studies found that chronic FX treatment decreased serotonin in platelet rich plasma at 6 weeks (Ko et al., 1997; Menys et al., 1996) while a single dose of FX (10 or 20 mg/kg) increased plasma serotonin levels at three hours (Bourdeaux et al., 1998). It has been suggested that serotonin in platelet free plasma is a good estimate of serotonin in the extracellular pool. Acute FX (10 mg/kg IP) increased plasma serotonin levels 520% 30 minutes after the

injection in rats, whereas chronic treatment (5mg/kg for 14 days) had no effect on plasma serotonin levels, although whole blood serotonin was reduced (Ortiz & Artigas, 1992). These studies suggest that chronic FX treatment prevents serotonin uptake by platelets resulting in lower serotonin levels in platelets, while acute treatment causes a short-lasting increase in plasma serotonin due to inhibition of serotonin uptake by the platelets.

1.5.6 Pharmacokinetics of Fluoxetine

1.5.6.1 Absorption

FX is well absorbed after oral administration with a time to maximum concentration (t_{max}) of between 6-8 h (Benfield et al., 1986; Altamura et al., 1994) and 4-8 h (Sommi et al., 1987; Saletu & Grunberger, 1985) depending on the blood sampling intervals used in the studies cited. Maximum plasma concentrations (C_{max}) after a 40 mg FX dose were 15-55 ng/ml (Altamura et al., 1994) and 5 ng/ml (Sommi et al., 1987). When administered with food, C_{max} decreases (Benfield et al., 1986) or does not change (Lemberger et al., 1985). The absolute bioavailability of FX has been determined in the beagle dog where the oral:intravenous $AUC_{0-\infty}$ is 72% for FX and 104% for NFX (Benfield et al., 1986; Bergstrom et al., 1988; Sommi et al., 1987). Steady state plasma concentrations are attained in about thirty days with 60 mg/d dosing (Altamura et al., 1994) but may be achieved within two weeks depending on the dose (Benfield et al., 1986). Steady state plasma concentration was reached with a 20 mg/d dose of FX in 6-8 weeks (Goodnick & Goldstein, 1998).

1.5.6.2 Distribution

The apparent volume of distribution, V_{dapp}, of FX is high, ranging from 20-42 l/kg (Benfield et al., 1986). FX is highly bound to plasma proteins, in the order of 94% or greater (Altamura et al., 1994; Sommi et al., 1987) with weak binding to α_1-glycoprotein (Preskorn, 1997). Tissue binding is reported to be high but no values have been determined.

1.5.6.3 Metabolism

It has been noted clinically that coadministration of FX and drugs such as monoamine oxidase inhibitors, neuroleptics, antiarrhythmics, TCAs and beta blockers results in adverse effects. Both FX and NFX inhibit several cytochrome P450 enzymes including CYP2D6, CYP3A3/4, CYP2C19 and CYP2C9/10 (Brosen & Skjelbo, 1991; Crewe et al., 1992; Otton et al., 1993; Preskorn, 1997; Alfaro et al, 1999, 2000). It is suggested that this inhibition may persist long after FX discontinuation due to the long half lives of both FX and particularly NFX (Otton et al., 1993; Sellers, 1993). The inhibition of CYP2D6 is an important issue not only in understanding drug interactions with FX but also the metabolism of FX itself. FX is a substrate for the CYP2D6 metabolic pathway and is demethylated to NFX, an active metabolite (Stevens & Wrighton, 1993). The formation of NFX influences the clinical efficacy of FX treatment because NFX is almost as potent as FX in inhibiting serotonin reuptake. The S-enantiomer of both FX and NFX is a more potent CYP2D6 inhibitor than the R-enantiomer (Stevens & Wrighton, 1993). von Moltke et al (1997A) have reported that FX is N-demethylated principally by CYP2C9 with

CYP2C19 and CYP3A3/4 playing only a minor role. However, both Stevens and Wrighton (1983) and von Moltke et al (1997A) used FX concentrations for their enzyme kinetic studies that are much higher than the therapeutic levels of the drug and in the range that causes inhibition of CYP2D6. A more recent study in our lab (Kim, 2000) used FX concentrations in the therapeutic range and found that FX demethylation is mediated primarily by CYP2C9, 2C18/19 and 2D6. Moreover, it is the inhibition of CYP2D6 with prolonged FX administration that results in the decrease in FX clearance and the appearance of stereoselective disposition, since FX demethylation by CYP2C9, 2C18/19 is stereoselective, whereas that mediated by CYP2D6 is not (Alfaro et al., 1999 , 2000; Kim, 2000).

1.5.6.4 Excretion

In a single dose administration of 30 mg ^{14}C-FX, 60% of the radioactivity was recovered in urine after 35 days and 12 or 16% in faeces over 28 days (Lemberger et al., 1985; Benfield et al., 1986). Both FX and NFX have long elimination half lives, 1-3 days for FX and 7-15 days for NFX (Lemberger et al., 1985; Bergstrom et al., 1988; Sommi et al., 1987). Further, multiple doses of FX increase its half-life to 2-7 days (Sommi et al., 1987). Clearance of FX decreases from 36-50 l/h after a single dose to 9-12l/h at steady state (Benfield et al., 1986).

1.6 Placental Transfer of Fluoxetine

Before considering the effects of pharmacological treatment of depression during pregnancy, we must consider whether or not the fetus is exposed to the drug.

Thus, a brief overview of placental transfer is included. Molecules with a molecular weight less than 1500 are capable of crossing the placenta by the process of simple diffusion at a rate determined by Ficks' diffusion equation:

$$Q/t = K.A\ (C_m - C_f)/L$$

Where Q/t is the rate of diffusion, C_m is maternal plasma concentration, C_f is fetal concentration, A is placental surface area, L is the transplacental distance and K is the diffusion constant (Ledward, 1996). It should also be noted that drugs crossing the placenta by this method do so by diffusion from a compartment with a high concentration to a compartment with a low concentration but only nonprotein bound drug can participate in this process. In addition, molecules which are highly fat-soluble and uncharged cross the placenta most extensively (Ledward, 1996).

Mothers (n=8) treated with 20.0±1.9 mg/d FX for 8.9±5.6 months had FX and NFX serum concentrations of 41.4±14.6 and 80.4±17.8ng/ml, respectively while the fetal (umbilical cord) concentrations were 45.4±13.6 and 84.4±19.8 ng/ml, respectively (Kim, 2000). In addition, rat studies have shown that a single dose of 12.5 mg/kg of radiolabelled FX administered to the dam on gestational days 12 and 18 resulted in placental transfer of both FX and NFX to the embryo and fetus (Pohland et al., 1989). The amount of radiocarbon in the fetal brain after FX administration on day 18 was approximately half of that found in the entire body of the fetus. Placental transfer was greater at 18 days than 12 days gestation. In addition, in both the embryo and fetus, the concentration of FX in the body was greatest at one hour postinjection, while at 24 hours postinjection the concentration

of NFX in the body was greatest. This result is in keeping with the expected metabolism of FX to its' active metabolite, NFX. Pharmacokinetic analysis after bolus infusion of FX in the sheep fetus found no formation of NFX in fetal arterial blood or accumulation in the amniotic cavity suggesting that the capacity of the fetus to metabolise FX is very limited (Kim, 2000). The long half-lives of these agents suggests that accumulation in embryonic and fetal tissue will occur with long-term treatment.

1.7 Effects of Fluoxetine on Pregnancy Outcome

First trimester exposure to FX in 128 women was compared to controls resulting in no significant differences in the incidence of major congenital anomalies, maternal weight gain, gestational age at birth, birth weight or route of delivery (Pastuszak et. al., 1993). An increase in spontaneous abortion was observed in the FX group but this was also observed in a group exposed to TCAs suggesting that the rise in spontaneous abortion may be due to either antidepressant drug therapy or the underlying pathology of depression. Administration of the McCarthy General Cognitive Index, Bayley Mental Development Index, and Reynell Language Scales to infants ranging from 16 to 86 months who were exposed to FX (n=55), TCAs (n=80) or no antidepressants (n=84) during the first trimester revealed no difference in neurodevelopment amongst the children (Nulman et. al., 1997). In addition, no difference in birth weight or gestational age at birth was reported. However, comparison of first versus third trimester exposure to FX found significant differences in pregnancy outcome. Pregnancies with late gestation FX exposure had

a greater incidence of preterm delivery, admission to special-care nursery, poor neonatal adaptation and decreased birth weight compared to the control group (Chambers et al., 1996). Several points should be made about this study. First, each group had a different number of subjects, early exposed n=100, late exposed n=73 and controls n=223. Second, only 4 of 73 subjects in the late gestation FX exposed group received FX only during the third trimester, most received FX throughout gestation. Third, as with the Pastuszak (1993) study, the control group was selected based on calls to the research group by pregnant women exposed to nonteratogenetic drugs or medical procedures. These women were on average younger, had had fewer therapeutic abortions, lower gravidity and, most significantly, did not suffer from depression. An industry-sponsored review of FX exposure in the third trimester regardless of exposure earlier in pregnancy and with no control group or follow-up found a greater frequency of prematurity than in the general population (Goldstein, 1995). Thus, it is plausible that FX-exposure may result in altered birth weights.

1.8 Serotonin Effects on Uterine and Umbilical Blood Flow

One potential mechanism for FX induced intrauterine growth restriction and preterm delivery is a decrease in uterine artery blood flow caused by increased plasma serotonin levels. Serotonin administration in pregnant sheep elicits dose-dependent decreases in uterine artery blood flow (Clark et al, 1980) and when administered to the fetus the compound causes umbilical vasoconstriction, hypertension and bradycardia (Berman et al, 1978). Moreover, long term decreases

in either uterine or umbilical blood flow result in fetal growth restriction (Daniel et al, 1989; Murotsuki et al, 1996; Lang et al, 2000). However, long-term elevations in serotonin levels would be required for a sustained decrease in uterine or umbilical blood flow. As noted above, while plasma serotonin levels increase with acute FX administration, they decrease with chronic drug exposure (Ortiz & Artigas, 1992; Menys et al, 1996; Ko et al, 1997; Bourdeaux et al, 1998). Thus altered uterine or umbilical perfusion with long-term FX therapy may not occur. However, the effects of FX or other SSRIs on uterine or umbilical blood flow have not been investigated in any species.

1.9 Effects of Prenatal Fluoxetine Exposure on Development

Prenatal exposure to FX has also been studied in rats. Pregnant rats were given 2.5 mg/kg FX in their drinking water from day 6 of gestation to delivery. This resulted in a decrease in B_{max} of [^{3}H]-imipramine binding in pups 25 days old but not at 90 days while similar treatment in adult rats had no effect on [^{3}H]-imipramine binding (Montero et al., 1990). A follow-up to this found that phosphoinositide hydrolysis was reduced in cortical slices of 25 day old pups exposed to FX prenatally while no effect was seen at 90 days of age (Romero et al., 1994). However, this decrease in phosphoinositide hydrolysis (i.e. the intracellular transduction system utilized by the $5HT_{2C}$ receptor) was not accompanied by any change in $5HT_{2C}$ receptor binding studies. Another series of studies involved administration of 10 mg/kg SC FX from gestational age 13 to 20 d in rats. Hypothalamic $5HT_{2A/2C}$ receptor density was reduced in male progeny at 70 postnatal days with a decrease

in ACTH release induced by (±)-[125]4-iodo,2,5-dimethoxyphenylisopropylamine, a $5HT_{2A/2C}$ agonist (Cabrera and Battaglia, 1994). No difference was observed in basal hormone levels or in $5HT_{2A/2C}$ receptor density or function in 28 postnatal day rats. Male offspring had significantly lower serotonin content in the frontal cortex at 28 postnatal days whereas at 70 days a decrease in serotonin content was observed only in the midbrain compared to saline control animals (Cabrera-Vera et al., 1997). The density of serotonin transporters was altered in the hypothalamus, hippocampus and amygdala of 28 postnatal day rats but not in 70 postnatal day rats (Cabrera-Vera and Battaglia, 1998).

1.10 Cardiovascular Effects of Fluoxetine

Systemic administration of FX decreased arterial pressure and heart rate in rabbits (Szabo et al., 1992) and dogs (Steinberg et al., 1986). In contrast, intracerebroventricular administration of either serotonin or FX increased arterial pressure and heart rate in rats although this was preceded by a decline in heart rate with serotonin injection (Lambert et al., 1975; Tsai & Lin, 1986). Peripheral infusion of 0.1 mg/kg/min FX for 50 min resulted in a decrease in platelet serotonin uptake, stroke volume and cardiac output as well as an increase in peripheral resistance. NFX caused similar cardiovascular effects but to a lesser extent with an increase in stroke volume and no change in peripheral resistance (Steinberg et. al, 1986). Human studies investigating cardiovascular effects of FX at doses of 20-80 mg/d have shown significant decreases in heart rate (Fisch, 1985) with no change in blood pressure (Roose et al., 1998).

1.11 Fluoxetine and Human Adult Sleep Patterns

Sleep is not merely the absence of wakefulness. Rather, cycling between slow wave sleep (SWS) and rapid eye movement (REM) sleep occurs throughout the sleep period. Parasympathetic activity predominates in SWS with decreased heart rate and blood pressure, increased gastrointestinal motility and muscle relaxation. Based on electroencephalogram (EEG) recordings, SWS is divided into four stages, with the arousal threshold being greatest in Stage 4. Upon falling asleep, the sleeper enters Stage 1 SWS and progresses through each stage to Stage 4 and then back to Stage 1. After about 90 min of sleep, the sleeper enters REM sleep defined by desynchronised, low-voltage (LV) fast activity, similar to that of wakefulness, on the EEG. Loss of muscle tone, slow and rolling eye movement with discrete rapid outbursts, increased heart rate variability, a high arousal threshold and dreaming characterise REM sleep. REM sleep consists of 20-25% of total sleep time while Stage 2 represents ~50% and Stages 3 and 4 each represent ~15% of total sleep time (Kelly, 1991).

In healthy human male adults, single dose FX administration leads to changes in sleep patterns. Although 20 and 40 mg doses did not alter sleep, a single 60 mg dose of FX decreased total sleep time, sleep efficiency (defined as total sleep time / time in bed) and REM sleep time as well as increased nocturnal awakenings, while all doses resulted in increased daytime sleep latencies (Nicholson & Pascoe, 1988). A subsequent study found that a 40 mg dose of FX taken in the morning increased REM latency (time to first REM onset) with no effect on other sleep

parameters (Saletu et al., 1991). Subjects receiving a single oral dose of 30, 60 or 75 mg FX exhibited increased power of the α and β components of the EEG waveform and decreased attention during the subsequent 10 hours (Saletu & Grunberger, 1985).

Patients suffering from major depressive disorders (n=34) were treated with 60 mg FX for 42 days with sleep polygraphic recordings performed before and after treatment. After FX treatment a decrease in REM sleep and an increase in the number of stage shifts and awakenings was observed (Kerkhofs et al., 1990). Seven subjects receiving FX (25 to 75 mg/day) for depression or bulimia nervosa exhibited an increase in eye movements in NREM sleep, increased REM latency, decreased sleep efficiency, and increases in Stage 1 sleep with decreased Stage 3 and 4 compared to a control group (Keck et al., 1991). The increase in eye movements during NREM sleep was proposed to be due to an increase in central arousal or a dissociation between eye movements, the electroencephalogram and electromuscular components of REM sleep control. Healthy volunteers administered 20 mg FX for 6 days showed an increase in REM latency, decreased REM sleep, and increased Stage 2 sleep as previously shown, but no change in eye movements during NREM sleep was observed (Vasar et al., 1994). This difference may be due to the short duration of treatment or may be due to differential response in healthy and depressed subjects. Further studies of eye movements during sleep have shown an increase in both the amplitude and frequency of eye movements especially during Stage 1 and REM sleep in 41 subjects receiving 20 mg/d FX for 4-5 wk (Armitage et al., 1995). In addition an increase in the amplitude of chin/cheek

electromuscular activity was observed in all stages of sleep in these subjects. These changes in sleep patterns may be due to an elevation in central nervous system arousal. The increased muscle activity may be due an increase in myoclonic activity and the increased eye movements may be due to a disinhibition of brainstem omnipause neurons which control saccadic eye movements (Schenck et al, 1992; Armitage et al., 1995). A similar study in six children treated with FX also found increased eye movements and muscle activity during some stages of sleep with no effect on REM latency (Armitage et al., 1997). A study in nine depressed subjects receiving 20 mg/d FX supported previous effects on sleep parameters while also showing increased arousals during NREM sleep and periodic limb movement disorder (Dorsey et al., 1996).

The changes observed in sleep parameters with FX treatment are not static throughout the course of therapy. Two studies have investigated the changes in sleep parameters over 8 and 10 wk periods. Within one week of treatment REM latency is increased with a small attenuation over time. In contrast sleep efficiency decreases while the number of awakenings increases with time (Rush et al., 1998; Trivedi et al., 1999). FX may have effects on sleep, which persist after discontinuation of treatment. Women studied at four weeks post treatment showed an increase in REM percentage and phasic REM activity (Buysse et al., 1999). Of course the long half-life of FX and NFX may have influenced these findings.

1.12 Fluoxetine and Sleep Patterns in Adult Animals

As in humans, acute FX exposure causes a decrease in REM sleep in cats, rats and Syrian hamsters (Slater et al., 1978; Pastel & Fernstrom, 1987; Gao et al., 1992). In rats, the decrease in REM sleep was due to a decrease in the number of REM sleep episodes rather than the length of these episodes; an increase in REM sleep latency was also observed with doses of 1.25, 2.5 and 5 mg/kg IP FX (Pastel & Fernstrom, 1987). An increase in SWS was observed over a 5 h period in cats with 0.5 mg/kg PO (Slater et al., 1978). Chronic exposure to FX caused an attenuation in the REM sleep suppressing effects of the drug at 7 and 14 days in the Syrian hamster (Gao et al., 1992). A similar effect was also observed during the second and third 5-day treatment periods in cats (Slater et al., 1978).

1.13 Serotonin and Fetal Behavioural States

Both the sheep and human fetus exhibit behavioural states in late gestation. These states are formed by the coincident occurrence of several activities. In the fetal sheep, behavioural states develop around 120 d gestation (term, 147 d) (Clewlow et al., 1983). Electrocortical (ECoG), electroocular (EoG) and electromuscular (EMG) activity define these states. The LV/REM behavioural state, as shown in Figure 1.3, is defined by the presence of LV, fast activity ECOG, eye movements and nuchal muscle atonia (Dawes et al., 1972). The high-voltage (HV)/NREM behavioural state is defined by the presence of HV, slow activity ECoG, no eye movements and nuchal muscle tone. The presence of a LV, fast activity ECoG, eye movements and nuchal muscle tone define wakefulness.

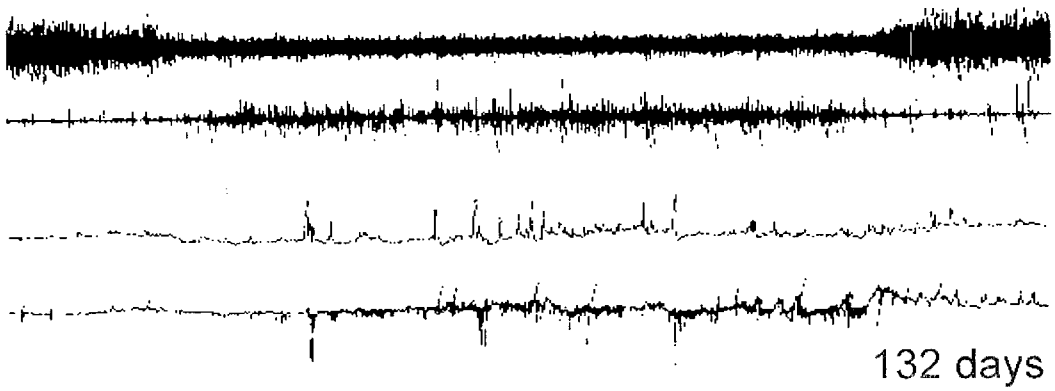


Figure 1.3. A strip chart recording from a 132-d gestation fetal sheep. Electrocortical activity is displayed in the top row followed by electroocular activity, heart rate and tracheal pressure. The middle section of the recording shows an episode of LV/REM behavioural state while the outer edges show the HV/NREM behavioural state.

Some controversy exists in the literature over the presence of wakefulness in the fetus (Parkes, 1991). Each state is also associated with a variety of concomitant behaviours. For example, fetal breathing movements (FBM) occur during the LV/REM behavioural state and periods of wakefulness (Dawes & Robinson, 1976). Fetal heart rate is higher during HV/NREM, while swallowing and voiding occur during LV/REM periods (Richardson, 1994; Wlodek et al., 1989).

When behavioural states develop, LV/REM behavioural state predominates (>50%) with HV/NREM behavioural state occurring about 40% of the time and only brief periods of arousal (10%) (Szeto & Hinman, 1985). By term, the incidence of LV/REM behavioural state decreases to 40%, mostly due to an increase in arousal (10%) (Richardson, 1994).

Similar to the situation in adults, fetal behavioural states are influenced by serotonergic neurotransmission. Acute intravenous administration of 5-hydroxytryptophan (5HTP), the precursor of serotonin, to the fetal lamb resulted in prolonged HV ECoG activity and an increase in fetal breathing movements (Quilligan et al., 1981) and blood pressure (Fletcher et al., 1988). Infusion of 5HTP into the cerebrospinal fluid bathing the fetal brainstem resulted in an increase in FBM but no change in HV ECoG activity (Morrison et al., 1997). It has been suggested that the effects on electrocortical activity of peripheral serotonin and 5HTP are due to the systemic effects of these drugs (Vanderwolf, 1988).

1.14 Effects of Serotonin on the Hypothalamus

The hypothalamus is an important regulatory component of the neuroendocrine system. Hormones are released by the hypothalamus into the hypophyseal portal circulation and act on the anterior pituitary gland. Hormones released from the pituitary enter the peripheral circulation system and act at target sites (see Figure 1.4). The adrenal cortex, which produces cortisol, is an important component of the hypothalamic-pituitary-adrenal (HPA) axis.

Serotonin and the HPA axis influence each other in many ways. Activation of serotonin receptors, 5-$HT_{1A/2A,C}$ in the paraventricular nucleus causes an increase in hypothalamic release of corticotropin releasing factor (CRF), increasing pituitary release of ACTH (van Praag 1996; Bagdy 1996; Fuller 1996). ACTH acts on the adrenal gland to increase cortisol release (Fuller 1992; Lewis & Sherman 1984). HPA axis feedback inhibition by cortisol is decreased by reduced hippocampal serotonin which decreases the number of glucocorticoid and mineralocorticoid receptors (van Praag, 1996). In addition, sustained increases in cortisol or chronic stress lead to decreased serotonin turnover and $5HT_{1A}$ receptor sensitivity. Serotonin neurons also play a role in the circadian rhythm of ACTH (Fuller, 1990).

1.15 Fluoxetine and Hypothalamic-Pituitary-Adrenal Axis Function

The effects of FX on neuroendocrine function have been studied in both acute and chronic administration paradigms in rats and humans. The results of these studies are difficult to reconcile as some have investigated basal hormone levels and

circadian rhythms while others have investigated hormone responses to serotonin challenge using a variety of agents. A single 10 mg/kg IP injection of FX in the rat increased CRF and vasopressin (AVP) in the hypophyseal circulation and increased plasma ACTH (Gibbs & Vale, 1983) while doses of 5-20mg/kg increased cortisol in rats (Fuller et al., 1976). In humans, 40 mg orally administered FX had no effect on cortisol or ACTH release (Torpy et al., 1997) while 80 mg caused an increase in cortisol (Bardeleben et al., 1989).

Rats treated with 10 mg/kg FX for 21 days exhibited attenuated responses in ACTH and cortisol to a $5HT_{1A}$ agonist (8-OH-DPAT) (Li et al., 1993; Li et al., 1994). These studies suggest that chronic FX treatment inhibits $5HT_{1A}$ receptor function causing reduced neuroendocrine responses to challenge. However, human studies have shown increased cortisol release in response to 5HTP challenge after 4 wk of 20-40 mg/d FX treatment (Meltzer et al., 1997). The difference in results between the rat studies and this human study may reflect 5HTP action on $5HT_2$ receptors. Obsessive-compulsive patients treated with 40 mg/d FX for 8 wk exhibited a nonsignificant decrease in cortisol but cortisol levels were much greater compared to controls (Monteleone et al., 1995).

1.16 Fetal Sheep Hypothalamic-Pituitary-Adrenal Axis Function

The HPA axis is of great interest in fetal sheep physiology (Figure 1.4). The maternal-fetal unit consists of a maternal and a fetal HPA axis with placental transfer of cortisol (Wood, 1994). Through most of gestation fetal cortisol and ACTH levels

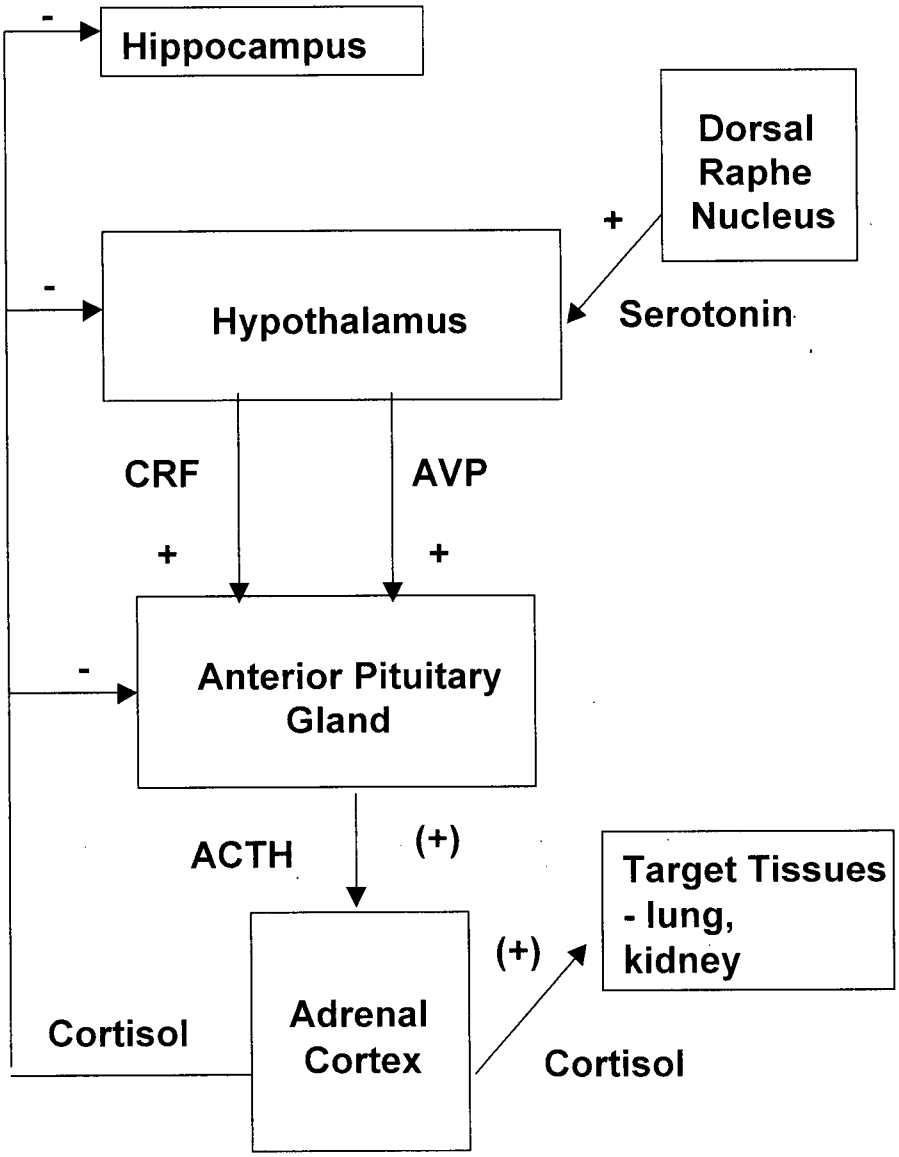


Figure 1.4. Schematic diagram describing the influence of serotonin on the hypothalamic-pituitary-adrenal axis and cortisol in the negative feedback control of this system. The plus and minus signs indicate positive and negative influences of serotonin, CRF (corticotropin releasing factor), AVP (arginine vasopressin), cortisol and ACTH (adrenocorticotrophic hormone).

are low with an exponential increase in late gestation (>130d of 147d term). Cortisol plays a very important role in development of the lung, liver, gastrointestinal tract and kidney as well as other tissues and organs. Cortisol stimulates pulmonary surfactant production which allows effective ventilation of the lung (Wood, 1994). In the fetal sheep, increased fetal plasma cortisol initiates parturition by increasing the estrogen to progesterone ratio resulting in uterine contractility. It has been suggested that ACTH may play a similar role in the primate (Wood, 1994). Thus, if FX has effects on the fetal HPA axis, there would be significant effects on fetal maturation and the onset of parturition.

1.17 Rationale

The antidepressant FX is a selective serotonin reuptake inhibitor, which increases serotonin neurotransmission. Serotonin is involved in the regulation of a variety of physiological systems including the sleep-wake cycle, circadian rhythms and the hypothalamic-pituitary-adrenal axis. Each of these systems also plays an important role in fetal development. Women are at greatest risk of suffering from depression during the childbearing years and thus may either become pregnant while taking an antidepressant or may require prescription for one during pregnancy. Compared to other antidepressant drugs (e.g. the TCAs), the SSRIs such as FX have fewer side effects. Because of this they are now frequently prescribed, especially during pregnancy. Since FX causes an acute increase in plasma serotonin levels, the drug may result in at least a transient reduction in uterine and/or umbilical blood flow. This in turn would reduce the delivery of oxygen and nutrients to the fetus, thereby reducing growth and/or eliciting preterm delivery. Moreover, since FX crosses the placenta, the fetus is directly exposed to the drug as well as to the effects of the drug on the mother. FX increases NREM sleep in human adults and thus may interfere with normal fetal neurodevelopment. FX also alters hypothalamic function in the adult. Thus it may result in changes in fetal hypothalamic regulation causing altered fetal maturation and preterm delivery. For these reasons, and the fact that these drugs are often used over periods of several weeks or months we believe that the infusion of FX over an eight-day period during late gestation in the sheep is a useful model for determining the effects of FX on

fetal physiology. Sheep are a commonly used species for the study of fetal physiologic functions in late gestation for a number of reasons. Of primary importance is the ability to study the fetal lamb for days or weeks in its normal intrauterine environment. The size of the ovine fetus also permits serial sampling of blood and other biological fluids thus permitting detail studies of drug disposition in both mother and fetus. Such sampling is not possible in humans or small animals, and while feasible in non-human primates, is much more difficult and expensive. Fetal physiologic and behavioural parameters can also be readily monitored and are similar to those in the human fetus under normal conditions and in response to cardiovascular and CNS drugs.

1.18 Objectives

To determine the effects of eight days of exposure to FX (achieved via continuous maternal IV infusion) during late gestation in the sheep fetus on:

a. fetal and maternal cardiovascular function including blood pressure, heart rate and uterine artery blood flow.

b. maternal and fetal plasma levels of serotonin

c. fetal behavioural states and breathing movements

d. HPA axis function by measuring cortisol and ACTH.

1.19 Hypotheses

a. FX administration to the ewe will at least transiently increase plasma serotonin levels in the ewe and fetus.

b. FX infusion will transiently decrease heart rate and arterial pressure in the ewe and fetus.

c. FX infusion will decrease uterine artery blood flow via effects on plasma serotonin levels. This will result in fetal hypoxemia.

d. If the FX-induced reduction in uterine blood flow is sustained, there will be a decrease in birth weight

e. The incidence of HV ECoG activity will increase and the incidence of LV ECoG and eye movements will decrease with FX infusion. Moreover, since FBM are tightly linked to LV ECoG in the sheep, FX will also decrease FBM

f. FX infusion will increase the activity of the HPA axis of the ewe and fetus, resulting in an increase in cortisol and ACTH plasma levels. If these alterations are sustained, there will be an increased incidence of preterm delivery.

In the sections of the thesis that follow, chapters 2, 3 and 4 are in the form of manuscripts that have been or will be submitted for publication, while chapter 5 provides an overall summary and conclusions. This is an acceptable format for a Ph.D. thesis at the University of British Columbia.

CHAPTER 2

EFFECT OF MATERNAL FLUOXETINE ADMINISTRATION ON UTERINE BLOOD FLOW, FETAL BLOOD GAS STATUS AND GROWTH

2.1 Introduction

Depression occurs in 5-15% of pregnant women while an additional 10-15% of women experience postpartum depression (O'Hara, 1984; Ledward, 1996; Weissman & Olfson, 1995). In a sample of 65 pregnant women, 31% exhibited depressive symptoms during at least one trimester (Da Costa et al., 2000). Depression during pregnancy results in negative pregnancy outcomes with an increase in admission to neonatal intensive care units, low birth weight and preterm delivery (Orr & Miller, 1995; Steer et al., 1992). In contrast, a study including only Caucasian women found no effect of depression on obstetrical complications such as preterm or induced labour, anaesthesia/analgesia in the first or second stage of labour, type of delivery and preterm delivery (Perkin et al., 1993). Both studies controlled for socio-economic class, although African American women experience more obstetrical complications than Caucasian women (Orr & Miller, 1995).

Although psychological therapy is equally effective as pharmacological treatment (Appleby et al., 1997), its onset of action is variable. Thus pharmacological treatment is often used to treat depression during pregnancy. Two studies of general practitioner records in the United Kingdom from 1992-1997 and 1991-1996 showed that antidepressants are prescribed 50-75% more in women than men (Lawrenson et al., 2000; Dunn et al., 1999). The selective serotonin reuptake inhibitors (SSRIs)

have fewer side effects than tricyclic acids and monoamine oxidase inhibitors and thus are frequently prescribed during pregnancy (Misri et al., 2000). In fact, SSRIs are prescribed twice as often in women as in men (Stewart, 1998) with fluoxetine (FX) being the most prescribed SSRI in Great Britain from 1991 to 1996 (Lawrenson et al., 2000).

It has been previously shown that first trimester in utero exposure to FX does not result in teratogenic effects in humans (Pastszak et al., 1993; Addis & Koren, 2000). Third trimester exposure to FX in humans has been associated with an increased incidence of preterm delivery, admission to special care nursery, poor neonatal adaptation and decreased birth weight (Chambers et al., 1996; Goldstein, 1995) while others have found no difference in birth weight, perinatal complications or neurobehavioural development (Nulman et al., 1997). It has been suggested that more data must be collected before there is a clear answer (Wisner et al., 1999). Most of these studies differentiate between patient populations receiving FX only during the first trimester versus those exposed to FX throughout gestation and have found differences in those exposed throughout pregnancy, referring to these as late exposed patients.

Chronic FX treatment enhances serotonin neurotransmission by inhibiting serotonin reuptake by the serotonin transporter. In addition, presynaptic inhibitory $5HT_{1A}$ and $5HT_{1D/1B}$ receptors are desensitised (Pineyro & Blier, 1999; Blier & de Montigny, 1998). Serotonin has been shown to cause contraction of the human and sheep umbilical artery (Karlsson et al., 1998; Berman et al., 1978). Injections of serotonin into the uterine artery in pregnant and nonpregnant sheep results in dose

dependent decreases in uterine artery blood flow ranging from 20-50% at doses of 1 to 10 μg (Clark et al., 1980). Whether FX interacts directly with serotonin receptors or through changes in plasma levels of serotonin, a decrease in uterine artery blood flow, either chronic or repeated, may be a mechanism for the possible negative effects of third trimester FX exposure. For this reason, we have chosen to measure uterine artery blood flow, blood gas status and fetal growth during maternal FX infusion in late gestation pregnant sheep.

2.2 Methods

2.2.1 Animals and Surgical Preparation

Twenty-nine time-bred Dorset/Suffolk cross pregnant sheep were surgically prepared between 118 and 122 d gestation (term, 147 d). All animal procedures were approved by the UBC Committee on Animal Care and conformed to the guidelines of the Canadian Council on Animal Care. Ewes were prepared for surgery with 2.4 mg of atropine sulphate IV approximately 10 min prior to transfer to the operating room. In the operating room, 1 g pentothal was injected via the jugular vein to induce anaesthesia. After intubation, anaesthesia was maintained with 1.5% isoflurane with ventilation at a rate of 13 breaths/min (tidal volume = 750 ml). The uterus was exposed with a midline incision in the maternal abdomen. After determination of fetal placement in the uterus, the uterus was incised and the upper body exposed for the implantation of polyvinyl catheters (Tygon, Akron, Ohio) with an inner diameter of 0.1 cm and an outer diameter of 0.175 cm. The trachea was exposed by a midline incision in the neck. Blunt dissection allowed appropriate

exposure for the insertion of a catheter between two rings of tracheal cartilage allowing the monitoring of fetal breathing movements and the withdrawal of tracheal fluid. Electrodes of Teflon®-coated stainless steel wire (Cooner, Chatsworth, CA) were implanted biparietally on the dura for fetal electrocortical recordings and through the lateral orbital ridge of the zygomatic bone of each eye for electroocular recordings. In addition, a reference electrode was sutured into the loose connective tissue over the occipital bone of the skull. The behavioural data recorded are reported in Chapter 3. After the attachment of an amniotic catheter to the fetal skin, the fetal head was returned to the abdominal cavity and the uterine incision sutured.

The lower body of the fetus was exposed through a second incision in the uterus allowing insertion of a catheter in a lateral tarsal vein, the right and left femoral arteries and attachment of a second amniotic catheter. Before suturing the uterus, 1 to 1.5 L of warmed saline was poured into the uterus to replace amniotic fluid lost during surgery. In 11 animals, a Transonic blood flow transducer (size 6R or 8R, Transonic Systems. Ithaca, NY) was placed around the main uterine artery of the uterine horn containing the operated fetus for the continuous measurement of uterine artery blood flow. The catheters, containing heparinised saline, and electrodes were exteriorised through the flank of the ewe. The maternal abdomen was closed in layers. Polyvinyl catheters were placed in the maternal femoral vein and artery and tunnelled to the same exteriorisation site as the fetal catheters or the jugular vein and carotid artery without tunnelling.

2.2.2 Experimental Protocol

Ewes were given 3-4 days to recover after surgery before connecting catheters to signal transducers and the polygraph recorder. Throughout this recovery period and during all of the experimental protocol, the ewes were maintained on a 12:12 lighting schedule with lights on at 06:00. One Control Day preceeded an eight-day continuous infusion of sterile water or FX. On Infusion Day 1, a bolus injection of 70 mg FX in 10 ml sterile water or 10 ml of sterile water was given over 2 minutes into the maternal venous catheter followed by continuous infusion of a 2.77 mg/ml FX solution or sterile water at a rate of 0.036 ml/min (control vehicle experiments) with a Harvard infusion pump. The drug infusion rate was 6.9 mg/d or 98.5 μg/d.kg maternal weight. At 07:00 each day, maternal (5 ml) and fetal (3 ml) blood samples were collected for analysis of FX and serotonin concentrations and blood gases. In addition, on the first day of the infusion, blood gas and FX samples were collected at 0 (07:00), 5, 15 and 30 min and 1, 2, 4, 6 and 12 h after the infusion began. Moreover, for reasons discussed below, maternal serotonin samples were taken at 0, 15 and 30 min, 1, 2, and 6 h following onset of FX administration in two additional animals that were not part of the overall study. Samples for serotonin analysis were collected in heparin rinsed syringes and gently placed in a 5 ml Vacutainer® with EGTA and glutathione (Amersham, Oakville, Ont.) and centrifuged for 10 min. Plasma was stored in polyvinyl Eppindorf® tubes at -20°C for one week and then at -80°C until analysis.

2.2.3 Blood Gas Analysis

Blood samples were analysed for pH, Pco_2 and Po_2 with an IL 1306 pH/blood gas analyser (Allied Instrumentation Laboratory, Milan, Italy) and temperature corrected to 39.5°C for fetal samples and 39°C for maternal samples. Hemoglobin and oxygen saturation were measured with an OSM-2 Hemoximeter (Radiometer, Copenhagen, Denmark). Glucose and lactate were determined with a glucose/lactate 2306 STAT plus analyser (YSI Inc., Yellow Springs, OH).

2.2.4 Physiologic Monitoring

Maternal and fetal arterial pressure and heart rate were continuously recorded using a Gould polygraph recorder that produced a strip chart record at a rate of 2.5 mm/min. Uterine artery blood flow was measured using a Transonic transit-time flow meter and also recorded on the polygraph recorder. A PCL-718 data acquisition card (Advantech Corp) converted the analogue data processed by the Gould recorder (model TA-4000, Gould Instrument Systems Inc, Valley View, OH) to digital data that was processed and stored using Labtech software (Labtech, Wilmington, MA).

2.2.5 Measurement of Fluoxetine Concentrations

A rapid, sensitive and selective chiral assay for FX and norfluoxetine (NFX) enantiomers using gas chromatography mass spectrometry with selective ion monitoring developed in our laboratory was used for FX and NFX analysis in plasma (Kim et al., 1995). Briefly, plasma samples were extracted using liquid-liquid

extraction with 7 ml of hexane:isopropanol (95:5v/v) conditioned with 0.05 M triethylamine. The (s)-(-)-N-trifluoroacetylproyl derivatives of FX and NFX isomers and the internal standard ((2-(diphenylmethoxy)-N-methylamine) were separated on a 20 m x 0.18 mm ID fused silica capillary column [DB-5MS (5% phenylmethylsilicone) with 0.18 μm film thickness (J&W Scientific, Folsom, CA, USA)] with a run time of 27 min. Plasma values are shown for the six animals for whom maternal and fetal blood samples were collected at all time points in the protocol from 07:00 on the Control day to 72 hrs after FX infusion ceased.

2.2.6 Measurement of Serotonin Concentrations

Samples were thawed on ice in the dark prior to serotonin extraction. Then 250 μl of plasma, 100 μl of 0.1 M perchloric acid, and 20 ul of 3,4-dihydroxybenzylamine (3mg/l) (the internal standard) were mixed and centrifuged for 55 minutes at 172,400 Xg at 4°C in a L7-55 ultracentrifuge equipped with a 50.2 Ti type rotor (Beckman Instruments, Palo Alto, CA) (de la Presa Owens & Innis, 1999). 100 μl of supernatant was pipetted into a low volume insert vial (Waters Div. LC Millipore, Milford, MA) and separated using a Waters Alliance 2690 HPLC separation module equipped with a refrigerated autosampler (Waters, Mississauga, Ontario, Canada). The analytical column was a Symmetry C18, 2.1 mm ID x 150 mm, coupled to a Sentry Symmetry C18, 3.9 mm ID 20 mm guard column (Waters, Milford, MA). The mobile phase consisted of octal sulphate sodium salt (125 mg/l), sodium acetate (6 g/l), EDTA (10 mg/l), glacial acetic acid (27 ml /l) and HPLC grade methanol (20 ml l/l). The mobile phase was filtered and degassed using a solvent

filtration apparatus with GV 0.22 μm (pore size) Millipore filters. The separation was performed under isocratic conditions with a column temperature of 32°C and a flow rate of 0.3 ml/min. Detection of serotonin and internal standard was performed with an electrochemical detector (EG&C Princeton Applied Res., Princeton, NJ, Model 400) with a glass carbon electrode cell block and reference electrode filled with a 3M NaCl/Sat AgCl solution. The working electrode potential was maintained at 775 mV and 5 nA for the range current.

2.2.7 Statistical Analysis

Blood gas, cardiovascular, uterine artery blood flow and serotonin data were analysed using repeated measures ANOVA followed by post hoc Fishers t tests to determine the effect of both time and treatment on changes in measurements from preinfusion values during the first 24 h after FX infusion and changes from Control Day on each Infusion Day, using Number Cruncher Statistical Software (Kaysville, Utah, USA). Birth weight and gestational age data were analysed using paired Student t tests. Results are presented as means plus or minus the standard error of the mean. Due to the length of the protocol and technical difficulties with polygraph recorders including severe electrical interference for four months, all parameters were not collected from all animals at all time points.

2.3 Results

2.3.1 Pregnancy Outcome

Gestational age at delivery did not differ between the control (140.5±1.6 d) and FX (141.9±1.5 d) groups as shown in Table 2.1. The birth weights of operated animals were not significantly different between the two groups with the average birth weight for the control group being 3662.8 ± 334.2 g and the FX group 3481.4 ± 218.8 g. There was no significant difference in the percentage of live births between the control group, 67%, and the FX group, 64%. Circumference (Figure 2.1) of the fetal head (A) and abdomen (B) and the ratios of head to abdominal circumference (C) and body weight to head circumference (D) were not significantly different between the control and FX groups at surgery or birth. This suggests that the FX group did not experience intrauterine growth restriction. Postnatal growth was monitored in both the operated control and FX lambs and their siblings (Figure 2.2). Although there were significant differences between the control and FX groups on postnatal days 2 and 5, the overall growth curves were similar.

2.3.2 Plasma Fluoxetine Levels

Maternal and fetal FX and NFX total (i.e. racemic) plasma levels in 6 animals from which blood samples were collected at all time points throughout the protocol and are shown in Figure 2.3. On Infusion Day 1, FX levels in both the ewe and fetus peaked at 5 min after the bolus infusion (173.3±31.4 ng/ml in the ewe and 26.8±3.9

Table 2.1. Gestational age at birth and birth weights of fetuses exposed to an eight day infusion of sterile water (n=15) or FX (n=14) during late gestation.

Control Ewe #	GA at Delivery (days)	Birth Weight (grams)	Number Of Fetuses	Fluoxetine Ewe #	GA at Delivery (days)	Birth Weight (grams)	Number Of Fetuses
7106[b]	144	3560	2	5142	139	2511	2
7239[b]	144	4480	1	5127[a]	146	4142	2
1149[a]	137	3715	1	4237[a]	148	3328	2
7208[a]	136	3150	1	1150[a]	-	3250	3
7140[ab]	148	3310	2	7103[ab]	143	-	1
7244[a]	147	5733	1	7132[ab]	145	4240	1
6117	142	4720	1	7126[b]	136	3010	2
8137[ab]	137	2550	2	7137[ab]	141	4100	2
4113[ab]	135	2200	3	4110[a]	137	3270	2
4148[a]	135	3210	3	106*[ab]	138	3770	2
7107*[b]	144	2410	2	320*[ab]	129	4490	1
7232*[a]	139	4700	1	7250*[ab]	144	3105	2
7213*[a]	139	3930	-	5146*[a]	131	-	3
330*[ab]	143	3000	2	8141*	127	-	2
7130*[b]	132	-	2				
Mean	140.5	3662.8	1.7	Mean	141.9	3481.4	1.9
SEM	1.6	334.2	0.3	SEM	1.5	218.8	0.2
Live births	10 of 15	% live birth	67	Live births	9 of 14	% live birth	64

*indicates fetuses which were born dead. Not included in mean values. 7213, died 1-4 days before delivery; 330 died between 0 and 9 days prior to delivery; 7130, died on Infusion day 6 and delivered 2 days later; 7107, difficult labour; 7232, died 0-10 days prior to delivery; 5146, died 2 days prior to delivery on Infusion day 3; 7250, died 3 days prior to delivery; 1150, ewe refused to stand so protocol ended on Infusion day 6 and returned to farm for delivery; 7103, born alive, birth weight unknown; 106, died just prior to delivery on Infusion day 8, twin survived; 320, fetus died on Infusion day 7 and delivered; 8141, died on Infusion 5 and delivered the following day.

All the animals in both groups provided data used in this Chapter.

[a] indicates animals that provided data used in Chapter 3

[b] indicates animals that provided data used in Chapter 4

No significant differences were observed in GA (gestational age), birth weight, or number of fetuses.

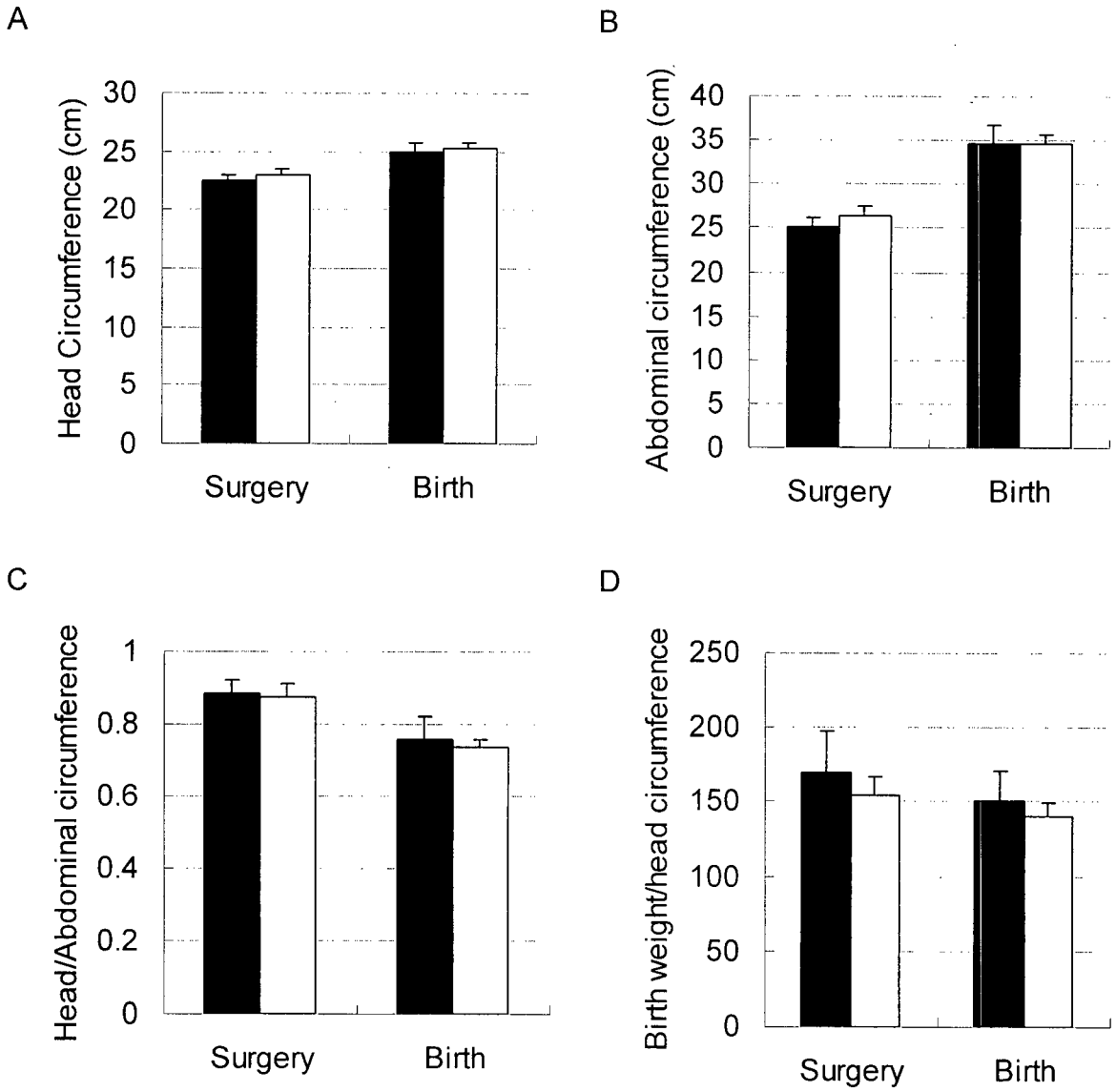


Figure 2.1. Intrauterine growth was monitored with measures of fetal size at surgery and birth including head circumference (A), abdominal circumference (B) and ratio of head to abdominal circumference (C) and birth weight to head circumference (D) in fetuses exposed to eight day maternal intravenous infusion of sterile water (closed bars, n=4, 7, 4 and 4, respectively) or FX (open bars, n=7, 7, 7 and 7, respectively) for eight days.

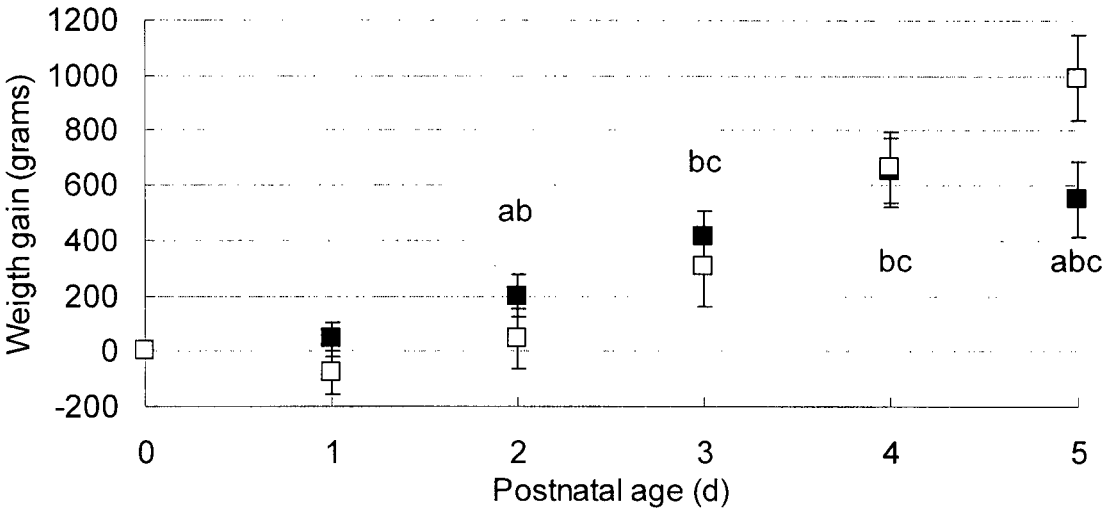


Figure 2.2. Postnatal weight gain in operated + sibling fetuses exposed prenatally to eight day maternal intravenous infusion of sterile water (closed squares, n= 14) or FX (open squares, n= 7). [a], significant difference between control and FX groups ($p<0.05$); significant difference ($p<0.05$) from day of birth in control [b] and FX group [c].

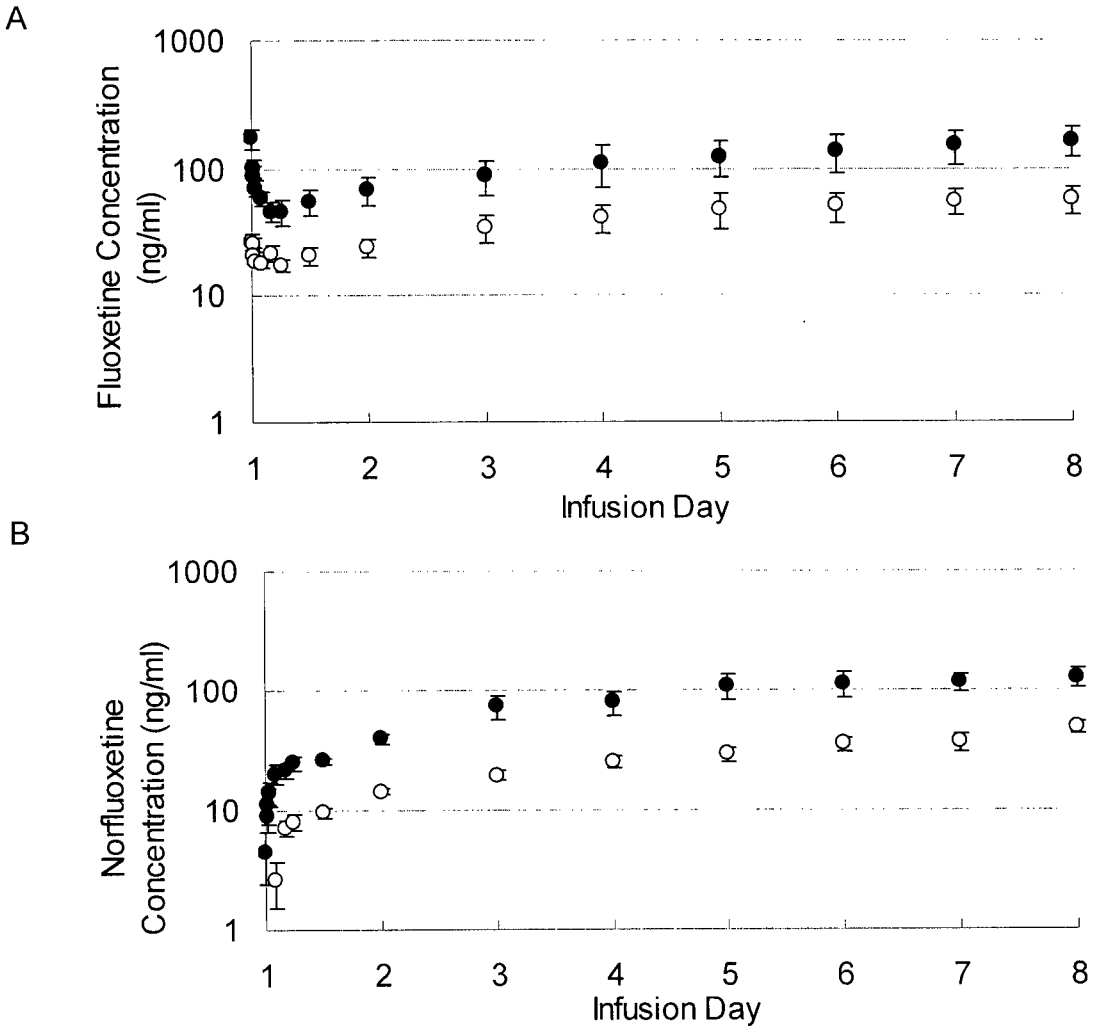


Figure 2.3. Maternal (closed circles, n=6) and fetal (open circles, n=6) FX (A) and norfluoxetine (B) arterial plasma concentrations at 5, 15, 30 mins and 1, 2, 4, 6 and 12 hours after maternal FX administration as well as at 07:00 on each day of the eight day infusion.

ng/ml in the fetus) and decreased during the first 6 h (46.9±10.4 ng/ml in the ewe and 17.4±1.7ng/ml in the fetus). NFX plasma levels increased over the initial six hour period from 4.5±2.1 ng/ml to 25.3±3.4 ng/ml in the ewe and 0 to 8±1.2 ng/ml in the fetus. Maternal and fetal FX levels progressively increased throughout the eight days of infusion peaking at 166.5±45 ng/ml on Infusion Day 7 in the ewe and at 58.9±14.9 ng/ml on Infusion Day 8 in the fetus. Maternal and fetal plasma NFX levels also increased progressively during the infusion period. Fetal FX plasma levels were 37.1±0.0% of maternal levels from 6 h after the infusion began until the infusion ended on Infusion Day 8.

2.3.3 Acute Physiologic Changes during Fluoxetine Infusion

Maternal and fetal blood gas and pH values during the first 24 h of vehicle or FX infusion are illustrated in Figures 2.4 and 2.5. In both groups, maternal arterial Po_2 increased slightly during the first 30 min of the infusion period, and in the control group, there was a small, but statistically significant increase in pH at 12 h. No other significant maternal changes were observed. Compared to the preinfusion values, fetal pH, Po_2 and oxygen saturation decreased in the FX group while Pco_2 increased ($p<0.05$). Fetal Po_2 fell 5.5±0.8 mmHg in the first five minutes after FX infusion began from 23.5±0.7 mmHg to 18.0± 1.1 mmHg. Po_2 returned toward the control value but remained slightly but significantly decreased for the whole of the first day. There were similar changes in fetal O_2 saturation (Figure 2.5), with an initial decrease from 60.3±5.3 to 41.9±8.5. at 5 min post-injection. The pH decrease in the FX group was not associated any change in base excess, which was 0.55±0.68

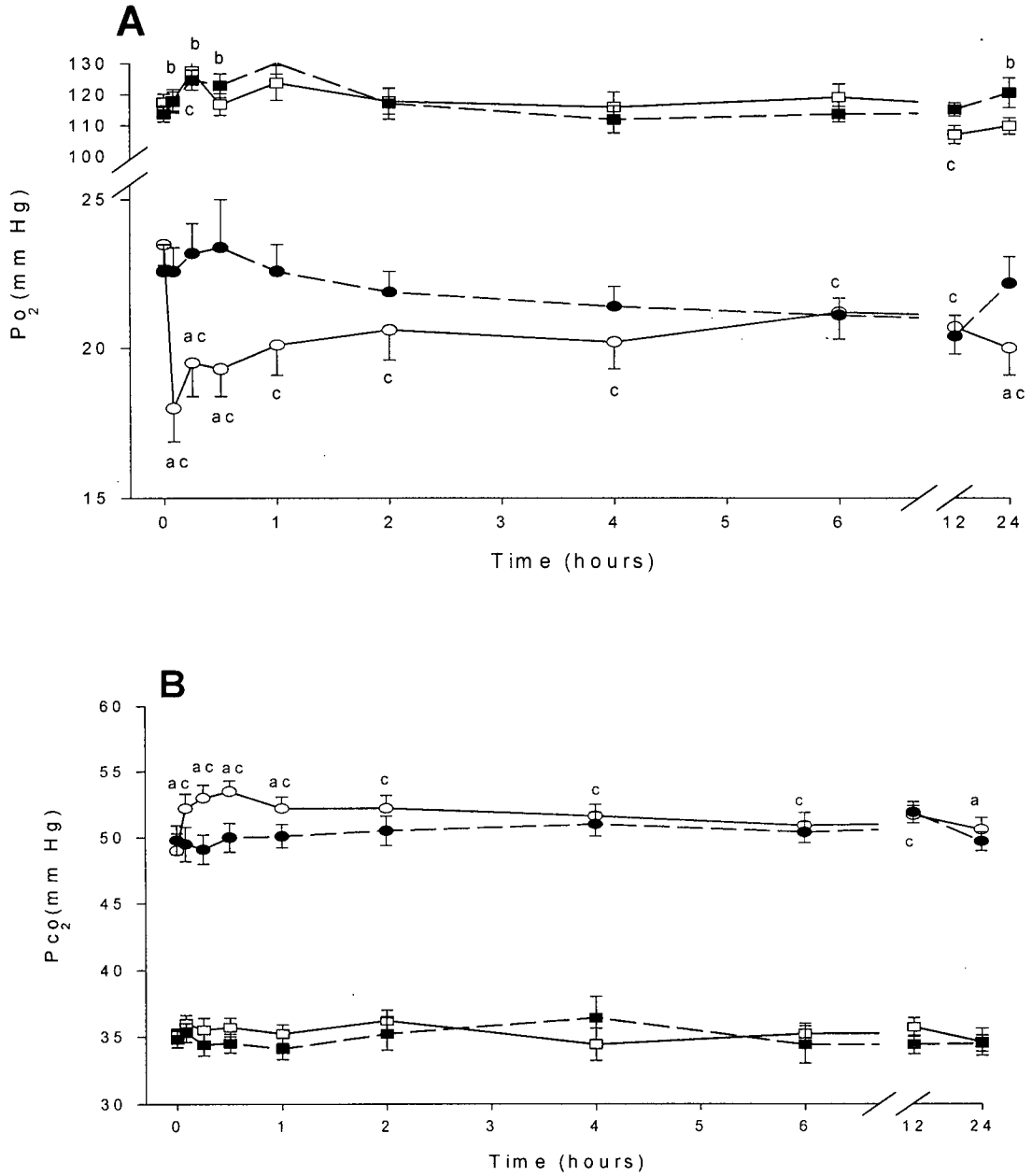


Figure 2.4. Maternal (squares) and fetal (circles) Po_2 (A) and Pco_2 (B) during the first 24 h of maternal intravenous sterile water (closed symbols, n=14) or FX (open symbols, n=13) infusion. [a], significant difference between control and FX groups ($p<0.05$); significant difference ($p<0.05$) from Control day in control [b] and FX group [c].

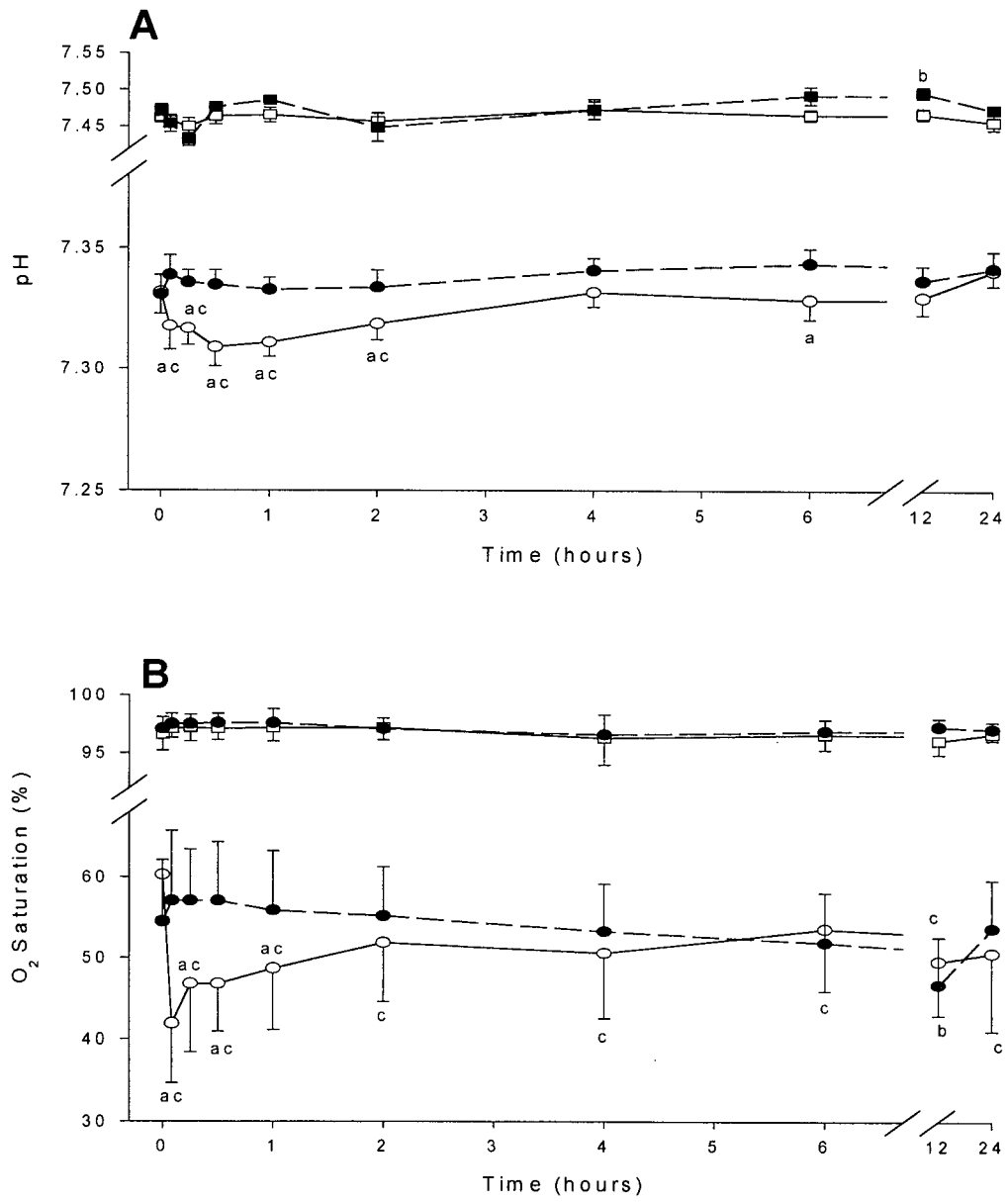


Figure 2.5. Maternal (squares) and fetal (circles) arterial pH (A) and O_2 saturation (B) during the first 24 hours of maternal sterile water (closed symbols, n=14) or FX infusion (open symbols, n=13). [a], significant difference between control and FX groups; significant difference from Control Day in control [b] and FX group [c] ($p<0.05$).

mEq/l prior to FX administration and 0.68±0.33 mEq/l at 30 min, i.e. the time of the maximum fall in pH. Thus the acidemia was entirely respiratory in nature. In the control group, fetal hemoglobin concentration decreased from 10.7±0.4 to 10.0±0.5 g% at 5 min and remained at 10.3-10.8 g% for the rest of the first day. Hemoglobin concentration in the FX fetuses (10.0±0.5) was not significantly altered. Figure 2.6 illustrates the changes in maternal and fetal glucose and lactate concentration during the first 24 h of the experiment. In both groups, the changes in maternal and fetal glucose concentration were parallel and the levels increased significantly following the feeding of the animals between the 1 and 2 h post-infusion times. However, in the FX group, there was a tendency for both maternal and fetal glucose concentrations to increase in the first 30 min of FX infusion. These changes were not statistically significant. Maternal lactate concentrations also increased in both groups, along with the rise in glucose. In addition, in the FX-exposed fetuses, there was a greater rise in the first hour of the infusion and the levels remained elevated above the control value for the remainder of the day.

Figure 2.7 illustrates the changes in maternal and fetal arterial pressure and heart rate during the first 24 h of the experiment. In the FX group, both fetal and maternal arterial pressure tended to be higher than the values in the control group for the entire 24 h, and for the ewe the differences between the groups were statistically significant at 4 and 6 h. There were however no consistent changes in either maternal or fetal values with time. Maternal heart rate tended to increase during the initial 6 h, but the changes were not significant. In the FX group fetal heart rate increased significantly from 162±6 bpm prior to drug infusion to 188±7 bpm at 1

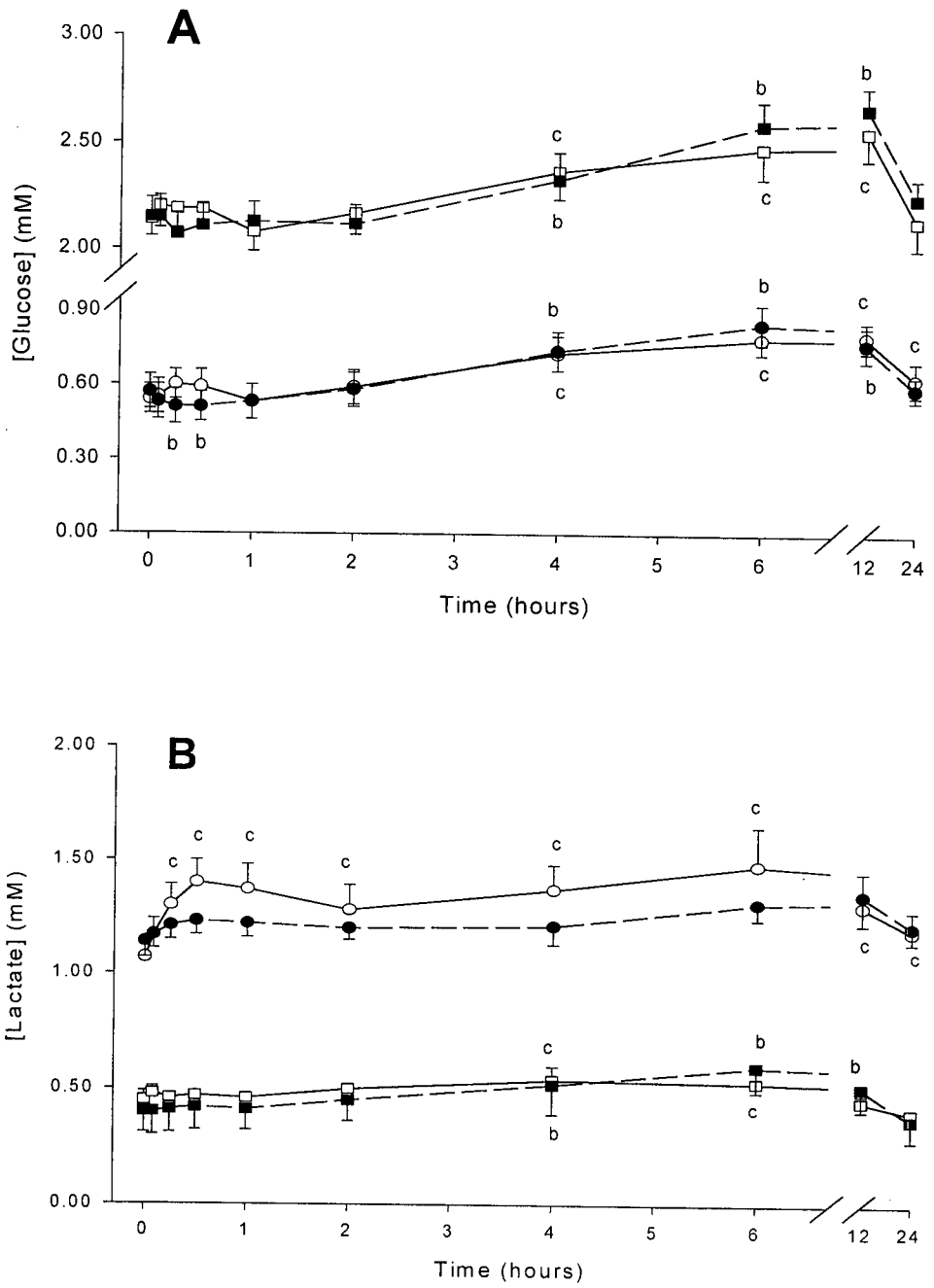


Figure 2.6. Maternal (squares) and fetal (circles) arterial glucose (A) and lactate (B) levels during the first 24 h of maternal sterile water (closed symbols, n=14) or FX (open symbols, n=13) infusion. Significant difference ($p<0.05$) from Control Day in control [b] and FX group [c].

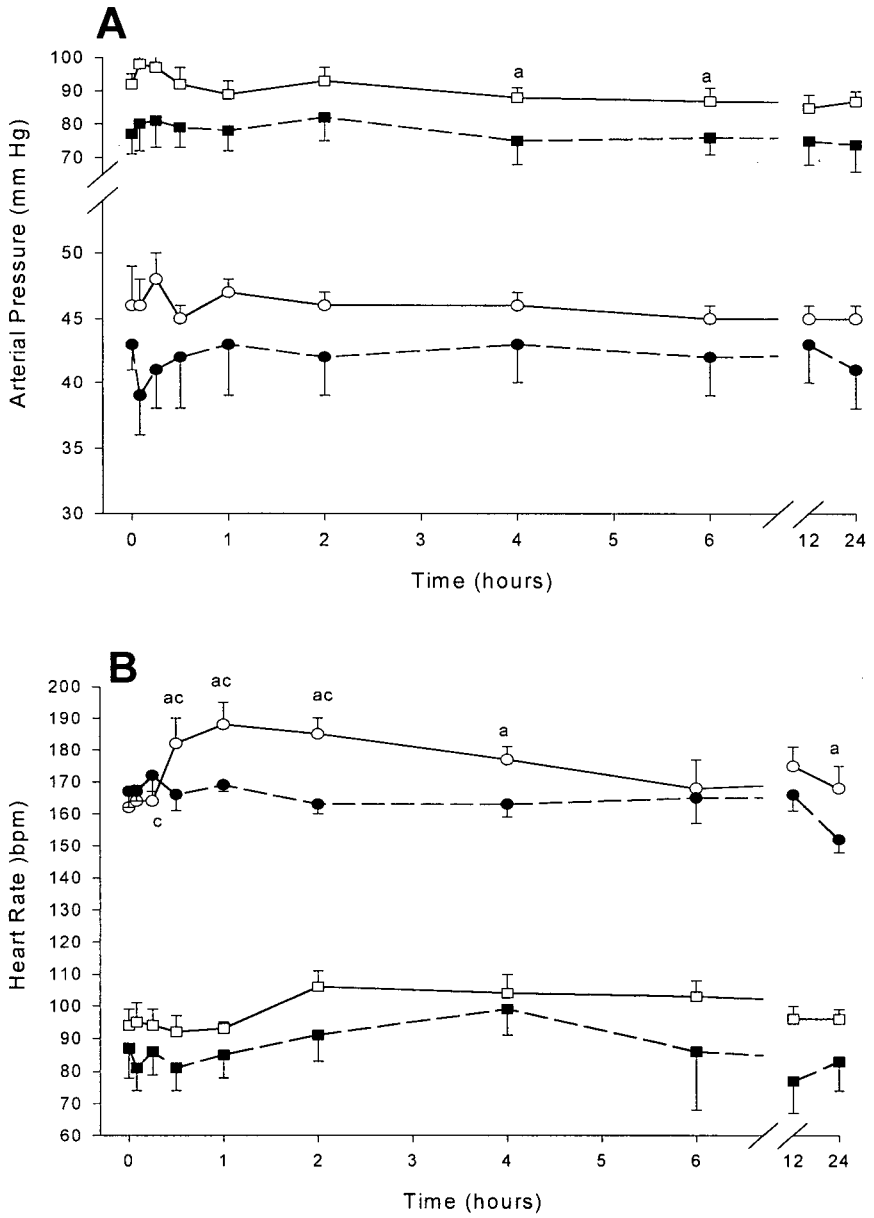


Figure 2.7. Maternal (squares) and fetal (circles) arterial pressure (A) and heart rate (B) during the first 24 h of maternal IV infusion of vehicle (closed symbols, n= 8 and 10 for maternal and fetal arterial pressure, n= 6 and 9 for maternal and fetal heart rate) or FX (open symbols, n= 12 and 12 for maternal and fetal arterial pressure, n= 7 and 8 for maternal and fetal heart rate). [a], significant difference between control and FX groups; significant difference ($p<0.05$) from Control Day in control [b] and FX group [c].

h. It remained elevated at 2 and 4 h. In comparison to the control group, fetal heart rate in the FX fetuses tended to be higher at every sampling point on the first day.

Absolute uterine artery blood flow decreased from 489±26 ml/min before FX infusion to 378±27 ml/min at 5 min after start of FX infusion with a return to 465±26 ml/min by 1 h (Figure 2.8A). This represents a fall to 74±6% of the control value at 5 min and 77±6% at 15 min with a return to the control by 1 h in the FX group ($p<0.05$). No significant changes in uterine artery blood flow were observed in the control group. In one animal, for which we had both uterine artery blood flow and FX concentration data, maternal FX concentration and uterine artery blood flow were negatively correlated ($y = -0.1388x + 97.064$, $R^2 = 0.7452$, n=4) over the first 30 min. Figure 2.9 illustrates the relationship between percent change in uterine artery blood flow and change in fetal arterial Po_2 and Pco_2 in the control and FX-exposed animals. In the control group there was no significant relationship between the uterine artery blood flow and either variable. In contrast, in the FX group, fetal Po_2 fell linearly with the decrease in uterine artery blood flow ($p<0.05$). There was no relationship between changes in uterine blood flow and fetal arterial Pco_2.

2.3.4 Daily Physiologic Changes with Fluoxetine Infusion

Blood gas status was monitored at 07:00 daily with the Control Day and Infusion Day 1 samples being preinfusion samples. No changes in maternal blood gas status were observed with FX infusion over this 8-day time period (Table 2.2). Fetal pH decreased on Infusion Days 1, 3, 6 and 7 in the FX group compared to the Control Day with the greatest decrease of –0.026±0.005 on Infusion Day 6 (Table

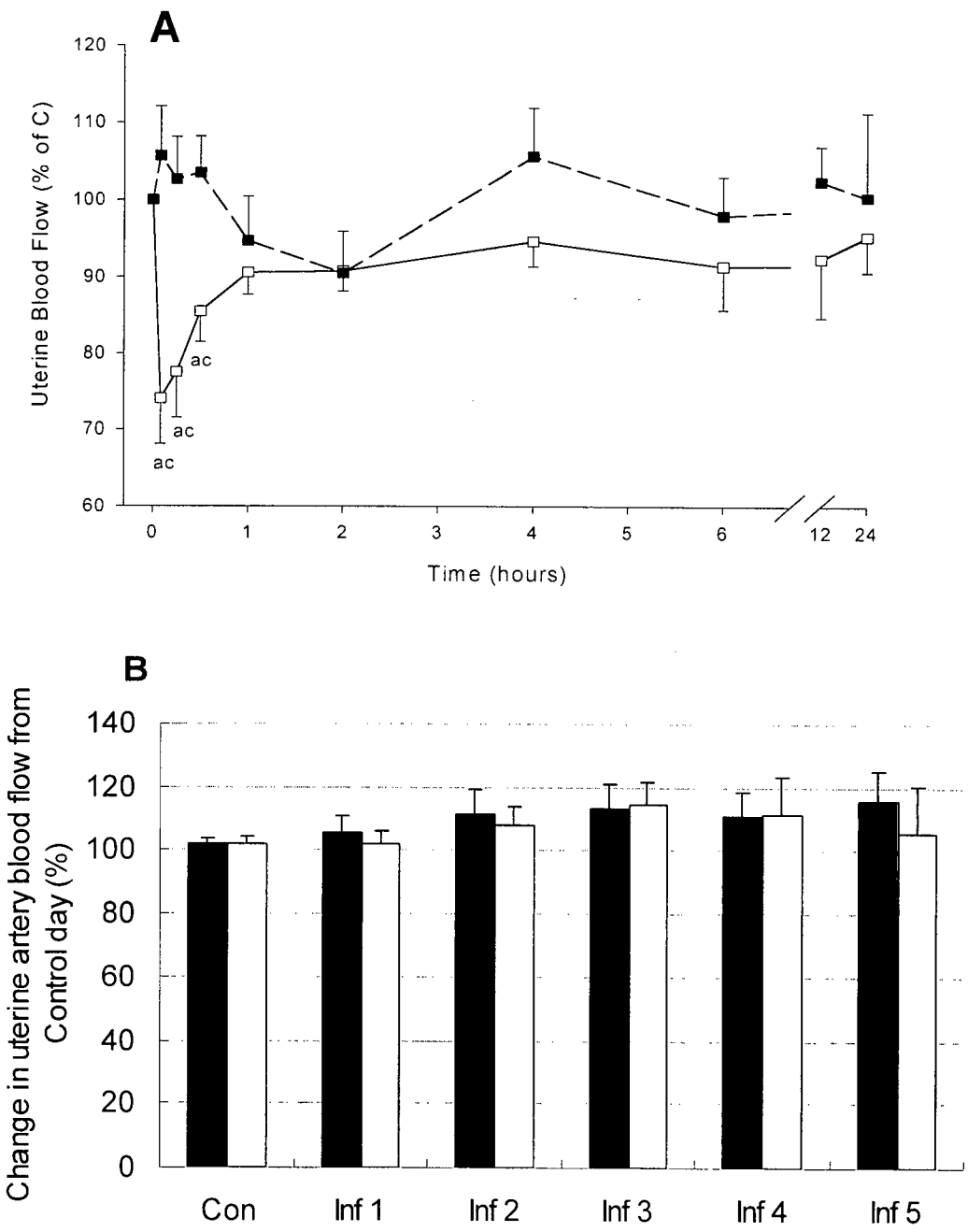
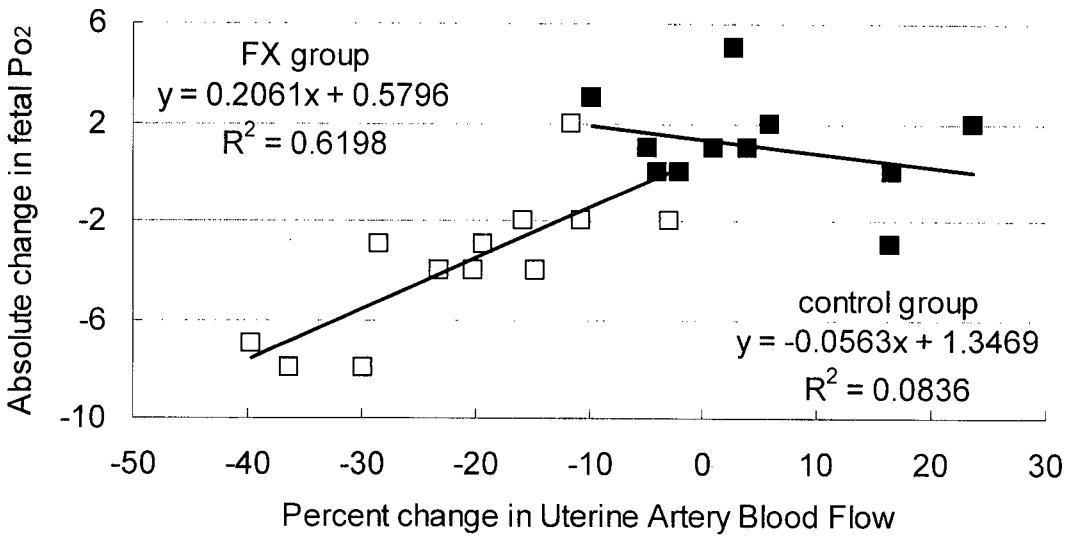


Figure 2.8. Percent change in uterine artery blood flow from Control day in sterile water (closed squares/bars, n=5) or FX (open squares/bars, n=6) treated groups during the first 24 h after infusion began (A) and during the first five days of the 8-day infusion period (B). The n value for the FX group decreased to 5 on day 3 and 4 on days 4 and 5. [a], significant difference between control and FX groups; significant difference from Control Day for the FX group [c]; Con, Control Day; Inf, Infusion Day.

A



B

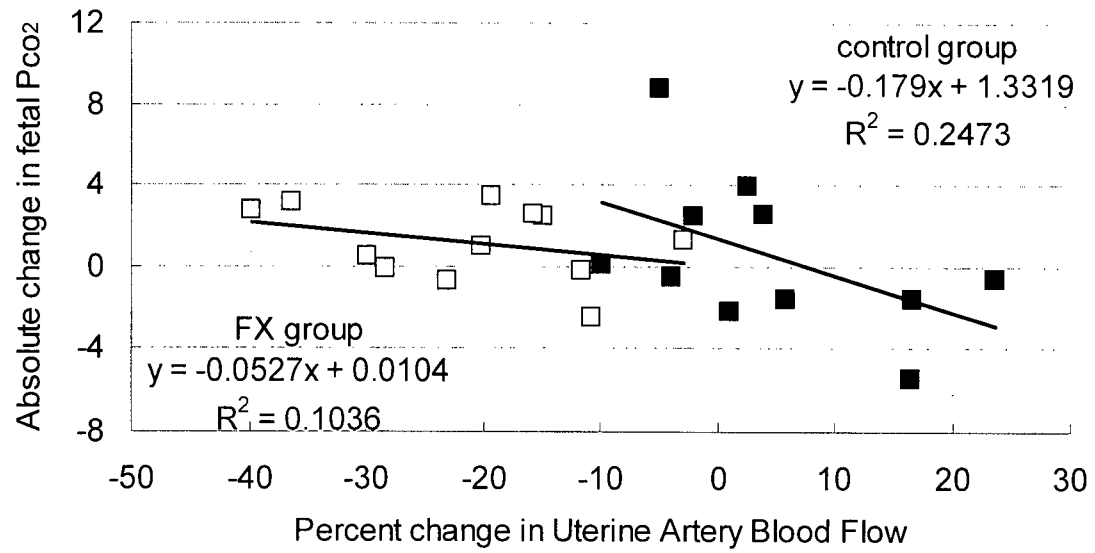


Figure 2.9. Relationship between the percent change in uterine artery blood flow and change in fetal Po_2 (A) and Pco_2 (B) in the control (closed squares) and FX (open squares) animals during the first 30 min of maternal intravenous infusion.

Table 2.2. Maternal arterial blood gas status at 07:00 on each day of an eight day maternal sterile water or FX intravenous infusion expressed as change from Control Day.

		Con	Inf 1	Inf 2	Inf 3	Inf 4	Inf 5	Inf 6	Inf 7	Inf 8
pH										
Con	Mean	7.468	0.002	0.007	0.000	-0.002	-0.012	-0.017	-0.017	-0.016
	SEM	0.009	0.009	0.009	0.008	0.011	0.010	0.015	0.010	0.012
FX	Mean	7.465	-0.001	-0.005	-0.011	-0.010	-0.007	-0.014	-0.015	-0.038
	SEM	0.012	0.010	0.018	0.008	0.009	0.012	0.010	0.015	0.012
P_{CO2}		mmHg								
Con	Mean	34.8	-0.1	-3.3	-2.4	0.3	0.8	0.7	0.8	-0.6
	SEM	0.8	0.9	2.9	2.9	0.9	0.7	1.0	0.8	0.8
FX	Mean	34.0	0.8	1.2	1.4	1.7	0.8	-0.4	0.1	0.6
	SEM	0.9	0.8	0.9	0.6	0.7	0.7	0.6	0.8	0.9
P_{O2}		mmHg								
Con	Mean	125.5	-7.6	-12.0	-15.9	-7.3	-6.0	0.6	-3.0	7.5
	SEM	5.1	4.1	14.1	13.2	5.1	5.1	4.3	7.7	6.0
FX	Mean	121.1	-4.1	-10.5	2.2	-2.9	-1.3	0.3	9.7	13.7
	SEM	5.1	5.4	6.2	5.3	7.0	9.4	8.2	8.7	8.7
Hemoglobin		g%								
Con	Mean	10.2	0.0	-1.1	-0.9	-0.6	-0.4	-0.6	-0.8	-0.4
	SEM	0.4	0.2	0.7	0.7	0.3	0.2	0.3	0.3	0.4
FX	Mean	9.6	0.4	0.4	0.4	0.5	-0.1	-0.1	-0.1	0.3
	SEM	0.2	0.3	0.2	0.4	0.4	0.5	0.5	0.4	0.7
O_2 saturation		%								
Con	Mean	95.9	1.3	-6.8	-6.8	1.3	1.5	1.8	2.4	2.4
	SEM	1.5	1.5	8.6	8.6	1.5	1.9	1.7	1.8	1.9
FX	Mean	97.3	0.3	-0.4	-0.5	-0.2	-0.6	0.1	0.0	-0.2
	SEM	0.2	0.2	0.3	0.3	0.3	0.4	0.4	0.3	0.5
Lactate		mmol/l								
Con	Mean	0.38	0.01	-0.05	-0.03	0.02	-0.05	-0.04	0.02	0.02
	SEM	0.02	0.02	0.03	0.04	0.06	0.03	0.05	0.03	0.06
FX	Mean	0.35	0.09	0.04	0.05	0.07	0.04	0.06	0.10	0.11
	SEM	0.02	0.04	0.01	0.03	0.02	0.02	0.02	0.06	0.07
Glucose		mmol/l								
Con	Mean	1.95	0.19	0.09	0.09	0.31	0.00	-0.21	0.10	0.01
	SEM	0.13	0.09	0.25	0.26	0.15	0.33	0.34	0.21	0.19
FX	Mean	2.25	0.04	0.08	0.08	0.07	-0.18	-0.09	-0.18	-0.20
	SEM	0.09	0.11	0.14	0.14	0.13	0.14	0.08	0.10	0.17
# of animals										
Con		12	12	11	11	12	10	10	10	9
FX		11	11	11	11	10	11	9	7	7

Note that Control and Infusion day 1 are preinfusion values.
Con, Control day; Inf, Infusion day.

Table 2.3 Fetal arterial blood gas status at 07:00 on each day of an eight day maternal sterile water or FX intravenous infusion expressed as change from Control Day.

		Con	Inf 1	Inf 2	Inf 3	Inf 4	Inf 5	Inf 6	Inf 7	Inf 8
pH										
Con	Mean	7.343	-0.013	-0.002	-0.016	-0.014	-0.007	-0.015	-0.012	-0.012
	SEM	0.008	0.005	0.009	0.011	0.008	0.010	0.015	0.014	0.013
FX	Mean	7.346	-0.017[c]	-0.011	-0.019[c]	-0.013	-0.009	-0.026[c]	-0.021[c]	-0.011
	SEM	0.007	0.008	0.007	0.007	0.004	0.005	0.005	0.007	0.009
P_{CO2}		mmHg								
Con	Mean	48.3	0.2	0.3[a]	-0.7[a]	-0.1	1.0	0.5	1.8	0.8
	SEM	1.3	0.9	0.9	1.0	1.1	0.9	1.0	1.1	1.0
FX	Mean	48.2	1.4	3.4	3.2	0.5	1.4	0.1	1.9	2.5
	SEM	1.2	1.2	1.6	1.5	1.3	1.1	1.2	1.2	2.0
P_{O2}		mmHg								
Con	Mean	23.0	-0.2	-0.3	-1.1	-1.3	-1.6	-1.7	-0.6	-1.5
	SEM	1.1	0.5	0.8	0.8	1.0	0.9	0.8	1.3	1.3
FX	Mean	23.4	-0.1	-2.4[c]	-1.1	-1.9	-1.6	-2.9[c]	-1.9[c]	-1.5
	SEM	0.7	0.6	0.9	0.8	0.6	0.7	0.9	0.8	1.0
Hemoglobin		g%								
Con	Mean	10.7	-0.2	-0.6	-0.9	-0.9	-0.8	-0.9	-1.9	-0.8
	SEM	0.6	0.3	0.3	0.3	0.3	0.2	0.3	1.2	0.3
FX	Mean	10.6	-0.3	-0.1	-0.1	-0.4	-0.7	-1.1	-0.7	-0.9
	SEM	0.5	0.3	0.4	0.4	0.4	0.3	0.4	0.4	0.5
O_2 saturation		%								
Con	Mean	56.7	-1.8	-2.7	-4.1	-6.8[b]	-7.0[b]	-7.7[b]	-8.5[b]	-12.7[b]
	SEM	2.9	1.7	2.0	2.1	2.8	2.8	2.3	4.0	3.8
FX	Mean	61.6	-1.2	-11.1[c]	-6.9	-14.0[c]	-6.7	-8.4[c]	-11.0[c]	-13.0[c]
	SEM	2.3	1.2	2.7	2.3	5.2	2.0	2.9	2.0	3.3
Lactate		mmol/l								
Con	Mean	1.0488	0.08	0.13	0.19[b]	0.19[b]	0.19[b]	0.13	0.36[b]	0.38[b]
	SEM	0.050645	0.06	0.05	0.07	0.10	0.08	0.07	0.21	0.11
FX	Mean	0.5395	0.11	0.13[c]	0.08	0.13	0.14[c]	0.13[c]	0.13[c]	0.14[c]
	SEM	0.072716	0.06	0.07	0.05	0.05	0.06	0.08	0.09	0.08
Glucose		mmol/l								
Con	Mean	0.577667	0.00	0.02	-0.01	0.00	-0.01	-0.01	0.00	-0.01
	SEM	0.052785	0.04	0.04	0.04	0.04	0.04	0.04	0.09	0.05
FX	Mean	0.649667	0.06	0.03	-0.06	-0.05	-0.08	-0.11	-0.08	-0.01
	SEM	0.055402	0.09	0.05	0.04	0.07	0.06	0.05	0.09	0.09
# of animals										
Con		15	15	15	15	14	14	13	10	10
FX		12	12	12	12	11	11	11	8	7

Note that Control and Infusion day 1 are preinfusion values.
[a], significant difference between control and FX groups ($p<0.05$); significant difference ($p<0.05$) from Control day in control [b] and FX group [c]
Con, Control day; Inf, Infusion day

2.3). Fetal Po_2 also decreased in the FX group on Infusion Days 2, 6 and 7 compared to the Control Day. The maximum decrease in Po_2 of –2.9±0.9 mmHg occurred on Infusion Day 6. Compared to Control Day, fetal lactate increased on Infusion Days 2, 5, 6, 7 and 8 in the FX group and on Infusion Days 3, 4, 5, 7 and 8 in the control group. Fetal oxygen saturation decreased on Infusion Days 2, 4, 6, 7 and 8 in the FX group and on Infusion Days 4 to 8 in the control group. The maximum decrease in oxygen saturation was observed on Infusion Day 4 in the FX group (-14.0±5.2 %) and on Infusion Day 8 in the control group (-12.7±3.8 %).

Daily average maternal arterial pressure and heart rate were not affected by maternal FX infusion (Table 2.4) nor was fetal arterial pressure (Table 2.5). Compared to the Control Day, there was a decrease in fetal heart rate on Infusion Days 2 to 7. A decrease in fetal heart rate was also observed in the control group on Infusion Days 4 to 8 compared to the Control Day. Fetal heart rate was significantly higher in the FX group compared to the control group on the Control Day of the experiment as well as Infusion Days 1, 7 and 8 (Table 2.5). This is consistent with the data presented in Figure 2.7 for fetal heart rate measurements at intervals during the first day.

Daily uterine artery blood flow was analysed for only the first 5 days of the protocol, as the sample was not large enough in the FX group beyond this time point for statistical analysis. No significant difference was observed in the percent change from the Control Day value of uterine artery blood flow in the control or FX group (Figure 2.8).

Table 2.4. Daily average maternal arterial pressure and heart rate during an eight-day maternal sterile water or FX intravenous infusion.

		Con	Inf 1	Inf 2	Inf 3	Inf 4	Inf 5	Inf 6	Inf 7	Inf 8
Maternal Arterial Pressure			mmHg							
Con	Mean	81	78	79	77	76	77	77	78	78
	SEM	7	6	6	5	5	4	5	3	3
	n	6	7	7	8	8	8	8	8	7
FX	Mean	87	88	87	85	85	85	87	87	87
	SEM	3	3	3	4	4	5	4	7	8
	n	12	12	12	11	10	10	8	5	5
		Con	Inf 1	Inf 2	Inf 3	Inf 4	Inf 5	Inf 6	Inf 7	Inf 8
Maternal Heart Rate			bpm							
Con	Mean	90	86	88	88	84	83	88	87	84
	SEM	8	7	10	7	7	7	7	8	9
	n	6	6	5	7	7	7	7	7	6
FX	Mean	106	101	101	100	102	102	103	106	108
	SEM	6	5	6	4	6	4	4	5	7
	n	8	8	8	8	8	8	7	6	6

Con, Control day; Inf, Infusion day.

Table 2.5. Daily average fetal arterial pressure and heart rate during an eight-day maternal sterile water or FX intravenous infusion.

		Con	Inf 1	Inf 2	Inf 3	Inf 4	Inf 5	Inf 6	Inf 7	Inf 8
Fetal Arterial Pressure			mmHg							
Con	Mean	44	43	43	44	42	44	44	46	43
	SEM	1	1	1	1	2	1	2	2	2
	n	8	8	8	9	9	9	9	9	8
FX	Mean	44	45	45	45	45	46	45	46	48
	SEM	1	1	1	1	1	1	1	1	1
	n	12	12	12	12	11	11	9	6	6
		Con	Inf 1	Inf 2	Inf 3	Inf 4	Inf 5	Inf 6	Inf 7	Inf 8
Fetal Heart Rate			bpm							
Con	Mean	164[a]	164[a]	164	163	159[b]	158[b]	155[b]	154[ab]	150[ab]
	SEM	4	3	3	4	4	3	3	4	6
	n	9	9	9	10	10	10	10	7	
FX	Mean	172	173	162[c]	164[c]	160[c]	158[c]	157[c]	162[c]	164
	SEM	4	5	6	5	5	4	6	7	8
	n	9	9	9	9	9	9	7	6	6

[a], significant difference between control and FX groups ($p<0.05$); significant difference ($p<0.05$) from Control day in control [b] and FX group [c]
Con, Control day; Inf, Infusion day.

2.3.5 Plasma Serotonin Levels

Daily samples for plasma serotonin estimation were collected in all control and FX exposed animals. However, the serotonin concentrations in these samples were very high and variable. I.e. much higher than levels reported for platelet-free plasma in other species (Fuller & Wong, 1987; Ortiz & Artigas, 1992; Alvarez et al., 1999a, 1999b). In additional studies in non-pregnant ewes, it was determined that the high serotonin concentrations were likely the result of serotonin release from platelets during the time the samples were being collected in the syringe. Rinsing the syringes with 0.5 ml of 1,000 U/ml heparin (Organon Teknika, Toronto, CA) prior to sample collection appeared to eliminate this problem and results in plasma levels in the low M range, consistent with published data. FX was then administered (IV bolus, 70 mg) to two additional pregnant ewes and maternal blood samples were collected for serotonin measurement at 0, 15 and 30 min and 2 and 6 h. Prior to FX infusion, the level averaged 1.6 nM. It increased to 3.9 nM at 15 min post-FX injection, which is a 144% increase from the preinfusion value. At 30 and 60 minutes, the levels were 2.4 and 1.9 nM, respectively. Levels at 2 and 6 h were 0.5 and 1.3 nM, respectively.

2.4 Discussion

This study is the first to examine the possible underlying mechanisms involved in the previously reported negative effects of FX exposure during third trimester in humans. Changes in these variables were measured both acutely and

during chronic exposure at clinically relevant plasma concentrations. The results of this study show that maternal intravenous infusion of FX decreases uterine artery blood flow transiently causing a decrease in oxygen supply to the fetus. This transient response lasts about 12 h with no chronic changes in uterine artery blood flow. Mild alterations of fetal blood gas status were observed throughout the eight days of FX infusion. No negative birth outcomes were observed in terms of birth weight, gestational age at delivery or intrauterine growth restriction in FX-exposed fetuses compared to control fetuses.

The maternal FX and NFX concentrations in the present study are similar to those reported in humans. The mean maternal FX levels fall within the adult human therapeutic range for the drug, which is 40-250 ng/ml (Krogh, 1995). In the fetus, the FX concentration averaged 58.9±14.9 ng/ml at the end of the infusion period. There are limited data on FX levels in the human fetus, although 2 case reports give concentrations at delivery of 26 and 129 ng/ml in 2 infants (Spencer, 1993; Mhanna et al., 1997). Our own study of 9 pregnant women on FX therapy found umbilical cord vein concentrations of 7.9-89.7 ng/ml (mean = 45.4±18.5 ng/ml) at delivery (Kim, 2000). This is similar to the mean value in the fetal lamb on Infusion Day 8. In both the ewe and fetus, the FX concentrations rose progressively during the eight-day infusion period. As discussed elsewhere (Kim, 2000), this may be due to FX-mediated inhibition of a sheep analogue of cytochrome P450 2D6, a key enzyme involved in the demethylation of the drug to NFX. The inhibition of CYP 2D6 results in a reduction in FX clearance and rise in FX plasma concentration. Kim et al. (2000) and others (Alfaro et al., 1999, 2000) have reported a similar phenomenon in the

human. NFX, the main FX metabolite is also pharmacologically active (Fuller et al., 1992) and NFX plasma levels in the human are similar to those of the parent drug (Benfield et al., 1986). This is similar to the findings in the current study.

The initial decrease in uterine artery blood flow during the first 30 min of FX infusion is significantly related to the decrease in fetal arterial Po_2 (Figure 2.9). This is similar to results obtained by Yaffe et al (1987) where a 25% reduction in uterine artery blood flow, achieved *via* partial occlusion of the uterine artery, deceased fetal arterial Po_2 by ~5 mm Hg. Thus it is most likely that the fall in Po_2 (and rise in Pco_2) observed during the first day of FX infusion are due to decreased uterine artery blood flow. Although it is possible that the drug also affected umbilical blood flow, this seems unlikely. With experimental reductions in umbilical blood flow, there is fetal hypertension and bradycardia (Itskovitz et al., 1983; Giussani et al., 1997), whereas in the FX-exposed fetuses, there was no change in arterial pressure and tachycardia during the period of hypoxemia.

The time course of the decrease in uterine artery blood flow is similar to the that for the changes in plasma serotonin concentration in the two animals in which serotonin measurements were made over the first 6 h of FX infusion. Since FX acts on platelets in a manner similar to its action on synapses, the inhibition of serotonin reuptake would prevent platelet serotonin uptake causing a transient increase in serotonin plasma levels. Plasma serotonin levels increase immediately after FX injection in humans and rats (Fuller & Wong, 1987; Ortiz & Artigas, 1992) and a similar phenomenon occurred in the current study. Serotonin concentration was increased by 144% at the first sampling point following onset of FX infusion.

However, this was at 15 min, whereas the maximum decrease in uterine blood flow was at 5 minutes post FX injection. Thus plasma serotonin concentration at this time may have been higher than the 15 min value. In a study involving acute injections of serotonin directly into the uterine vasculature (Clark et al., 1980), a ~20% reduction in uterine blood flow was achieved with a 1 μg injection.

Fetal Pco_2 was increased in the FX-exposed fetuses during the first 12 h of maternal FX infusion. In studies involving experimental reduction of uterine artery blood flow, a tendency toward an increase in Pco_2 has also been observed (Yaffe et al., 1987; Bocking et al., 1988; Harding et al., 1981). In addition, significant increases in fetal Pco_2 occur with spontaneous uterine contractures (Llanos et al., 1986). Since we did not observe any changes in base excess in the FX group, the decrease in pH observed in the FX group is due to the rise in Pco_2. Maternal and fetal glucose concentrations rose at 4 h post infusion in both the control and FX groups due to feeding between the 1 and 2 h samples. The increases in lactate at these time points are due to the increase in glucose (Slater & Mellor, 1981), but the significant rise in fetal lactate concentration in the FX group after FX infusion is consistent with a decrease in Po_2 (Towell et al., 1987). The main cardiovascular effect observed was the increase in fetal heart rate during the first 6 h of FX infusion. Comparison of Figures 2.6 and 2.7 show that the time course for the tachycardia is similar to the time course for the hyperlactic acidemia. Experimental lactic acidemia, similar to that observed with acute reductions in uterine artery blood flow, resulted in increased fetal Pco_2 and sustained tachycardia (Bocking et al., 1991). No changes in maternal or fetal arterial pressure were observed over the first 24-h of the

experiment in both groups; however maternal arterial pressure was consistently higher in the FX group than in the control group for the entire experimental period. However, the reason for this difference is not clear.

Chronic FX exposure caused only minor alterations of fetal blood gas status. It should be noted that fetal Po_2 (although not significant in the control group) and oxygen saturation decreased throughout the eight-day infusion period in both the control and FX groups, although the magnitude of the fall tended to be greater in the FX group. Over this time period, uterine artery blood flow in the FX and control groups are not different. Thus the reduced fetal oxygenation over this time period is not due to reduced uterine blood flow. We (Kumar et al., 2000) and others (Hedriana et al., 1995) have found that weight-normalised umbilical blood flow decreases progressively in late gestation in the sheep, and a similar phenomenon occurs in the human (St. John Sutton et al, 1990). This is associated with a progressive fall in vascular Po_2 in the human fetus (Soothill et al., 1986), and this is also likely to be the case in the fetal lamb (Bell et al., 1986). This would explain the fall in fetal oxygenation during the experimental period in the two groups of fetuses in the current study. The decrease in fetal heart rate in both the control and FX groups over the eight days of infusion is consistent with previous findings of decreased fetal heart rate with increasing gestational age (Boddy et al., 1974; Tan, 1997). An increase in fetal arterial pressure has been observed between 100 and 140 days gestation in the sheep (Boddy et al., 1974) with a greater rate of increase in the week before labour (Tan, 1997). We did not observe an increase in fetal arterial pressure in the control or FX groups over the eight-day infusion period but we did

observe a decrease in fetal heart rate. This may be explained by the fact that fetal arterial pressure increases at a rate of 0.46 mmHg/d while fetal heart rate decreases at a rate of 0.67 beats/d from 100 to 140 d gestation (Boddy et al., 1974).

Uterine artery blood flow is not reduced throughout the eight days of FX infusion. This finding is consistent with the effects of chronic FX treatment on serotonin levels. Chronic FX treatment has been shown to cause a decrease in plasma and platelet serotonin levels in humans and mice (Alvarez et al., 1999a; Alvarez et al., 1999b). At 24 h after exposure to FX, platelet serotonin was slightly lower than controls and decreases with continued treatment in mice while brain tissue levels are higher at 24 h and decrease to below control values with continued treatment (Alvarez et al., 1999b). In humans, FX treatment significantly decreased platelet and plasma serotonin levels within 2 weeks of treatment initiation and this continued for 12 weeks (Alvarez et al., 1999a). Platelet serotonin is decreased by half after 3 days of FX (10 mg/kg IP) administration in rats (Fuller & Wong, 1987).

Thus, the major effects of FX on the fetus in the current study occurred on the first day consisting of transient hypoxemia, respiratory acidemia, hyperlactic acidemia and tachycardia of ~6 h duration. Changes of this duration and magnitude would not be expected to affect either gestational length or fetal growth. This is consistent with the lack of differences in gestational length, birth weight or postnatal weight gain in the control and FX groups. Thus, the results are not consistent with the Chambers et al. (1996) and Goldstein (1995) studies that found an increase in preterm delivery and lower birth weight in fetuses exposed to FX during the third trimester. It is possible that the severity of the underlying depression requiring

pharmacological treatment into the third trimester may have resulted in preterm delivery and decreased birth weight observations in those human studies. Differences in lifestyle such as increased smoking and alcohol consumption rather than a direct pharmacological effect of FX may also have been responsible for these observations (Goldstein et al., 1997). Alternatively, it is possible that exposure to FX throughout most or all of pregnancy may be required to result in preterm delivery or reduced birth weight. The observations in human studies may be due to a cumulative effect over the entire gestational period.

The FX-elicited transient changes in fetal Po_2 and Pco_2 are similar in magnitude to those occurring with contractures and fetal skeletal muscle activity (Llanos et al., 1986; Jansen et al., 1979; Sunderji et al., 1984). However, it should be noted that contractures last about 5 min whereas the FX-induced decrease in uterine artery blood flow was significant for 30 min while Po_2 decreased for 24 h. Pulsatile administration of oxytocin to pregnant ewes from 96 d gestation to term increased the frequency of contractures from ~1.3 /h to ~3.2/h. In addition to decreased fetal Po_2, this increase in the frequency of contractures resulted in accelerated cardiovascular and neurological maturation and attenuated the ACTH and cortisol responses to hypoxia (Shinozuka et al., 2000; Shinozuka et al., 1999; Shinozuka et al., 2000b; Owiny et al., 1995). Thus, repeated FX-induced alterations in blood gas status over most or all of pregnancy may impact on fetal development.

At this time, it is unclear whether the initial FX-elicited alterations in fetal blood gas status are due to the high levels of FX created by the loading dose or due to the initial exposure to the drug. However, for several reasons we do not think it is the

high level of FX. First, although maternal FX concentrations are related to the change in uterine artery blood flow, fetal FX concentration is not related to the initial changes in blood gases. Second, maternal and fetal FX concentrations on Infusion Day 8 are similar to the initial values. Third, preliminary data in the lab show that 6 h maternal intravenous FX infusion on three consecutive days decreased uterine artery blood flow transiently on each day. Plasma serotonin increases 30 min after initial FX injection and decreases after 7 d of chronic administration (Ortiz & Artigas, 1992; Fuller & Wong, 1987). Serial measurements of plasma serotonin have not been performed with repeated daily FX treatment to determine the time course of daily changes in plasma serotonin. If in fact there are daily FX effects, then the cumulative effect on fetal oxygenation would be comparable to those elicited by increasing contracture frequency in the studies of Owiny et al (1995) and Shinozuka et al (1999, 2000, 2000b). The fetus could be exposed to FX-elicited decreases in Po_2 for all or most of pregnancy. Moreover, a recent study has shown that preventing the rise in uterine artery blood flow that normally occurs from 115 to 138 d gestation results in decreased fetal and placental weight (Lang et al., 2000).

Although changes in fetal blood gas status due to FX exposure were mild and short lasting, chronic treatment throughout pregnancy may result in negative effects due to repeated hypoxic events. Thus it is valuable to continue investigating the effects of in utero exposure to antidepressants such as FX in children at later stages of development and into adulthood.

CHAPTER 3

FETAL BEHAVIOURAL STATE CHANGES FOLLOWING MATERNAL FLUOXETINE INFUSION IN SHEEP

3.1 Introduction

Depression occurs in 5-15% of all pregnancies (O'Hara, 1984; Ledward, 1996; Weissman and Olfson, 1995). Pharmacotherapy may be necessary during pregnancy as depression itself has been shown to lead to negative pregnancy outcomes such as an increase in admission to neonatal intensive care units, low birth weight and preterm delivery in some populations (Orr and Miller, 1995; Steer et al., 1992). The selective serotonin reuptake inhibitor (SSRI), fluoxetine (FX), is currently the most widely prescribed antidepressant (Lawrenson et al., 2000) and is often prescribed during pregnancy.

Many studies in men and nonpregnant women have shown that FX alters sleep. In acute studies, a 60 mg dose decreased rapid eye movement (REM) sleep, total sleep time and sleep efficiency index (Nicholson and Pascoe, 1988). A subsequent study found that a 40 mg dose of FX taken in the morning increased REM latency with no effect on other sleep parameters (Saletu et al., 1991). Chronic treatment, 4-5 weeks, at doses as low as 20 mg per day have been shown to decrease REM sleep, increase REM latency, decrease sleep efficiency index and increase nonrapid eye movement (NREM) sleep (Rush et al, 1998; Trivedi et al., 1999; Kerkhofs et al., 1990). Increased eye movements in NREM sleep (Keck et al., 1991; Dorsey et al., 1996) as well as increased electroocular (EOG) activity count

and amplitude and electromuscular (EMG) activity in REM, Stage 1, 2 and slow wave sleep (SWS)(Armitage et al., 1995) have been observed. Additionally, altered sleep patterns, increased REM sleep and SWS, were observed 4 weeks after treatment ended (Buysse et al., 1999) either due to the long half-life of FX and its' N-demethylated metabolite, norfluoxetine (NFX), or long-term sleep alterations caused by treatment with this agent. Studies in rats have shown that FX is capable of crossing the placenta (Pohland et al., 1989). In addition, fetal clearance of serotonin may be decreased due to inhibition of the placental serotonin transporter by FX (Padbury et al., 1997). As a result, the fetus is exposed to FX and fetal behavioural states may be altered by maternal treatment with FX.

Pharmacological agents such as ethanol (Patrick et al., 1985), morphine and methadone (Umans and Szeto, 1983) have previously been shown to alter fetal behavioural state with maternal administration. Both the human (Nijhuis et al., 1982) and sheep (Dawes et al., 1972) fetus exhibit behavioural states in late gestation. Fetal behavioural states develop in the late gestation sheep at about 120 days (term ~ 145 d) (Clewlow et al., 1983). The sheep fetus cycles between two behavioural states with each cycle lasting about 30-40 minutes (Clapp et al., 1980). The low-voltage (LV)/REM behavioural state is characterised by the presence of LV fast electrocortical (ECoG) activity, eye movements and a lack of nuchal muscle tone and predominates at this stage of development (Richardson, 1994). The presence of high-voltage (HV) slow ECoG activity, the absence of eye movements and the presence of nuchal muscle tone characterise HV/NREM behavioural state. Fetal

breathing movements (FBM) occur only during LV/REM behavioural state but are not used to define this state.

Previous work has shown that intravenous infusion of L-5-hydroxytryptophan, the precursor to serotonin, in the sheep fetus results in an increase in HV ECoG activity, fetal breathing movements (Quilligan et al., 1981) and blood pressure (Fletcher et al., 1988). In addition, intracisternal administration of L-5-hydroxytryptophan in the fetal lamb increased FBM with little effect on ECoG activity or eye movements (Morrison et al., 1997). These studies suggest that as FX is capable of crossing the placenta, it may alter fetal behavioural states in a manner similar to that observed in adult species. Thus the purpose of this study is to determine if maternal treatment with FX results in behavioural state changes in the fetus.

3.2 Methods

3.2.1 Animals and Surgical Preparation

Twenty-two time bred Dorset/Suffolk cross pregnant sheep were surgically prepared between 118 and 122 d gestation (term, 147 d). The University of British Columbia Animal Care Committee approved experimental protocols and procedures performed on the sheep conformed to the guidelines of the Canadian Council on Animal Care. Surgical procedures have been described in detail previously (Rurak et al, 1988). Briefly, anaesthesia was induced with injection of 1 g pentothal via the

jugular vein. After intubation, anaesthesia was maintained with 1.5% isoflurane. The uterus was incised and the fetus exposed for the implantation of polyvinyl catheters (Tygon, Akron, OH) in the trachea, femoral arteries, lateral tarsal vein and amniotic cavity. Electrodes of Teflon-coated stainless steel wire (Cooner, Chatsworth, CA) were implanted biparietally on the dura for fetal ECoG recordings and through the lateral orbital ridge of the zygomatic bone of each eye for EoG recordings. A reference electrode was sutured into the loose connective tissue over the occipital bone of the skull. In eleven animals, a Transonic blood flow transducer was placed around the main uterine artery of the uterine horn containing the operated fetus for the continuous measurement of uterine artery blood flow (Data discussed in Chapter 2). The catheters, containing heparinised saline, and electrodes were exteriorised through the flank of the ewe. Polyvinyl catheters were placed in the maternal femoral vein and artery and tunnelled to the same exteriorisation site as the fetal catheters or the jugular vein and carotid artery without tunnelling. All catheters were flushed daily with approximately 2 ml of sterile 0.9% sodium chloride containing 12 units of heparin per millilitre to maintain their patency. Ampicillin (500 mg) was administered into the amniotic cavity and intramuscularly to the ewe at surgery and for 3 post-operative days. Sheep were housed in holding pens with other sheep and free access to food and water after surgery.

3.2.2 Experimental Protocol

Ewes were given three or four days to recover after surgery before connecting catheters to signal transducers and electrodes to amplifiers and then either a Gould TA4000 thermal array (Gould Inc., Valley View, OH, USA) or Grass

K2G (Astro-Med, Montreal, PQ) polygraph recorder. One Control Day proceeded an eight-day continuous infusion of sterile water or FX. At 07:00 each day, blood samples were collected for analysis of FX, NFX and blood gases and pH. The ewes were maintained in a 12:12 lighting schedule with lights on at 06:00. At 07:00 on Infusion Day 1, a bolus injection of 70 mg FX in 10 ml sterile water or sterile water was given over 2 minutes into the maternal femoral or jugular vein catheter. This injection was immediately followed by a continuous infusion of a 2.77 mg/ml FX solution or sterile water at a rate of 0.036 ml/min with a Harvard infusion pump. All parameters could not be collected from each animal due to a four-month period of severe electrical interference and the breakdown of one of our two polygraph recorders.

3.2.3 Blood Gas Analysis

Arterial blood samples were analysed for pH, partial pressure of carbon dioxide (Pco_2), partial pressure of oxygen (Po_2) and base excess with an IL 1306 pH/blood gas analyser (Allied Instrumentation Laboratory, Milan, Italy) and temperature corrected to 39.5°C for fetal samples and 39°C for maternal samples. Hemoglobin and oxygen saturation were measured with an OSM-2 Hemoximeter (Radiometer, Copenhagen, Denmark).

3.2.4 Evaluation of Fetal Behavioural States

Biophysical data was collected with a polygraph recorder, which produced a strip chart recording at a rate of 2.5 mm/min (Gould) or 3 mm/min (Grass). Each strip chart recording was divided into 3 hr blocks for the 9 days of the experiment and 1 hr

blocks for the 24 h before and after the FX/vehicle infusion began; the duration of each activity was analysed during that period and the percent time calculated. The mean duration and number of LV and HV episodes was calculated on the Control and Infusion 1 Days between 7:00 and 19:00. ECoG activity was analysed by visual inspection and divided into periods of LV (<50 μV), intermediate-voltage (50-100 μV) and HV (100-200 μV) (Morrison et al., 1997). Eye movement activity was present when an episode of EOG activity had an amplitude greater than 50 μV and lasted longer than 30 seconds. Repeated negative deflections in tracheal pressure greater than 2 mmHg for longer than 30 sec were defined as FBM (Morrison et al., 1997).

3.2.5 Fluoxetine and Norfluoxetine Analysis

A rapid, sensitive and selective chiral assay for FX and NFX enantiomers using gas chromatography mass spectrometry with selective ion monitoring developed in our laboratory was used for FX and NFX analysis in plasma (Kim et al., 1995). Briefly, plasma samples were extracted using liquid-liquid extraction with 7 ml of hexane:isopropanol (95:5v/v) conditioned with 0.05 M triethylamine. The (s)-(-)-N-trifluoroacetylproyl derivatives of FX and NFX isomers and the internal standard ((2-(diphenylmethoxy)-N-methylamine) are separated on a 20 m x 0.18 mm ID fused silica capillary column [DB-5MS (5% phenylmethylsilicone) with 0.18 μm film thickness (J&W Scientific, Folsom, CA, USA)] with a run time of 27 min. Plasma values are shown for the six animals for whom maternal and fetal blood samples were collected at all time points in the protocol from 07:00 on the Control Day to 72 h after FX infusion ceased.

3.2.6 Statistical Analysis

Behavioural activity, blood gas and cardiovascular data were analysed using three way and two way repeated measures ANOVA followed by post hoc Fishers t tests to determine the effect of both time and treatment on changes in measurements from control values using Number Cruncher Statistical Software (Kaysville, UT).

3.3 Results

3.3.1 Pregnancy Outcome

There were 10 and 12 animals in the control and FX groups, respectively. Gestational age at birth was not significantly different between the two groups with control animals delivering at 139.7±1.9 d and FX treated animals delivering at 142.5±1.5 d. In addition, no difference in birth weight was observed (3359±377grams for the control group and 3599±203 for the FX group). Survival to Infusion Day 8 was similar in the two groups (60% control group; 58% FX group). Animals who did not reach Infusion Day 8 of the experiment either delivered prematurely or died in utero.

3.3.2 Maternal and Fetal Fluoxetine and Norfluoxetine Concentrations

Maternal and fetal FX and NFX concentrations are reported in detail elsewhere (Chapter 2). Briefly, the maternal plasma FX level peaked at 173.3 ±31.5 ng/ml 5 min following the bolus drug injection and then declined to 46.9±10.4 ng/ml at 6 h of infusion. Thereafter the drug concentration rose progressively for the remainder of the infusion period to 106.1±43.3 ng/ml on Infusion Day 8. Plasma FX concentration in the fetus showed a similar time course to that in the ewe. However, the fetal level was consistently lower, being 58.9±1.49 ng/ml on Infusion Day 8. The overall fetal/maternal concentration ratio was 0.36±0.02. Maternal and fetal NFX concentrations were similar to those of the parent drug.

3.3.3 Maternal and Fetal Blood Gas Status

Maternal Po_2 (control group, 125.1±4.7 mmHg; FX group, 121.0±5.1 mmHg), Pco_2 (control group, 34.2±0.6 mmHg; FX group, 34.0±0.9 mmHg) and pH (control group, 7.469±0.009; FX group, 7.465±0.012) were not significantly different in the control and FX animals on the Control Day. There were no significant changes in any of these variables during the experimental period. The corresponding values for the fetus for the first 24 h of the infusion period are given in Figure 3.1. In the control group, there were no changes in the variables. In contrast in the FX group, there were significant decreases in Po_2 and pH, and an increase in Pco_2. The maximum fall in Po_2 (23.5±0.5 to 18.0±1.1 mmHg) occurred at 5 min post-bolus injection, whereas the maximum changes in pH (7.332±0.009 to 7.309±0.008) and Pco_2 (49.0±1.3 to 53.5±0.8 mmHg) occurred at 30 min. The acidemia was largely

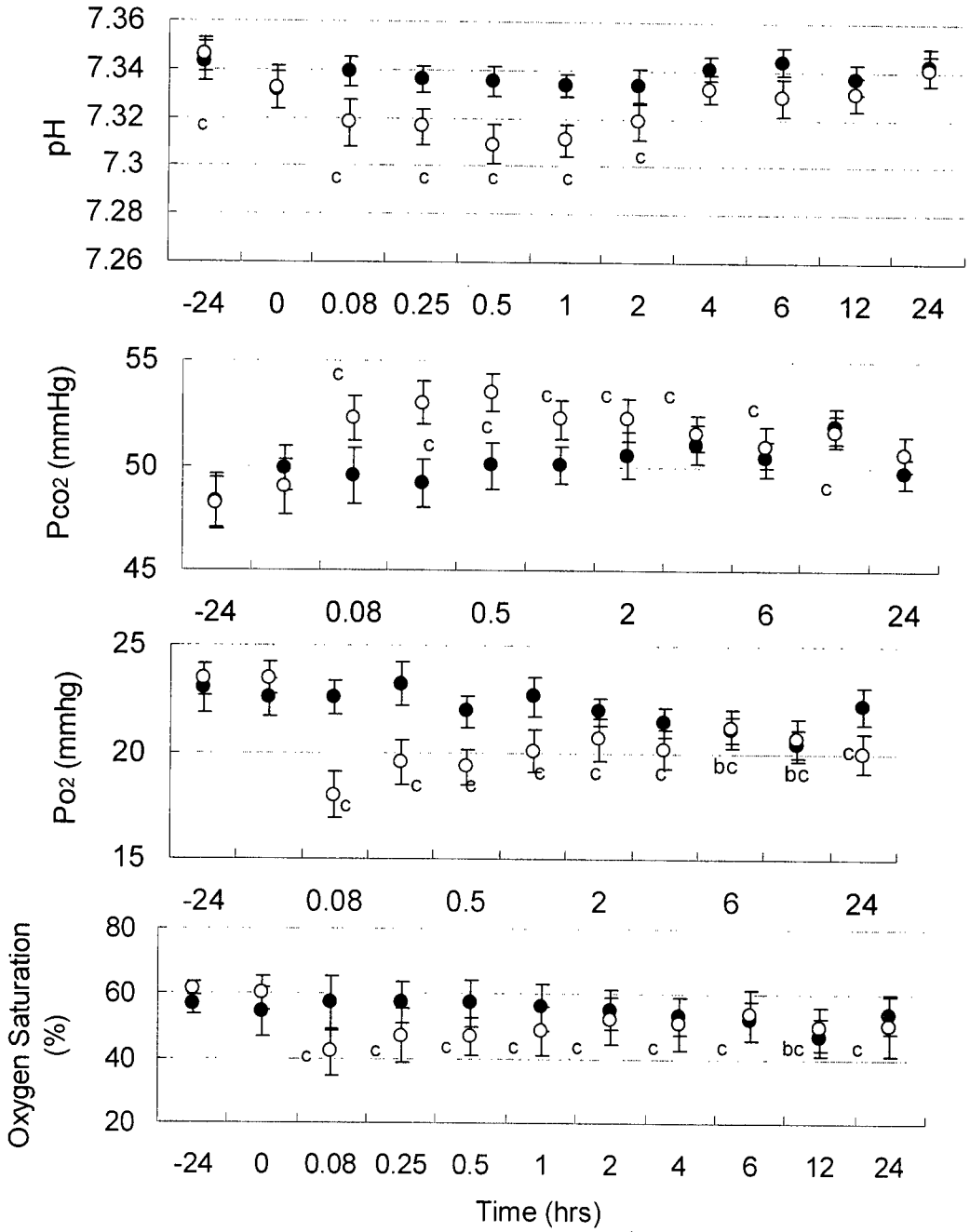


Figure 3.1. Fetal blood gas changes in arterial pH, Pco_2, Po_2 and oxygen saturation during the 24 hours before and after maternal intravenous FX (open circles, n=12) and sterile water (closed circles, n=10) infusion including samples at 5, 15 and 30 minutes, 1, 2, 4, 6, and 12 hours after infusion. Significant difference from –1 hr in the control [b] and FX group [c].

Table 3.1. Fetal blood gas status of animals at 07:00 daily. Absolute value is shown for the Control Day with the change from Control Day shown for each Infusion Day.

		Con	Inf 1	Inf 2	Inf 3	Inf 4	Inf 5	Inf 6	Inf 7	Inf 8
pH										
Con FA	Mean	7.340	-0.012	0.004	-0.011	-0.009	0.001	-0.012	-0.010	-0.004
N=10	SEM	0.009	0.006	0.009	0.014	0.008	0.011	0.017	0.018	0.011
FX FA	Mean	7.346	-0.017[c]	-0.011	-0.019[c]	-0.013	-0.009	-0.026[c]	-0.021[c]	-0.011
N=12	SEM	0.007	0.008	0.007	0.007	0.004	0.005	0.005	0.007	0.009
P_{CO2}		MmHg								
Con FA	Mean	48.1	-0.6	0.3	-1.6	-0.4	0.3	0.4	1.5	1.0
N=10	SEM	1.6	0.9	0.9	1.0	1.1	1.0	0.8	1.1	0.7
FX FA	Mean	48.2	1.4	3.4	3.2	0.5	1.4	0.1	1.9	2.5
N=12	SEM	1.2	1.2	1.6	1.5	1.3	1.1	1.2	1.2	2.0
P_{O2}		MmHg								
Con FA	Mean	22.7	-0.3	0.3	-0.6	-1.3	-0.4	-1.5	-0.3	-1.6
N=10	SEM	1.4	0.6	1.0	0.9	1.1	0.8	1.0	1.5	1.6
FX FA	Mean	23.4	-0.1	-2.4[c]	-1.1	-1.9	-1.6	-2.9[c]	-1.9[c]	-1.5
N=12	SEM	0.7	0.6	0.9	0.8	0.6	0.7	0.9	0.8	1.0
Base Excess										
Con FA	Mean	1.3	-1.1	0.7	-1.2	-0.4	0.6	0.1	1.4	0.2
N=10	SEM	0.5	0.7	0.6	1.3	0.5	0.4	0.3	0.8	0.9
FX FA	Mean	1.0	-0.5	0.7	0.0	-0.4	0.1	-1.2	-0.2	-0.1
N=12	SEM	0.7	0.7	0.4	0.6	0.5	0.5	0.8	0.8	0.6
Oxygen saturation		%								
Con FA	Mean	54.8	-1.6	-0.9	-2.7	-6.7b	-4.1	-7.7b	-7.7[b]	-12.6[b]
N=10	SEM	3.4	1.9	2.2	2.4	3.3	2.5	2.7	4.8	4.7
FX FA	Mean	61.6	-1.2	-11.1[c]	-6.9	-14.0[c]	-6.7	-4.7[c]	-11.0[c]	-13.0
N=12	SEM	2.3	1.2	2.7	2.3	5.2	2.0	5.1	2.0	3.3

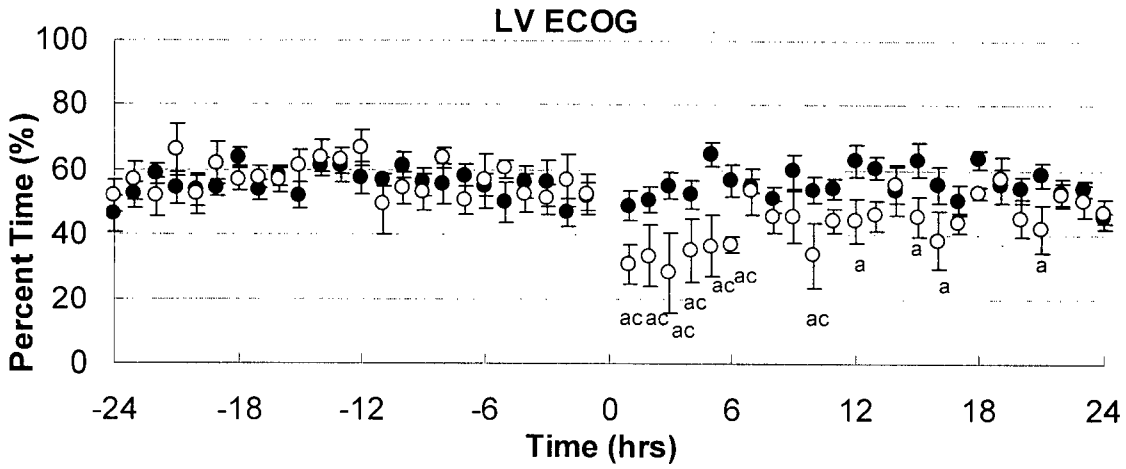
Significant difference ($p<0.05$) from Control day in control [b] and FX group [c].
Inf, Infusion; Con, Control.

respiratory although there was a slight, but significant increase in base deficit (0.55±0.68 mEq/l to 1.61±0.34 mEq/l). Table 3.1 gives the daily blood gas variables for each day of the infusion period, based upon the samples taken at 07:00 daily. The values are expressed as the change from the Control Day (i.e. day 0, pre-infusion values) to account for the decrease in animal numbers that occurred towards the end of the experiment. In both groups, Po_2 and pH tended to decrease during the infusion period. However, for the control fetuses, the changes were not statistically significant, whereas in the FX group, they were of larger magnitude and statistically significant. Similar changes occurred with O_2 saturation. The largest changes in both variables (-0.026±0.005 for pH and -2.9±0.9 mmHg for Po_2) occurred on FX Infusion Day 6. There were no changes in fetal arterial Pco_2.

3.3.4 Fetal Behavioural State Variables

Figure 3.2 illustrates the hourly averages of percent time spent in LV and HV ECoG activity over the 24 h preceding and following the start of maternal sterile water or FX administration. Similar plots for fetal REM and breathing activity are given in Figure 3.3. In the FX group, the percent time spent in LV ECoG decreased, from 52.5±5.9% to a minimum of 28.1±12.5% at 3 h after the start of the infusion. This was significantly below the corresponding values in the control group and this situation persisted for most of Infusion Day 1. HV ECoG incidence significantly increased in the FX-exposed lambs, from 39.1±2.9% to 68.7±14.1% at 3 h of infusion, and this persisted for at least 9 hours. A significant decrease in average

A



B

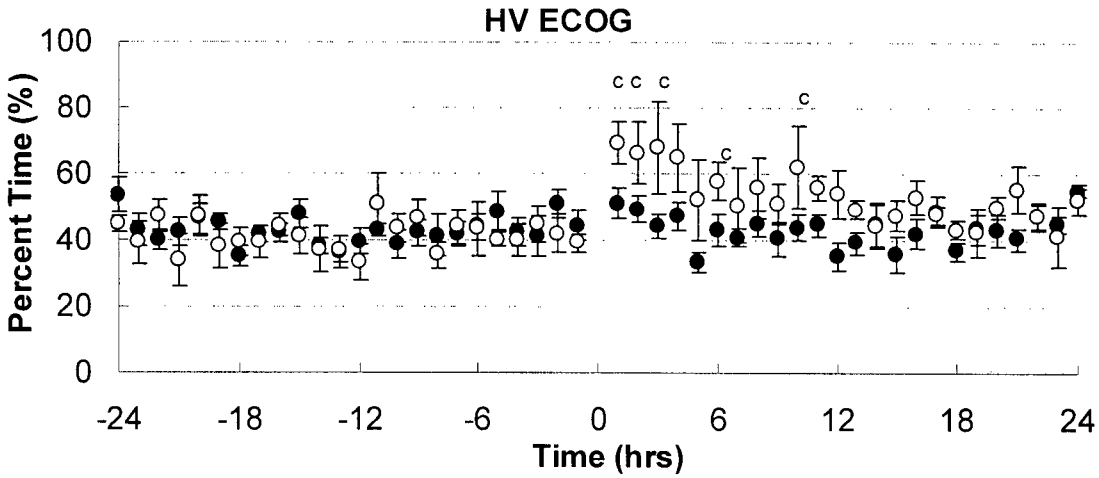
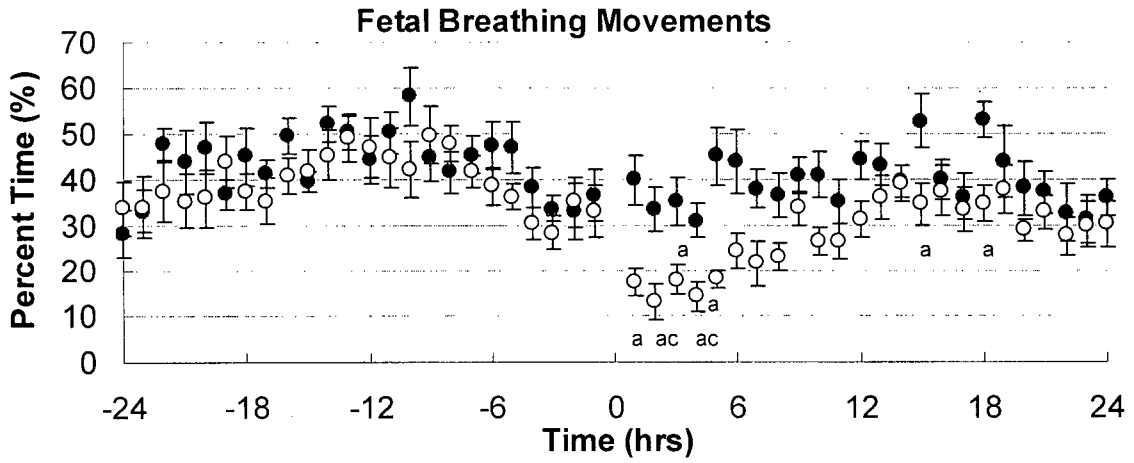


Figure 3.2. Percent time spent in LV (A) and HV (B) electrocortical (ECoG) activity each hour for the 24 h before and after maternal intravenous FX (open circles, n=6) and sterile water (closed circles, n=6) infusion. [a], between group differences ($p<0.05$); [c], differences from –1 h in the FX group ($p<0.05$).

A



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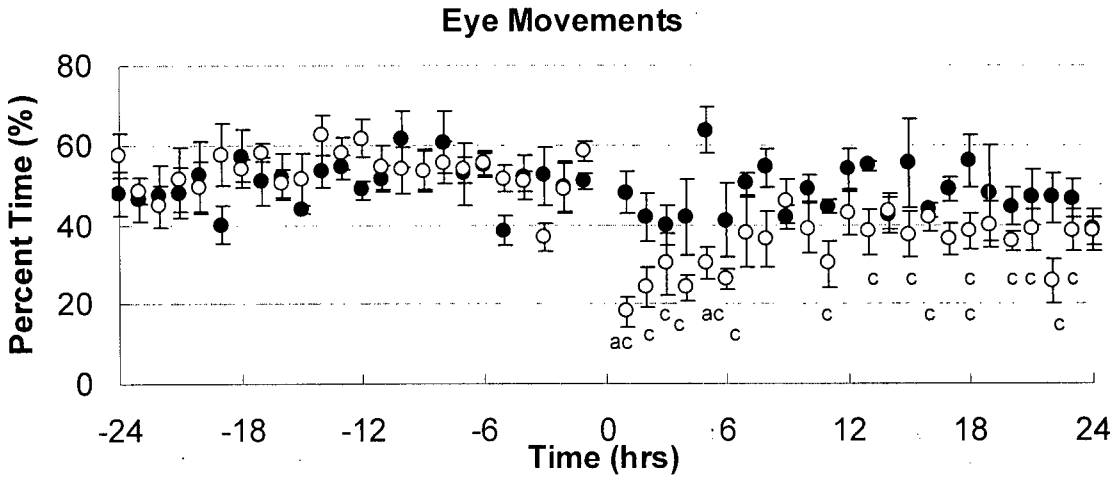


Figure 3.3. Percent time spent in fetal breathing movements (A) and eye movements (B) during each hour for the 24 h before and after maternal intravenous FX (open circles, n=9 and 5 respectively) and sterile water (closed circles, n=13 and 6 respectively) infusion. [a], between group differences ($p<0.05$); [c], differences from –1 h in the FX groups ($p<0.05$).

Table 3.2. Average duration and number of LV and HV ECOG episodes and LV-HV cycle length from 07:00 to 19:00 on the Control Day and Infusion Day 1 with maternal intravenous infusion of sterile water (n=5) or FX (n=5).

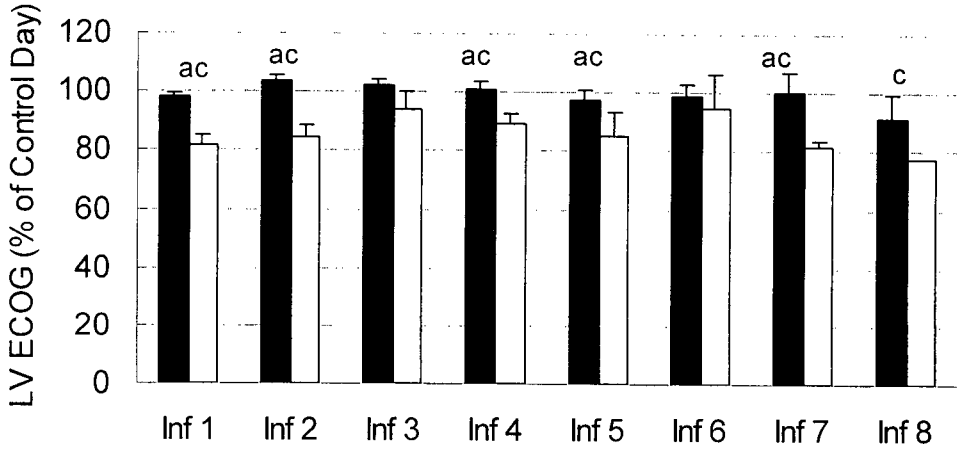
		Con		Inf 1
Duration of LV ECOG Episodes			min	
Con	Mean	14.2		14.2
	SEM	0.7		0.6
FX	Mean	14.8		12.3[c]
	SEM	0.8		1.0
Duration of HV ECOG Episodes			min	
Con	Mean	11.0		11.2
	SEM	0.5		0.8
FX	Mean	10.5		22.4[ac]
	SEM	0.5		4.7
Length of LV-HV Cycle			min	
Con	Mean	25.2		25.0
	SEM	1.1		1.1
FX	Mean	25.9		29.8[ac]
	SEM	1.1		1.8
Number of LV Episodes			min	
Con	Mean	6.2		6.4
	SEM	0.2		0.3
FX	Mean	6.0		4.7[ac]
	SEM	0.3		0.5
Number of HV Episodes			min	
Con	Mean	6.2		6.4
	SEM	0.2		0.3
FX	Mean	6.0		4.7[ac]
	SEM	0.3		0.5

[a], significant difference between control and FX groups ($p<0.05$); significant difference ($p<0.05$) from Control day in FX group [c].
Inf, Infusion; Con, Control.

duration of LV episodes and increase in HV episode duration accompanied these changes in the overall incidence of the ECoG patterns (Table 3.2). The overall cycle length (i.e. HV to LV or LV to HV) also increased significantly, and there was a significant decrease in the number of LV and HV episodes. In the control group, these variables were unchanged. The incidence of REM decreased in the FX group (Figure 3.3B), from 58.3±2.5 to 30.7±7.7% in the first hour and remained below the pre-infusion value for the rest of Infusion Day 1. There was a similar fall in FBM (Figure 3.3A), which declined from 33.2±5.7 to 18.8±5.7% during the first 6 h of drug administration. No such changes occurred in the control group. However, a significant ($p<0.05$) diurnal rhythm was observed in fetal breathing movements in both groups, as has previously been reported (Boddy et al., 1973).

Daily averages of fetal behavioural state data over the entire eight-day infusion period are shown in Figures 3.4 and 3.5. Again the daily averages are expressed as change from the Control Day because of a decrease in animal numbers toward the end of the experiment. In the control animals there were no changes in the incidence of LV and HV ECoG and REM at any point during the infusion period. In contrast, in the FX group the incidences of LV and REM were significantly reduced and the incidence of HV significantly increased in comparison to the Control Day. In addition, the mean change from Control Day over the entire infusion period was significantly different between the control and FX groups for both LV ECoG (control group, -0.9±1.3%; FX group, -14±2.2%) and HV ECoG (3.1±1.7%; FX group, 17.3±3.2%). In both groups, the incidence of FBM decreased

A



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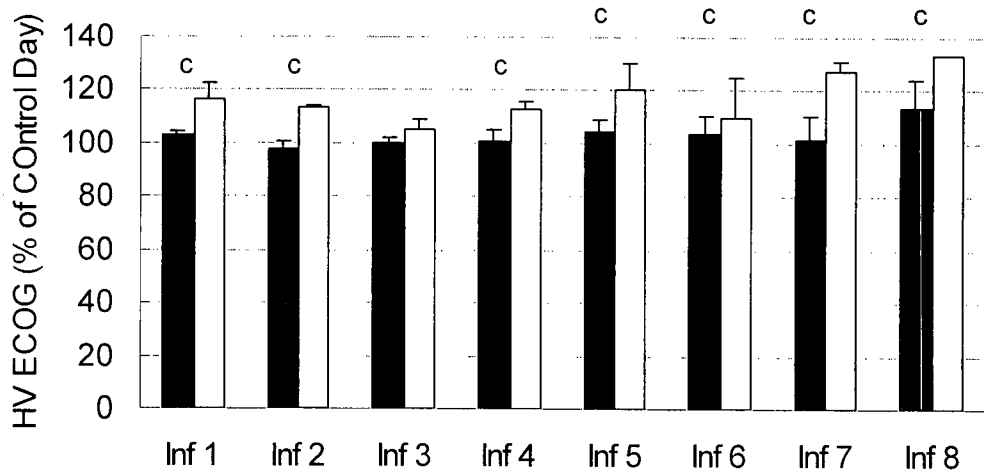
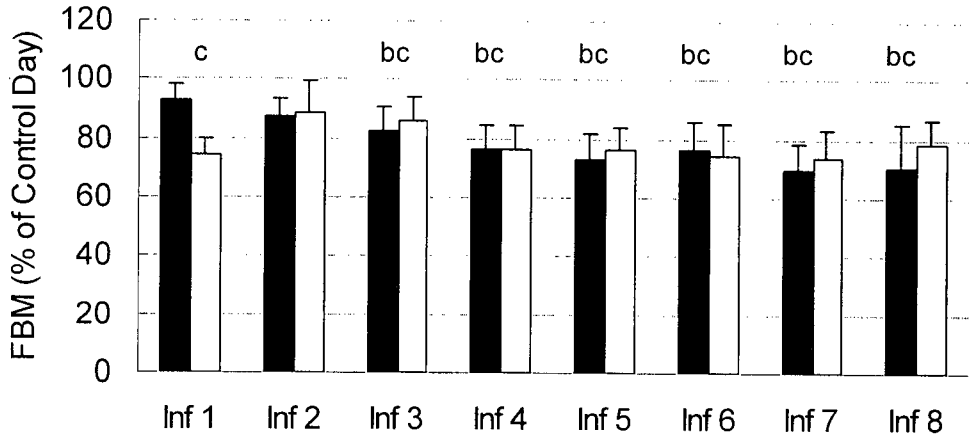


Figure 3.4. Incidence of LV (A) and HV (B) ECoG activity, expressed as a percentage of the values on the control day, during eight days of maternal intravenous infusion of FX (open bars, n=6) and sterile water (closed bars, n=6) beginning at 07:00 on Infusion day 1. [a], between group differences ($p<0.05$); [c], differences from the Control Day in the FX groups ($p<0.05$); Inf, Infusion Day.

A



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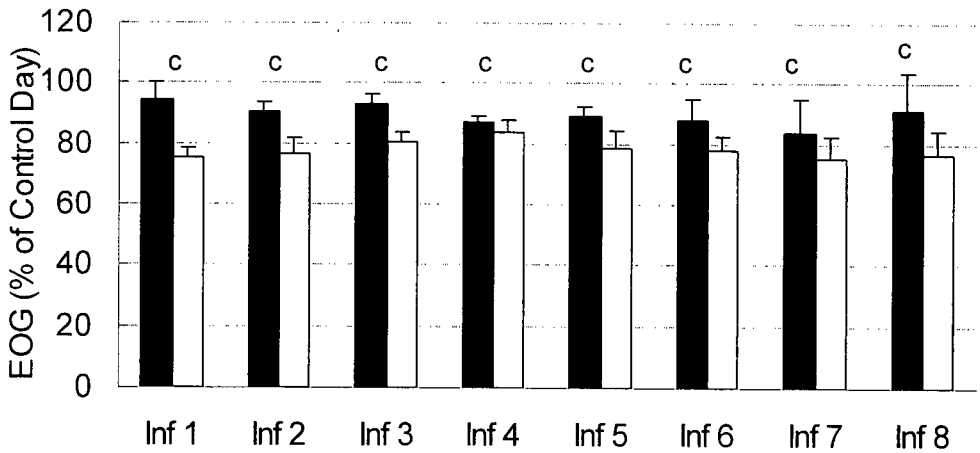

Figure 3.5. Incidence of fetal breathing movements (A) and eye movements (B), expressed as a percentage of the values on the control day, during an eight day maternal intravenous infusion of FX (open bars, n=12 and 5 respectively) and sterile water (closed bars, n=8 and 6 respectively) beginning at 07:00 on Infusion day 1. Differences from Control Day in the FX [c] and control [b] groups; Inf, Infusion Day

progressively during the infusion period, and other than on the Control Day, there were no differences between the groups.

3.4 Discussion

This study appears to be the first that has examined the effects of a SSRI on fetal behavioural state. Fetal exposure to FX was achieved via continuous maternal IV infusion of the drug. This is of course different from the normal oral route of administration of the drug in humans. However, the maternal FX and NFX concentrations that occurred in the present study are similar to those reported in humans. The mean maternal FX levels fall within the adult human therapeutic range for the drug, which is 40-250 ng/ml (Krogh, 1995) (see Chapter 2 for detailed discussion).

Maternal FX administration resulted in alterations in fetal behaviour that persisted throughout the 8-day infusion period. However, these changes were greatest in the first 24 h of the infusion, when there were significant decreases in LV ECoG, eye movements and fetal breathing movements and a corresponding increase in HV ECoG. There were also alterations in the average duration of HV and LV states and a significant increase in the average cycle length, i.e. the frequency of ECoG switching decreased. In contrast no such changes were observed in the sterile water infused animals. On the subsequent days, there were the same alterations in the incidence of LV and HV ECoG patterns in the FX-exposed fetuses. In both groups, the incidence of FBM decreased significantly over the experimental

period, a phenomenon that has been previously reported (Berger et al, 1986). Thus while the drug has acute, transient effects on both ECoG patterns and FBM, longer-term exposure affects only the former variable.

The initial FX-elicited alterations in fetal behavioural parameters on Infusion Day 1 were associated with transient decreases in fetal arterial Po_2, pH and oxygen saturation and an increase in Pco_2, changes that did not occur in the sterile water infused animals. In contrast, in both groups, Po_2 and oxygen saturation were significantly decreased on most of the subsequent days of the experimental period, although the magnitude of the decrease tended to be greater in the FX group. In Chapter 2 it was shown that the initial FX-induced alterations in fetal blood gas and acid-base status are associated with a transient decrease in uterine artery blood flow, likely caused by an initial rise in plasma serotonin concentration (Ortiz & Artigas, 1992; Bourdeaux et al., 1998). However, on Infusion Days 2 to 8 of the protocol, uterine artery blood flow in the FX and control groups are not different (see Chapter 2 for further discussion).

Acute hypoxemia is a potent inhibitor of FBM and other forms of skeletal and smooth muscle activity in the fetal lamb, and if the hypoxemia is severe enough there can also be inhibition of REM sleep (Boddy et al., 1974, Koos et al., 1987, Natale et al., 1981). However, we do not believe that either the initial or longer-term hypoxemia in the current study was responsible for the alterations in fetal ECoG patterns and breathing activity in the FX group. In these animals, the maximal fall in fetal arterial Po_2, which occurred at 5 min following the start of FX administration was 5.9±0.9 mmHg. Koos et al (1987) have shown that hypoxemia of this magnitude has

no effects on FBM, HV and LV ECOG and REM, whereas with more severe hypoxemia (ΔP_{O2} ~7.4 mmHg), FBM and REM, but not LV ECoG, were significantly reduced. Thus the magnitude of the hypoxemia in the FX-exposed fetuses seems insufficient to have caused the observed alterations in fetal behaviour and breathing.

Consequently, the effect of FX on fetal behavioural states is more likely due to either a direct effect of FX on state control or an indirect effect of FX due to an increase in central nervous system serotonin level that alters state control. The alterations in LV and HV ECoG incidence are similar to the reported effects of the drug in adult humans (Nicholson and Pascoe, 1988; Kerkhofs et al., Rush et al., 1998; Trivedi et al., 1999), which have been attributed to drug-elicited increased brain serotonergic neourotransmission. Other FX effects on sleep in adults such as increased REM sleep latency were not monitored in the present study or in the case of total sleep time are not relevant as the fetus is asleep most or all of the time (Parkes, 1991). Similar effects of FX on REM sleep occur in cats (Slater et al., 1978), Syrian hamsters (Gao et al., 1992) and rats (Pastel and Fernstrom, 1987), and in the latter species, the decrease in REM sleep is due to a reduction in the number of REM sleep episodes, rather than the length of these episodes. In the current study, both the number and duration of LV ECoG episodes was decreased, but this difference from the rat results could be due to the proportion of time that the fetal lamb spends in LV/REM sleep which is much greater than in an adult rat. Overall then, the effects of FX on fetal behavioural parameters appear similar to the actions of the drug on the analogous parameters in adults.

The transient decrease in FBM on Infusion Day 1 in the FX group is different from the stimulation of FBM that occurs with either systemic or central administration of L-5-hydroxytryptophan, the precursor of serotonin, in the fetal lamb (Quilligan et al., 1981; Fletcher et al., 1988; Morrison et al., 1997). However, this difference could be due to peripheral effects of L-5-hydroxytryptophan when it is given systemically (Vanderwolf, 1988). Moreover, in the study with central administration the compound was given locally in the brainstem (Morrison et al., 1997), whereas the FX effects of sertononinergic neurotransmission are likely more widespread in the brain, and thus could involve the regions in the rostral pons, caudal mesoncephalon and the posterior thalamus that are involved in the episodic pattern of fetal breathing and its inhibition by acute hypoxemia (Dawes et al., 1983; Gluckman and Johnston, 1987; Koos et al., 1998, 2000). Normally the hypoxia-elicited decrease in FBM is associated with a reduction in phasic REM (Koos et al., 1987), and in the current study this association was present on Infusion Day 1 (Figure 3.3). Adenosine causes similar effects on FBM and REM with increased brain adenosine levels during hypoxemia being partly responsible for the hypoxemia-induced inhibition of FBM and REM (Koos & Matsuda, 1990; Koos et al., 1994). Moreover, with prolonged hypoxemia and adenosine administration, the initial inhibition of FBM and REM is not sustained, and it has been suggested that this is due to downregulation of adenosine receptors (Koos et al., 1988: Bocking et al., 1988; Koos et al., 1990). In the current study, the FX-elicited inhibition of FBM did not extend beyond the first day, similar to the effects of hypoxemia and adenosine. However, REM appeared to be reduced for the entire 8-day infusion period; thus there was a dissociation of FBM

and REM. Koos et al (2000) have recently reported that lesions in the medial thalamus of the fetal lamb result in a similar dissociation of the effects of adenosine on these variables: the inhibition of FBM by adenosine is abolished, whereas the inhibition of REM is only blunted. This area of the thalamus (i.e. containing the parafasicular nuclear complex), receives fibres from raphe nuclei and hypothalamic sectors involved in sleep regulation (Koos et al, 2000). In adults, FX enhances serotonin actions in both of these brain regions (Owens and Nemeroff, 1994); thus similar effects in the fetus could have affected the thalamic centres involved in FBM and REM.

In summary, maternal FX administration in pregnant sheep resulted in decreases in LV ECoG, REM and FBM, and in the case of the former two parameters the effect persisted for the 8-day duration of the experiment. Whether these alterations would continue with a longer administration period similar to the clinical use of SSRIs to treat depression in pregnancy remains to be determined. In addition, the mechanisms underlying these effects are largely unknown. Given the likely importance of REM sleep for brain development during the fetal and postnatal period (Roffwarg et al., 1966; Richardson, 1994), further work in both areas is warranted.

CHAPTER 4

EFFECTS OF CHRONIC FLUOXETINE ON THE MATERNAL AND FETAL HYPOTHALAMIC-PITUITARY-ADRENAL AXIS

4.1 Introduction

In utero exposure to higher than normal levels of glucucorticoids may result in alterations in long-term developmental outcomes due the role of gluococorticoids in the growth and maturation of many organ systems during the perinatal period (Wood, 1994). For example, high levels of glucocorticoids in utero have been linked to low birth weight and hypertension in adulthood (Edwards et al., 1993). Studies show that prenatal stress and repeated dexamethasone treatment both cause an increase in glucocorticoids and result in long-term developmental outcomes. In rats, prenatal stress decreases the number of glucocorticoid receptors in the hippocampus, increases hypothalamic-pituitary-adrenal axis sensitivity and increases emotionality (Takahashi, 1998). Prenatal stress during pregnancy in rhesus monkeys leads to increased basal adrenocorticotropin hormone (ACTH) and cortisol levels in offspring and increased ACTH in response to stressful conditions (Clarke et al., 1994). Late gestation dexamethasone treatment in rats results in decreased survival during neonatal hypoxia challenge (Kauffman et al., 1994), and decreases birth weight and results in adult hypertension (Benediktsson et al., 1993) and a similar phenomenon has been reported in humans (Doyle et al., 2000).

Hypothalamic-pituitary-adrenal axis regulation of cortisol release is influenced by the neurotransmitter serotonin. These actions are mediated by $5HT_{1A/2A/2C}$ and

$5HT_3$ receptors in the paraventricular nucleus of the hypothalamus (Fuller, 1996; Kageyama et al., 1998; Bagdy, 1996). Serotonin microinjection into the paraventricular nucleus of conscious rats increases mRNA expression of corticotropin-releasing factor in the paraventricular nucleus and proopriomelanocortin mRNA in the anterior pituitary as well as short term increases in plasma adrenocorticotropin (Kageyama et al., 1998). Serotonin releasing drugs such as chloroamphetamine and fenfluramine also increase corticosterone in rats (Fuller & Snoddy, 1980) and ACTH and cortisol in humans (Lewis & Sherman, 1984).

FX is a selective serotonin reuptake inhibitor (SSRI) that causes an increase in serotonergic neurotransmission. Acute FX injections (10 mg/kg IP) in the rat increase cfos expression, corticotropin releasing factor mRNA expression and corticotropin-releasing factor receptor mRNA expression in the paraventricular nucleus (Torres et al., 1998). In addition, acute FX injection increases corticotropin-releasing factor in the hypophysial portal plasma and plasma ACTH (Gibbs & Vale, 1983) and corticosterone (Fuller et al., 1976). However, no change in basal ACTH and cortisol plasma concentration was observed in humans treated with 20 mg FX for 20 days (Berlin et al., 1998). Studies of chronic FX treatment have focussed on the neuroendocrine responses to challenge using either 5-hydroxytryptophan or a $5HT_{1A}$ agonist. An attenuated ACTH and cortisol/corticosterone response after chronic FX treatment occurs with $5HT_{1A}$ activation in humans (Berlin et al., 1998; Lerer et al., 1999) and rats (Raap et al., 1999; Li et al., 1993b; Li et al., 1994).

Our studies (Kim, 2000) and those of Pohland et al (1989) show that FX crosses the placenta and thus may have similar effects in the fetus as in the adult on serotonin and hypothalamic-pituitary-adrenal axis function. However, few studies have investigated the interaction between serotonin and the fetal hypothalamic-pituitary-adrenal axis. Intravenous infusion of 5-hydroxytryptophan, the precursor of serotonin which is capable of crossing the blood brain barrier, to the sheep fetus at 110 and 130 days gestation caused an increase in serotonin in hypothalamic tissue (Richards & Kendall, 1987). However, no studies have assessed the effects of FX or other SSRIs on endocrine function in the fetus. This study investigates the effect of chronic maternal FX infusion on fetal hypothalamic-pituitary-adrenal axis function in pregnant sheep.

4.2 Methods

4.2.1 Animals and Surgical Preparation

Eighteen time-bred Dorset/Suffolk cross pregnant sheep were surgically prepared between 118 and 122 d gestation (term, 147 d). The University of British Columbia Animal Care Committee approved experimental protocols and procedures performed on the sheep conformed to the guidelines of the Canadian Council on Animal Care. Surgical procedures have been described in detail in Chapter 2. Briefly, anaesthesia was induced with 1.0 g pentothal to allow intubation and maintained with 1.5% isoflurane. A midline incision in the maternal abdomen allowed access to the uterus to expose the fetus for implantation of polyvinyl catheters (Tygon, Akron, OH) in the trachea, both femoral arteries, lateral tarsal vein and the

amniotic cavity. Electrodes consisting of Teflon-coated stainless steel wire (Cooner, Chatsworth, CA) were implanted biparietally on the dura for fetal electrocortical recordings and through the lateral orbital ridge of the zygomatic bone of each eye for electroocular recordings. Before suturing the uterus, 1 to 1.5 L of warmed saline was poured into the uterus to replace amniotic fluid lost during surgery. In ten animals, a Transonic blood flow transducer was placed around the main uterine artery of the uterine horn containing the operated fetus for the continuous measurement of uterine blood flow. These data were reported in Chapter 2. The catheters, containing heparinised saline, and electrodes were exteriorised through the flank of the ewe. Polyvinyl catheters were placed in the maternal femoral vein and artery and tunnelled to the same exteriorisation site as the fetal catheters or the jugular vein and carotid artery without tunnelling.

4.2.2 Experimental Protocol

Ewes are given three or four days to recover after surgery. One Control Day preceded 8 days of infusion of either sterile water or FX and 4 days of monitoring post infusion. At 07:00 each day, blood samples were collected for analysis of blood gases (1 ml) and cortisol and ACTH levels (1.5 ml) in EDTA Vacutainers® and centrifuged for 10 mins at 8,000 rpm. Plasma was stored in polyvinyl Eppendorf® tubes and stored at -20°C for two weeks and then at -80°C in 75μl and 225 μl aliquots. Plasma (75μl and 225 μl aliquots) was also collected for a circadian rhythm study of melatonin and prolactin. Insufficient plasma was available for measurement of all four hormones at 07:00 on Infusion days 4, 5 and 6. As a result, ACTH plasma

concentrations were not measured on Infusion Days 4 and 6 and cortisol was not measured on Infusion Day 5. Ewes were housed in a 12:12 light:dark cycle (lights on at 06:00) with feeding once daily between 08:30 and 09:00. At 07:00 on Infusion day 1, a bolus injection of 70 mg FX in 10 ml sterile water was given over 2 min into the maternal femoral vein catheter followed by continuous infusion of a 2.77 mg/ml FX solution at a rate of 0.036 ml/min with a Harvard infusion pump.

4.2.3 Blood Gas Analysis

Blood samples were analysed for pH, partial pressure of carbon dioxide (Pco_2) and partial pressure of oxygen (PO_2) with an IL 1306 pH/blood gas analyser (Allied Instrumentation Laboratory, Milan, Italy) and temperature corrected to 39.5°C for fetal samples and 39°C for maternal samples. Hemoglobin and oxygen saturation were measured with an OSM-2 Hemoximeter (Radiometer, Copenhagen, Denmark).

4.2.4 Hormone Assays

4.2.4.1 Adrenocorticotropin Hormone

Plasma concentrations of immunoreactive ACTH were determined using an ^{125}I radioimmunoassay kit (ICN Biomedicals, Seven Hills, NSW, Australia). 100 μl of experimental plasma was vortexed and incubated for 16-24 hrs at 2-8°C with 200 μl each of ACTH antiserum and ^{125}I ACTH. 500 μl of precipitating complex was added and the mixture was then vortexed and incubated for 15-20 minutes at 20-25°C in a water bath. Samples were centrifuged for 20 minutes and the supernatant aspirated. A gamma scintillation counter was used to count the radioactivity in each tube

(Riastar, Packard, Canberra, Australia). The intra-assay coefficient of variation was <10% and the interassay coefficient of variation was 14.6%. The rabbit antihuman ACTH (1-39) had a cross reactivity of <0.01% with α-MSH (α-melanocyte-stimulating hormone), β- endorphin, α-lipotrophin and β-lipotropin and the sensitivity of the assay was 7 pg/ml (product information supplied by ICN Biomedicals) (Adams et al., 1999).

4.2.4.2 Cortisol

Total cortisol plasma concentration determination was performed using an ^{125}I radioimmunoassay kit (Orion Diagnostica, Espo, Finland). 100 µl of unknown plasma sample was mixed with 100 µl of distilled water and 2 ml of dichloromethane for extraction of cortisol into the organic layer (Bocking et al., 1986). The average efficiency of recovery of ^{125}I cortisol was 90%. After allowing tubes to dry down, 100 µl of buffer, cortisol antiserum (rabbit) and ^{125}I label were added to each tube, and incubated at 37°C for 1 hour. One millilitre of polyethylene glycol was added to each tube, followed by centrifugation at 4,000 rpm for 30 min (6°C). The supernatant was aspirated and the tubes counted on a gamma counter (Riastar, Packard, Canberra). The sensitivity of the assay was 0.39 nmol/l. The rabbit cortisol antibody was <1% cross reactive with cortisone and 17-hydroxyprogesterone and <0.01% with aldosterone, pregnenolone, estradiol and progesterone. The inter-and intra- assay coefficients of variation were always less than 10%.

4.2.5 Statistical Analysis

Blood gas, cardiovascular and hormone data were analysed using repeated measures ANOVA followed by post hoc Fishers t tests to determine the effect of both time and treatment on changes in measurements from Control Day using NCSS Statistical Software (Kaysville, Utah). Results are presented as means plus or minus the standard error of the mean.

4.3 Results

4.3.1 Fetal Outcome

Samples from a total of 10 control and 9 FX treated animals were analysed for cortisol and ACTH plasma concentrations. Animals were excluded from analysis in this paper if the fetal plasma concentration of cortisol was greater than 15 nmol/l on the Control Day as they may have begun the normal prepartum increase in cortisol. Thus data presented represents 8 control animals and 7 FX treated animals. On Infusion Day 8 mean gestational age was 135.4±0.7 in the control group (n=8) and 135.5±1.0 in the FX group (n=7) and the number of days prior to delivery in the control group was –7.1±1.6 and –6.3±1.3 in the FX group. These values for the two groups are not significantly different.

4.3.2 Fetal Blood Gas Status

In the FX group, fetal arterial Po_2 was below the Control Day value (23.0±1.1 mmHg) on Infusion Days 2 to 8 and on Days 2, 5, 6 and 7 the changes were statistically significant ($p<0.05$) as indicated in Table 4.1. The maximum drop in Po_2 of 3.3±0.9 mmHg occurred on Infusion day 6. A decrease in pH was also observed in the FX group ($p<0.05$) on Infusion Days 4, 6 and 7 compared to the Control Day (7.344±0.007 mmHg), with the maximum decrease (0.029±0.007) occurring on Infusion Day 6. Fetal oxygen saturation decreased on Infusion Days 2, 4, 6, 7 and 8 in the FX group ($p<0.05$) with a maximum drop of 12.8±4.3% on Infusion Day 2. A decrease in oxygen saturation ($p<0.05$) was also observed in the control group on Infusion Days 4, 5, 6, 7 and 8 compared to the Control Day (56.8±5.1%) with the maximum drop of 9.8±2.6% on Infusion Day 8. This was associated with a trend for decreased Po_2, but the changes were not statistically significant.

4.3.3 Plasma ACTH and Cortisol Levels

Maternal and fetal ACTH plasma concentrations are shown in Figure 4.1. In both groups maternal ACTH decreased with infusion especially on Infusion Days 2, 3 and 8 but reached statistical significance only in the FX treated ewes on these days. Maximum changes in maternal ACTH occurred on Infusion Day 2 (-26.7±11.9 pg/ml, control group ($p>0.05$); -38.0±11.9 pg/ml, FX group ($p<0.05$)) in the ewe. Fetal ACTH increased from 37.0±7.9 pg/ml on Control day to 75.7±20.1 on Infusion day 7 ($p<0.05$) in the FX group with no other significant changes. Although not significant, fetal ACTH is increased in both groups on Infusion Day 8.

Table 4.1. Blood gas status of animals at 07:00 daily when cortisol and ACTH samples were collected. Absolute value is shown for the Control Day, with change from Control Day shown thereafter.

		Con	Inf 1	Inf 2	Inf 3	Inf 4	Inf 5	Inf 6	Inf 7	Inf 8
pH										
Con FA	Mean	7.347	-0.013	-0.007	-0.015	-0.021	-0.019	-0.026	-0.021	-0.011
(n=8)	SEM	0.014	0.007	0.016	0.013	0.013	0.015	0.026	0.023	0.017
FX FA	Mean	7.344	-0.007	-0.016	-0.016	-0.017[c]	-0.009	-0.029[c]	-0.026[c]	-0.012
(n=7)	SEM	0.007	0.007	0.008	0.008	0.002	0.006	0.007	0.005	0.012
pCO2	mmHg									
Con FA	Mean	48.4	0.5	0.5	0.6	1.0	1.7	1.8	2.0	1.1
(n=8)	SEM	1.1	1.2	1.3	1.3	1.3	1.3	1.3	1.7	1.3
FX FA	Mean	49.3	0.9	3.3	0.9	1.6	0.5	0.5	1.6	1.9
(n=7)	SEM	1.3	1.3	1.4	1.5	1.8	1.3	1.1	1.6	1.2
pO2	mmHg									
Con FA	Mean	22.5	-0.4	-0.9	-1.3	-0.3	-1.5	-1.0	0.0	-0.4
(n=8)	SEM	1.9	0.6	0.9	1.3	1.6	1.6	1.1	1.8	0.8
FX FA	Mean	23.0	0.1	-3.0[c]	-0.6	-1.6	-2.1[c]	-3.3[c]	-2.4[c]	-1.3
(n=7)	SEM	1.1	0.7	1.3	0.9	0.6	0.7	0.9	0.6	0.9
Hemoglobin	g%									
Con FA	Mean	11.0	0.0	-0.7	-0.9	-0.7	-1.0	-1.0	-2.8	-1.0
(n=8)	SEM	1.0	0.3	0.6	0.5	0.4	0.3	0.4	1.9	0.4
FX FA	Mean	10.7	0.0	0.4	0.0	0.2	-0.3	-0.3	-0.2	0.1
(n=7)	SEM	0.9	0.4	0.3	0.4	0.4	0.4	0.3	0.5	0.4
Oxygen Saturation	%									
Con FA	Mean	56.8	-3.1	-3.8	-5.4	-6.3[b]	-7.6[b]	-8.4[b]	-7.3[b]	-9.8[b]
(n=8)	SEM	5.1	1.7	2.5	2.8	4.2	4.1	1.7	4.1	2.6
FX FA	Mean	59.1	0.4	-12.8[c]	-4.0	-8.4[c]	-6.7	-11.0[c]	-11.8[c]	-9.6[c]
(n=7)	SEM	3.1	1.0	4.3	2.0	2.1	2.7	3.8	2.1	2.6

Significant difference ($p<0.05$) from Control Day where [b] is control groups and [c] is FX group. Con, Control day; Inf Infusion day.

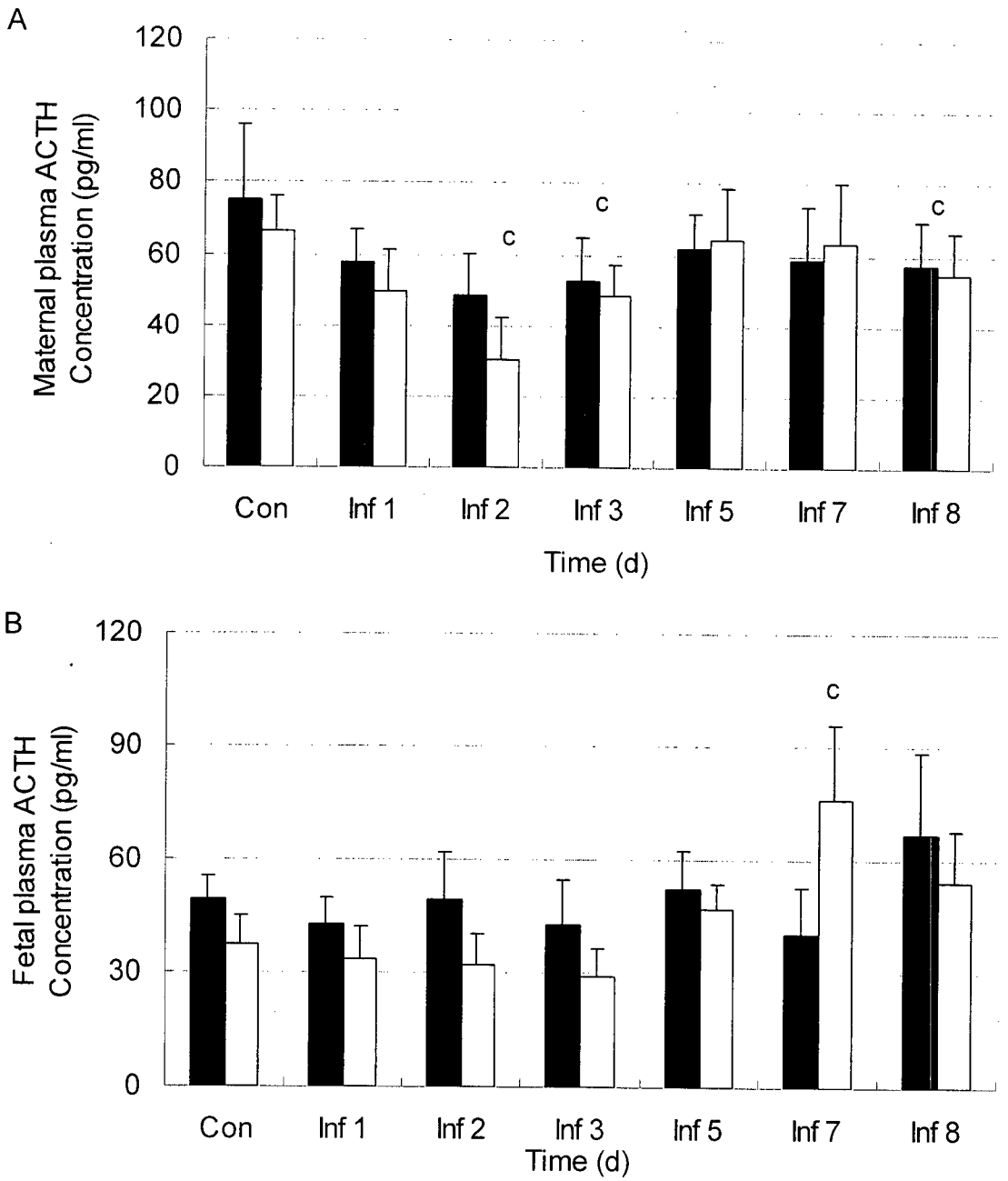

Figure 4.1. Maternal (A) and fetal (B) plasma ACTH concentrations at 0700 on the Control and eight sterile water (closed bars, n=8) or FX (open bars, n=7) Infusion days. [c], significant difference from control value for the FX group. Con, Control day; Inf Infusion day.

Maternal cortisol tended to increase without reaching statistical significance on Infusion Days 7 and 8 in the FX group (Figure 4.2). Fetal cortisol plasma concentrations increased on Infusion Days 6, 7 and 8 compared to the Control Day in both the control and FX group. The maximum change in cortisol occurred on Infusion Day 7 in both groups with an increase of 27.0±15.7 nmol/l in the control groups and 58.5±16.2 nmol/l in the FX group. The change in fetal cortisol levels on Infusion Day 7 and 8 was significantly greater in the FX group than the control group ($p<0.05$). Figure 4.3 shows that no significant difference was observed in the fetal to maternal ratio of plasma cortisol concentrations between the control and FX group or in each group. However, on Infusion day 7, the ratio in the FX group was significantly increased in comparison to the Control (pre-infusion) day (Figure 4.3A). Moreover, when the fetal-maternal cortisol ratio is plotted for the early (Control Day to Infusion 2) and late (Infusion days 5-8) phases of the infusion period, the ratio in both groups is significantly increased in the late period (Figure 4.3 B). This suggests that the prepartum cortisol rise in the fetus is of fetal origin .

Comparison of fetal ACTH and cortisol values on Control Day, Infusion Days 1 and 2 versus Infusion Days 5, 6, 7 and 8 was calculated to illustrate differences in these values early and late in the protocol (see Figure 4.4). In the FX group, there was a significant increase in maternal cortisol on Infusion Days 5 - 8 (46.8±11.6 nmol/l) compared to Control - Infusion Day 2 (24.7±3.4 nmol/l). However, this was not accompanied by any change in maternal ACTH. In the FX-exposed fetuses,

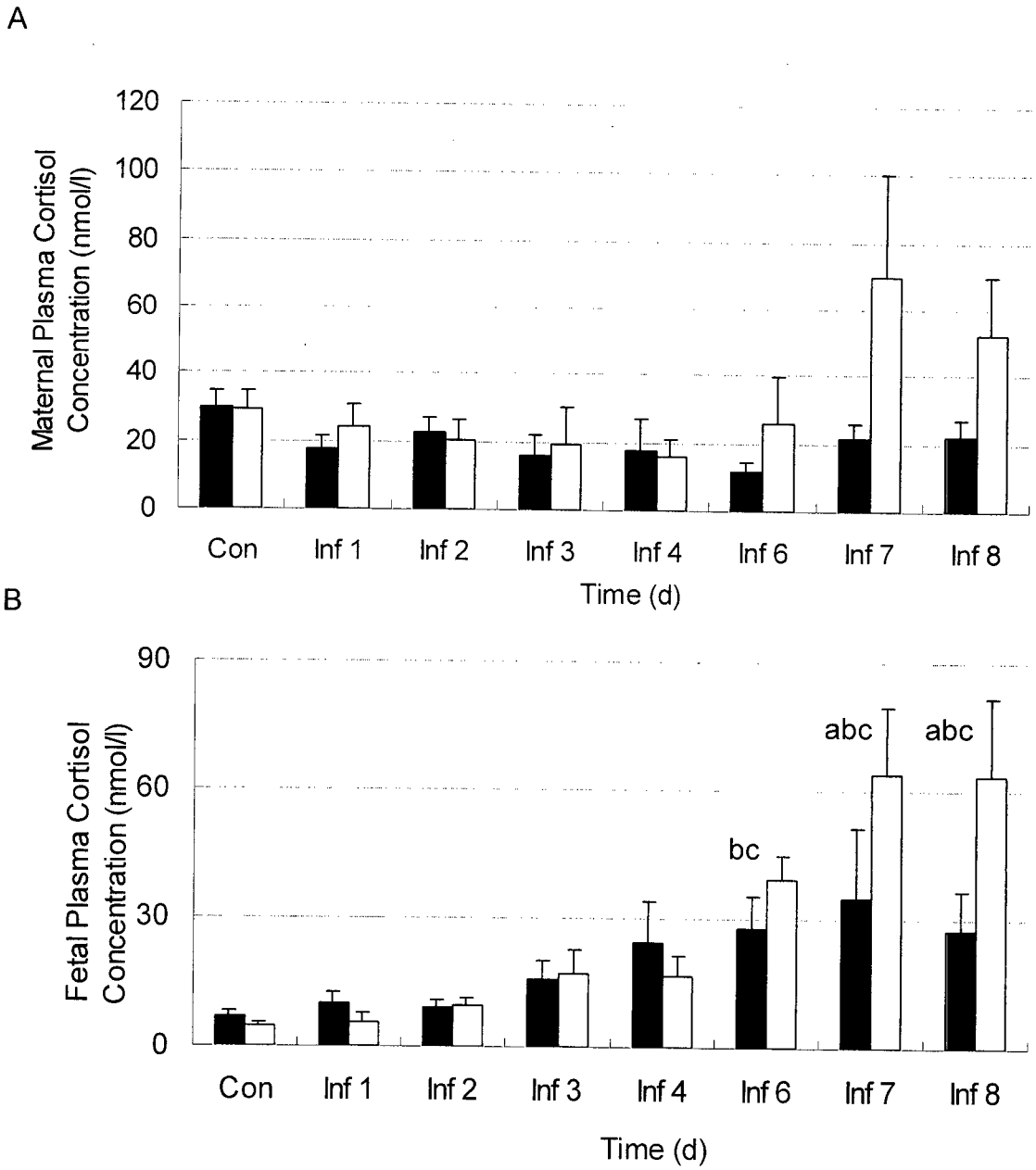

Figure 4.2. Maternal (A) and fetal (B) plasma cortisol concentrations at 07:00 on the Control and eight sterile water (closed bars, n=8) or FX (open bars, n=7) Infusion days. [a], between group differences ($p<0.05$); significant difference ($p<0.05$) from control value where [b] is control group and [c] is FX group. Con, Control day; Inf Infusion day.

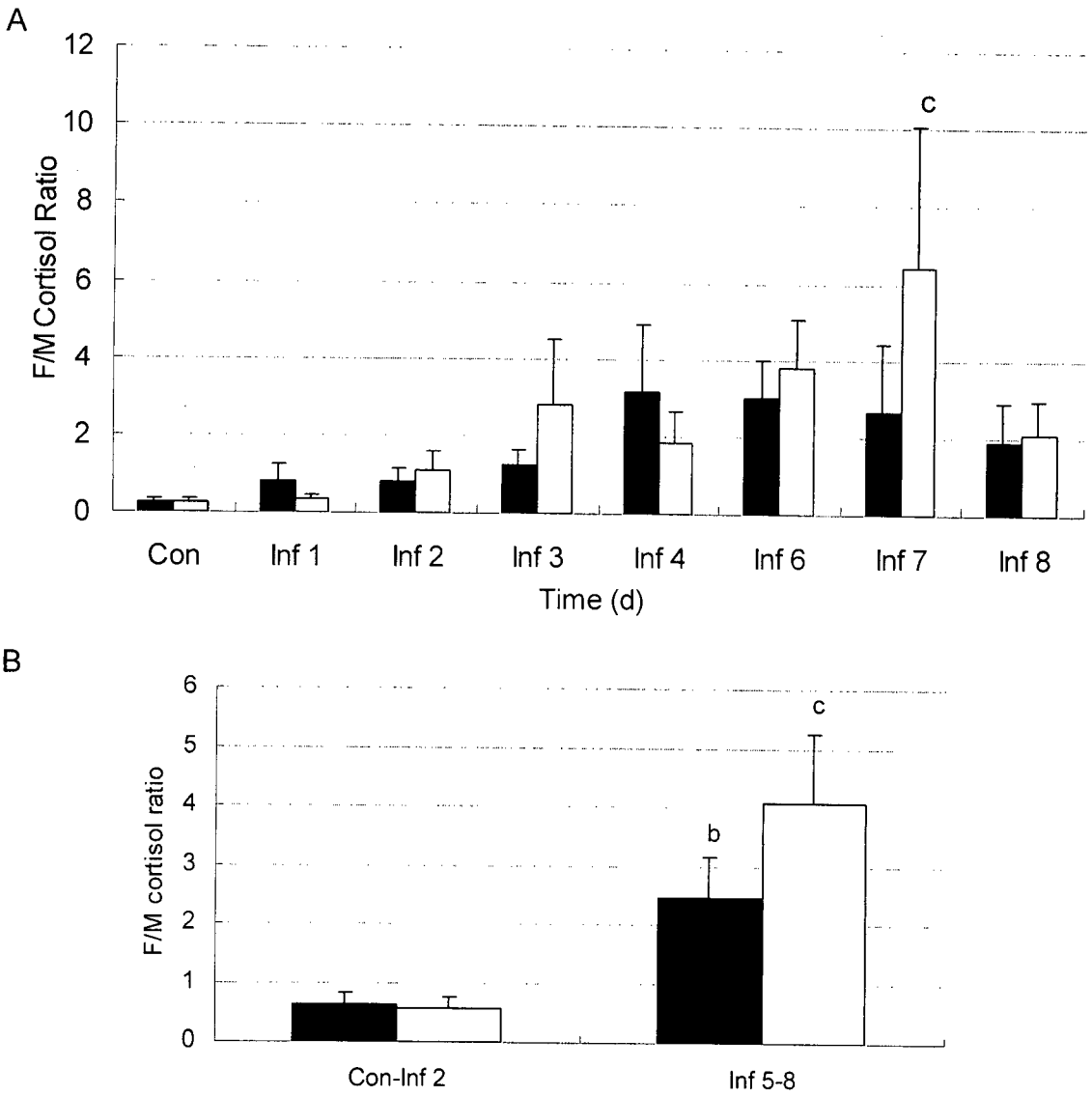

Figure 4.3. Fetal plasma cortisol concentration divided by maternal cortisol concentration at 07:00 on the Control and eight maternal infusion days (A) of sterile water (closed bars, n=8) or FX (open bars, n=7). Comparison of the F/M cortisol ratio early and late in the infusion period illustrates the prepartum increase in cortisol of fetal origin (B). Significant difference ($p<0.05$) from control value where ^{b} is control group and ^{c} is FX group. Con, Control day; Inf Infusion day.

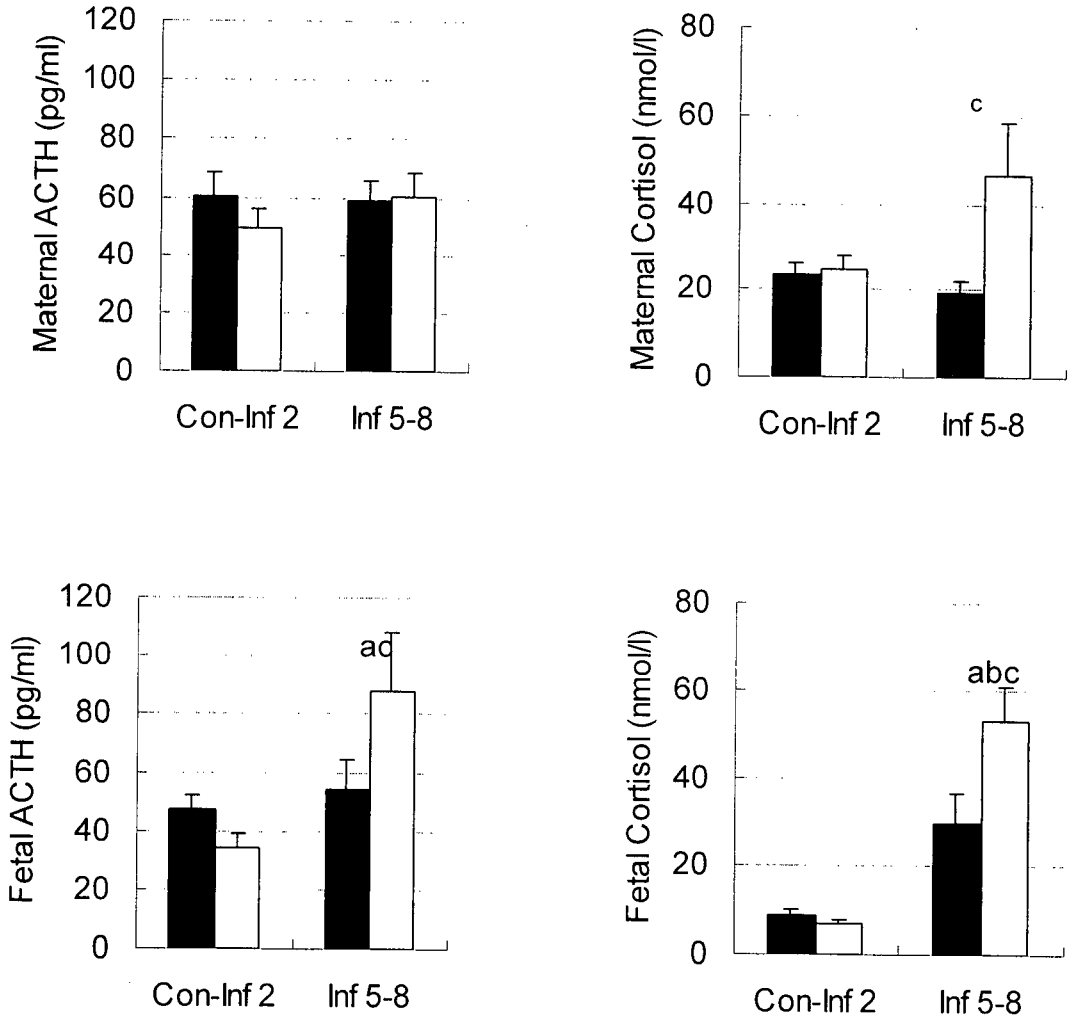

Figure 4.4. Mean of all maternal and fetal ACTH and cortisol plasma concentration data points on the Control, Infusion 1 and 2 days versus Infusion 5, 6, 7 and 8 days of an eight day maternal sterile water (closed bars, n=8) or FX (open bars, n=7) infusion. [a], between group differences ($p<0.05$); significant difference ($p<0.05$) from control value where [b] is control group and [c] is FX group. Con, Control day; Inf Infusion day.

there was an increase in ACTH from 34.3±4.6 nmol/l on Control day – Infusion Day 2 to 87.4±21.1 nmol/l on Infusion Days 5 – 8 which was significantly different from the control group. However, no such change was observed in the vehicle infused fetuses. In contrast, fetal cortisol values rose in both the control and FX groups on Infusion Days 5 - 8 (29.8±6.6 nmol/l, control group; 53.2±7.4 nmol/l, FX group) compared to Control - Infusion Day 2 (8.7±1.2 nmol/l, control group; 6.8±1.0 nmol/l, FX group) with a significantly greater increase in the FX group ($p<0.05$).

Changes in fetal ACTH and cortisol values were plotted for the FX group separating the male and female fetuses (Figure 4.5). Male fetuses exposed to FX exhibited an increase in ACTH on Infusion Days 7 and 8 while female fetuses did not. In addition, the increase in cortisol began in the male fetuses on Infusion Day 4 and the female fetuses on Infusion Day 6 with no significant difference in the magnitude of the increase. A similar analysis could not be conducted in the control group because of the high female to male ratio (6:2).

4.4 Discussion

Our results show that eight-day maternal intravenous infusion of FX tends to increase maternal cortisol levels after four days of infusion but decreases maternal ACTH significantly on Infusion Days 2, 3 and 8. Fetal ACTH levels increase late in the FX infusion period while fetal cortisol levels rise earlier and to a greater degree than in the control group.

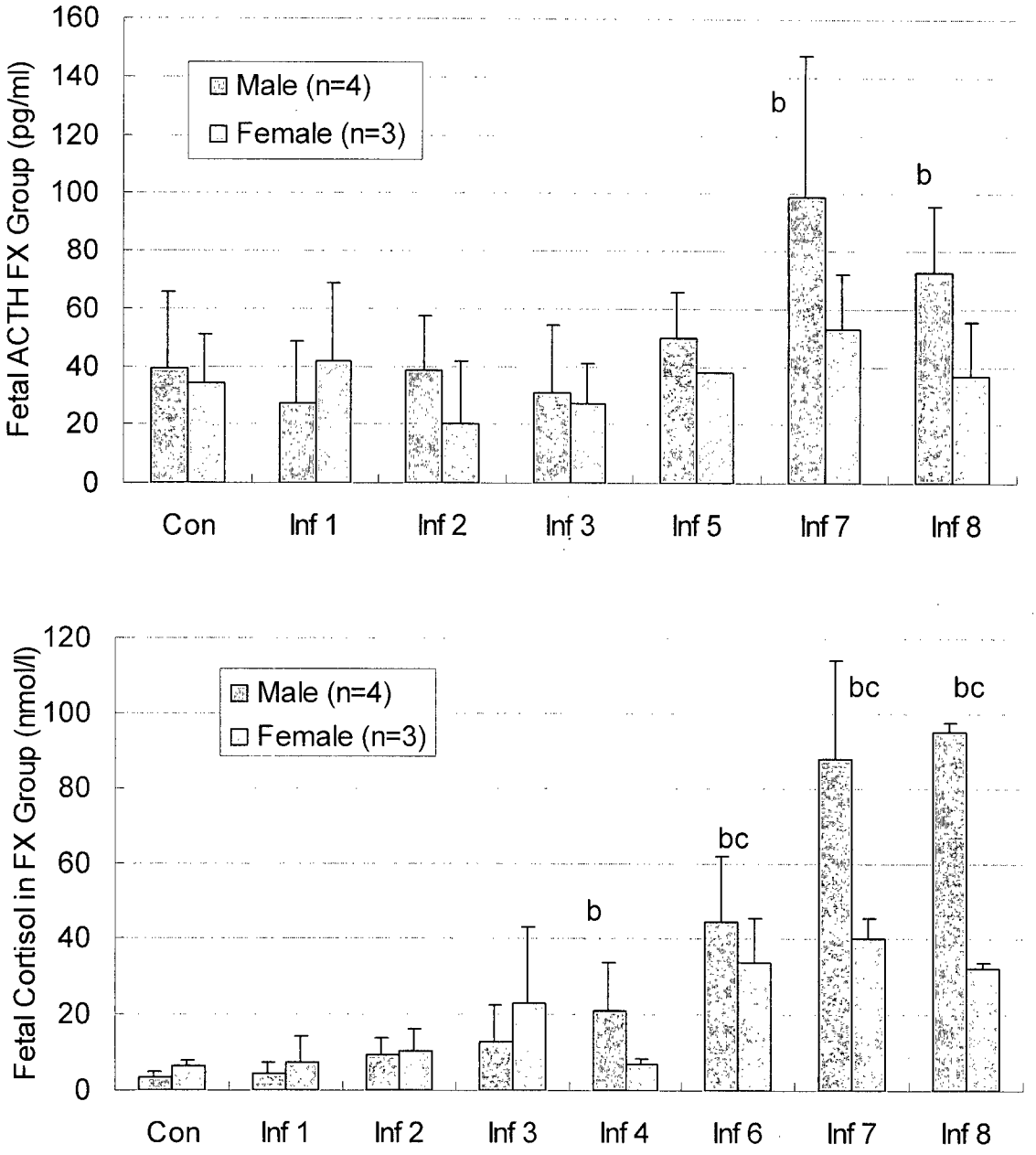

Figure 4.5. Fetal ACTH and cortisol values for male and female fetuses exposed to an eight day maternal intravenous infusion of FX.. Significant difference ($p<0.05$) from control value where [b] is males and [c] is females. Con, Control day; Inf Infusion day.

The increase in fetal cortisol on Infusion Days 6, 7 and 8 was greater in the FX group than in the control group while fetal ACTH rose on only Infusion Day 7. An increase in cortisol levels is expected in late gestation with an increase of 1 ng/ml/day beginning nine days prior to delivery (Kitts et al., 1984; Magyar etal., 1980). In the current study, this corresponded to Infusion Days 5 and 4 in the control and FX-exposed fetuses, respectively. Fetal cortisol plasma concentration nine days before delivery was 8.9±3.4 nmol/l in the control group and 6.0±1.8 nmol/l in the FX group. The expected range of fetal cortisol plasma levels nine days before delivery is 3.3 to 6.2 ng/ml (Mellor et al., 1977; Rose et al., 1982; Kitts et al., 1984; Magyar et al., 1980).

Fetal ACTH plasma levels increase progressively between 110 and 140 days gestation while fetal cortisol levels remain low between 110 to 120 days increasing between 125-140 days gestation (Norman, 1985). McMillen (1995) found that fetal ACTH values double between 120-136 days and 140-143 days gestation. Fetal ACTH did not increase in the control group but doubled in the FX group. It is possible that FX treatment induced a normal increase in ACTH at an earlier gestational age (mean 134.5±1.0 days) in the FX group. The pituitary response to ovine corticoptropin releasing factor is greater between 125-130 days than 135-140 days gestation while adrenal responsiveness to ACTH increases over late gestation (Norman, 1985). Fetal lambs (120 d gestation) treated with ovine corticotropin releasing factor every 4 hours for 7 days showed an initial increase in basal ACTH on the first two days. Cortisol levels also increased but the increase was greater on days 5-7 of the infusion showing that the ACTH response to corticotropin releasing

factor decreased while adrenal sensitivity increased (Brooks, 1987). This may be due to changes in the negative feedback of ACTH with increased cortisol plasma levels. Increased serotonin levels in the hippocampus with acute FX treatment may have increased gluocorticoid receptors in the hippocampus causing an increase in negative feedback on the hypothalamic-pituitary-adrenal axis. This could account for the decrease in maternal ACTH with FX treatment.

Hypoxemia and acidemia are potent stimuli for ACTH and cortisol release in the fetus, particularly with acute perturbations in these parameters (Boddy et al., 1974; Bocking et al., 1986; Challis et al., 1986; Towell et al., 1987; Akagi & Challis, 1990; Hooper et al., 1990; Murotsuki et al., 1996; Gagnon et al., 1997). As noted in Table 4.1, there were significant decreases in fetal arterial Po_2 in the FX groups at several points during the infusion period, with the maximum fall of 3.3±0.9 mmHg occurring on Infusion Day 6, i.e. when fetal plasma cortisol was beginning to rise. However, for at least two reasons, it seems unlikely that this modest degree of hypoxemia could have been involved in the cortisol increase. Firstly, published studies of chronic fetal hypoxemia whether achieved experimentally (Murotsuki et al., 1996, Gagnon et a.l, 1997) or occurring spontaneously (Kerr et al., 1992) have not found elevated fetal cortisol levels. Secondly, in the current study, arterial Po_2 in the FX-exposed fetuses was significantly decreased on Infusion Day 2 (by 3.0±1.3 mmHg), yet fetal cortisol was not elevated on this day. However, as reported in Chapters 2 and 3, there was a greater fall in Po_2 (5.9±0.9 mmHg) on the first day of the infusion, although samples for ACTH and cortisol measurement were not collected serially over this time. Akagi and Challis (1990) examined the threshold for

ACTH and cortisol responses to acute hypoxemia in fetal lambs at 125-129 and 134-147 days gestation. With mild hypoxemia (4.6-5.3 mmHg Po_2 drop), there was no change in the plasma level of either hormone, whereas with moderate hypoxemia (8.3-8.8 mmHg Po_2 drop), the concentrations of both ACTH and cortisol increased significantly. They concluded that the threshold for these endocrine responses to hypoxemia is between 5 and 8 mmHg. In contrast, Towell et al (1987) reduced fetal arterial P_{O2} by 4.8 mmHg for 24 h and found a significant rise in cortisol concentration. This suggests that the initial fall in Po_2 in the FX-exposed fetuses could have led to a transient rise in fetal plasma cortisol concentration. However, with the sampling protocol employed, any such increase would not have been detected as the blood samples for ACTH and cortisol estimation on Infusion Day 1 were collected before the start of FX infusion. The fetal cortisol levels on Infusion Day 2 in the two groups are not different, indicating the if any rise did occur in the FX group on Infusion Day 1, it did not persist.

The increase in fetal cortisol levels in the FX group seems best explained by the increase in fetal ACTH levels. The increase in ACTH is likely due to the effects of FX-induced increases in serotonin levels or serotonergic neurotransmission on the hypothalamic release of corticotropin releasing factor. We were not able to measure serotonin levels in the brain or in the hypothalamus but fetal treatment with L-5-hydroxytryptophan, the precursor to serotonin that is capable of crossing the blood brain barrier, increases hypothalamic serotonin content (Richards & Kendall, 1987). As a selective serotonin reuptake inhibitor, FX increases extracellular serotonin levels acutely and serotoninergic neurotransmission chronically. Activation of

serotoninergic receptors ($5HT_{1A}$, $5HT_{2A}$, $5HT_{2C}$ or $5HT_3$) increase the release of corticotropin releasing factor in adult species resulting in increased plasma ACTH and cortisol levels (Fuller, 1996).

Changes in fetal plasma ACTH and cortisol concentrations were not observed during the first five days of FX treatment despite high FX levels. This may be related to serotonin receptor function. As noted in the Introduction (Chapter 1), one of the effects of the rise in extracellular serotonin elicited by FX and other SSRIs is to downregulate the $5HT_{1A}$ autoreceptors that inhibit the firing of serotonergic neurones in the raphe nucleus. However, in adults, this appears to require 2-3 weeks of FX treatment (Lanfumey & Hamon, 2000). In the fetus, the development of the brain serotonin system begins early in gestation with serotonin immunoreactive perikarya in the 110 d gestation sheep fetus identical to that of the newborn, with well developed neuritic processes (Tillet, 1988). During development, the receptors may be more susceptible to downregulation than in the adult, accounting for the increase in fetal ACTH and cortisol observed around the sixth day of FX infusion. Increased serotonin levels during pregnancy in rats through a tryptophan-enriched diet or 5-methoxytryptophan treatment delays serotonin axon outgrowth and/or decreases collateral sprouting and synapse formation as well as receptor downregulation (Heuther et al., 1992; Whitaker-Azmitia et al., 1987).

Gluococorticoids play an important role in the maturation of the lung, heart, kidney and gut (Seckl, 1998). Women at risk of or in premature labour are routinely treated with synthetic glucocorticoids such as betamethasone and dexamethasone to increase the development of specific tissues in the fetus, especially the lung

(Seckl, 1998). Prenatal exposure to increased glucocorticoids may program effects in the brain by altering glucocorticoid receptor gene expression in the hippocampus (Seckl, 1997). $5HT_2$ receptors on the hippocampal neurons may directly regulate glucocorticoid receptor gene expression. Serotonin increases glucocorticoid receptor expression in primary hippocampal cell culture (Meaney, 2000). The hippocampus is a site of negative feedback by cortisol in the hypothalamic-pituitary-adrenal axis, which also includes the hypothalamus and pituitary gland. Prenatal exposure to dexamethasone increases mineralocorticoid and glucocorticoid receptor mRNA in the CA1-2 region of the hippocampus (Dean & Matthews, 1999). Ten-day corticosterone treatment in rats reduces somatodendritic $5HT_{1A}$ receptor function (McAlister-Williams et al.,1999) which also occurs with FX treatment. Chronic treatment with corticosterone in rats or FX in humans reduces the hypothermic response to a $5HT_{1A}$ agonist which is thought to be mediated through the somatodendritic $5HT_{1A}$ receptors (Young et al., 1994; Lerer et al., 1999). This link between chronic glucocorticoid and FX treatment may explain a potential mechanism for altering development with prenatal exposure to FX.

An intriguing result of the current study is the greater increase in plasma ACTH and cortisol in the male fetuses compared to the female fetuses in the FX group. Studies of prenatal exposure to dexamethasone in guinea pigs and both FX and cocaine in rats show differences between male and female offspring. Prenatal exposure to cocaine (15 mg 2X/d SC) potentiated the ACTH response to a $5HT_{1A}$ agonist in 28 day old male rats but not female offspring (Battaglia & Cabrera, 1994) while late gestation dexamethasone treatment in guinea pigs, increased fetal plasma

cortisol levels in females and decreased cortisol levels in males (Dean & Mathews, 1999). Our FX group was small with 4 males and 3 females. However, it appears that male fetuses had a greater cortisol response to maternal FX infusion than female fetuses.

In summary, maternal FX administration for eight days increases fetal plasma ACTH and cortisol concentrations towards the end of the infusion period. In addition there is a sex difference in the response, with the hormone increases being greater in males. Thus, the potential for long-term alterations of the hypothalamic-pituitary-adrenal axis exists with prenatal exposure to FX. A longer FX exposure period, earlier in gestation, before the normal rise in cortisol could distinguish the effects of FX versus the effects of gestation. An increase in cortisol would be expected but the magnitude and length of increase and the impact on the timing of delivery would be of interest.

Chapter 5

SUMMARY AND CONCLUSIONS

5.1 Summary

The overall objective of this study was to investigate the effects of maternal FX treatment on the fetus. Studies in humans suggested that first trimester FX exposure did not affect fetal outcome while third trimester exposure negatively influenced fetal outcome. To this end, we exposed late gestation sheep fetuses to FX at clinically relevant concentrations and measured fetal response in terms of blood gas status, cardiovascular function, fetal behavioural state and HPA axis function. FX was administered to the ewe with a loading dose followed by continuous intravenous infusion in an attempt to reach steady state plasma concentration levels in the human therapeutic range within a reasonable time period. This was successful in that the maternal and fetal FX levels are in the range reported for human pregnancies associated with FX therapy. However, the infusion regimen differs from the clinical setting in two ways. First, human fetuses in third trimester FX exposure groups were actually exposed to FX throughout pregnancy so our protocol does not directly mimic the clinical setting. Second, humans receive FX as a tablet once daily with FX levels peaking 4-8 h after dosing. Despite the differences between the clinical setting and the research laboratory, the data gained in this study contributes valuable knowledge to the determination of the risks and benefits of antidepressant treatment during pregnancy.

The following conclusions can be drawn from the results of late gestation maternal intravenous infusion of FX for eight days.

1. Maternal FX administration alters uterine artery blood flow and fetal blood gas and acid-base status over the first few hours of drug infusion. The decrease in uterine perfusion is likely due to a transient rise in plasma serotonin concentration, due to FX-mediated inhibition of serotonin uptake by platelets. Although umbilical blood flow was not measured, the lack of fetal hypertension and bradycardia following the start of the infusion suggests that umbilical vasoconstriction due to a FX-mediated increase in fetal plasma serotonin levels did not occur. Thus the transient fall in fetal arterial Po_2 (~ -6 mmHg) and rise in Pco_2 (~3 mmHg) were likely entirely due to the fall in uterine artery blood flow, while the acidemia was of respiratory origin (i.e. due to the increased Pco_2). These blood gas and acid-base changes are similar in magnitude to those that occur normally during gestation as a result of uterine contractures and fetal skeletal muscle activity. However, they last for a substantially longer time period (~ 2-6 h) compared to the changes of several minutes duration that occur during individual contractures or vigorous bouts of fetal activity. Thus if daily administration of FX results in changes of similar duration and magnitude each day following drug dose (as suggested by preliminary results in our lab), there could be negative consequences on fetal growth and development. This issue warrants further investigation.

2. Fetal behavioural state was significantly altered during the first 6 h of FX infusion. Since we began the FX infusion during the circadian trough of LV/REM behavioural state, the incidence of LV ECoG, eye movements and FBM were at the lowest values normally observed during the day. However, they decreased further with the onset of FX infusion. The incidence of LV ECoG, and eye movements remained lower than observed in the control group throughout the FX infusion period. If these changes in fetal behavioural state persist with longer periods of FX administration, there could be long term alterations in neurobehavioral function, as has been observed with long-term clomipramine and clonidine administration in neonatal rats (Mirmiran, 1986). The changes in fetal behavioural state were likely due to an increase in serotonin and/or serotonergic neurotransmission. Microdialysis probes implanted in the fetal cortex or lateral ventricle would have allowed sampling of extracellular fluid and cerebrospinal fluid. Analysis of serotonin concentrations in these fluids would provide direct evidence of the effects of FX on fetal serotonin brain levels. In the absence of this highly technical method, determination of fetal plasma serotonin may have provided a marker of changes in extracellular serotonin caused by FX infusion. Although plasma serotonin concentrations may not remain high throughout the infusion period, increased serotonergic neurotransmission due to disinhibition of somatodendritic $5HT_{1A}$ receptors caused by FX may account for the sustained decrease in the incidence of some behavioural state parameters.

3. A decrease in maternal basal plasma ACTH concentrations was observed on Infusion Days 2, 3 and 8 with no change in maternal cortisol concentration.

Despite this, maternal cortisol levels were higher at the end of the infusion period than at the beginning. Fetal ACTH increased on Infusion Day 7 in FX-exposed fetuses. FX-exposed fetuses exhibited a rise in cortisol which was greater than the normal prepartum rise. The fetal increase in ACTH is due to increased serotonin neurotransmission activating postsynaptic $5HT_{1A}$ or $5HT_{2A}$ receptors in the paraventricular nucleus causing an increase in hypothalamic release of CRF which acts on the pituitary to signal the release of ACTH. Testing the function of the HPA axis by treating the ewe or fetus with $5HT_{1A}$ and/or $5HT_{2A}$ receptor agonists would have been interesting to determine if the attenuation of the ACTH response observed in adults pretreated with FX also occurs in the fetus. The increase in plasma ACTH accounts for the increase in fetal cortisol. In addition, adrenal sensitivity to ACTH may have been enhanced in the FX-exposed fetuses. Fetal ACTH and cortisol responses to FX infusion were greater in males than females. Although of equal gestational age, there may be a gender difference in serotonin receptor function in the paraventricular nucleus, the effectiveness of FX in altering serotonin system function or adrenal sensitivity.

4. Measures of fetal outcome such as intrauterine growth, birth weight, gestational age at delivery and postnatal weight gain were not affected by late gestation exposure to FX. An increase in preterm delivery and decreased birth weight in human fetuses exposed to FX in late gestation has been reported (Goldstein, 1995; Chambers et al., 1996). The mechanism of initiation of parturition in humans and sheep differs and thus may explain different effects of FX on gestation length in humans. Alternatively, it may have been more clinically

relevant to treat the sheep with FX throughout gestation. This protocol would be labour intensive and lengthy. Mini pumps could be implanted subcutaneously in the ewes prior to fertilisation and reloaded with FX or vehicle as required. Alternatively, pulsatile FX treatment throughout gestation may better mimic human dosing. Pulsatile oxytocin treatment from 96 d gestation to delivery increased the frequency of normal contractures from 1.3/h to 3.18/h, accelerating fetal cardiovascular development, ECoG maturation and attenuating the ACTH and cortisol response to hypoxia. The effects of the long term changes in the intrauterine environment are more likely due to the decrease in Po_2 than the compression of the fetus (Shinozuka et al., 2000; Shinozuka et al., 2000b; Shinozuka et al., 1999). In addition, as far as we know, our ewes were not depressed. The underlying pathology of depression itself results in negative fetal outcomes, although, most human studies of FX exposure during pregnancy have included control groups with normal, nondepressed pregnant women.

5.2 Potential Long-term Outcomes

The studies described in this thesis did not focus on the long-term outcomes of prenatal FX exposure. This could have been accomplished in several ways.

1. Epidemiological studies in humans have found that the placental to birth weight ratio is an important predictor of adult disease. Low birth weight fetuses with large placentas are at increased risk of hypertension in adulthood (Seckl, 1998). Measurement of placental weight would have

allowed us to investigate this parameter and suggest potential adult outcomes of FX exposure.

2. With the appropriate work force and time frame a study of our prenatally exposed fetuses at 3 and 12 months of age to monitor blood pressure, growth, neuroendocrine responses to serotonin challenge and serotonin pathways would increase our knowledge substantially.

3. Measurement of serotonin levels throughout the protocol would allow pharmacodynamic modelling of the effects of FX. The easiest compartment to access serotonin is the plasma but analysis of the samples is technically difficult. Sampling from the cerebrospinal fluid compartment is technically more difficult, significantly lengthening surgery with the implantation of microdialysis probes, but sample analysis is easier and the values may be more representative of the therapeutic effects.

4. It would be interesting to correlate FX concentration with physiological parameters. Due to economical and time limitations, FX analysis was performed only when samples were collected at all time points. It would have been interesting to analyse more samples for FX in order to do more comparisons. Most studies of prenatal pharmacological exposure focus on fetal responses or adult outcomes. Combining both time points would allow integration of previous studies and increase their predictive value.

The sheep model is not conducive to these long-term studies due to the time expenditure required with a gestation of 145 days and an additional 8-12 months to

maturity. A better model for studying prenatal alterations on adult health may be the guinea pig that is a prenatal developer with a gestational age of 67 days but matures more quickly. Fetal monitoring, although technically difficult, can be performed in guinea pigs and the offspring can then be followed. Rats have been more commonly used in prenatal exposure studies but they are postnatal developers. Gestational day 13 to birth is a period of rapid differentiation, proliferation and axonal outgrowth in the serotonin system in rats (Cabrera-Vera & Battaglia, 1998). Despite this a great deal of brain development including that of sleep states occurs postnatally when the prenatally exposed offspring are housed with nontreated dams. Drugs with a long half-life, such as FX would continue to influence postnatal development but those with short half-lives would not.

5.3 Concluding Remarks

Fetal outcome is determined by a host of physiological variables, most of which are currently beyond our control. Depression clearly impacts maternal and fetal well being and for this reason pharmacological treatment is frequently indicated. Human studies find no teratogenetic effects, minor effects on birth outcome and no neurocognitive developmental effects in children prenatally exposed to FX. Animal studies demonstrate that prenatal FX exposure induces permanent alterations in the serotonin system in adulthood. These results combined with the results described in this thesis, show that the potential for long term effects of prenatal exposure to FX exist. Further long-term comparative study is required to determine if the

consequences of prenatal exposure to FX outweigh the benefits of controlled maternal depression.

CHAPTER 6

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