AN ABSTRACT OF THE DISSERTATION OF

John R. Jaeger for the degree of Doctor of Philosophy in Animal Science presented on November 14, 2005.
Title: Quantities of Prostaglandins in Whole and Extended Bovine Semen and Their Potential Effect on Fertility Following Insemination.

Abstract approved:

_____

Timothy DelCurto

Our objectives were to determine 1) the concentration of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) in whole and extended bovine semen, 2) if concentrations of prostaglandins in extended bovine semen are correlated to fertility, and 3) if $PGF_{2\alpha}$ administered at the time of artificial insemination would improve conception rate. Concentration of $PGF_{2\alpha}$ tended to be only slightly greater for whole compared to extended semen. To elucidate why $PGF_{2\alpha}$ levels were comparable, semen was extended at eight dilution rates. Prostaglandin $F_{2\alpha}$ in sub-samples, collected during extension, decreased at higher dilution rates and later steps of extension. To quantify $PGF_{2\alpha}$ synthesized during extension, quantities of $PGF_{2\alpha}$ in semen and the diluent were subtracted from each step. Higher dilution rates reduced the final amount of $PGF_{2\alpha}$ synthesized. Initial $PGF_{2\alpha}$ concentration was greater in whole semen compared to seminal plasma; however, when extended at three dilution rates quantity of $PGF_{2\alpha}$ synthesized during extension was greater for semen compared to seminal plasma, indicating less disparity than for original samples. Concentrations of prostaglandin E_2 (PGE_2) and $PGF_{2\alpha}$ in extended semen and the ratio of PGE_2 to $PGF_{2\alpha}$ were compared to a fertility rating. The ratio of PGE_2 to $PGF_{2\alpha}$ was not correlated to the fertility rating. Semen was extended to contain either 0, 500, or 5000 pg/ml of exogenous

$PGF_{2\alpha}$. Exogenous $PGF_{2\alpha}$ did not affect post-thaw motility or proportion of normal spermatozoa. *In vitro* fertilization did not differ between treatments. Analysis of cleavage rate per embryo revealed a bull x semen treatment interaction. First service conception rate of cows inseminated with the $PGF_{2\alpha}$-enhanced semen was affected by semen treatment and technician. Beef heifers inseminated with subfertile semen and dairy cows inseminated with normal fertility semen and treated with $PGF_{2\alpha}$ after insemination displayed higher conception rates compared to control animals. These data suggest that, although extension reduces the concentration of many seminal components, $PGF_{2\alpha}$ synthesis during extension results in concentrations similar to whole semen. Seminal prostaglandin concentrations may be related to fertilizing capability of the semen, and exogenous $PGF_{2\alpha}$ administered at the time of insemination may improve conception rate.

Quantities of Prostaglandins in Whole and Extended Bovine Semen and Their Potential Effect on Fertility Following Insemination

by
John R. Jaeger

A DISSERTATION

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Doctor of Philosophy

Presented November 14, 2005
Commencement June 2006

Doctor of Philosophy dissertation of John R. Jaeger presented on November 14, 2005.

APPROVED:

Major Professor, representing, Animal Science

Head of the Department of Animal Science

Dean of the Graduate School

I understand that my dissertation will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request.

John R. Jaeger, Author

ACKNOWLEDGEMENTS

I would like to express appreciation to my parents, Henry and Joan Jaeger, for instilling me with an appreciation of learning and agriculture. Their continual support and encouragement to pursue a higher education and advanced degrees is truly valued.

I must also express gratitude to my committee, Dr. DelCurto, Dr. Stormshak, Dr. Menino, Dr. Males, and Dr. Mathews, for their belief in my abilities, guidance during my doctoral program, and providing opportunities to improve my presentation and teaching skills. Special acknowledgement is extended to Chad Mueller for agreeing to serve on my graduate committee for the final oral defense. My gratitude is extended to Select Sires for contribution of the whole, extended, and prostaglandin $F_{2\alpha}$-enhanced semen utilized during this research. Special thanks go to Ben Krahn and Clint Sexton for their efforts to supply adequate animal numbers, assist with data collection and provide excellent animal management. The assistance of senior research team students Jesse Roth and Seth Reno was invaluable.

I must convey my esteem for Dr. Fredrick Stormshak and the gratitude I feel for the initial training in scientific research and preparation of manuscripts that I received during my Master of Science program. His wisdom, insights, and wit provided significant direction and further education during my doctoral program.

Dr. John Brethour, my first supervisor following completion of my Master of Science degree, provided me with a unique opportunity to conduct research in addition to my normal duties. He also continually voiced the importance of obtaining a PhD and encouraged me to pursue this degree as soon as I was ready. Also, appreciation is expressed to Dr. Kenneth Olson for allowing me to participate in numerous research projects at the Kansas State University Agriculture Research Center in Hays. These projects provided me with a further understanding of applied experimental design and presentation of research results, both written and oral. He is a steadfast friend that has also been supportive during my doctoral program, and always has time for a discussion of current achievements, “breakthroughs”, and disappointments.

My longtime friend, supervisor, and major professor, Dr. Timothy DelCurto, also provided me the capacity to conduct research in addition to my normal duties and

the opportunity to participate in numerous research projects outside my area of expertise. These research projects made me much more aware of the interconnective nature of biological sciences; I have no doubt that this understanding will significantly improve the quality of my research throughout my career. Dr. DelCurto's professionalism, teaching style, research endeavors, and personal character are all inspiring and are assets to be emulated. Additionally, during the course of the many phases of my life, Dr. DelCurto and his family have been a continual source of support and encouragement.

I also applaud my daughter, Cora, for her pleasant demeanor, just right timing of hugs, patience during data collection and late nights at the office, and support during my doctoral program. I must also recognize my daughter Amelia for her not so perfect timing in attempting to be born on the day of my oral preliminary examination.

Foremost, I must recognize my wife, Elizabeth. If not for a chance meeting, during the artificial insemination of cattle, it is likely that I would not have pursued a doctoral degree. It was in my attempts to convince her to attend Oregon State University that I realized the time was right for me to return to school for a doctoral program. Her support has been invaluable and her encouragement priceless. She has provided numerous insights into the interpretation of the research results, and is always willing to be a sounding board and editor. No matter the situation, her even demeanor has provided me with the foundation to excel at whatever task was at hand.

CONTRIBUTION OF AUTHORS

Dr. J.M. DeJarnette provided significant intellectual contributions to the design and experimental protocols utilized in examining the effect of exogenous prostaglandin $F_{2\alpha}$ on semen motility and function, and of prostaglandin $F_{2\alpha}$-enhanced semen on female fertility. Dr. A.R. Menino supplied laboratory space and expertise to determine the effect of prostaglandin $F_{2\alpha}$-enhanced semen on the *in vitro* fertilization of *in vitro* matured oocytes. Dr. T. DelCurto offered input for the interpretation and presentation of these data.

TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

TABLE OF CONTENTS (Continued)

TABLE OF CONTENTS (Continued)

LIST OF FIGURES

LIST OF APPENDIX TABLES

LIST OF APPENDIX TABLES (Continued)

QUANTITIES OF PROSTAGLANDINS IN WHOLE AND EXTENDED BOVINE SEMEN AND THEIR POTENTIAL EFFECT ON FERTILITY FOLLOWING INSEMINATION

GENERAL INTRODUCTION

Artificial insemination (AI) utilizing commercially available bovine semen has been available to beef producers for more than 60 years. However, only 13% of beef operations utilize AI (NAHMS, 1998), resulting in only 5% of the 33.8 million beef cows in the U.S. being exposed to AI (USDA, 2005). In contrast, 78% of the 9.1 million head of dairy cows in the U.S. are exposed to AI (USDA, 2005). Because dairy cows are maintained in confinement, it is not surprising that a greater proportion of dairy cows are inseminated artificially. Although beef cattle are more likely to be managed in extensive conditions, surprisingly, large operations with 300 or more cows are more likely to use AI (37% of operations) than operations with 50 or fewer cows (12% of operations; NAHMS, 1998). This suggests that cow accessibility may not be the principal factor in determining whether AI is employed.

Producers cite 50-70% conception rates following AI (Jaeger et al., 1992; Werth et al., 1996; DeJarnette et al., 2001) as being unacceptable; however, calving rate following a single natural service is only 50-60% (Parkinson, 2004). In addition, most producers (39%) cited excessive time and labor as the main reason for not incorporating AI into their operation (USDA, 1998). Regardless of the reason, failure of beef producers to utilize AI results in a loss of superior genetics from bulls with proven progeny performance. If conception rate to AI could be improved at minimal

expense and without adding additional labor, more producers may consider capturing the superior genetics available from proven AI sires.

Administration of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) to the female, by injection or via $PGF_{2\alpha}$-supplemented semen, at the time of insemination has improved conception rate in sheep (Gustafsson et al., 1975), rabbits (Spilman et al., 1973), swine (Peña et al., 1998; Peña et al., 2000; Willenburg et al., 2003), and cattle (Prinzen et al., 1991). Further evaluation of exogenous $PGF_{2\alpha}$ administration at the time of insemination of cattle will perhaps result in identification of an inexpensive and low-labor method of using $PGF_{2\alpha}$ to improve fertility following artificial insemination.

OVERVIEW OF PROSTAGLANDINS

Kurzrok and Lieb (1930) first observed that human seminal plasma caused relaxation of isolated uterine strips from fertile women and stimulation of uterine strips collected from women with a history of infertility. The active substances in semen were named prostaglandins because they were presumed to be secreted from the prostate gland (von Euler, 1936). Later it was found that the prostate preparations had been contaminated with secretions from the seminal vesicles, and that they in fact are the major source of prostaglandins in seminal plasma (Eliasson, 1959). Lipid extracts from seminal vesicles were prepared and the fraction containing lipid-soluble acids was found to be responsible for smooth mucle contrations; further studies indicated that this lipid-soluble acid contained a double bond and its solubility properties indicated it to be a hydroxy acid (von Euler, 1939). Bergström and

coworkers (1964) elucidated the structures of prostaglandins and demonstrated that they were produced from an essential fatty acid, arachidonic acid. At nearly the same time, Van Dorp and coworkers (1964) also described the biosynthesis of prostaglandins. Isolated active compounds from extracts obtained from sheep prostate glands were designated prostaglandin E (Bergström and Sjövall, 1960a) and prostaglandin F (Bergström and Sjövall, 1960b). The discoveries of numerous prostaglandins has since occurred and all of these unsaturated hydroxyl fatty acids are composed of a 5-membered ring on a 20-carbon skeleton, and are derivatives of a 20-carbon parent substance called prostanoic acid.

Prostaglandins are nearly ubiquitous throughout the mammalian body. Therefore, prostaglandins are produced by most cells and act as autocrine and paracrine lipid mediators or hormones (acting at or adjacent to their site of synthesis). Prostaglandins belong to a class of compounds called eicosanoids. The other compounds belonging to this classification are thromboxanes and leukotrienes. These are all lipids that are characterized by their shared metabolic origin, powerful physiological effects, low tissue levels, and rapid metabolism. These compounds are not stored, but are synthesized *de novo* from membrane-released arachidonic acid when cells are activated by specific stimuli.

BIOSYNTHESIS OF PROSTAGLANDINS

The biosynthesis of prostaglandins can be divided into three steps: 1) release of arachidonic acid from membrane phospholipids; 2) oxygenation of arachidonate to yield PGH_2, a prostaglandin endoperoxide that serves as a precursor to other

prostaglandins; and 3) depending on the enzymes present in a cell, the conversion of PGH to other prostaglandins or to thromboxane A_2 (Mathews et al., 2000).

A number of enzymes regulate cellular levels of arachidonic acid and keep it esterified until it is mobilized by phospholipase (PLA_2). Arachidonic acid is the product of PLA_2. It has been demonstrated that the release of arachidonic acid from membranes is primarily regulated by type IV cytosolic PLA_2 ($cPLA_2$), because cells lacking $cPLA_2$ are generally devoid of eicosanoid synthesis (Funk, 2001). Translocation of $cPLA_2$ to the nuclear and endoplasmic reticulum membranes and the Golgi apparatus is coordinated by cell-specific and agonist-dependent events (Evans et al., 2001). At the nuclear membrane and endoplasmic reticulum, arachidonic acid released by $cPLA_2$ is exposed to prostaglandin H synthase (PGHS, also designated as COX for cyclooxygenase) and is metabolized to an intermediate prostaglandin H_2 (PGH_2). Prostaglandin H synthase exists as two isoforms that are called PHGS-1 or COX-1, and PGHS-2 or COX-2 (Smith et al., 2000). In basic terms, COX-1 is responsible for basal, constitutive prostaglandin synthesis, and COX-2 is important for inflammatory and induced prostaglandin synthesis (Smith et al., 2000). Both cyclooxygenase enzymes are present on the luminal surface of the endoplasmic reticulum, and the inner and outer membranes of the nuclear membrane (Otto and Smith, 1994; Morita et al., 1995; Spencer et al., 1998), but COX-2 appears to be more concentrated within the nuclear membrane (Morita et al., 1995).

The intermediate PGH_2 is metabolized to other prostaglandins or thromboxane A_2 by downstream enzymes in a cell-specific fashion (Funk, 2001). Microsomal prostaglandin E synthase (PGE-S), or PGH-PGE isomerase, is responsible for the

synthesis of prostaglandin E_2 (PGE_2; Jakobsson et al., 1999). Prostaglandin F synthase (PGF-S) catalyzes the reduction of prostaglandin D_2 (PGD_2), PGH_2, and various carbonyl compounds yielding prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) as the reaction product (Watanabe et al., 1985). In the bovine endometrium, aldoketoreductase 1B5 has been identified as the PGF-S catalyzing the conversion of PGH_2 to $PGF_{2\alpha}$ (Madore et al., 2003).

MECHANISMS OF PROSTAGLANDIN ACTION

Prostaglandin Transporters

Although prostaglandins are generally regarded as hydrophobic compounds, they do not easily permeate the plasma membrane (Narumiya and FitzGerald, 2001). The first carrier demonstrated to promote the transport of PGE_2 and $PGF_{2\alpha}$ was identified and characterized by Kanai and coworkers (1995). Prostaglandins are released from cells primarily by facilitated transport through a prostaglandin transporter that belongs to the organic ion transporter family, but may also be released by uncharacterized transporters (Schuster, 1998). Both the human (Lu and Schuster, 1996; Naoaki et al., 1996) and the rat (Itoh et al., 1996; Naoaki et al., 1996) prostaglandin transporter bind $PGF_{2\alpha}$, PGE_2, PGE_1, and PGD_2 with high affinity. It has been suggested that the human (Naoaki et al., 1996; Chan et al., 1998) and rat (Naoaki et al., 1996) prostaglandin transporter may not only be responsible for release of prostaglandins from the cell, but may also be involved with metabolic clearance by transporting prostaglandins into the cell for intracellular termination. This loss of

biologic activity has been demonstrated to be due to cellular uptake and oxidation. In fact, only a single passage through the lungs is required for inactivation (Ferreira and Vane, 1967; Piper et al., 1970; Anderson and Eling, 1976). Prostaglandin transporter function appears to be regulated by the transporter interacting with the prostaglandin receptor, but transporter regulation is independent of the receptor's ability to catalyze the activation of adenylate cyclase (Vezza et al., 2001).

Prostaglandin Receptors

There are at least nine prostanoid receptors. These receptors are highly conserved in mammals and splice variants are divergent only on the carboxy terminus (Narumiya et al., 2001). These receptors belong to the G protein-coupled receptor family, which is characterized by their seven transmembrane domains. Each prostaglandin receptor is encoded by different genes (Narumiya et al., 1999).

Prostanoid receptors identified to date include two prostaglandin D_2 receptors (DP1 and DP_2; Hirai et al., 2001; Monneret et al., 2001), four subtypes of the PGE receptor (EP1, EP2, EP3, and EP4), the PGF receptor (FP), the prostaglandin I receptor (IP), and the thromboxane A_2 receptor designated as TP (Narumiya and FitzGerald, 2001). The DP_1, EP2, EP4, and IP receptors mediate an increase in cAMP levels and are termed relaxant receptors, while TP, EP1, and FP receptors induce calcium mobilization and comprise a contractile receptor subgroup (Narumiya and FitzGerald, 2001). The DP_2 receptor belongs to a separate receptor subgroup and is a member of chemoattractant receptors (Hirai et al., 2001; Monneret et al., 2001).

Prostaglandin $F_{2\alpha}$ Receptor Downstream Signaling

Prostaglandin $F_{2\alpha}$ binds to the FP receptor, which activates phospholipase C (PLC) in a pertussis toxin-insensitive manner (Gusovsky, 1991; Nakao et al., 1993; Quarles et al., 1993), suggesting that the FP receptor interacts with the G_q family of guanine nucleotide-binding proteins (GTP-binding proteins). Signal transduction of the α_c-subunit involves activation of membrane bound PLC. Activated phospholipase C catalyzes the hydrolysis of membrane-bound phosphatidylinositol 4,5-bisphosphate (PIP_2) to form two second messengers, diacylglycerol (DAG) and inositol 1,4,5-triphosphate (IP_3; Leung et al., 1986; Davis et al., 1987; Norman and Litwack, 1997). These second messengers work in concert to activate protein kinase C (PKC). Second messenger IP_3, released from the membrane, interacts with its own receptor (Spat et al., 1986) located on the interior membrane of the endoplasmic reticulum, resulting in the opening of Ca^{2+} channels and increased cytosolic concentration of these ions (Berridge and Irvine, 1984; Norman and Litwack, 1997). Second messenger DAG, along with Ca^{2+} released from the endoplasmic reticulum, activates PKC (Norman and Litwack, 1997). Active PKC phosphorylates serine and threonine residues on substrate proteins.

Prostaglandin $F_{2\alpha}$ has been demonstrated to have diverse physiological actions including hypertrophy of cardiac myocytes (Karmazyn, 1989; Kunapuli et al., 1998), vascular smooth muscle contraction (Csepli and Csapo, 1975), myometrial contractions (Patil et al., 1980; Yu et al., 1993; Hirsbrunner et al., 2003), and stimulation of labor and parturition (Sugimoto et al., 1997).

ROLE OF PROSTAGLANDINS IN FEMALE REPRODUCTION

Although prostaglandins were first identified in semen, the majority of subsequent research has examined the role of $PGF_{2\alpha}$ in female reproduction, primarily focusing on luteolysis of the corpus luteum (CL).

Regression of the Corpus Luteum by Prostaglandin $F_{2\alpha}$

Overview of corpus luteum development

After ovulation, the follicular granulosa cells undergo morphological changes and begin to luteinize after exposure to a surge of luteinizing hormone (LH). The process of luteinization involves the exponential growth of granulosa cells and differentiation of the follicle wall. The CL was observed to contain the same cells as the follicle (Corner, 1919). However, Corner (1919) also observed that after rupture of the follicle the membrana propria, which separates the thecal and granulosa cells, breaks down. Theca interna cells undergo hyperplasia, dispersing between the granulosa cells that are undergoing hypertrophy (Corner, 1919). Theca and granulosa cells migrate inward to form the CL, which steadily increases in size following ovulation. The CL becomes the dominant "gland" in the ovary during diestrus, secreting massive quantities of progesterone. Progesterone prepares the uterus for embryonic attachment, and inhibits ovulation and male receptivity.

Overview of luteal oxytocin

Oxytocin (OT) is classified as a peptide hormone, consisting of nine amino acids. Oxytocin is traditionally considered a product of magnocellular neurons located

in the hypothalamus, and is transported down long axons to be stored in the posterior pituitary until release. Hypothalamic oxytocin has two primary modes of action, milk ejection and uterine contractions during parturition. However, OT can also stimulate uterine contractions in nonpregnant ewes demonstrating the presence of oxytocin receptors in the uterus of this species (Gilbert et al., 1992).

Wathes and Swann (1982) hypothesized that OT was also an ovarian hormone. Peptides extracted from luteal tissue and assayed for OT indicated the presence of this peptide. In the luteal tissue, it was determined that ovarian OT represents about 15% of the total hypothalamo-neurohypophyseal store of hormone in nonpregnant ewes during the luteal phase and roughly 0.2% during pregnancy (Wathes and Swann, 1982).

Luteal OT is temporarily stored in the bovine and ovine corpora lutea in granular form during the luteal phase of the cycle. However, within 10 minutes of administering $PGF_{2\alpha}$ to the ewe or cow, there is an increase in OT secretion (Flint and Sheldrick, 1982; Wathes et al., 1983). Flint and coworkers (1990) also reported that OT is secreted by the CL of ruminants during the luteal phase of the estrous cycle. Secretion of luteal OT comes from the large luteinized granulosa cells in the cow and ewe (Swann et al., 1984; Fields and Fields, 1986; Theodosis et al., 1986). It has been demonstrated that luteal OT secretion is stimulated by $PGF_{2\alpha}$ and appears to be critical for luteal regression in the ewe (Flint et al., 1990).

Intramuscular injection of a synthetic $PGF_{2\alpha}$, cloprostenol, to intact and ovariectomized ewes resulted in a transient rise in plasma OT in intact ewes within 10

min of $PGF_{2\alpha}$ administration that lasted up to 40 min (Flint and Sheldrick, 1982). However, this increase in OT secretion was absent in ovariectomized ewes. Administration of $PGF_{2\alpha}$ was unable to stimulate secretion of OT by the posterior pituitary in the ovariectomized ewes, confirming that the CL was the source of this hormone during the luteal phase of the estrous cycle (Flint and Sheldrick, 1982; Schams et al., 1982).

Luteolysis of the corpus luteum by prostaglandin $F_{2\alpha}$

Luteolysis of the corpus luteum is affected by numerous hormones, proteins and enzymes. The following information briefly describes the principal mechanisms controlling luteolysis.

In ruminants, trophoblastic proteins, or interferon-τ, are produced by the blastocyst and are present in the uterus begining about day 13 to 21 of gestation (Flint, 1995). Interferon-τ apparently serves as the maternal recognition factor by binding to uterine endometrial cells and inhibiting oxytocin receptor synthesis to promote survival of the CL (Flint, 1995). Arosh and coworkers (2004) reported that high physiological levels of interferon-τ decreased the pulsatile secretion of endometrial $PGF_{2\alpha}$ and also resulted in increased endometrial PGE_2 production. These authors speculated that establishment of pregnancy may depend not only on inhibition of endometrial $PGF_{2\alpha}$ release to prevent luteolysis, but also upon increased endometrial production of PGE_2 to stimulate progesterone production by the corpus luteum. In fact, infusion of PGE_2 from day 10 to 17 in the uterine horn ipsilateral, but not contralateral, to the corpus luteum maintains luteal function in sheep (Magness et al.,

1981). Prostaglandin E_2 has also repeatedly been demonstrated to stimulate progesterone production by the ovine (Fitz et al., 1984; Kim et al., 2001; Weems et al., 2002) and bovine (Del Vecchio et al., 1995) CL.

Estrogen, which increases in concentration near the time of luteolysis (Cox et al., 1971; Barcikowski et al., 1974), also plays a role by enhancing uterine $PGF_{2\alpha}$ synthesis (Barcikowski et al., 1974) and amplifying the effect of OT on uterine $PGF_{2\alpha}$ synthesis in sheep (Sharma and Fitzpatrick, 1974).

Oxytocin receptors have been identified in the pituitary, hypothalamus, mammary tissues, and uterus (Zingg et al., 1998). Activation of uterine oxytocin receptors causes the production and release of uterine $PGF_{2\alpha}$ from arachidonic acid precursors (Watanabe et al., 1985). Roberts and McCracken (1976) demonstrated the sheep uterus was capable of releasing $PGF_{2\alpha}$ in response to OT stimulation. In turn, $PGF_{2\alpha}$ has been demonstrated to stimulate the release of luteal OT from the bovine (Schallenberger et al., 1984; Orwig et al., 1994; Salli et al., 2000) and ovine (Flint and Sheldrick, 1982; Wathes et al., 1983) CL. In ruminants it appears that uterine $PGF_{2\alpha}$ and luteal OT act in concert through a "double positive feedback loop" to promote regression of the CL (Roberts and McCracken 1976; Roberts et al., 1976; McCracken et al., 1996). Previous research confirmed that administration of $PGF_{2\alpha}$ to cows and ewes results in an increase in plasma OT levels, and in some studies was associated with the onset and promotion of luteal regression (Flint and Sheldrick, 1982; Wathes et al., 1983; Flint et al., 1990; Orwig et al., 1994; Salli et al., 2000).

Role of Prostaglandins in Ovulation

Species displaying spontaneous ovulation typically ovulate in response to hormonal changes with regular frequency; however, mating has been demonstrated to advance the timing of ovulation in rats (Aron et al., 1966; Zarrow and Clark, 1968), sheep (Parsons et al., 1967), and swine (Signoret et al., 1972). Additional mating stimuli via a vasectomized boar significantly improved conception rate of gilts compared to gilts serviced by only an intact boar (Mah et al., 1985). The effects of mating stimuli could be mediated by altering release of pituitary gonadotropins that occur in the first 8 h after the onset of estrus (Niswender et al., 1970). Randel and coworkers (1973) reported an advanced preovulatory surge of luteinizing hormone following cervical and clitoral stimulation, resulting in an earlier ovulation. Although the onset of ovulation may be initiated by luteinizing hormone, it appears to be mediated by prostaglandins.

The bovine ovary has been reported to respond to exogenous $PGF_{2\alpha}$ *in vitro* with rhythmic contractions that were more pronounced in samples collected from nonpregnant compared to pregnant cows (Singh et al., 1979). Contractile response to $PGF_{2\alpha}$ has also been reported for the ovary of the rabbit (Virutamasen et al., 1972) and human (Coutinho and Maia, 1971), and the follicular wall of sheep (O'Shea and Phillips, 1974). It has been hypothesized that these ovarian contractions may assist in the expulsion of the oocyte from the follicle.

Prostaglandin E_2 and $PGF_{2\alpha}$ concentrations are greater in follicular fluid collected from *in vitro* perfused rabbit ovaries that were exposed to luteinizing hormone (Koos et al., 1983). *In vitro* perfused rat ovaries displayed fewer ovulations

when treated with indomethacin compared to ovaries treated with forskolin or dibutyryl cAMP + isobutylmethacin, but addition of PGE_2 to the indomethacin + forskolin group overcame this inhibition (Brännström et al., 1987). In addition, these researchers reported that luteinizing hormone, forskolin, or dibutyryl cAMP + isobutylmethacin increased PGE_2 concentrations and this increase was associated with the cAMP-induced ovulations. In a series of experiments, Holmes and coworkers (1983) reported that $PGF_{2\alpha}$ was also capable of inducing ovulation in rabbit ovaries perfused *in vitro*. This was accomplished by demonstrating that indomethacin completely blocked ovulation in 4 of 5 ovaries tested, but indomethacin + $PGF_{2\alpha}$ restored ovulations, and ovulation was significantly reduced in ovaries treated with luteinizing hormone + indomethacin, but this inhibition was also overcome by addition of $PGF_{2\alpha}$. Schmidt and colleagues (1986) reported similar responses for combinations of PGE_2, indomethacin and luteinizing hormone treatments in rabbit ovaries perfused *in vitro*.

Systemic administration of prostaglandin synthetase inhibitors, indomethacin and asprin, blocks ovulation in rats (Armstrong and Grinwich, 1972; Orczyk and Behrman, 1972). Armstrong and coworkers (1974) concluded that $PGF_{2\alpha}$ was the most important prostaglandin for ovulation as determined by the ability of *in vivo* intrafollicular injections of indomethacin, or antisera to PGE_2 or $PGF_{2\alpha}$ to prevent luteinizing hormone-induced ovulations.

Prostaglandin $F_{2\alpha}$ is essential for follicle rupture, as demonstrated by the blockade of ovulation by the administration of $PGF_{2\alpha}$ antiserum, and the accumulation

of lysosomes (Bjersing and Cajander, 1975). Lysosomal enzymes have been associated with the granulosa cells of the follicle (Zoller and Weisz, 1980) and the deterioration of collagen fibers at the apex of the follicle (Cajander and Bjersing, 1976; Okamura et al., 1980) at the time of ovulation.

Whether prostaglandins in seminal plasma can be transported from the site of deposition to the follicle to induce a more prompt ovulation remains to be demonstrated. It is possible that seminal prostaglandins could be transported by the same vascular countercurrent exchange system as described for the transport of uterine $PGF_{2\alpha}$ to the corpus luteum (Bonnin et al., 1999).

Role of Prostaglandins in Parturition

Although mice lacking the receptor for $PGF_{2\alpha}$ display a normal estrous cycle, ovulation, fertilization rate, and embryo implantation, they are unable to deliver normal fetuses at term (Sugimoto et al., 1997). Prostaglandin E_2 and $PGF_{2\alpha}$ are considered the major prostaglandins in mediating myometrial contractions associated with the onset of labor in sheep (Liggins et al., 1971), humans (Karim, 1972; MacDonald et al., 1991), rats (Gu et al., 1990), and marsupials (Renfree et al., 1994). In sheep, fetal plasma PGE_2 levels begin to increase near 105 days of gestation (Fowden et al., 1987) and further elevation occurs during labor (Thorburn, 1991; Thorburn et al., 1991). In contrast, plasma levels of $PGF_{2\alpha}$ remain fairly constant throughout pregnancy in sheep (Liggins et al., 1971; Liggins et al., 1973) and women (Green et al., 1974; Mitchell et al., 1978), but rise significantly during spontaneous labor at term in sheep (Fowden et al., 1987) and women (Mitchell et al., 1978; Sellers

et al., 1981). Maternal fluids contain a high concentration of $PGF_{2\alpha}$, and the placenta of sheep (Liggins et al., 1973) and the decidua of women (MacDonald et al., 1991) are considered the source of $PGF_{2\alpha}$ during labor. In addition, during labor, concentrations of $PGF_{2\alpha}$ increase to a greater extent than do concentrations of PGE_2 in sheep (Liggins et al., 1973).

Role of Prostaglandins in Prevention of Uterine Infection

Short exposure to luteal or exogenous progesterone down-regulates immune functions in ewes and can result in the uterus shifting from a state of resistance to becoming susceptible to infections (Seals et al., 2002b). When postpartum ewes were challenged with uterine treatments of bacteria and concomitantly assayed for blood levels of progesterone, PGE_2, and $PGF_{2\alpha}$, Lewis (2003) concluded that progesterone tends to be uterine immunosuppressive and enhancing $PGF_{2\alpha}$ production may improve the ability of the uterus to resist infections. Seals and coworkers (2002b) also reported that progesterone administered to postpartum ewes suppressed uterine immunity. Similar results were reported for dairy cattle, and these authors speculated that changes in uterine eicosanoid production may affect the ability of the uterus to prevent uterine infections (Seals et al., 2002a). Subclinical infections following insemination, coinciding with increasing progesterone production by the developing corpus luteum, may result in reduced fertilization or embryonic mortality. In fact, the bactericidal activity of neutrophils from ovariectomized mares is increased by $PGF_{2\alpha}$ (Watson, 1988), and $PGF_{2\alpha}$ and leukotriene B_4, another eicosanoid, were demonstrated to be chemoatractant to neutrophils *in vitro* (Hoedmaker et al., 1992). In addition to the

enhanced uterine motility stimulated by $PGF_{2\alpha}$ present in bovine semen, this prostaglandin may also assist in provoking the uterus to combat bacteria that may be introduced at the time of copulation or artificial insemination. These data again suggest an important role for the concentration of seminal prostaglandins in fertility.

Stimulation of Prostaglandin Synthesis in the Female Reproductive Tract

Chaudhuri (1971) first proposed that intrauterine devices (IUDs) may cause the release of prostaglandins by mildly traumatizing the human endometrium. In fact, the presence of an IUD has been associated with an increased production of prostaglandin $F_{2\alpha}$ in sheep (Spilman and Duby, 1972), rabbits (Saksena and Harper, 1974), rats, hamsters (Saksena et al., 1974), and mice (Lau et al., 1974) in the portion of the endometrium that is in close contact with the IUD. When sheep possessing an IUD were treated with indomethacin, an inhibitor of prostaglandin production, $PGF_{2\alpha}$ concentrations in the endometrial area near the IUD were reduced (Spilman and Duby, 1972). In many of these animal studies it was also demonstrated that the uterine venous blood concentrations of $PGF_{2\alpha}$ were increased by at least 10-fold compared to animals not possessing an IUD (Spilman and Duby, 1972; Saksena et al., 1974). Insertion of an IUD has also been demonstrated to cause infiltration of neutrophils and macrophages (Tatum, 1972). Zurrier and coworkers (1974) reported that macrophages could be a significant source of prostaglandins. In fact, Myatt and coworkers (1975) found that the concentration of $PGF_{2\alpha}$ was approximately 250 μg in macrophages that were adhered to IUDs removed from human patients.

The advent of the controlled intravaginal drug-releasing (CIDR®) device, that is impregnated with progesterone and used for estrous synchronization of cattle and sheep, more than likely causes prostaglandin production to occur within the vagina of these ruminant species. If high levels of $PGF_{2\alpha}$ are detrimental to sperm function, one would expect to observe a decrease in conception rate following use of these devices. However, first service conception rate to artificial insemination following the use of CIDR devices has consistently been reported to be enhanced compared to animals estrous synchronized without these devices (Lamb et al., 2001; Lucy et al., 2001; Stevenson et al., 2003; Post et al., 2005).

PROSTAGLANDIN CONCENTRATION IN SEMEN

Although prostaglandins were first identified in seminal plasma and $PGF_{2\alpha}$ occurs in greater concentrations in this fluid than anywhere else in mammals, little research has examined the role of this eicosanoid in semen. To date, there appears to be no convincing argument to explain the high concentrations of $PGF_{2\alpha}$ in semen.

Concentration of Seminal Prostaglandins among Species

von Euler (1936) examined the accessory glands of the monkey, horse, bull, sheep, pig, dog, cat, rabbit and guinea pig, and fresh semen from the bull and horse. He was unable to identify any prostaglandin-like effect in any of the preparations except for the vesicular gland collected from sheep. Eliasson (1959) confirmed the previous work of von Euler; however, he also examined semen from the horse and

goat, and identified prostaglandin activity in goat semen. However, the bioassays utilized at that time were not very sensitive.

The concentrations of prostaglandins in semen from men with normal fertility have been determined to range from 15-20 μg/ml for PGE_1 and PGE_2, and 2.2-2.4 μg/ml for $PGF_{2\alpha}$ (Bygdeman, 1969). Vlachos et al. (1973) first confirmed the presence of prostaglandins E and F in bull semen. Subsequent, more sensitive, assays revealed that prostaglandin concentration in semen collected from the pig and dog (Poyser, 1974), bull (Voglmayr, 1973), and rat (Ventura and Freund, 1973) were less than 5 ng/ml. Unfortunately, these researchers were unable to separate the different classes of prostaglandins. Bygdeman and Holmberg (1966) reported sheep semen contained 31 μg/ml PGE_2 and 7 μg/ml $PGF_{2\alpha}$. Semen collected from the horse and rabbit were reported to contain 21 and 71 ng/ml PGE_2, respectively (Poyser, 1974). The sperm-rich fraction of the boar ejaculate was reported to contain 59 pg/ml $PGF_{2\alpha}$ (Cheng et al., 2003). Gas chromatography revealed the concentrations of PGE_1 and E_2 contained in bovine semen were 395 ± 225 and 487 ± 407 ng/ml, respectively (Mai and Kinsella, 1980). The wide range of values from this study suggests significant variability between animals or else that this method is not accurate for measuring seminal prostaglandin concentrations. Using thin layer chromatography, Ledwozyw and coworkers (1986) reported that pooled bovine semen contained approximately 1170 ng/ml prostaglandin of the F series. The concentration of $PGF_{2\alpha}$ in the semen collected from water buffalo was reported to be 1.931 ng/ml (Reddy et al., 1982).

TRANSPORT OF SPERMATOZOA

The transportation of spermatozoa from the site of ejaculation to the site of fertilization in the mammalian oviduct has long been considered an uncomplicated progression of motile cells, aided by smooth muscle contractions and cilial activity. However, transport of spermatozoa from the site of deposition, by natural service or artificial insemination, is a critical component of the reproductive process.

Effect of Prostaglandins on Sperm Motility

Effect of endogenous prostaglandins on sperm motility

Mai and Kinsella (1980) reported a constant 1:1 ratio between PGE_1 and PGE_2 in bovine semen, and there was no relationship between PGE and sperm motility; however, high sperm counts were normally associated with low PGE levels. In ejaculates collected from fertile and infertile men, $PGF_{2\alpha}$ was negatively correlated with motility in normal men and was always higher in men with disturbed fertility, and PGE_2 was elevated only in men with persisting varicocele, low sperm counts and severely impaired motility (Schlegel et al., 1981). In contrast, treatment of mouse sperm with indomethacin, an inhibitor of prostaglandin synthesis, resulted in a decrease in motility and *in vitro* fertilization rates that could be overcome by the addition of $PGF_{2\alpha}$ (Hayashi et al., 1988). When cyclooxygenase inhibitors were added to mouse semen there was no effect on motility, but this treatment resulted in decreased *in vitro* fertilization, and when added to guinea pig semen it reduced the acrosome reaction (Joyce et al., 1987). However, this diminished fertilization and acrosome reaction was overcome by the addition of a mixture of $PGF_{2\alpha}$ and PGE_2.

Effect of exogenous prostaglandins on sperm motility

Addition of $PGF_{2\alpha}$ to bovine semen during the extension process, with final concentrations of $PGF_{2\alpha}$ calculated to be either 75, 225 or 675 μg/ml, resulted in a significant reduction in post-thaw motility (Abbitt et al., 1977). However, these levels are over a million-fold greater than the endogenous concentration of $PGF_{2\alpha}$ previously reported for bovine semen. In contrast, addition of increasing amounts of $PGF_{2\alpha}$ to fresh boar semen, resulting in final concentrations of either 25, 50 or 100 μg/ml $PGF_{2\alpha}$, caused no change in sperm motility (Maes et al., 2003). Likewise, addition of $PGF_{2\alpha}$ to ram semen had no post-thaw effect and improved conception of ewes inseminated with this $PGF_{2\alpha}$-enhanced semen (Gustafsson et al., 1975).

Cohen and coworkers (1977) found that $PGF_{2\alpha}$ added to human semen at levels 10-fold greater than endogenous levels caused decreased motility, and similar results were reported by Didolkar and Roychowdhury (1980). Likewise, when 2,500 ng/ml of $PGF_{2\alpha}$ was added to semen collected from subfertile men, sperm motility was improved; however, when a superfluous amount (25,000 ng/ml) was added to similar semen this effect was reversed, resulting in no net improvement in sperm motility (Grunberger et al., 1981).

The endogenous oxygen consumption of washed cauda epididymal spermatozoa or the oxidative and glycolytic activities of washed ejaculated bovine spermatozoa are not affected by exogenous $PGF_{2\alpha}$ (Voglmayr, 1973). Only 19-hydroxy PGE, but not prostaglandins of the E or F series, depressed human spermatozoa respiration and it had no effect on the production of lactate (Kelly, 1977).

However, there is evidence that PGE_1 and $PGF_{2\alpha}$ act at an intracellular level by interacting with the adenylate cyclase-cAMP system (Ramwell and Shaw, 1971). In fact, in most adenylate cyclase systems in which prostaglandin synthesis and degradation occur, PGE stimulates accumulation of cAMP; but, $PGF_{2\alpha}$ either has no effect or causes only a slight reduction in formation of this nucleotide (Marsh and LeMaire, 1974). Bovine sperm motility has been suggested to be at least partially controlled by cAMP and/or cGMP (Garbers et al., 1971). Furthermore, the cAMP content in spermatozoa has been demonstrated to be an accurate and sensitive indicator of sperm motility (Tash and Mann, 1973).

Testing the effect of prostaglandins on spermatozoa is confounded by the fact that ejaculated spermatozoa have already been in contact with seminal fluid prostaglandins. Although many researchers wash spermatozoa after collection, endogenous prostaglandins most likely have already exerted their effects. Pento and coworkers (1970) found that the rate of glycolysis was significantly increased in epididymal sheep spermatozoa after exposure to either PGE_1 or $PGF_{2\alpha}$; however, washed ejaculated sheep spermatozoa failed to display this increase.

Prostaglandin $F_{2\alpha}$-Induced Myometrial and Oviductal Contractions

Seminal prostaglandins were first reported to cause an effect within the female reproductive tract over four decades ago, when Sandberg and coworkers (1963) found that prostaglandins extracted from human semen caused an increase in the tone and amplitude of contractions in the proximal fallopian tube. Later, addition of only $PGF_{2\alpha}$ was found to cause an increase in muscular activity of the rabbit oviduct *in*

vitro, but treatment with PGE_2 suppressed spontaneous activity and often completely abolished oviductal muscle activity (Spilman and Harper, 1973). Coutinho and Maia (1971) reported that although prostaglandins of the E and F series exert opposing effects on the human uterus *in vitro*, they both exert stimulation of the myometrium *in vivo*. Prostaglandin $F_{2\alpha}$ has also been reported to elicit contractions of myometrium collected from sows (Yu et al., 1993), and cows during the proestrus (Patil et al., 1980) and diestrus (Hirsbrunner et al., 2003) phases. Patil and coworkers (1980) also noted that spontaneous uterine contractility was most pronounced during the follicular phase of cows. Stolla and Schmid (1990) found differences for *in vivo* myometrial contractility between natural $PGF_{2\alpha}$ and its synthetic analogs, with the greatest response occurring after administration of natural $PGF_{2\alpha}$ to diestrus cows followed by proestrus and metestrus cows. It is interesting to note that these researchers reported that none of the types of $PGF_{2\alpha}$ utilized elicited a response when administered to cows in estrus. Prostaglandin $F_{2\alpha}$, but not PGE_2, administered s.c. immediately before AI or added to semen before AI resulted in an increased fertilization rate of rabbits that underwent utero-tubal ligation 2.5-3 h after insemination compared to control animals receiving no exogenous $PGF_{2\alpha}$ (Spilman et al., 1973). These data suggest that seminal prostaglandins are capable of eliciting uterine and oviductal contractions that can promote the movement of sperm from the site of deposition to the site of fertilization.

Prostaglandins also appear to play a role in the movement of embryos from the site of fertilization to the uterus. Weber and coworkers (1991) reported that continuous intraoviductal infusion, but not intramuscular injection, intrauterine

infusion or intraperitoneal administration, of PGE_2 hastened oviductal transport of equine embryos to the uterus, and suggested that selective oviductal transport of embryos to the uterus between day 5 and 6 after ovulation is the result of localized action of embryonic PGE_2 on the oviduct.

EFFECT OF SEMINAL PROSTAGLANDINS ON MALE FERTILITY

Prostaglandin $F_{2\alpha}$ concentration in whole semen collected from water buffalo appears to be positively correlated to total and live sperm concentration, but this relationship did not exist for PGE_2 (Reddy et al., 1982). There has been no reported relationship between the concentration of human seminal $PGF_{2\alpha}$ and sperm concentration or morphology (Hawkins and Labrum, 1961; Hawkins, 1968). However, prostaglandin concentrations in human semen do seem to be correlated with fertility when control patients are compared to patients in which infertility cannot otherwise be explained, and prostaglandin concentrations in this semen are not correlated with sperm count or motility (Kelly, 1978). In contrast, either lower or higher than normal PGE_2 seminal concentrations are associated with decreased sperm concentration and motility (Isodori et al., 1980). In addition, PGE_2 concentration appears to play an important role in male reproduction, in that men with idiopathic infertility have lower seminal PGE_2 concentrations compared to fertile men (Bygdeman et al., 1970; Collier et al., 1975).

EFFECT OF OTHER SEMINAL COMPONENTS ON FERTILITY

Researchers have made numerous attempts to link other components of the bovine ejaculate to fertility with varying degrees of success.

Lipocalin-Type Prostaglandin D Synthase

Killian and coworkers (1993) examining bovine seminal plasma identified two proteins that predominated in higher-fertility bulls and two other proteins that predominated in lower-fertility bulls. The protein that significantly contributed to the regression model used to predict bull fertility occurred in 3.5-fold greater concentrations in bulls with above average fertility than in bulls of average or below average fertility. This protein was later identified as lipocalin-type prostaglandin D synthase (PGD-S) and was found to be a major protein secreted by epididymal epithelial cells into the lumen of the tubule in several mammalian species including the bull (Gerena et al., 1998), ram and stallion (Fouchécourt et al., 1999), human (Gerena et al., 1998), mouse (Hoffmann et al., 1996), and rat (Tokugawa et al., 1998). Further research demonstrated PGD-S is localized in elongating spermatids and Sertoli cells of the seminiferous tubules, varying with stage of the spermatogenic cycle (suggesting a role in both development and maturation of sperm), and is associated with the plasma membrane of the ejaculated sperm head (Gerena et al., 2000). These researchers concluded that the enzyme was bifunctional, acting as a PGD_2 producing enzyme as well as an intracellular transporter of retinoids or other lipophilic substances to maintain the blood-testis and blood-epididymal barriers. In fact rat PGD-S has been demonstrated to bind retinal and retinoic acid with affinities similar

to other retinoic binding proteins (Tanaka et al., 1997). Lipocalin-type prostaglandin D synthase, also referred to as β-trace, demonstrates highly specific expression at blood-tissue barriers in mice such as the blood-cerebrospinal fluid, blood-retina, blood-aqueous humor, and blood-testis barrier, further suggesting a potential role for this lipocalin in transport across or maintenance of these barriers (Hoffmann et al., 1996). In addition, another lipocalin, epididymal retinoic acid-binding protein, has been demonstrated to bind retinoic acid, which is a well-known regulator of gene expression and a morphogen. Epididymal retinoic acid-binding protein binds to the sperm plasma membrane and is believed to be important for sperm maturation (Newcomer, 1993). Also in support of these findings, sodium selenite, an inhibitor of PGD-S (Islam et al., 1991), administered to rats resulted in lesions involving the advanced stages of spermatogenesis (Nebbia et al., 1987). Although there was no statistical correlation, PGD-S was observed to be greater in semen obtained from bulls with normal or high fertility and was low or undetectable in bulls of all ranges of fertility (Fouchécourt et al., 2002).

Cytokines

Cytokines, which modulate various reactions of the immune response and include interleukins, have been identified in human seminal plasma (Dousset et al., 1997). It could be assumed that the local action of cytokines would be to modulate the male immune response to spermatozoa antigens or protection of the spermatozoa from the female immune system (Kelly, 1995). Immunosuppression is important because sperm cells express surface antigens that do not occur on somatic or premeiotic germ

cells (Diekman and Goldberg, 1995) and contact of these antigens with immunocompetent cells may result in an autoimmune reaction (Shulman, 1995; Bronson, 1999). Normally, the blood-testis barrier would prevent this reaction (Johnson and Setchell, 1968; Turek, 1999), but autoantigens have been identified on the surface of germ cells that are just about to enter meiosis in mice (Yule et al., 1988) and rats (Saari et al., 1996).

Anti-sperm antibodies have been speculated to reduce human sperm quality (Matson et al., 1988; Mazumdar and Levine, 1998; Zeyneloglu and Yarali, 2002). In fact, sperm-bound IgAs have been demonstrated to be associated with poor human cervical mucus penetration (Clarke, 1988). Corticosteroid treatment of infertile men resulted in reduced number of antisperm antibodies, improved sperm motility and sperm count, and increased conception rate (Drobnis and Overstreet, 1992; Skau and Folstad, 2004). Denison and coworkers (1999) demonstrated *in vitro* that human semen was capable of stimulating the release of interleukin-10 in the female reproductive tract, suggesting that this anti-inflammatory cytokine may promote sperm survival.

Measurement of cytokines, interleukins 6 and 10, and tumor necrosis factor alpha, in bovine seminal plasma revealed that the level of interleukin 10 was correlated to individual sperm motility, but not gross sperm motility (Vera et al., 2003). In addition, human seminal plasma is capable of suppressing lymphocyte proliferation and raising intracellular cAMP levels; the combination of PGE and 19-hydroxy PGE are as effective as whole seminal plasma in raising cAMP, but are not as effective in inhibiting lymphocyte proliferation (Kelly et al., 1994).

Relaxin

Relaxin, originally identified as a hormone of pregnancy and best known for its role in parturition (Sherwood et al., 1975; Bagna et al., 1991), has also been detected in the seminal plasma from humans (Loumaye et al., 1980; DeCooman et al., 1983) boars (Juang et al., 1990; Sasaki et al., 2001) and bulls (Kohsaka et al., 2003). It has been demonstrated that boar relaxin is produced in the seminal vesicles (Kohsaka et al., 1992), secreted into the seminal plasma (Sasaki et al., 2001), and binds to the sperm after ejaculation (Kohsaka et al., 2001). The concentration of relaxin in seminal plasma is correlated to the percentage motile boar sperm and has been suggested to be a useful predictor of the fertilizing ability of boar semen (Sasaki et al., 2001). Bovine seminal plasma concentration of relaxin was also found to be significantly correlated with the proportion of spermatozoa displaying the greatest motility (Kohsaka et al., 2003).

Heparin-Binding Proteins

Sperm from high fertility bulls has been reported to have a greater binding affinity for heparin (Marks and Ax, 1985), a sulfated glycosaminoglycan, which has been demonstrated to induce the *in vitro* acrosome reaction of bovine spermatozoa (Lenz et al., 1982; Lenz et al., 1983a, b). Nass and coworkers (1990) found several different heparin-binding proteins in all of the accessory glands collected from rats and bulls, but the major source of bovine seminal plasma heparin-binding proteins was attributed to the seminal vesicle.

These heparin-binding proteins have been found to bind to spermatozoa following ejaculation (Miller et al., 1990), and may therefore play a role in fertilization by enabling heparin-like glycosaminoglycans that have been identified in the female reproductive tract (Lee and Ax, 1984) to induce capacitation. When heparin-binding protein was added to epididymal spermatozoa it stimulated acrosome reactions (Miller et al., 1990). Bulls examined to test the affinity of a specific heparin-binding protein for sperm membranes and seminal fluid, revealed that bulls with the greatest affinity for heparin-binding protein-B5 in sperm membranes, but not in seminal fluid, had greater fertility (higher non-return rate) than bulls with other heparin-binding protein-B5 profiles (Bellin et al., 1994).

EFFECT OF PROSTAGLANDIN $F_{2\alpha}$ ADMINISTRATION TO FEMALES AT TIME OF INSEMINATION

Numerous research projects have examined the effect of exogenous $PGF_{2\alpha}$ administered near the time of insemination. In all cases, despite administration method, $PGF_{2\alpha}$ treatment has resulted in improved reproductive performance. Unfortunately, little information exists about the effects of $PGF_{2\alpha}$ administration at the time of insemination of cattle.

Injection of Prostaglandin $F_{2\alpha}$ at Insemination

Injection of $PGF_{2\alpha}$ into the vulvar mucosa of sows significantly increased the farrowing rate of sows during periods of low fertility, summer months, and also slightly increased litter size throughout the year (Peña et al., 1998). Injection of

$PGF_{2\alpha}$ to rabbits inseminated with low numbers of sperm resulted in significantly greater sperm numbers in the oviducts 2.5 h after insemination and fertilization rate compared to control rabbits (Hawk et al., 1982). Injection of $PGF_{2\alpha}$ at the time of AI results in increased sperm numbers in the reproductive tract in rabbits (Spilman et al., 1973), and ewes (Edqvist et al., 1975). Intravenous $PGF_{2\alpha}$ injection immediately after AI of cows resulted in a significantly higher conception rate compared to cows receiving an intravenous injection of bi-distilled water after AI (Prinzen et al., 1991).

Addition of Prostaglandin $F_{2\alpha}$ to Semen before Insemination

Addition of 5 mg $PGF_{2\alpha}$ to an AI low-dose (0.5×10^9 sperm/80 ml) unit of boar semen immediately before insemination resulted in an increase of myometrial contractions, but had no effect on amplitude or duration of contractions (Willenburg et al., 2003). Apparently due to increased number of myometrial contractions, these authors reported a trend for more sperm numbers in the uteri of treated animals. This treatment had no effect on pregnancy rate, but resulted in a greater number of fetuses per pregnancy. A similar response, of increased sperm numbers in the reproductive tract, was reported for ewes inseminated with $PGF_{2\alpha}$-supplemented semen (Edqvist et al., 1975).

Addition of $PGF_{2\alpha}$ to ram (Gustafsson et al., 1975) and boar (Peña et al., 2000; Horvat and Bilkei, 2003; Kos and Bilkei, 2004) semen improved the fertility of females inseminated with this $PGF_{2\alpha}$-supplemented semen. In the only study where $PGF_{2\alpha}$ was added to bovine semen, it resulted in a significant decrease in post-thaw

motility and this semen was unfortunately not used to inseminate cows (Abbitt et al., 1977).

STATEMENT OF THE PROBLEM

Quality of beef products has become an increasingly important component of production practices. The proportion of consumers demanding lean beef and a consistent product continues to increase. Although AI offers the potential to choose sires that will supply proven genetics, few beef producers have embraced this technology. This is due primarily to the producer's perception that AI is too time and labor intensive and that conception rate following AI is unacceptable although it is similar to a single natural service. Producers consider an AI conception rate of 50-70% unacceptable, but calving rate following a single natural service is only 50-60% (Parkinson, 2004). Regardless of the reason, failure to use AI results in a loss of superior genetics. If conception to AI could be improved, additional producers may consider capturing the superior genetics from proven AI sires.

Transport of sperm from the site of deposition to the site of fertilization is a critical component of the reproductive process, and is influenced by sperm flagellar motion, ciliary beats, and myometrial contractions. Ewes displaying inhibited sperm transport were found to have a decreased number of uterine contractions moving toward the oviducts and an increased number moving away from the oviducts (Hawk and Echternkamp, 1973). This suggests that myometrial contractions play a significant role in the fertilization process, and $PGF_{2\alpha}$ has been demonstrated to stimulate *in vitro* bovine (Patil et al., 1980) and porcine (Yu et al., 1993) myometrial contractions. Furthermore, addition of $PGF_{2\alpha}$ to extended boar semen or extender alone also increased *in vitro* myometrial contractility (Cheng et al. 2001). These data

demonstrate that addition of $PGF_{2\alpha}$ to semen can stimulate myometrial contractions and suggest that sperm transport and fertility could potentially be improved.

Unfortunately there is no information regarding the effect of intramuscular injection of $PGF_{2\alpha}$ at the time of insemination of cattle on fertility and the only experiment examining the addition of $PGF_{2\alpha}$ to bovine semen during extension used elevated concentrations that resulted in decreased post-thaw motility. Perhaps addition of $PGF_{2\alpha}$ at only slightly greater than endogenous levels may prevent reduction in post-thaw motility and still improve fertility. Regrettably, there have been large variations in previous studies examining the concentration of $PGF_{2\alpha}$ in bovine semen.

The experiments of the present dissertation were conducted to determine: 1) the quantity of $PGF_{2\alpha}$ present in bovine semen and whether endogenous $PGF_{2\alpha}$ concentration in bovine semen was correlated to fertility, 2) whether $PGF_{2\alpha}$ could be added to bovine semen during extension without altering subsequent post-thaw motility, 3) whether fertility of cows inseminated with $PGF_{2\alpha}$-supplemented semen would be improved, and 4) whether intramuscular injection of $PGF_{2\alpha}$ at the time of insemination would improve the fertility of beef females that had previously been exposed to estrous synchronization.

ENDOGENOUS PROSTAGLANDIN $F_{2\alpha}$ CONCENTRATIONS IN BOVINE WHOLE SEMEN, SEMINAL PLASMA AND EXTENDED SEMEN, AND THEIR POTENTIAL EFFECT ON FEMALE FERTILITY

J.R. Jaeger, and T. DelCurto

Journal of Animal Science

1111 N. Dunlap Ave.

Savoy, IL 61874

ABSTRACT

Four experiments were conducted to quantify prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) in bovine semen, seminal plasma and extended semen by enzyme immunoassay. In experiment 1, $PGF_{2\alpha}$ was measured in paired samples of whole and extended semen from beef (n = 6) and dairy bulls (n = 18). Levels of $PGF_{2\alpha}$ did not differ between beef and dairy (273.8 ± 42.8 vs. 210.3 ± 18.5 pg/ml, respectively; P = 0.12), but tended to be greater for whole compared to extended semen (255.5 ± 29.8 vs. 194.5 ± 17.0 pg/ml, respectively; P = 0.08). In experiment 2, to elucidate why $PGF_{2\alpha}$ levels in extended semen were comparable to whole semen, semen from dairy bulls (n =7) was extended at eight dilutions (1:10, 1:15, 1:20, 1:25, 1:30, 1:35, 1:40 and 1:80). Semen was extended using a diluent consisting of two fractions: A (egg yolk based) and B (glycerol based). Samples collected after semen addition to fraction A and after each addition of fraction B resulted in four sub-samples. Prostaglandin $F_{2\alpha}$ in sub-samples decreased at higher dilution rates and later steps of extension (P < 0.001). To quantify $PGF_{2\alpha}$ synthesized during extension, amounts of $PGF_{2\alpha}$ in semen and fractions A and B (52.8 and 87.7 pg/ml, respectively) were subtracted from each step. Higher dilution rates reduced the final amount of $PGF_{2\alpha}$ synthesized (P < 0.001). In experiment 3, paired samples of whole semen and seminal plasma from dairy bulls (n = 7) were extended at three dilutions (1:15, 1:20 and 1:25). Initial $PGF_{2\alpha}$ concentration was greater in whole semen compared to seminal plasma (430.0 ± 37.2 vs. 62.2 ± 15.0 pg/ml, respectively; P < 0.001). During extension, $PGF_{2\alpha}$ synthesis resulted in less disparity than for original samples, but amount synthesized was greater for semen

compared to seminal plasma (194.5 ± 15.8 vs. 150.5 ± 10.9 pg/ml, respectively; P = 0.03) and was not affected by dilution rate (P = 0.41). In experiment 4, concentrations of prostaglandin E_2 (PGE_2) and $PGF_{2\alpha}$ and the ratio of PGE_2 and $PGF_{2\alpha}$ were compared to a Relative Fertility Rating based on non-return data. The ratio of PGE_2 and $PGF_{2\alpha}$ was not correlated to the Relative Fertility Rating (P = 0.15). These data suggest that, although extension reduces the concentration of many seminal components, $PGF_{2\alpha}$ synthesis during extension results in concentrations similar to whole semen and seminal prostaglandin concentrations may be related to fertilizing capability of the semen.

Key words: bovine, bull fertility, semen, prostaglandin $F_{2\alpha}$, prostaglandin E_2

INTRODUCTION

Transport of sperm from the site of deposition is a critical component of the reproductive process. Hawk and Echternkamp (1973) reported that ewes with inhibited sperm transport display a decreased number of uterine contractions moving toward the oviducts. Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) stimulates bovine (Patil et al., 1980) and porcine (Yu et al., 1993) myometrial contractions. Addition of $PGF_{2\alpha}$ to extended boar semen or extender alone also increased *in vitro* myometrial contractility (Cheng et al. 2001).

Injection of $PGF_{2\alpha}$ at the time of AI increased embryo number in rabbits (Spilman et al., 1973) and farrowing rate of sows (Peña et al., 1998). Addition of $PGF_{2\alpha}$ to semen improved pregnancy rates of ewes (Gustafsson et al., 1975), and

farrowing rate and litter size of sows (Peña et al., 2000). Ewes injected with $PGF_{2\alpha}$ at breeding or inseminated with $PGF_{2\alpha}$-supplemented semen display more spermatozoa in all reproductive tract segments (Edqvist et al., 1975).

Addition of $PGF_{2\alpha}$ to ram semen had no post-thaw effect, and this $PGF_{2\alpha}$-enhanced semen improved conception rate (Gustafsson et al., 1975). In contrast, addition of $PGF_{2\alpha}$ to bovine semen during extension caused a dose-dependent decrease in post-thaw motility (Abbitt et al., 1977).

Thin layer chromatography revealed that pooled bovine semen contained approximately 1170 ng/ml prostaglandin of the F series (Ledwozyw et al., 1986). However, radioimmunoassay of bull genital tract secretions revealed 0.17 ng/ml of $PGF_{2\alpha}$ (Voglmayr, 1973).

Due to the equivocal $PGF_{2\alpha}$ concentrations previously reported for bovine semen and lack of data for quantity of $PGF_{2\alpha}$ in extended bovine semen, the amount of $PGF_{2\alpha}$ that could be added to bovine semen during extension to enhance fertility is ambiguous. Therefore, the objectives of the current study were to determine the endogenous concentrations of $PGF_{2\alpha}$ in whole and extended semen, and determine if the concentrations of $PGF_{2\alpha}$ and PGE_2 in extended bovine semen are correlated to female fertility.

MATERIALS AND METHODS

Experiment 1

Semen was collected from beef (n = 6) and dairy bulls (n = 18) at an AI collection facility (Select Sires Inc., Plain City, OH) using an artificial vagina. Following semen collection, a 1-ml aliquot of whole semen was harvested and immediately frozen. The remaining semen was extended following the normal protocol for this AI collection facility. Following shipment to our laboratory, samples of whole and extended semen were thawed in a 35° C water bath for 2 min, dispensed into 1.5-ml microcentrifuge tubes, centrifuged at 15,000 x G, and the resulting supernatant was frozen at -20° C until analysis for $PGF_{2\alpha}$ concentration.

Experiment 2

Semen was collected from dairy bulls (n = 7) at an AI collection facility (Select Sires Inc., Plain City, OH) using an artificial vagina. Following collection, a 1-ml aliquot of whole semen was harvested and immediately frozen. Following shipment to the laboratory, semen was thawed in a 35° C water bath for 2 min and extended at eight dilutions (1:10, 1:15, 1:20, 1:25, 1:30, 1:35, 1:40, and 1:80) regardless of initial sperm concentration. Semen was extended using a diluent consisting of two fractions: A (egg yolk based) and B (glycerol based) following an industry protocol. This protocol requires a four-minute equilibration period after semen addition to fraction A and following each addition of fraction B. Samples collected after addition of semen to fraction A and after each addition of fraction B

resulted in four sub-samples. Sub-samples were centrifuged at 15,000 x G and the resulting supernatant was frozen at -20° C until analysis for $PGF_{2\alpha}$ concentration.

Experiment 3

Semen was collected from dairy bulls (n = 7) at an AI collection facility (Select Sires Inc., Plain City, OH) using an artificial vagina. Following collection, a 1-ml aliquot of whole semen was harvested and immediately frozen. An additional 1.5-ml aliquot of whole semen was centrifuged at 15,000 x G and the resulting supernatant was harvested and immediately frozen. Following shipment to the laboratory, whole semen and seminal plasma samples were thawed in a 35° C water bath for 2 min and extended at three dilutions (1:15, 1:20, and 1:25), regardless of initial sperm concentration, following the protocol described in Experiment 2. The resulting sub-samples were centrifuged at 15,000 x G, and the resulting supernatant was harvested and frozen at -20° C until analysis for $PGF_{2\alpha}$ concentration.

Experiment 4

Frozen extended semen from dairy bulls (n = 16) was obtained from an AI collection facility (Select Sires Inc., Plain City, OH). The frozen extended semen was thawed in a 35° C water bath for 2 min, dispensed into 1.5-ml microcentrifuge tubes, centrifuged at 15,000 x G, and the resulting supernatant was harvested and immediately frozen until analysis for $PGF_{2\alpha}$ and prostaglandin E_2 (PGE_2) concentrations.

Data for non-return rates were obtained from three sources: Estimated Relative Conception Rate (Dairy Records Management Systems, North Carolina State University, Raleigh, NC and Iowa State University, Ames, IA;); Holstein Bull Fertility (AgriTech Analytics, Visalia, CA;); and Relative Breeding Efficiency (Select Sires, Plain City, OH;). Each of these data sets contained the number of services for each bull (ranging from 218 to 26,945) and the deviation from the average non-return rate for all bulls within the data set. Using the number of services for each bull, a weighted deviation was calculated and bulls were assigned a Relative Fertility Rating ranging from 1 to 3 (1 being a high non-return rate, 2 being a moderate non-return rate, and 3 being a low non-return rate). The correlations between the concentration of $PGF_{2\alpha}$, PGE_2, and the ratio of PGE_2 to $PGF_{2\alpha}$ to the Relative Fertility Rating were determined.

Prostaglandin $F_{2\alpha}$ Assay

Concentration of $PGF_{2\alpha}$ in whole and extended semen and in sub-samples collected during the extension of whole semen and seminal plasma were determined by use of $PGF_{2\alpha}$-acetylcholinesterase (AChE) Competitive Enzyme Immunoassay (Cayman Chemical Company, Ann Arbor, MI). The sensitivity of the assay was 8 pg/ml, and the intra- and interassay coefficients of variation were 7.5 and 17.1%, respectively.

Prostaglandin E_2 Assay

Concentration of PGE_2 in seminal plasma obtained from extended semen was determined by use of PGE_2-acetylcholinesterase (AChE) Competitive Enzyme Immunoassay (Cayman Chemical Company, Ann Arbor, MI). The sensitivity of the assay was 15 pg/ml, and the intra- and interassay coefficients of variation were 5.5 and 12.0%, respectively.

Statistical Analysis

Prostaglandin $F_{2\alpha}$ concentrations in whole and extended semen (Experiment 1) were analyzed by analysis of variance in SAS (SAS Inst. Inc., Cary, NC). Concentration of $PGF_{2\alpha}$ in sub-samples collected during the extension of whole semen and seminal plasma (Experiments 2 and 3) were analyzed as repeated measures (step of extension process) by analysis of variance in SAS using the proc mixed statement. The statistical model consisted of treatment (whole semen vs. seminal plasma), dilution rate, step of extension process, and their interactions. To quantify the amount of $PGF_{2\alpha}$ synthesized during extension of whole semen and seminal plasma (Experiments 2 and 3), the quantity of $PGF_{2\alpha}$ present in semen and the quantity of $PGF_{2\alpha}$ contributed by fractions A and B of the diluent was subtracted from each respective dilution step before final analysis. Direct comparison of final $PGF_{2\alpha}$ concentrations following extension of whole semen or seminal plasma were analyzed by analysis of variance and difference between means was determined with pair-wise t-tests. The correlation between $PGF_{2\alpha}$, PGE_2, or PGE_2:$PGF_{2\alpha}$ to the Relative Fertility Rating was determined using the PROC CORR procedure of SAS. The critical alpha

level was set at 0.05 and P-values ranging from 0.06 to 0.10 were considered tendencies.

RESULTS

Experiment 1

The concentration of $PGF_{2\alpha}$ in whole semen was slightly greater in samples collected from beef bulls compared to the concentration present in samples collected from dairy bulls (273.8 ± 29.8 vs. 210 ± 18.5 pg/ml, respectively); however, this difference was not statistically different (P = 0.12). In addition, the average concentration of $PGF_{2\alpha}$ in whole semen tended to be only slightly greater (P = 0.08) than the average concentration of $PGF_{2\alpha}$ in extended semen (255.5 ± 29.8 vs. 194.5 ± 17.0 pg/ml, respectively).

Experiment 2

Prostaglandin $F_{2\alpha}$ concentration in sub-samples collected during the extension process decreased at higher dilution rates and later steps of extension (P < 0.001; Figure 2.1), suggesting that a dose response does exist. To quantify the amount of $PGF_{2\alpha}$ synthesized during extension of whole semen, the amounts of $PGF_{2\alpha}$ present in semen (255.5 pg/ml) and fractions A and B (52.8 and 87.7 pg/ml, respectively) of the diluent were subtracted from each sub-sample. Analysis of variance for the quantity of $PGF_{2\alpha}$ present following completion of semen dilution revealed that higher dilution rates reduced the total amount of $PGF_{2\alpha}$ synthesized during the extension process (P < 0.001; Figure 2.2).

Experiment 3

Initial concentration of $PGF_{2\alpha}$ in whole semen was significantly greater ($P < 0.001$) than for seminal plasma (430.0 ± 37.2 vs. 62.2 ± 15.0 pg/ml, respectively). Following extension of these paired samples of whole semen and seminal plasma at 3 dilution rates (1:15, 1:20, and 1:25), $PGF_{2\alpha}$ synthesis during the extension process resulted in less disparity than was observed in the original samples. As was observed in Experiment 2, after the quantity of $PGF_{2\alpha}$ contributed from whole semen or seminal plasma and from fractions A and B of the diluent were subtracted, dilution rates of 1:10, 1:15 and 1:20 had no effect on the amount of $PGF_{2\alpha}$ synthesized during the extension process, except for seminal plasma at the 1:20 dilution rate which tended ($P = 0.08$) to result in less $PGF_{2\alpha}$ synthesis (Figure 2.3). However, when means for $PGF_{2\alpha}$ concentrations in extended whole semen and seminal plasma were pooled for all dilution rates, the amount of $PGF_{2\alpha}$ synthesized during extension was greater ($P = 0.03$) for semen compared to seminal plasma (194.5 ± 15.8 vs. 150.0 ± 10.9 pg/ml, respectively).

Experiment 4

Concentration of PGE_2 in extended bovine semen was not correlated ($P = 0.98$) to the Relative Fertility Rating and the concentration of $PGF_{2\alpha}$ in extended bovine semen was not correlated ($P = 0.15$) to the Relative Fertility Rating (Figure 2.4), but the slope of the line suggested that seminal $PGF_{2\alpha}$ concentration and the Relative Fertility Rating may be negatively correlated. However, one bull sampled had a low Relative Fertility Rating and displayed the highest concentrations of $PGF_{2\alpha}$ and PGE_2

of all bulls measured. When data for this bull were removed from the data set, concentration of $PGF_{2\alpha}$ no longer appeared to be negatively correlated to the Relative Fertility Rating ($P = 0.62$). The ratio of PGE_2:$PGF_{2\alpha}$ concentrations in extended semen was also not correlated ($P = 0.15$) to the Relative Fertility Rating (Figure 2.5).

DISCUSSION

Quantity of $PGF_{2\alpha}$ present in whole semen collected from beef and dairy bulls was similar; therefore, data for these bulls were pooled and seminal $PGF_{2\alpha}$ concentrations averaged 255.5 pg/ml. This concentration is greater than was previously reported for bovine semen (170 pg/ml; Voglmayr, 1973), but is almost 8-fold less than was reported for semen collected from water buffalo (1931 pg/ml; Reddy et al., 1982).

Comparison of $PGF_{2\alpha}$ concentrations in whole and extended semen, collected from beef and dairy bulls, yielded a smaller difference than had been expected. Although $PGF_{2\alpha}$ concentration in whole semen did tend to be slightly greater than was observed in extended semen, based on an average concentration of 255.5 pg/ml $PGF_{2\alpha}$ in whole semen observed in this study, following extension of bovine semen at the average industry dilution rate of 1:20 a concentration of approximately 10-20 pg/ml would be expected to be present in the extended semen. This augmentation of $PGF_{2\alpha}$ concentration present in the extended semen could not be attributed to the diluent. Concentration of $PGF_{2\alpha}$ in the diluent was determined to be 52.8 pg/ml for fraction A and 87.7 pg/ml for fraction B.

Previous researchers have reported that when $PGF_{2\alpha}$ was added to semen before insemination of females it improved the fertility of ewes (Gustafsson et al., 1975), and sows (Peña et al., 2000). Because administration of $PGF_{2\alpha}$ to cattle (Patil et al., 1980), swine (Yu et al., 1993) and rabbits (Spilman and Harper, 1973) elicits an increase in myometrial contractions, it could be assumed that this increase in fertility would be due to an increased movement of spermatozoa from the site of deposition to the site of fertilization. However, it appears that superfluous quantities of $PGF_{2\alpha}$ may have detrimental effects on sperm motility. In a previous study, when $PGF_{2\alpha}$ was added to the glycerol fraction (fraction B), and final concentrations in extended bovine semen were calculated to be either 0, 75, 225, or 675 μg/ml $PGF_{2\alpha}$, post-thaw motility of this semen following a 2 h incubation was 19.3, 17.8, 13.6, and 5.8%, respectively (Abbitt et al., 1977). If the observed phenomenon of $PGF_{2\alpha}$ synthesis during semen extension also occurred during this previous study, final concentrations of $PGF_{2\alpha}$ would have been nearly a million-fold greater than endogenous levels observed for whole semen. This superfluous amount of $PGF_{2\alpha}$ could have resulted in the depressing effect on sperm motility. In addition, when 2,500 ng/ml $PGF_{2\alpha}$ was added to sperm collected from subfertile men, sperm motility was enhanced; however, when 25,000 ng/ml $PGF_{2\alpha}$ (25 μg/ml), which exceeded physiological levels, was added to similar semen this effect was reversed, resulting in no net improvement in sperm motility (Grunberger et al., 1981). Also in human semen, exogenous $PGF_{2\alpha}$ has been found to inhibit (Cohen et al., 1977) or stimulate (Schlegel et al., 1981) spermatozoa function and the outcome appears to be influenced by the quantity of exogenous

$PGF_{2\alpha}$ added. These data suggest that $PGF_{2\alpha}$ may have paradoxical effects on sperm function, wherein addition of $PGF_{2\alpha}$ at levels only slightly above endogenous levels may have positive effects on sperm motility, and addition of $PGF_{2\alpha}$ at significantly greater levels than found endogenously may have detrimental effects on sperm function. In fact, similar findings occurred for human endogenous PGE seminal concentrations, wherein sperm concentration and motility were significantly reduced when PGE concentrations were either increased or decreased with respect to normal levels (Isidori, et al., 1980).

Because the concentration of $PGF_{2\alpha}$ in extended semen measured during Experiment 1 was greater than expected, semen was extended at eight dilution rates regardless of initial sperm concentration to elucidate whether the quantity of semen initially added to the diluent would result in a dose-dependent response for quantity of $PGF_{2\alpha}$ produced during the extension process. Prostaglandin $F_{2\alpha}$ concentration in sub-samples collected during the later steps of the extension process and at higher dilution rates decreased, suggesting that a dose response does exist. When the quantity of $PGF_{2\alpha}$ contributed by the diluent and whole semen was subtracted from the quantity of $PGF_{2\alpha}$ present in the extended semen it revealed that in fact higher dilution rates did result in reducing the total amount of $PGF_{2\alpha}$ synthesized during the extension process. However, there was no difference in quantity of $PGF_{2\alpha}$ synthesized in extended semen diluted at 1:15, 1:20 or 1:25, which is inclusive of the range in which bovine semen is normally diluted. This suggests that sperm concentration, which determines semen dilution rate, will most likely not have an effect on the final concentration of $PGF_{2\alpha}$

present in extended semen unless the semen being extended is collected from a bull with abnormally low or high sperm concentration.

Nevertheless, dilution rate will affect the quantity of seminal plasma present in the extended semen. The amount of supplementary seminal plasma present has been demonstrated to result in improved viability and motility of fresh rabbit sperm (Castellini et al., 2000), to accelerate binding of zona pellucida proteins to fresh boar spermatozoa (Harkema et al., 2004), and to enhance heterogeneity and viability of frozen-thawed ram semen (Ollero et al., 1997). Quantity of supplementary seminal plasma in extended semen has also been demonstrated to improve post-thaw motility, produce additional capacitated sperm, generate fewer acrosome reacted cells and improve pregnancy rate following cervical insemination of frozen-thawed ram semen (Maxwell et al., 1999). When bovine seminal plasma was added to caudal epididymal sperm, it resulted in improved *in vitro* penetration of zona-free oocytes after heterospermic insemination (Henault et al., 1995). Addition of seminal plasma to bovine semen diluted to low cell numbers per insemination dose improved the post-thaw viability of the semen (Garner et al., 2001). In addition, complete or partial removal of seminal plasma before extension of bovine semen had deleterious effects on post-thaw motility and number of intact acrosomes (Bass et al., 1983; Kalloo et al., 2003).

It is likely that the factor(s) responsible for these improvements in post-thaw measurements are due to seminal plasma proteins. Ollero and coworkers (1997) found that the seminal plasma fraction >10 kDa had the same effect on improving post-thaw heterogeneity and viability of ram sperm as did whole seminal plasma. A seminal

plasma protein of approximately 20 kDa was reported to be responsible for reverting cold-shock damage to ram sperm membrane (Barrios et al., 2000). Bovine epididymal fluid has been found to contain five proteins ranging in size from 36 to 50-52 kDa that improve post-thaw motility (Reyes-Moreno et al., 2002). Other unidentified factors, including $PGF_{2\alpha}$, that influence post-thaw spermatozoa function may also be present in seminal plasma. Joyce and coworkers (1987) reported that cyclooxygenase inhibitors added to mouse spermatozoa had no effect on motility, but decreased *in vitro* fertilization. In addition, these researchers found that these inhibitors decreased the acrosome reaction of guinea pig sperm, but this inhibition did not occur when a mixture of $PGF_{2\alpha}$ and PGE_2 was also added.

The initial concentration of $PGF_{2\alpha}$ in whole semen was significantly greater than for seminal plasma. However, following extension of the whole semen and seminal plasma, $PGF_{2\alpha}$ synthesis during the extension process resulted in less difference in $PGF_{2\alpha}$ concentrations than was observed in the original samples. As was observed in Experiment 2, after the quantity of $PGF_{2\alpha}$ contributed from whole semen or seminal plasma and from fractions A and B of the diluent were subtracted, dilution rates of 1:10, 1:15 and 1:20 had no effect on the amount of $PGF_{2\alpha}$ synthesized during the extension process, except when seminal plasma was extended at the 1:20 dilution rate and resulted in less $PGF_{2\alpha}$ synthesis. However, when means for whole semen and seminal plasma were pooled for all dilution rates, the amount of $PGF_{2\alpha}$ synthesized during extension was greater for whole semen compared to seminal plasma. Altogether, these data suggest that the majority of factors responsible for $PGF_{2\alpha}$

synthesis during the extension process are associated with the spermatozoa, but some must also be associated with seminal plasma. In fact, Shalev and coworkers (1994) reported that cyclooxygenase 1, or prostaglandin H synthase-1, which catalyzes the conversion of arachidonic acid to prostaglandin H_2, is localized in the apical region of the spermatozoa head, the post-acrosomal region and the mid-pieces of the tail, and demonstrated that intact bovine spermatozoa can synthesize prostaglandins. Chicken egg yolk, the major component in Fraction A of the diluent, contains phospholipids which are composed of 80% (w/w) phosphatidylcholine and 15% (w/w) phosphatidylethanolamine (Yoon and Kim, 2002), and bovine seminal plasma lipids are composed of 30% phosphatidylcholine and 10.5% phosphatidylethanolamine and bovine spermatozoa lipids are composed of 35.6% phosphatidylcholine and 20.0% phosphatidylethanolamine (Pursel and Graham, 1967), both of which can be acted on by phospholipase A_2 (PLA_2) to yield arachidonate. Phospholipase A_2 has been identified in ram spermatozoa membranes (Hinkovska et al., 1987), and bovine spermatozoa have been reported to contain protein kinase C (PKC) activity, which activates PLA_2, comparable to that observed for human spermatozoa (Breitbart et al., 1992).

Bovine spermatozoa exposed to increasing concentrations of arachidonic acid resulted in concomitant linear increases of $PGF_{2\alpha}$ and PGE_2 concentrations in the media (Shalev et al., 1994). Melittin, which activates PLA_2 via PKC activation (Shier, 1979), increased prostaglandin production in the absence of exogenous arachidonic acid in bovine semen (Shalev et al., 1994), indicating that activation of PLA_2 may be the limiting step in the release of arachidonic acid and subsequent prostaglandin

synthesis. In contrast, bovine spermatozoa exposed to both melittin and staurosporin, an inhibitor of PKC (Sako et al., 1988; King and Rittenhouse, 1989), resulted in decreased prostaglandin synthesis, demonstrating that PLA_2 activation by PKC is a necessary step in bovine spermatozoa prostaglandin synthesis (Shalev et al., 1994). Additionally, these researchers reported that the inhibitory effect of staurosporin was not observed when exogenous arachidonic acid was present, further demonstrating that increased prostaglandin synthesis normally occurs through release of arachidonic acid from membrane lipids. With the existence of both prostaglandin precursors and the rate-limiting enzyme for prostaglandin biosynthesis present during the semen extension process it is not surprising that prostaglandin synthesis did occur during the extension of bovine semen.

Prostaglandin E_2 concentration in extended bovine semen was not correlated to the Relative Fertility Rating (derived from non-return data), and as the concentration of $PGF_{2\alpha}$ in extended semen samples decreased the Relative Fertility Rating improved, suggesting that $PGF_{2\alpha}$ concentrations in extended bovine semen may be related to bull fertility. Similar findings were reported for ejaculates collected from fertile and infertile men; $PGF_{2\alpha}$ was negatively correlated with motility in normal men and was always higher in men with disturbed fertility, and PGE_2 was elevated only in men with persisting varicocele, low sperm counts and severely impaired motility (Schlegel et al., 1981). In contrast, $PGF_{2\alpha}$ concentration in whole semen collected from water buffalo did seem to be positively correlated to total and live sperm concentration, but this relationship did not exist for PGE_2 (Reddy et al., 1982). The

ratio of PGE_2 to $PGF_{2\alpha}$ did appear to be correlated to the Relative Fertility Rating and perhaps the ratio of these two opposing prostaglandins is more important than the individual concentration of either $PGF_{2\alpha}$ or PGE_2. In fact, when $PGF_{2\alpha}$ or PGE_2 were administered individually or in combination to cows during diestrus, the greatest increase in intrauterine pressure occurred when these prostaglandins were administered in combination (Hirsbrunner et al., 2003). Reddy and coworkers (1982) reported a positive correlation between water buffalo seminal $PGF_{2\alpha}$ concentration and total and live sperm concentrations. While there has been apparently only one animal study examining the relationship of seminal prostaglandins to fertility, the hypothesis that fertility is related to seminal prostaglandin concentration can be further supported in part by the results of several human studies. Although there has been no reported relationship between the concentration of human seminal $PGF_{2\alpha}$ and sperm concentration or morphology (Hawkins and Labrum, 1961; Hawkins, 1968), prostaglandin concentration in human semen does seem to be correlated with fertility when control patients are compared to patients in which infertility cannot otherwise be explained, and prostaglandin concentration in this semen is not correlated with sperm count or motility (Kelly 1978). In contrast, either lower or higher than normal PGE seminal concentrations are associated with decreased sperm concentration and motility (Isodori, et al., 1980). In addition, PGE concentration appears to play an important role in male reproduction, in that men with "idiopathic" infertility have lower seminal PGE concentrations compared to fertile men (Bygdeman et al., 1970; Collier et al., 1975).

Numerous researchers have attempted to develop predictive measures of bull fertility based on semen parameters. Attempts to correlate bovine sperm motility to fertility have been variable with some researchers noting significant correlations (Linford et al., 1976; Kjæstad, et al., 1993; Correa et al., 1997), but not by other researchers (Graham et al., 1980; Januskauskas et al., 1996b). However, the proportion of morphologically abnormal bovine spermatozoa has been reported to be correlated with fertility (Wood et al., 1986; Soderquist et al., 1991). Analysis of frozen-thawed bovine semen by measuring post-thaw linear motility, concentration of motile spermatozoa after swim-up and absolute sperm binding to the zona pellucida of homologous oocytes were correlated with non-return rates after field AI; however, combined analysis of sperm linear-motility patterns, swim-up separated sperm motility and absolute zona pellucida binding assay provide better assessment of the fertilizing capacity of AI bull semen (Zhang et al., 1998; 1999; Larssen and Rodríguez-Martínez, 2000). In addition, under the assumption that only bovine spermatozoa with intact membranes will be viable, membrane integrity and fertility have been found to be highly correlated by researchers conducting osmotic tests (Correa et al., 1997), but not by those using fluorophores (Januskauskas and Rodriguez-Martinez, 1995; Januskauskas et al., 1996a).

Although numerous sperm cell and seminal plasma characteristics have been correlated with bull fertility, few of these measurements are employed on a regular basis. Even though the national pregnancy rate for beef cows is approximately 75%, only 17.3% of beef operations semen-tested their bulls and 9.8% conducted a scrotal measurement (NAHMS, 1997). The Society for Theriogenology promotes screening

breeding bulls using a standardized breeding soundness examination which includes body condition, structural soundness, assessment of accessory sex glands, scrotal circumference, and analysis of sperm for abnormalities and motility (Chenoweth et al., 1993). Further research should be conducted to compare the numerous techniques previously discussed in a single experiment to determine which method(s) can best be utilized economically to predict bull fertility. Perhaps examination of additional bulls to validate the correlation of seminal prostaglandin concentrations to non-return rates will allow this method to emerge as a preferred method to predict bull fertility. However, currently little is known about whether season and bull age may affect seminal prostaglandin concentrations, or whether seminal prostaglandin concentrations decrease with subsequent ejaculations.

In conclusion, seminal plasma $PGF_{2\alpha}$ in extended bovine semen does not appear to be limiting due to $PGF_{2\alpha}$ synthesis that occurs during the dilution process. Dilution rate did not affect the quantity of $PGF_{2\alpha}$ that was synthesized during the extension process, indicating that bulls with higher than average spermatozoa concentrations should not have lower fertility due to lower seminal plasma $PGF_{2\alpha}$ concentration by having their semen subjected to higher dilution rates. The majority of the factors responsible for $PGF_{2\alpha}$ synthesis appear to be associated with the sperm cell, although they apparently occur in the seminal plasma in adequate concentrations to result in synthesis of similar quantities of $PGF_{2\alpha}$ as for whole semen. The improvement in fertility observed in other species when $PGF_{2\alpha}$ is added to extended semen or administered to the female at the time of insemination was not evident when

seminal plasma concentration of $PGF_{2\alpha}$ was compared to non-return data. In fact, endogenous seminal plasma $PGF_{2\alpha}$ concentrations appeared to decrease slightly as the conception rate increased. Many of the methods reviewed in this paper that attempted to relate a single factor like sperm cell or seminal plasma characteristics to fertility, but usually multiple factors play a role in male fertility. Likewise, rather than a single prostaglandin affecting sperm transport in the female, the ratio of two opposing prostaglandins ($PGF_{2\alpha}$ and PGE_2) may play a significant role in fertility. Further research should be conducted to determine whether exogenous $PGF_{2\alpha}$ could be administered to beef cattle at the time of insemination or added to extended semen to enhance conception rates.

IMPLICATIONS

These data suggest that $PGF_{2\alpha}$ is synthesized during extension of bovine semen, resulting in levels of $PGF_{2\alpha}$ in extended semen similar to those observed in whole semen following collection with an artificial vagina. This documentation of the actual concentration of $PGF_{2\alpha}$ present in whole and extended semen will assist in determining the quantity of $PGF_{2\alpha}$ that could be added to extended bovine semen to potentially enhance conception rates. Seminal prostaglandin concentrations in extended bovine semen may be an indicator of the fertilizing capability and bull fertility.

LITERATURE CITED

Abbitt, B., G.E. Seidel, Jr., and W.E. Berndtson. 1977. Effect of tris (hydroxymethyl) aminomethane salt of prostaglandin $F_{2\alpha}$ on post-thaw motility of bovine spermatozoa. J. Dairy Sci. 60:1991-1993.

Barrios, B., R. Pérez-Pé, M. Gallego, A. Tato, J. Osada, T. Muiño-Blanco. 2000. Seminal plasma proteins revert cold-shock damamge on ram sperm membrane. Biol. Reprod. 63:1531-1537.

Bass, J.W., P.C. Molan, and P. Shannon. 1983. Factors in seminal plasma that affect the viability and motility of spermatozoa. Reprod. Fertil. 68:275-280.

Breitbart, H., J. Lax, R. Rotem, and Z. Naor. 1992. Role of protein kinase C in the acrosome reaction of mammalian spermatozoa. Biochem. J. 281:473-476.

Bygdeman, M., B. Fredricsson, K. Svonbirg, and B. Samuelsson. 1970. The relationship between fertility and prostaglandin content of seminal fluid in men. Fertil. Steril. 21:622-629.

Castellini, C., P. Lattaioli, M. Moroni, and A. Minelli. 2000. Effect of seminal plasma on the characteristics and fertility of rabbit spermatozoa. Anim. Reprod. Sci. 63:275-282.

Cheng, H., G.C. Althouse, and W.H. Hsu. 2001. Prostaglandin $F_{2\alpha}$ added to extended boar semen at processing elicits *in vitro* myometrial contractility after 72 hours of storage. Theriogenology. 55:1901-1906.

Chenoweth, P.J., F.M. Hopkins, J.C. Spitzer, and R.E. Larsen. 1993. Guidelines for using the bull breeding soundness evaluation form. Pages 1-5 in Theriogenology Handbook B-10. Society for Theriogenology, Hastings, NE.

Cohen, M.S., M.J. Colin, M. Golimbu, and R.S. Hotchkiss. 1977. The effects of prostaglandins on sperm motility. Fertil. Steril. 28:78-85.

Collier, J.G., R.J. Flower, and S.L. Stanton. 1975. Seminal prostaglandins in infertile men. Fertil. Steril. 26:868-871.

Correa, J.R., M.M. Pace, and P.M. Zavos. 1997. Relationships among frozen-thawed sperm characteristics assessed via the routine analysis, sperm functional tests and fertility of bulls in an artificial insemination program. Theriogenology 48:721-731.

Edqvist, S., S. Einarsson, and B. Gustafsson. 1975. Effect of prostaglandin $F_{2\alpha}$ on sperm transport in the reproductive tract of the ewe. Acta Vet. Scand. 16:149-151.

Garner, D.L., C.A. Thomas, C.G. Gravance, C.E. Marshall, J.M. DeJarnette, and C.H. Allen. 2001. Seminal plasma addition attenuates the dilution effect in bovine sperm. Theriogenology 56:31-40.

Graham, E.F., M.K.L. Schmehl, B.K. Evenson, and D.S. Nelson. 1980. Problems with laboratory assays. Pages 1-8 in Proc. 8th Tech. Conference on Artificial Insemination and Reprod., National Assoc. Anim. Breeders, WI

Grunberger, W., U. Maier, and G. Lunglmayr. 1981. Effect on prostaglandin F 2 alpha on sperm motility in vitro. Reproduccion 5:141-144.

Gustafsson, B., S. Edqvist, and S. Einarsson. 1975. The fertility of deep-frozen ram semen supplemented with $PGF_{2\alpha}$. Acta Vet. Scand. 16:468-470.

Harkema, W., B. Colenbrander, B. Engel, and H. Woelders. 2004. Effects of exposure of epididymal boar spermatozoa to seminal plasma on the binding of zona pellucida proteins during in vitro capacitation. Theriogenology 61:215-226.

Hawk, H.W., and S.E. Echternkamp. 1973. Uterine contractions in the ewe during prostaglandin-regulated oestrus. J. Reprod. Fertil. 34:347-349.

Hawkins, D.F. 1968. Relevance of prostaglandins to problems of human subfertility. Pages 1 in Prostaglandins Symposium of the Worchester Foundation for Experimental Biology P.W Ramwell, and J.E. Shaw eds. John Wiley, New York.

Hawkins, D.F., and A.H. Labrum. 1961. Semen prostaglandin levels in fifty patients attending a fertility clinic. J. Reprod. Fertil. 2:1-10.

Henault, M.A., G.J. Killian, J.F. Kavanaugh, and L.C. Griel, Jr. 1995. Effect of accessory sex gland fluid from bulls of differing fertilities on ability of cauda epididymal sperm to penetrate zona-free bovine oocytes. Biol. Reprod. 52:390-397.

Hinkovska, V.T., A.B. Momchilova, D.H. Petkova, and K.S. Koumanov. 1987. Phospholipase A_2 activity in ram spermatozoa plasma membranes. Int. J. Biochem. 19:569-572.

Hirsbrunner, G., B. Knutti, U. Küpfer, H. Burkhardt, and A. Steiner. 2003. Effect of prostaglandin E_2, DL-cloprostenol, and prostaglandin E_2 in combination with D-cloprostenol on uterine motility during diestrus in experimental cows. Anim. Reprod. Sci. 79:17-32.

Isidori, A., D. Conte, G. Laguzzi, P. Giovenco, and F. Dondero. 1980. Role of seminal prostaglandins in male fertility. I. Relationship of prostaglandin E and 19-OH prostaglandin E with seminal parameters. J. Endocrinol. Invest. 3:1-4.

Januskauskas, A., and H. Rodrigues-Martinez. 1995. Assessment of sperm viability by measurement of ATP, membrane integrity and motility in frozen/thawed bull semen. Acta Vet. Scand. 36:571-574.

Januskauskas, A., L. Söderquist, H.G. Häärd, M.C. Häärd, N. Lundeheim, and H. Rodrigues-Martinez. 1996a. Estimation of sperm viability in frozen-thawed semen from Swedish A.I. bulls. Zentralbl Veterinarmed A. 43:281-287.

Januskauskas, A., L. Söderquist, H.G. Häärd, M.C. Häärd, N. Lundeheim, and H. Rodrigues-Martinez. 1996b. Influence of sperm number per straw on the post-thaw sperm viability and fertility of Swedish Red and White AI bulls. Acta Vet. Scand. 37:461-470.

Joyce, C.L., N.A. Nuzzo, L. Wilson, Jr., and L.J.D. Zaneveld. 1987. Evidence for a role of cyclooxygenase (prostaglandin synthetase) and prostaglandins in the sperm acrosome reaction and fertilization. J. Androl. 8:74-82.

Kalloo, F.A., N.A. Wani, M.Z. Khan, and G.M. Wani. 2003. Effects of partial to complete removal of seminal plasma on cryopreservation of bovine semen. Indian J. Anim. Sci. 73:63-65.

Kelly, R.W. 1978. Prostaglandins in semen: Their occurrence and possible physiological significance. International J. Andrology. 1:188-200.

King, W.G., and S. Rittenhouse. 1989. Inhibition of protein C by staurosporine promotes elevated accumulation of inositol triphosphates and tetrabiphosphate in human platelets exposed to thrombin. J. Biol. Chem. 264:6070-6074.

Kjæstad, H., E. Ropstad, and K. Andersen Berg. 1993. Evaluation of spermatological parameters used to predict the fertility of frozen bull semen. Acta Vet. Scand. 34:299-303.

Larssen, B., and H. Rodríguez-Martínez. 2000. Can we use in vitro fertilization tests to predict semen quality? Anim. Reprod. Sci. 60-61:327-336.

Ledwozyw, A., R. Pruszkowska, and A. Kadziolka. 1986. Isolation and identification of prostaglandins of the E and F series in testes and semen of lowland-black-white breed of bulls. Acta Physiologica Hungarica. 68:11-17.

Linford, E., F.A. Glover, C. Bishop, and D.L. Stewart. 1976. The relationship between semen evaluation methods and fertility in the bull. J. Reprod. Fertil. 47:283-291.

Maxwell, W.M.C., G. Evans, S.T. Mortimer, L. Gillan, E.S. Gellatly, and C.A. McPhie. 1999. Normal fertility in ewes after cervical inseminiation with frozen-thawed spermatozoa supplemented with seminal plasma. Reprod. Fertil. Dev. 11:123-126.

National Animal Health Monitoring System. 1997. Part 1: reference of 1997 beef cow-calf management practices. USDA-APHIS, Washington, DC.

Ollero, M., J.A. Cebrian-Perez, and T. Muiño-Blanco. 1997. Improvement of cryopreserved ram sperm heterogeneity and viability by addition of seminal plasma. J. Androl. 18:732-739.

Patil, R.K., S.N. Sinha, S. Einarsson, and I. Settergren. 1980. The effect of prostaglandin $F_{2\alpha}$ and oxytocin on bovine myometrium *in vitro*. Nord. Vet. Med. 32:474-479.

Peña, F.J., J.C. Domínguez, B. Alegre, and J. Peláez. 1998. Effect of vulvomucosal injection of $PGF_{2\alpha}$ at insemination on subsequent fertility and litter size in pigs under field conditions. Anim. Reprod. Sci. 52:63-69.

Peña, F.J., J.C. Domínguez, J. Pelá, and B. Alegre. 2000. Intrauterine infusion of $PGF_{2\alpha}$ at insemination enhances reproductive performance of sows during the low fertility season. The Vet. J. 159:259-261.

Pursel, V.G., and E.F. Graham. 1967. Phospholipids of bovine spermatozoa and seminal plasma. J. Reprod. Fert. 14:203-211.

Reddy, B.B., A.S.N. Murty, V.S.N. Rao, P.N. Rao, and K.S. Rao. 1982. Seminal prostaglandins E_2 and F_2 alpha in Murrah buffalo bulls. J. Nuclear Agri. Biol. 11:126-127.

Reyes-Moreno, C., M. Boilard, R. Sullivan, and M.-A. Sirard. 2002. Characterization and indentification of epididymal factors that protect ejaculated bovine sperm during in vitro storage. Biol. Reprod. 66:159-166.

Sako, T., A.I. Tauber, A.Y. Jeng, S.H. Yupsa, and P.M. Blumberg. 1988. Contrasting actions of staurosporine, a protein kinase C inhibitor, on human neutrophils and primary mouse epidermal cells. Cancer Res. 48:4646-4650.

Schlegel, W., S. Rotermund, G. Farber, and E. Nieschlag. 1981. The influence of prostaglandins on sperm motility. Prostaglandins 21:87-99.

Shalev, Y., M. Shemesh, T. Levinshal, S. Marcus, and H. Breitbart. 1994. Localization of cyclooxygenase and production of prostaglandins in bovine spermatozoa. J. Reprod. Fertil. 101:405-413.

Shier, W.T. 1979. Activation of high levels of endogenous phospholipase A_2 in cultured cells. Proc. Natl. Acad. Sci. 76:195-199.

Soderquist, L., L. Janson, K. Larsson, and S. Einarsson. 1991. Sperm morphology and fertility in A.I. bulls. Zentralbl Veterinarmed A. 38:534-543.

Spilman, C.H., A.E. Finn, and J.F. Norland. 1973. Effect of prostaglandins on sperm transport and fertilization in the rabbit. Prostaglandins. 4:57-64.

Spilman, C.H., and J.K. Harper. 1973. Effect of prostaglandins on oviduct motility in estrous rabbits. Biol. Reprod. 9:36-45.

Voglmayr, J.K. 1973. Prostaglandin $F_{2\alpha}$ concentration in genital tract secretions of dairy bulls. Prostaglandins 4:673-678.

Wood, P.D., J.A. Foulkes, R.C. Shaw, and D.R. Melrose. 1986. Semen assessment, fertility and the selection of Hereford bulls for use in AI. J. Reprod. Fertil. 76:783-795.

Yoon, T.H., and I.H. Kim. 2002. Phosphatidylcholine isolation from egg yolk phospholipids by high-performance liquid chromatography. J. Chromatogr. A. 949:209-216.

Yu, H., D.-H. Shin, and W.H. Hsu. 1993. Effects of oxytocin (OT), vasopressin (VP), and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) on porcine myometrial contractility *in vitro* and their dependence on Ca^{2+} influx. Biol. Reprod. 48(Suppl.):162. (Abstr.)

Zhang, B.R., B. Larsson, N. Lundeheim, and H. Rodriguez-Martinez. 1998. Sperm characteristics and zona pellucida binding in relation to field fertility of frozen-thawed semen from dairy AI bulls. Int. J. Androl. 21:207-216.

Zhang, B.R., B. Larsson, N. Lundeheim, M.G.H. Håård, and H. Rodriguez-Martinez. 1999. Prediction of bull fertility by combining in vitro assessments of frozen-thawed semen from young dairy bulls entering an AI-programme. Int. J. Androl. 22:253-260.

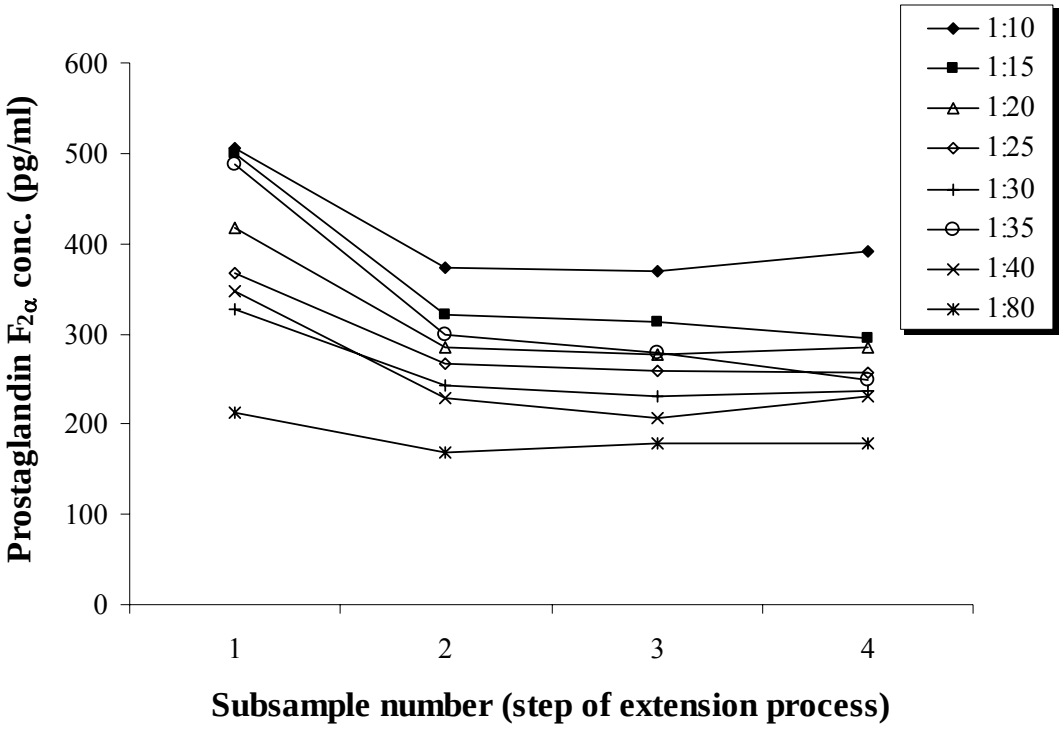

Figure 2.1. Prostaglandin $F_{2\alpha}$ concentrations in sub-samples following each addition of diluent during extension of whole semen at eight dilution rates (1:10, 1:15, 1:20, 1:25, 1:30, 1:35, 1:40, and 1:80). Dilution rate x dilution step interaction ($P < 0.001$).

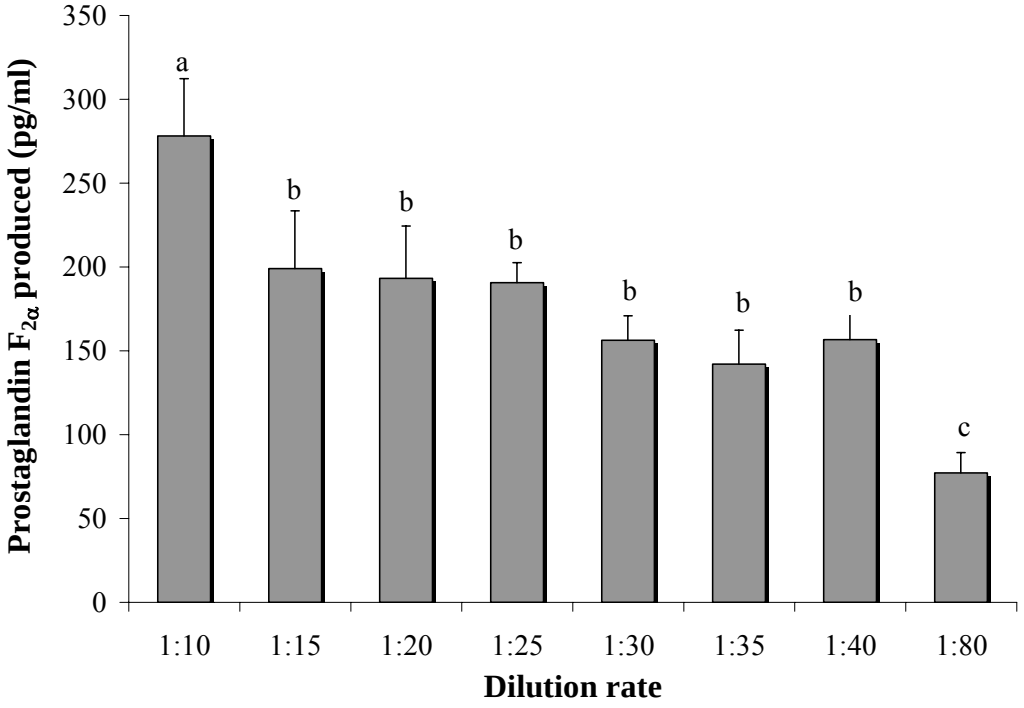

Figure 2.2. Mean $PGF_{2\alpha}$ synthesis during extension of bovine semen at eight dilution rates. Bars lacking a common letter are different ($P < 0.001$).

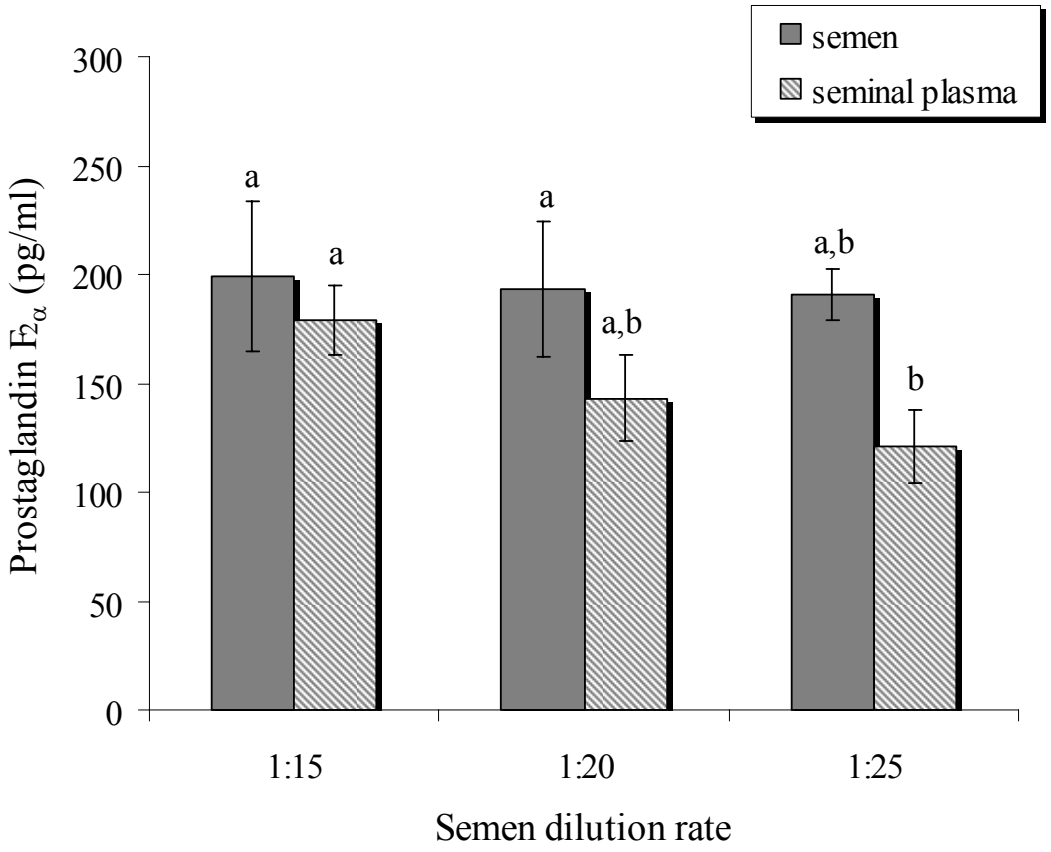

Figure 2.3. Mean $PGF_{2\alpha}$ synthesis during extension of bovine whole semen or seminal plasma at three dilution rates. Bars lacking a common letter tended to be different ($P < 0.08$).

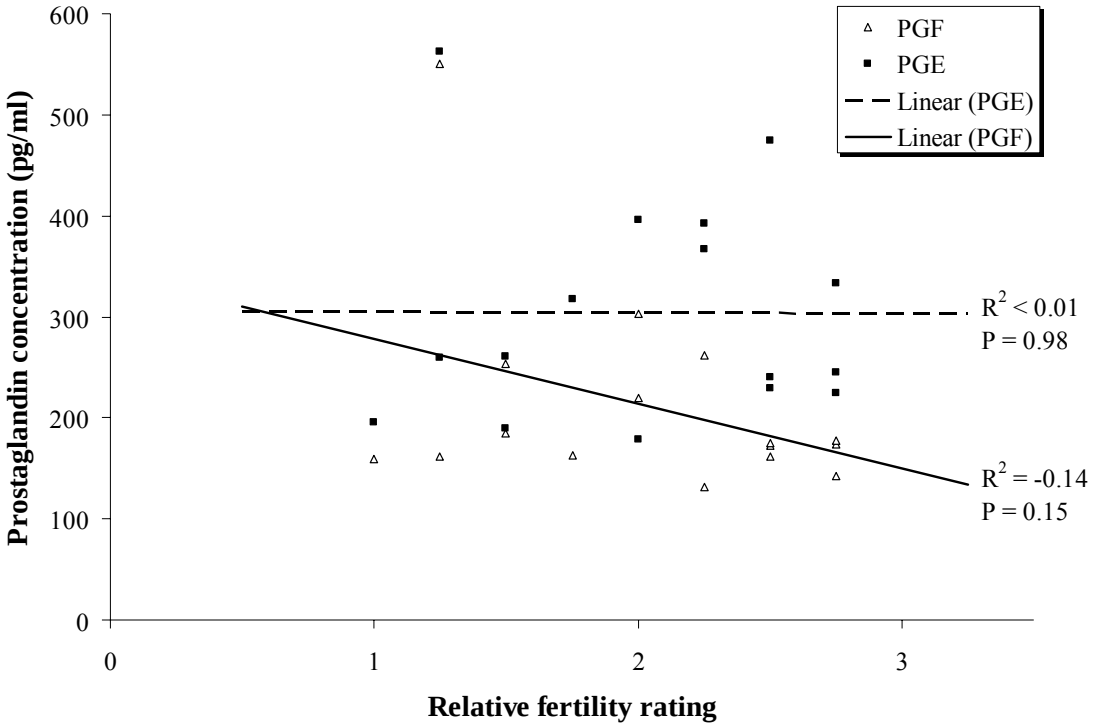

Figure 2.4. Correlation of prostaglandins in extended bovine semen to non-return deviation data used to construct a relative fertility rating (number of services for each bull ranged from 218 to 26,945).

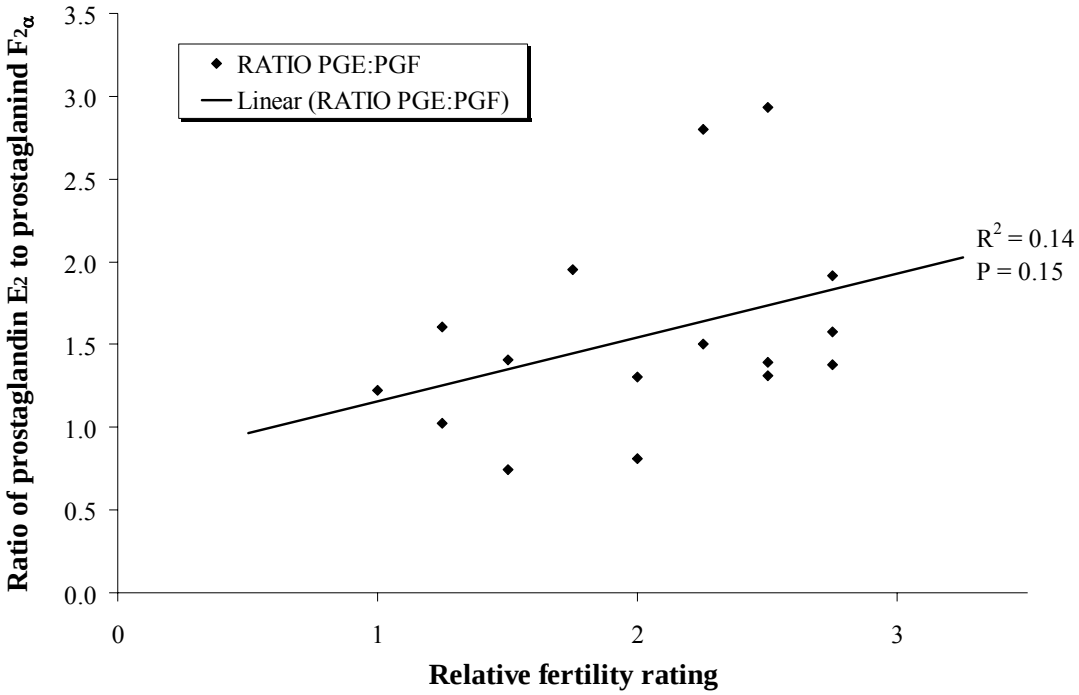

Figure 2.5. Correlation of the ratio of concentration of prostaglandin E_2 and prostaglandin $F_{2\alpha}$ in extended bovine semen to non-return deviation data used to construct a relative fertility rating (number of services for each bull ranged from 218 to 26,945).

IMPROVEMENT OF CONCEPTION RATE IN CATTLE RECEIVING EXOGENOUS PROSTAGLANDIN $F_{2\alpha}$ ADMINISTERED AT THE TIME OF INSEMINATION

J.R. Jaeger, J.M. DeJarnette, A.R. Menino, and T. DelCurto

Journal of Animal Science

1111 N. Dunlap Ave.

Savoy, IL 61874

ABSTRACT

Three experiments were conducted to determine whether exogenous prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) administered to cattle via $PGF_{2\alpha}$-supplemented semen or intramuscular injection would improve conception rate following artificial insemination. In experiment 1, semen collected from two Angus bulls was extended to contain 10 x 10^6 motile sperm and 0, 500, or 5000 pg/ml exogenous $PGF_{2\alpha}$. Following thawing, no differences were observed for proportion of normal spermatozoa or motility among $PGF_{2\alpha}$ treatments. *In vitro* fertilization rates did not differ (P = 0.44) between the 0, 500, and 5000 pg/ml $PGF_{2\alpha}$ treatments (17.6 ± 2.0, 13.4 ± 2.0, and 15.4 ± 2.4%, respectively). However, analysis of cleavage rate per embryo revealed a bull x semen treatment interaction (P = 0.09). Analysis of first service conception rate of cows (n = 420) estrous synchronized with the Cosynch+CIDR system and mass mated with the $PGF_{2\alpha}$-enhanced semen revealed a treatment x technician interaction (P = 0.01). In experiment 2, virgin beef heifers (n = 18) were estrous synchronized with the Cosynch+CIDR and mass mated with 30% post-thaw motility and assigned to receive either an i.m. injection of $PGF_{2\alpha}$ or no treatment immediately after insemination. Treatment with $PGF_{2\alpha}$ after the first insemination only resulted in a higher first service conception rate (P = 0.12), conception to AI rate (P = 0.12), and overall pregnancy rate (P = 0.02). In experiment 3, dairy cows (n = 62) were assigned to receive either no treatment or an injection of $PGF_{2\alpha}$ immediately after insemination. Significantly more (P = 0.08) cows that received $PGF_{2\alpha}$ conceived to AI compared to

control cows. These data suggest that exogenous $PGF_{2\alpha}$ administered at the time of insemination may improve conception rate following artificial insemination.

Key words: cattle, heifer, prostaglandin $F_{2\alpha}$, conception rate

INTRODUCTION

Artificial insemination (AI) utilizing commercial semen has been available to beef producers for 50-60 years. However, only 13% of beef operations use AI (NAHMS, 1998), resulting in AI of only 5% of 33.8 million U.S. beef cows (USDA, 2005). Although beef cattle often are managed in extensive conditions, large operations with ≥300 cows are more likely to use AI (37% of operations) than operations with ≤50 cows (12% of operations; NAHMS, 1998). This suggests that cow accessibility is not the primary factor in determining whether AI is employed. Producers cite AI conception rates of 50-70% as being unacceptable; however, calving rate following a single natural service is only 50-60% (Parkinson, 2004). Most producers (39%) cite time and labor requirements for not using AI (USDA, 1998). Regardless of the reason, failure to use AI results in a loss of superior genetics. If AI conception rate could be improved, additional producers may consider capturing the superior genetics from proven AI sires.

Previous research established that injection of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) at breeding increased embryo number of rabbits (Spilman et al., 1973) and farrowing rate of sows (Peña et al., 1998). Ewes injected with $PGF_{2\alpha}$ at breeding or inseminated with $PGF_{2\alpha}$-supplemented semen display more spermatozoa in all reproductive tract

segments (Edqvist et al., 1975). Addition of $PGF_{2\alpha}$ to semen improved pregnancy rate of ewes (Gustafsson et al., 1975), and farrowing rate and litter size of sows (Peña et al., 2000). Prostaglandin $F_{2\alpha}$ added to ram semen before freezing had no post-thaw effect on spermatozoa and improved subsequent conception of ewes (Gustafsson et al., 1975). In contrast, addition of $PGF_{2\alpha}$ to bovine semen during extension resulted in a dose-dependent decrease in post-thaw motility (Abbitt et al., 1977).

Our objectives were to determine whether 1) *in vitro* fertilization and cleavage rate are affected by $PGF_{2\alpha}$-enhanced semen, 2) insemination of beef cattle with $PGF_{2\alpha}$-enhanced semen would improve conception rate, and 3) administration of $PGF_{2\alpha}$ to cattle at the time of AI would improve conception rate.

MATERIALS AND METHODS

Experiment 1

To determine if first service conception rate could be improved with $PGF_{2\alpha}$-enhanced semen, semen was collected from two Angus bulls (Bull A and Bull B) at a commercial AI facility (Select Sires Inc., Plain City, OH) and extended with a commonly used industry diluent consisting of two fractions, an egg yolk based fraction and glycerol based fraction. Dinoprost tromethamine (ProstaMate®; Phoenix Scientific, St. Joseph, MO) was added to glycerol fraction of the diluent before semen extension, to provide 0, 500, or 5000 pg/ml of active $PGF_{2\alpha}$ per 0.5 ml straw. The quantity of $PGF_{2\alpha}$ added to the $PGF_{2\alpha}$-enhanced treatments during the extension process was approximately 2- and 20-fold greater than endogenous bovine semen

$PGF_{2\alpha}$ concentrations previously determined in our laboratory by enzyme immunoassay (unpublished data). To further elucidate whether addition of $PGF_{2\alpha}$ to extended semen would improve fertility a low-dose sperm number/straw was utilized; therefore, semen was diluted to 10 x 10^6 sperm/ml rather than the normal 20 x 10^6 sperm/ml. Following extension and freezing, semen from both bulls and each treatment was thawed in a 35° C water bath and examined for proportion of normal sperm and motility at 0 min and after incubation at 38° C for 180 min after thawing.

In vitro maturation and fertilization

Extended frozen-thawed semen from each bull and each treatment was examined for *in vitro* fertilization rate by exposing semen to *in vitro* matured oocytes (~90 oocytes/treatment). To achieve *in vitro* bovine cumulus-oocyte complex maturation, bovine ovaries were collected from a local abattoir and transported to the laboratory in Dulbecco's Phosphate Buffered Solution (DPBS) at approximately 30^o C with no more than a 6 h delay between collection and oocyte extraction. Oocytes were recovered from ovaries by utilizing a slashing and rinsing technique that allowed cumulus cells to stay intact (Wiemer et al., 1991; Watson et al., 1994). During the slashing, ovaries were rinsed frequently with DPBS to wash all exposed oocytes into a 50-ml conical Falcon collection tube. Oocytes were allowed to settle for 10 min, at which time the lower 10 ml of collected wash fluid was removed from the Falcon tube using a pipette. This fluid was dispensed into culture plates and oocytes were reclaimed from the rinse medium by aspiration and washed three times in M2 medium (Watson et al., 1994). Viable oocytes (oocytes surrounded by 3-4 layers of cumulus

cells) were transferred to a culture plate (denuded oocytes were discarded with the ovarian and follicular debris remaining in the dish) containing eight 50-µl microdrops of filter-sterilized maturation medium comprised of TCM 199 (Gibco BRL, Rockville, MD) pyruvate (28 µg/ml), 10% heat treated estrus cow serum (HTECS), luteinizing hormone (LH; 1 µg/ml), follicle stimulating hormone (FSH; 0.5 µg/ml), and estradiol (E_2; 1 µg/ml). After oocytes were placed in the maturation medium, the dishes were placed in a 5% CO_2 in air incubator at 38° C for 24 h. After placing the oocytes in the maturation medium, a bovine oviductal epithelial cell (BOEC) culture was constructed similar to that described by Wiemer and coworkers (1991). Oviductal epithelial cells from excised cow tracts were removed, washed under a sterile hood, and placed in a culture dish containing culture medium for 24 h. Following incubation, BOEC had amassed into "worm"-shaped structures and were washed two times in TCM 199 containing pyruvate (28 µg/ml), 100 µM β-mercaptoethanol (BME), and 10% heat treated fetal calf serum (HTFCS; Sigma, St. Louis, MO). After 24 h of *in vitro* maturation, oocytes were transferred into six different culture plates (one culture plate for each bull and $PGF_{2\alpha}$ treatment) containing eight 50-µl microdrops of fertilization medium (Watson et al., 1994), washed two times in clean microdrops of fertilization medium, and then placed into a third microdrop for fertilization.

To accomplish *in vitro* fertilization, semen from each bull and treatment was exposed to a "swim-up" procedure to select only the most motile sperm. This procedure consisted of thawing a straw of semen from each bull and treatment in a 35° C water bath for 1 min and then dispensing the semen into warmed 35 x 10 mm culture dishes. Following placement in the culture dish, 200 µl of semen from each

bull and treatment was dispensed into 1.5-ml cryotubes containing 1 ml of pre-warmed (38° C) Sperm TL solution (Parrish et al., 1986) by sliding the pipette tip down the side of the cryotube and slowly dispensing the semen under the Sperm TL. Cryotubes were placed in an incubator for 1 h, after which the top 800 µl of semen was removed and placed in a conical bottom Falcon tube for centrifugation at 1000 rpm for 5 min. The top 600 µl of supernatant was removed and 2 ml of warm Sperm TL was added to the tube and mixed gently. This mixture was again centrifuged at 1000 rpm for 5 minutes. The top 2100 µl of supernatant was then carefully removed, leaving only about 100 µl in the bottom of the tube. This remaining 100 µl was gently mixed and 5 µl of this solution was mixed with 95 µl of eosin stain and 5 µl of this mixture was placed on a hemocytometer to determine sperm concentration. The volume of sperm solution added to the fertilization droplet was calculated to provide 50,000 sperm cells. The total volume of sperm mixture added to each fertilization droplet was approximately 12 µl. After sperm was added to the fertilization drop containing the prepared oocytes, the plates were placed in a 5% CO_2 incubator at 38^{o}C for 18 h. The following day, oocytes and zygotes were aspirated from the fertilization drops, placed into a microcentrifuge tube containing 1 ml of warm (38° C) M2 medium, and vortexed for two minutes to denude the oocytes and embryos of surrounding cumulus cells. To aid in the retrieval of all embryos, the tubes were shaken to move embryos to the bottom of the tube. Medium within the tube was removed with a Pasteur pipet and placed in a warm 35 x 10 mm culture dish. To assure that all embryos were removed from the tubes, 1 ml M2 media was added to the tube and then removed and placed in the same culture dish. Zygotes were then examined for remaining cumulus cells and

denuded zygotes were moved to zygote culture dishes. Zygote culture dishes consisted of three separate microdrops consisting of 50 µl citrate modified synthetic oviductal fluid, and essential and nonessential amino acids (cmSOFaa), similar to that used by Watson and coworkers (2000) except polyvinyl alcohol was replaced with 6 mg/ml BSA. Oocytes and zygotes were washed through the first two microdrops and placed in the third and final microdrop which contained the BOEC worms. The two microdrops previously used as wash droplets were removed and the dishes were placed in a 5% CO_2 incubator at 38^{o}C. Oocytes and zygotes were observed at 24-h intervals for fertilization rate and stage of development. Every 24 hours thereafter (up to 192 hours), the multiple culture dishes were observed for fertilization rate, number of cleavages and embryonic development.

In vivo fertilization

To assess *in vivo* fertility of $PGF_{2\alpha}$-enhanced semen, 432 crossbred spring-calving beef cows located at two experiment stations (Union and Burns) were assigned randomly to be inseminated with semen collected from either bull and from one of the three $PGF_{2\alpha}$ treatments. Prior to insemination, all cows were estrous synchronized using the Cosynch plus CIDr system as described by Lamb and coworkers (2001). Cows were inseminated by one of two highly experienced technicians or one moderately experienced technician 60 h after $PGF_{2\alpha}$ (Lutalyse; Pfizer, New York, NY) injection and CIDR removal, and each cow received a 100 µg GnRH (Cystorelin, Merial, Deluth, GA) i.m. injection immediately after insemination. Ten days after insemination, cows were exposed to fertile bulls for the remaining 50 days of the 60

day breeding season. Pregnancy diagnosis occurred 60 days after the completion of the breeding season at the Burns station and 108 days after completion of the breeding season at the Union station. First service conception rate was determined by calving date and cows giving birth 10 days after the expected calving date were removed from the data set resulting in 420 observations.

Experiment 2

To determine whether exogenous $PGF_{2\alpha}$ would improve fertility following insemination with sub-fertile semen, 18 crossbred virgin heifers were estrous synchronized using the Cosynch plus CIDR system (Lamb et al., 2001). Heifers were inseminated by an experienced technician with 30% post-thaw motile semen 60 h after 25 mg $PGF_{2\alpha}$ (Lutalyse; Pfizer, New York, NY) i.m. injection and CIDR removal, and received a 100 μg GnRH (Cystorelin, Merial, Duluth, GA) i.m. injection immediately after insemination. Heifers were assigned randomly to receive either no further treatment or a 25 mg $PGF_{2\alpha}$ (Lutalyse; Pfizer, New York, NY) i.m. injection immediately after the post-insemination GnRH injection. Heifers were then observed for estrus twice daily using an epididectomized bull for 21 d and heifers displaying estrus were re-inseminated with the 30% post-thaw motility semen . All heifers were then exposed to a fertile bull for the remaining 25 days of the 45 day breeding season. First service conception rate were determined by rectal palpation 35 days and again 60 days after the end of the breeding season.

Experiment 3

To determine if exogenous $PGF_{2\alpha}$ administered by i.m. injection at the time of insemination would improve the fertility of dairy cows, multiparous Holstein and Jersey cows in moderate body condition were assigned randomly to receive either no treatment (n = 33) or a 25 mg i.m. injection of $PGF_{2\alpha}$ (Lutalyse; Pfizer, New York, NY) immediately after insemination (n = 29) by an experienced technician. Cows were bred either 12 h after displaying a spontaneous estrus or following estrous synchronization with Ovsynch (Pursley et al., 1995). First service conception rate, conception to AI, and final pregnancy rate were determined either by ultrasonography 35 days after insemination or by rectal palpation 45 days after insemination.

Statistical Analysis

Proportion of normal sperm, motility at 0 and 180 min post-thaw, *in vitro* fertilization rate and cleavage rate per embryo were analyzed by analysis of variance in SAS (SAS Inst. Inc., Cary, NC). First service conception rates, conception to AI and pregnancy rates were analyzed using the CATMOD procedure of SAS. The critical alpha for data subjected to analysis of variance was 0.05, and P-values between 0.06 and 0.10 were considered tendencies. For data analyzed as a categorical model the critical alpha was 0.10 and P-values from 0.10 to 0.15 were considered tendencies.

RESULTS

Proportion of post-thaw normal sperm did not differ between treatments and averaged 80.0, 81.5, and 78.0% normal sperm for the 0, 500, and 500 pg/ml $PGF_{2\alpha}$ semen treatments, respectively. However, the proportion of normal sperm did differ between bulls (85.7 ± 1.8% vs. 74.0 ± 1.7%, P = 0.05). Exogenous $PGF_{2\alpha}$ had no effect (P > 0.20) on post thaw motility at 0 or 180 min post-thaw and averaged 77.9 ± 1.1% and 32.9 ± 2.1%, respectively. Significant differences for motility were observed between the two bulls at both 0 and 180 min post-thaw. Immediately after thawing, motility for Bull A was significantly greater (P < 0.001) than for Bull B (80.4 vs. 75.4%, respectively). This difference remained evident at 180 min after thawing, whereas motility for Bull A was 30.1% compared to 28.7% for Bull B (P = 0.04).

In vitro fertilization rate did not differ (P = 0.44) between the 0, 500, and 500 pg/ml $PGF_{2\alpha}$ semen treatments and averaged 17.6 ± 2.0, 13.4 ± 2.0, and 15.4 ± 2.4%, respectively. However, analysis of cleavage rate per embryo revealed a bull x treatment interaction (P = 0.09; Figure 3.1).

Although differences existed between bulls for post-thaw motility and proportion of normal sperm, these differences did not have an effect on first service conception rate and bull was removed from the statistical model. In addition, first service conception rate did not differ between locations, for days postpartum, or for cow age and these factors were also removed from the model. Final analysis of data for first service conception rate of cows inseminated with the $PGF_{2\alpha}$-enhanced semen revealed a technician x treatment interaction (P = 0.01; Figure 3.2). However, it did

appear that exogenous $PGF_{2\alpha}$ added to low-dose semen during the dilution process did not have an effect on first service conception rate with similar proportions of cows conceiving to insemination with 0, 500, or 5000 pg/ml $PGF_{2\alpha}$-enhanced semen (49.7 ± 4.1, 44.4 ± 4.0, and 51.4 ± 4.2, respectively).

More heifers injected with $PGF_{2\alpha}$ immediately after insemination with 30% post-thaw motility semen conceived to first service compared to control heifers (44.4 ±16.6 vs. 0%, respectively; P = 0.12). Although $PGF_{2\alpha}$-treated heifers displaying estrus during the next 21 days were re-inseminated with the same sub-fertile semen and did not receive $PGF_{2\alpha}$ at the time of the second insemination, conception to AI during the first 22 days of the breeding season still tended to be greater for those heifers that received $PGF_{2\alpha}$ following their initial insemination compared to control heifers (55.6 ± 16.6 vs. 22.2 ± 13.9%, respectively; P = 0.12). Surprisingly, final pregnancy rate was significantly (P = 0.02) greater for heifers that had received a single $PGF_{2\alpha}$ injection following their first insemination compared to control heifers (88.9 ± 10.5 vs. 44.4 ± 16.6%).

Cow age and breed, days postpartum, number of previous services, and whether cows were inseminated after estrous synchronization or displaying a spontaneous estrus did not have an effect on conception rate to AI and were removed from the model before final analysis. A significantly (P = 0.08) greater proportion of cows receiving $PGF_{2\alpha}$ immediately after insemination conceived compared to control cows (53.3 ± 12.9 vs. 25.0 ± 9.7%, respectively).

DISCUSSION

Prostaglandin $F_{2\alpha}$ has been demonstrated to remain bioactive after the freezing process, eliciting the same myometrial contractility after storage in extended fresh and frozen semen (Cheng et al., 2001). Exogenous $PGF_{2\alpha}$ added to semen during the dilution process had no effect on the proportion of post-thaw normal sperm, but the proportion of normal sperm did differ between bulls. However, the post-thaw normal sperm values were similar to previous reports for Holstein and Brahman bulls (Yates et al., 2003). Post-thaw motility, averaging 79.8%, was not affected by addition of exogenous $PGF_{2\alpha}$ during the dilution process and was comparable to previous reports for extended frozen-thawed bovine semen (Reyes-Moreno et al., 2002; Kalloo et al., 2003; Yates et al., 2003), but significantly greater than reported by Abbitt and coworkers (1977) and Zhang and coworkers (1999). Differences in post-thaw motility have been attributed to cooling rate (Woelders et al., 1997), type of diluents and additives utilized (Foote et al., 2002, Foote and Kaproth, 2002), and processing method (Thun et al., 2002). Most notably, in this study, exogenous $PGF_{2\alpha}$ was not detrimental to post-thaw motility. In a previous study, addition of $PGF_{2\alpha}$ to bovine semen during the extension process, with final concentrations of $PGF_{2\alpha}$ calculated to be either 75, 225 or 675 μg/ml, resulted in a significant reduction in post-thaw motility (Abbitt et al., 1977). However, the midrange of these levels was approximately a million-fold greater than the endogenous concentration of seminal $PGF_{2\alpha}$ previously measured in our laboratory by enzyme immunoassay (unpublished data). The endogenous oxygen consumption of washed cauda epididymal spermatozoa or the

oxidative and glycolytic activities of washed ejaculated bovine spermatozoa were not affected by exogenous $PGF_{2\alpha}$ (Voglmayr, 1973). Only 19-hydroxy PGE, but not prostaglandins of the E or F series, depressed human spermatozoa respiration and it had no effect on the production of lactate (Kelly, 1977). However, there is evidence that PGE_1 and $PGF_{2\alpha}$ act at an intracellular level by interacting with the adenylate cyclase-cAMP system (Ramwell and Shaw, 1971). In fact, in most adenylate cyclase systems in which prostaglandins synthesis and degradation occur, PGE_2 stimulates accumulation of cAMP; but, $PGF_{2\alpha}$ either has no effect or causes only a slight reduction in formation of this nucleotide (Marsh and LeMaire, 1974). Bovine sperm motility has been suggested to be at least partially controlled by cAMP and/or cGMP (Garbers et al., 1971). Furthermore, the cAMP content in spermatozoa has been demonstrated to be an accurate and sensitive indicator or sperm motility (Tash and Mann, 1973). Testing the effect of prostaglandins on spermatozoa is confounded by the fact the ejaculated spermatozoa have already been in contact with seminal fluid prostaglandins. Although many researchers wash spermatozoa after collection, endogenous prostaglandins most likely have already exerted their effects. Pento and coworkers (1970) found that the rate of glycolysis was significantly increased in epididymal sheep spermatozoa after exposure to either PGE_1 or $PGF_{2\alpha}$; however, washed ejaculated sheep spermatozoa failed to display this increase in glycolysis.

Addition of increasing amounts of $PGF_{2\alpha}$ to fresh boar semen, resulting in final concentrations of either 25, 50 or 100 μg/ml $PGF_{2\alpha}$, caused no change in sperm motility (Maes et al., 2003). Likewise, addition of $PGF_{2\alpha}$ to ram semen had no post-

thaw effect and improved conception of ewes inseminated with this $PGF_{2\alpha}$-enhanced semen (Gustafsson et al., 1975). When cyclooxygenase inhibitors were added to mouse semen there was no effect on motility, but the treatment resulted in decreased *in vitro* fertilization, and when added to guinea pig semen the acrosome reaction was decreased (Joyce et al., 1987). However, this diminished fertilization and acrosome reaction was overcome by the addition of a mixture of $PGF_{2\alpha}$ and PGE_2. Furthermore, there is a positive correlation between water buffalo seminal $PGF_{2\alpha}$ concentration and total and live sperm concentrations (Reddy et al., 1982). Kelly (1978) reported that prostaglandin concentrations in human semen also seem to be correlated with fertility, when control patients are compared to patients in which infertility cannot be otherwise explained, but prostaglandin concentration in this semen was not correlated with sperm count or motility. In contrast, Cohen and coworkers (1977) found that $PGF_{2\alpha}$ added to human semen at levels 10-fold greater than endogenous levels caused decreased motility, and similar results were reported by Didolkar and Roychowdhury (1980). Likewise, when 2,500 ng/ml of $PGF_{2\alpha}$ was added to semen collected from sub-fertile men, sperm motility was improved; however, when a superfluous amount (25,000 ng/ml) was added to similar semen this effect was reversed, resulting in no net improvement in sperm motility (Grunberger et al., 1981). Treatment of mouse sperm with indomethacin, an inhibitor of prostaglandin synthesis, resulted in a decrease in motility and *in vitro* fertilization rates that could be overcome by the addition of $PGF_{2\alpha}$; likewise, oocyte degeneration and polyspermic eggs increased after indomethacin treatment, but were improved by $PGF_{2\alpha}$ supplementation (Hayashi et al.,

1988). Therefore, it seems that in addition to sperm function, $PGF_{2\alpha}$ may also play a role in fertilization via the oocyte. Based on these data, it appears prostaglandins may have paradoxical effects on sperm function, whereas addition of prostaglandins at levels only slightly above endogenous levels may have no effect or a positive influence on sperm function, and addition at significantly higher levels may be detrimental. In fact, Aitken and Kelly (1985) found that human semen exposed to increasing concentrations of PGE_1 displayed a higher penetration rate of zone-free oocytes, but increasing concentrations of PGE_2 resulted in bell-shaped dose-response curve, and $PGF_{2\alpha}$ had no effect on the penetrating ability of the spermatozoa.

In vitro fertilization rate was not affected by amount of exogenous $PGF_{2\alpha}$ added to semen during the dilution process and was lower than rates reported by previous researchers (Thomas and Seidel, 1993; Keskintepe and Brackett, 1996; Watson et al., 2000). Lower fertilization rates than had previously been reported were most likely due to laboratory technician inexperience and were not affected by quantity of exogenous $PGF_{2\alpha}$ that had been added to semen.

In contrast to previous reports when $PGF_{2\alpha}$ was added to semen and pregnancy rates were improved in ewes (Gustafsson et al., 1975), and swine (Peña et al., 2000), semen utilized in the current study containing 500 or 5000 pg/ml supplementary $PGF_{2\alpha}$ did not significantly improve first service conception rate and resulted in a treatment x technician interaction. Conception rates for the two experienced technicians appeared to increase slightly with increasing semen $PGF_{2\alpha}$ concentrations. However, the less experienced technician attained the highest conception rates for

semen containing 0 and 5000 pg/ml of added $PGF_{2\alpha}$, and the lowest conception rate for semen containing 500 pg/ml added $PGF_{2\alpha}$. Because exogenous $PGF_{2\alpha}$ was not detrimental to the proportion of normal sperm or sperm motility after thawing and a slight increase in conception rate with $PGF_{2\alpha}$-enhanced semen was obtained by the experienced technicians, further research should be conducted to elucidate the quantity of $PGF_{2\alpha}$ that could be added to extended bovine semen without impairing post-thaw characteristics and potentially improve conception to AI.

Prostaglandin $F_{2\alpha}$ has been reported to elicit contractions in myometrium collected from sows (Yu et al., 1993), and cows during the proestrus (Patil et al., 1980) and diestrus (Hirsbrunner et al., 2003) phases. Patil and coworkers (1980) also noted that spontaneous uterine contractility was most pronounced during the follicular phase. Prostaglandin $F_{2\alpha}$ added to boar semen resulted in increased myometrial contractions in sows inseminated with this semen (Cheng et al., 2001). Willenburg and coworkers (2003) also reported increased number, but not amplitude or duration, of myometrial contractions in sows following insemination with $PGF_{2\alpha}$-supplemented semen.

Stolla and Schmid (1990) found differences for *in vivo* myometrial contractility between natural $PGF_{2\alpha}$ and its synthetic analogs, with the greatest response occurring after administration of natural $PGF_{2\alpha}$ to diestrus cows followed by proestrus and metestrus cows. But, it is interesting that these researchers reported that none of the types of $PGF_{2\alpha}$ utilized elicited a response when administered to cows in estrus. Prostaglandin $F_{2\alpha}$, but not PGE_2, administered s.c. immediately before AI or

added to semen before AI resulted in an increased fertilization rate of rabbits that underwent utero-tubal ligation 2.5-3 h after insemination compared to control animals receiving no exogenous $PGF_{2\alpha}$ (Spilman et al., 1973).

The oviducts of sexually mature animals display waves of contractility during all stages of the estrous cycle, but are usually the greatest in frequency and often amplitude near the time of ovulation (Hunter, 1977). Peristaltic waves of contraction in the isthmus proceed primarily toward the ampullary-isthmic junction, assisting spermatozoa movement to the site of fertilization, but the contractions tend to fade beyond the ampullary-isthmic junction (Blandau and Gaddum-Rosse, 1974). Prostaglandin E_2 caused contractions in the quarter of the fallopian tube proximal to the uterus and relaxation of the distal three quarters (Sandberg et al., 1963; Sandberg et al., 1964). In fact, the products of ovulation have been demonstrated to stimulate the movement of spermatozoa from the proximal isthmus to the site of fertilization (Harper, 1973). Hunter and coworkers (1983) found that the concentration of $PGF_{2\alpha}$ in samples collected from oviduct isthmus arterioles in gilts displaying estrus was greater than in corresponding peripheral samples, and speculated that it originated from the very high concentrations of $PGF_{2\alpha}$ present in the preovulatory and recently collapsed Graafian follicles, arriving at the isthmus arterioles by counter-current exchange from the ovarian vein to the oviductal artery. This counter-current exchange is opposite to that described by McCracken and coworkers (1971) in which $PGF_{2\alpha}$ released from the luminal epithelial cells of the uterus is transferred from the uterine vein to the ovarian artery. Intravenous administration of either PGE_2 or $PGF_{2\alpha}$

provoked uterine contractions in women, but these prostaglandins had opposing effects on fallopian tube activity, with PGE_2 causing a significant inhibition and $PGF_{2\alpha}$ causing a stimulation of motility (Coutinho and Maia, 1971). Based on these data, it could be surmised that $PGF_{2\alpha}$-induced uterine contraction would improve sperm transport.

Although not statistically significant, conception rate of heifers in the current study receiving an i.m. injection $PGF_{2\alpha}$ immediately after AI with sub-fertile (30% post-thaw motility) semen was dramatically improved compared to control heifers. Similarly, but using semen of normal fertility, Edqvist and coworkers (1975) found that $PGF_{2\alpha}$ either administered i.m. at the time of AI or added to diluted semen increased the number of sperm that could be recovered from the uterus and oviducts compared to ewes that did not receive exogenous $PGF_{2\alpha}$. Hawk and coworkers (1982) also reported increased numbers of spermatozoa in the oviducts of rabbits 2 h following AI and administration of $PGF_{2\alpha}$.

The fertility of repeat breeder sows is improved by the addition of $PGF_{2\alpha}$ to the semen before insemination (Horvat and Bilkei, 2003). Rather than improved sperm transport due to uterine contractions, Bilkei (1995) speculated that exogenous $PGF_{2\alpha}$ reaches the ovarian follicles by counter-current exchange and induces a more prompt ovulation. Injection of $PGF_{2\alpha}$ into the vulvar mucosa of sows improved farrowing rates during the low fertility season, but had little effect during the remainder of the year (Peña et al., 1998). Therefore, it appears that exogenous $PGF_{2\alpha}$ administered to animals with impaired fertility at the time of insemination may be more beneficial than

for animals with normal fertility. Low fertility and extended number of days open continues to plague dairy producers. In the current study, dairy cows receiving an i.m. injection of $PGF_{2\alpha}$ at the time of insemination displayed over a 2-fold increase in conception rate. In a somewhat similar study, cows receiving a 50 μg i.v. injection of $PGF_{2\alpha}$ (Estrumate, Schering-Plough Coopers, Wellington, NZ) diluted to 2 ml in bi-distilled water immediately after AI displayed a significantly higher conception rate compared to cows receiving a 2 ml i.v. injection of bidistilled water after AI (64.1 vs. 48.9%, respectively; Prinzen et al., 1991).

These data suggest that exogenous $PGF_{2\alpha}$ administered to cattle at the time of insemination may benefit sperm transport and improve conception. Further research should be conducted to validate the responses observed in the current research and determine if the method of estrous synchronization will influence the effectiveness of post-insemination $PGF_{2\alpha}$ administration.

IMPLICATIONS

Results of the current research suggest that administration of $PGF_{2\alpha}$ at the time of insemination of cattle may improve reproductive performance, similar to that previously reported for rabbits, sheep, and swine. This cost effective treatment to improve first service conception rate could significantly promote the use of artificial insemination among beef herds and improve the reproductive performance of dairy cows.

LITERATURE CITED

Abbitt, B., G.E. Seidel, Jr., and W.E. Berndtson. 1977. Effect of tris (hydroxymethyl) aminomethane salt of prostaglandin $F_{2\alpha}$ on post-thaw motility of bovine spermatozoa. J. Dairy Sci. 60:1991-1993.

Aitken, R.J., and R.W. Kelly. 1985. Analysis of the direct effects of prostaglandins on human sperm function. J. Reprod. Fertil. 73:139-146.

Bilkei, G. 1995. Herd health strategy for improving the reproductive performance of pigs. Hung. Vet. J. 10:766-768

Blandau, R.J., and P. Gaddum-Rosse. 1974. Mechanism of sperm transport in pig oviducts. Fertil. Steril. 25:61-67.

Cheng, H., G.C. Althouse, and W.H. Hsu. 2001. Prostaglandin $F_{2\alpha}$ added to extended boar semen at processing elicits *in vitro* myometrial contractility after 72 hours of storage. Theriogenology 55:1901-1906.

Cohen, M.S., M.J. Colin, M. Colimbu, and R.S. Hotchkiss. 1977. The effects of prostaglandins on sperm motility. Fertil. Steril. 28:78-85.

Coutinho, E.S., and H.S. Maia. 1971. The contractile response of the human uterus, fallopian tubes, and ovary to prostaglandins in vivo. Fertil. Steril. 22:539-543.

Didolkar, A.K., and D. Roychowdhury. 1980. Effects of prostaglandins E-1, E-2, F-1a and F-2a on human sperm motility. Andrologia 12:135-140.

Edqvist, S., S. Einarsson, and B. Gustafsson. 1975. Effect of prostaglandin $F_{2\alpha}$ on sperm transport in the reproductive tract of the ewe. Acta Vet. Scand. 16:149-151.

Foote, R.H., C.C. Brockett, and M.T. Kaproth. 2002. Motility and fertility of bull sperm in whole milk extender containing antioxidants. Anim. Reprod. Sci. 71:13-23.

Foote, R.H., and M.T. Kaproth. 2002. Large batch freezing of bull semen: effect of time of freezing and fructose on fertility. J. Dairy Sci. 85:453-456.

Garbers, D.L., W.D. Lust, N.L. First, and H.A. Lardy. 1971. Effects of phosphodiesterase inhibitors and cyclic nucleotides on sperm respiration and motility. Biochemistry 10:1825-1831.

Grunberger, W., U. Maier, and G. Lunglmayr. 1981. Effect on prostaglandin F 2 alpha on sperm motility in vitro. Reproduccion 5:141-144.

Gustafsson, B., S. Edqvist, and S. Einarsson. 1975. The fertility of deep-frozen ram semen supplemented with $PGF_{2\alpha}$. Acta Vet. Scand. 16:468-470.

Harper, M.J.K. 1973. Stimulation of sperm movement from the isthmus to the site of fertilization in the rabbit oviduct. Biol. Reprod. 8:369-377.

Hawk, H.W., B.S. Cooper, and H.H. Conley. 1982. Effect of acetylcholine, prostaglandin $F_{2\alpha}$ and estradiol on number of sperm in the reproductive tract of inseminated rabbits. J. Anim. Sci. 55:891-900.

Hayashi, S., Y. Noda, and T. Mori. 1988. Analysis of the role of prostaglandins in the fertilization process. Eur. Obstet. Gynecol. Reprod. Biol. 29:287-297.

Hirsbrunner, G., B. Knutti, U. Küpfer, H. Burkhardt, and A. Steiner. 2003. Effect of prostaglandin E_2, DL-cloprostenol, and prostaglandin E_2 in combination with D-cloprostenol on uterine motility during diestrus in experimental cows. Anim. Reprod. Sci. 79:17-32.

Horvat, G., and G. Bilkei. 2003. Exogenous prostaglandin $F_{2\alpha}$ at time of ovulation improves reproductive efficiency in repeat breeder sows. Theriogenology 59:1479-1484.

Hunter, R.H.F. 1977. Function and malfunction of the fallopian tubes in relation to gametes, embryos and hormones. Europ. J. Obstet. Gynec. Reprod. Biol. 7:267-283.

Hunter, R.H.F., B. Cook, and N.L. Poyser. 1983. Regulation of oviduct function in pigs by local transfer of ovarian steroids and prostaglandins: a mechanism to influence sperm transport. Europ. J. Obstet. Gynec. Reprod. Biol. 14:225-232.

Joyce, C.L., N.A. Nuzzo, L. Wilson, Jr., and L.J.D. Zaneveld. 1987. Evidence for a role of cyclooxygenase (prostaglandin synthetase) and prostaglandins in the sperm acrosome reaction and fertilization. J. Androl. 8:74-82.

Kalloo, F.A., N.A. Wani, M.Z. Khan, and G.M. Wani. 2003. Effects of partial to complete removal of seminal plasma on cryopreservation of bovine semen. Indian J. Anim. Sci. 73:63-65.

Kelly, R.W. 1977. Effect of seminal prostaglandins on the metabolism of human spermatozoa. J. Reprod. Fertil. 50:217-222.

Kelly, R.W. 1978. Prostaglandins in semen: their occurrence and possible physiological significance. Int. J. Androl. 1:188-200.

Keskintepe, L., and B.G. Brackett. 1996. In vitro development competence of in vitro-matured bovine oocytes fertilized and cultured in completely defined media. Biol. Reprod. 55:333-339.

Lamb, G.C., J.S. Stevenson, D.J. Kesler, H.A. Garverick, D.R. Brown, and B.E. Salfen. 2001. Inclusion of an intravaginal progesterone insert plus GnRH and prostaglandin $F_{2\alpha}$ for ovulation control in postpartum suckled beef cows. J. Anim. Sci. 79:2253-2259.

Maes, D.G.D., B. Mateusen, T. Rijsselaere, S. De Vliegher, A. Van Soon, and A. de Kruif. 2003. Motility characteristics of boar spermatozoa after addition of prostaglandin $F_{2\alpha}$. Theriogenology 60:1435-1443.

Marsh, J.M., and W.J. LeMaire. 1974. Cyclic AMP accumulation and steroidogenesis in the human corpus luteum: effect of gonadotropins and prostaglandins. J. Clin. Endocrinol. Metab. 38:99-106.

McCracken, J.A., D.T. Baird, and J.R. Goding. 1971. Factors affecting the secretion of steroids from the transplanted ovary in the sheep. Recent Progr. Horm. Res. 27:527-582.

National Animal Health Monitoring System. 1998. Part III: reference of 1997 beef cow-calf production management and disease control. USDA-APHIS, Washington, DC.

Parkinson, T.J. 2004. Evaluation of fertility and infertility in natural service bulls. Vet. J. 168:215-229.

Parrish, J.J., J.L. Susko-Parrish, M.L. Leibfried-Rutledge, E.S. Crister, W.H. Eyestone, and N.L. First. 1986. Bovine *in vitro* fertilization with frozen-thawed sperm. Theriogenology 25:591-600.

Patil, R.K., S.N. Sinha, S. Einarsson, and I. Settergren. 1980. The effect of prostaglandin $F_{2\alpha}$ and oxytocin on bovine myometrium in vitro. Nord. Vet.-Med. 32:474-479.

Peña, F.J., J.C. Domínguez, B. Alegre, and J. Peláez. 1998. Effect of vulvomucosal injection of $PGF_{2\alpha}$ at insemination on subsequent fertility and litter size in pigs under field conditions. Anim. Reprod. Sci. 52:63-69.

Peña, F.J., J.C. Domínguez, J. Pelá, and B. Alegre. 2000. Intrauterine infusion of $PGF_{2\alpha}$ at insemination enhances reproductive performance of sows during the low fertility season. The Vet. J. 159:259-261.

Pento, J.T., R.J. Cenedella, and E.K. Inskeep. 1970. Effects of prostaglandins E_1 and $F_{2\alpha}$ upon carbohydrate metabolism of ejaculated and epididymal spermatozoa in vitro. J. Anim. Sci. 30:409-411.

Prinzen, R., F. Allgayer, U. Bartz, and E. Huber. 1991. Effects of prostaglandin $F_{2\alpha}$ on conception results of heifers and cows. Tierärtzliche Umschau 1:27-35.

Pursley, J. R., M. O. Mee, and M. C. Wiltbank. 1995. Synchronization of ovulation in dairy cows using $PGF_{2\alpha}$ and GnRH. Theriogenology 44:915.

Ramwell, P., and J.E Shaw. 1971. The biological significance of the prostaglandins. Ann. NY Acad. Sci. 180:10-13.

Reddy, B.B., A.S.N. Murty, V.S.N. Rao, P.N. Rao, and K.S. Rao. 1982. Seminal prostaglandins E_2 and F_2 alpha in Murrah buffalo bulls. J. Nuclear Agri. Biol. 11:126-127.

Reyes-Moreno, C., M. Boilard, R. Sullivan, and M.-A. Sirard. 2002. Characterization and identification of epididymal factors that protect ejaculated bovine sperm during in vitro storage. Biol. Reprod. 66:159-166.

Sandberg, F., A. Ingelman-Sundberg, and G. Rydén. 1963. The effect of prostaglandin E_1 on the human uterus and the fallopian tubes in vitro. Acta Obstet. Gynecol. Scand. 42:269-278.

Sandberg, F., A. Ingelman-Sundberg, and G. Rydén. 1964. The effect of prostaglandin E_2 and E_3 on the human uterus and fallopian tubes in vitro. Acta Obstet. Gynecol. Scand. 43:95-102.

Spilman, C.H., A.E. Finn, and J.F. Norland. 1973. Effect of prostaglandins on sperm transport and fertilization in the rabbit. Prostaglandins 4:57-64.

Stolla, R., and G. Schmid. 1990. Effects of natural and synthetic PGF2 alpha preparations on the uterine contractility of cattle. Berl. Munch. Tierarztl. Wochenschr. 103:198-202.

Tash, J.S., and F.R.S. Mann. 1973. Adenosine 3':5'-cyclic monophosphated in relation to motility and sequence of spermatozoa. Proc. R. Soc. Lond. Biol. 184:109-114.

Thomas, W.K., and G.E. Seidel, Jr. 1993. Effects of cumulus cells on culture of bovine embryos derived from oocytes matured and fertilized in vitro. J. Anim. Sci. 71:2506-2510.

Thun, R., M. Hurtado, and F. Janett. 2002. Comparison of Biociphos-Plus® and TRIS-egg yolk extender for cryoperservation of bull semen. Theriogenology 57:1087-1094.

USDA. 1998. Reproductive technology in beef cow-calf herds. Available : http://www.aphis.usda.gov/vs/ceah/ncahs/nahms/beefcowcalf/beef97/bf97repro.pdf. Accessed Sept. 8, 2005.

USDA. 2005. Cattle. National Agricultural Statistics Service. Available : http://usda.mannlib.cornell.edu/reports/nassr/livestock/pct-bb/catl0705.pdf. Accessed Sept. 7, 2005.

Voglmayr, J.K. 1973. Prostaglandin $F_{2\alpha}$ concentration in genital tract secretions of dairy bulls. Prostaglandins 4:673-678.

Watson, A.J., P. De Sousa, A. Caveney, L.C. Barcroft, D. Natale, J. Urquhart, and M.E. Westhusin. 2000. Impact of bovine oocyte maturation media on oocyte transcript levels, blastocyst development, cell number, and apoptosis. Biol. Reprod. 62:355-364.

Watson, A.J., P.H. Watson, D. Warnes, S.K. Walker, D.T. Armstrong, and R.F. Seamark. 1994. Preimplantation development of in vitro-matured and in vitro-fertilized ovine zygotes: comparison between coculture on oviduct epithelial cell monolayers and culture under low oxygen atmosphere. Biol. Reprod. 50:715-724.

Wiemer, K.E., A.J. Watson, V. Polanski, A.I. McKenna, G.H. Fick, and G.A. Schultz. 1991. Effects of maturation and coculture treatments on the developmental capacity of early bovine embryos. Mol. Reprod. Dev. 30:330-338.

Willenburg, K.L., G.M. Miller, S.L. Rodriguez-Zas, and R.V. Knox. 2003. Influence of hormone supplementation to extended semen on artificial insemination, uterine contractions, establishment of a sperm reservoir, and fertility in swine. J. Anim. Sci. 81:821-829.

Woelders, H., A. Mathiijs, and B. Engel 1997. Effects of trehalose, and sucrose, osmolarity of the freezing medium, and cooling rate on viability and intactness of bull sperm after freezing and thawing. Cryobiology 35:93-105.

Yates, J.H., J.E. Chandler, A.L. Canal, and J.B. Paul.2003. The effect of nocturnal sampling on semen quality and the efficiency of collection in bovine species. Theriogenology 60:1665-1667.

Yu, H., D.-H. Shin, and W.H. Hsu. 1993. Effects of oxytocin (OT), vasopressin (VP), and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) on porcine myometrial contractility in vitro and their dependence on Ca^{2+} influx. Biol. Reprod. 48(Suppl.):162. (Abstr.)

Zhang, B.R., B. Larsson, N. Lundeheim, M.G.H. Håård, and H. Rodriguez-Martinez. 1999. Prediction of bull fertility by combined *in vitro* assessments of frozen-thawed semen from young dairy bulls entering and AI-programme. Int. J. Androl. 22:253-260.

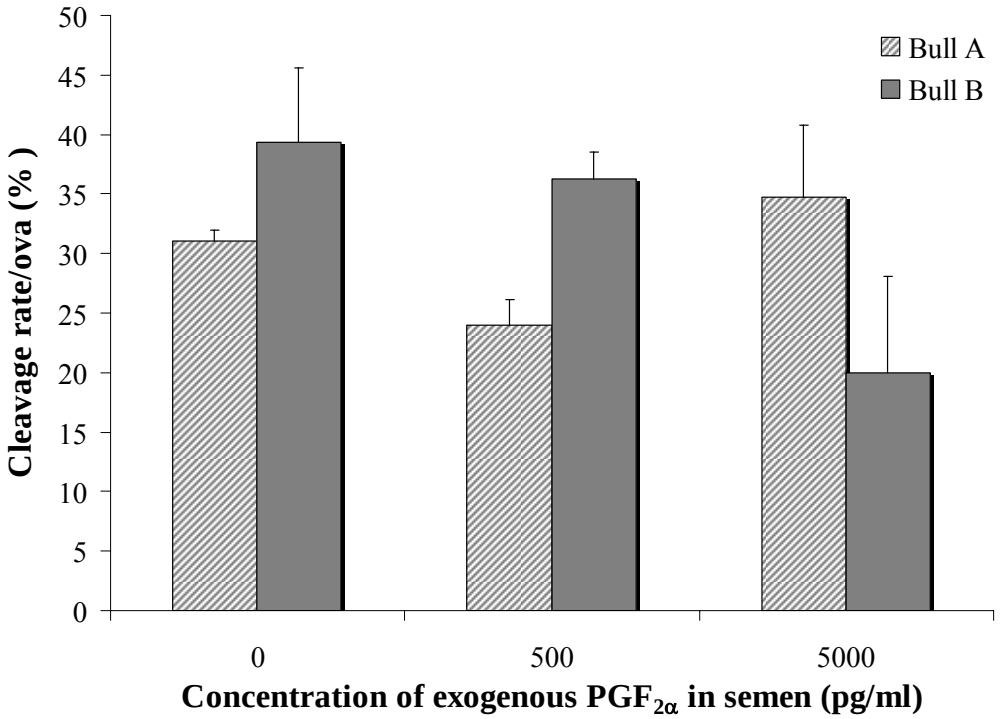

Figure 3.1. Cleavage rate of ova following fertilization with semen extended to contain either 0, 500, or 5000 pg/ml prostaglandin $F_{2\alpha}$. Semen treatment x bull interaction (P = 0.09).

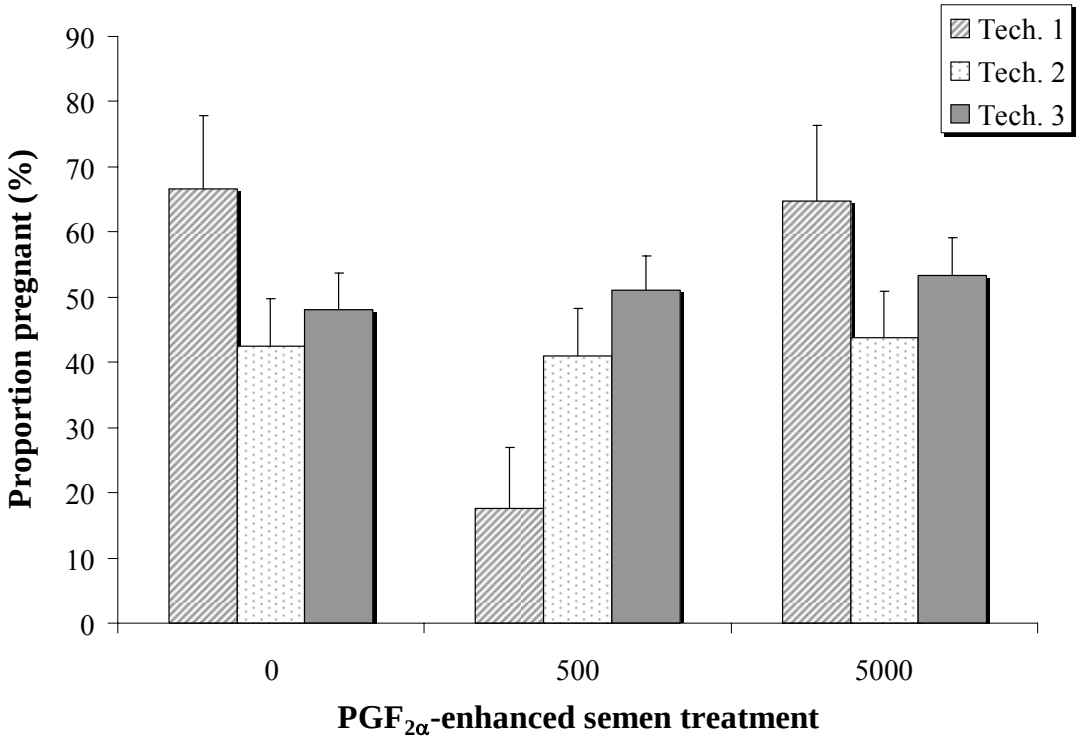

Figure 3.2. Proportion of cows conceiving to insemination with semen containing either 0, 500, or 5000 pg/ml exogenous $PGF_{2\alpha}$. Semen treatment x technician interaction (P = 0.01).

GENERAL CONCLUSION

Endogenous concentration of $PGF_{2\alpha}$ in semen collected from beef and dairy bulls in this study averaged 255.5 ± 29.8 pg/ml as determined by enzyme immunoassay. This value is on the lower range of $PGF_{2\alpha}$ concentrations previously reported for the bull (0.170 pg/ml, Voglmayr, 1973; 1170 pg/ml, Ledwozyw et al., 1986), and 7.5-fold less than previously reported for the water buffalo (Reddy et al., 1982). These inconsistent values for seminal $PGF_{2\alpha}$ concentrations suggest that further evaluation is needed. In addition, in this study, as well as previous studies, a small number of bulls were utilized and little is known about the variability between individuals. In fact, although not statistically significant (P = 0.12), seminal concentrations of $PGF_{2\alpha}$ tended to be greater in semen collected from beef compared to dairy bulls (273.8 ± 29.8 vs. 210 ± 18.5 pg/ml, respectively). Additionally, season of year and bull age may affect seminal prostaglandin concentrations, and seminal prostaglandin concentrations may decrease with subsequent ejaculations.

Observation of a significantly higher $PGF_{2\alpha}$ concentration in extended bovine semen, than was calculated to be present, led to the observation of $PGF_{2\alpha}$ synthesis during the extension process. It is not surprising that $PGF_{2\alpha}$ synthesis occurred considering that the necessary precursors and enzymes are present in semen and the diluent. Shalev and coworkers (1994) reported that cyclooxygenase 1, or prostaglandin H synthase-1, which catalyzes the conversion of arachidonic acid to prostaglandin H_2, is localized in the apical region of the spermatozoa head, the post-

acrosomal region and the mid-piece of the tail, and demonstrated that intact bovine spermatozoa can synthesize prostaglandins. Chicken egg yolk, the major component in Fraction A of the diluent utilized in this study, contains phospholipids which are composed 80% phosphatidylcholine and 15% phosphatidylethanolamine (Yoon and Kim, 2002). Bovine seminal plasma lipids are composed of 30% phosphatidylcholine and 10.5% phosphatidylethanolamine, and bovine spermatozoa lipids are composed of 35.6% phosphatidylcholine and 20.0% phosphatidylethanolamine (Pursel and Graham, 1967). Both of these phospholipids can be acted on by phospholipase A_2 (PLA_2) to yield arachidonate. Phospholipase A_2 has been identified in ram spermatozoa membranes (Hinkovska et al., 1987), and bovine spermatozoa have been reported to contain protein kinase C (PKC) activity, which activates PLA_2, comparable to that observed for human spermatozoa (Breitbart et al., 1992). The observation of $PGF_{2\alpha}$ synthesis during semen extension has not been reported in other species and, if absent, may explain why improved fertility has been observed following addition of $PGF_{2\alpha}$ to ram (Gustafsson et al., 1975) and boar (Peña et al., 2000; Horvat and Bilki, 2003; Kos and Bilkei, 2004) semen.

Although dilution rate, in the normal range for bovine semen, did not affect the quantity of $PGF_{2\alpha}$ synthesized during the extension process, the quantity of seminal plasma in the diluted semen may have an effect on post-thaw spermatozoa characteristics. The amount of supplementary seminal plasma present has been demonstrated to affect viability and motility of fresh rabbit sperm (Castellini et al., 2000), binding rate of zona pellucida proteins to fresh boar spermatozoa (Harkema et al., 2004), heterogeneity and viability of frozen-thawed ram semen (Ollero et al.,

1997), and post-thaw motility, number of capacitated sperm, number of acrosome reacted cells and pregnancy rate following cervical insemination of frozen-thawed ram semen (Maxwell et al., 1999). Supplementary bovine seminal plasma added to caudal epididymal sperm resulted in improved *in vitro* penetration of zona-free oocytes after heterospermic insemination (Henault et al., 1995). Addition of seminal plasma to bovine semen diluted to low cell numbers per insemination dose also improved the post-thaw viability of this semen (Garner et al., 2001). Since bovine seminal plasma contains a significant source of phospholipids, supplementary addition of this fluid may also increase prostaglandin synthesis during extension.

Because fertility of other species has been improved when $PGF_{2\alpha}$ was added to semen before insemination, it would be expected that the initial concentration of $PGF_{2\alpha}$ in extended bovine semen would be related to fertility. However, the concentration of $PGF_{2\alpha}$ in extended bovine semen was not correlated to a "Relative Fertility Rating" derived from non-return data, but the slope of the line suggested that increasing seminal $PGF_{2\alpha}$ concentrations may be correlated to lower fertility. Similar findings were reported for ejaculates collected from fertile and infertile men, wherein $PGF_{2\alpha}$ was negatively correlated with motility in normal men and was always higher in men with disturbed fertility (Schlegel et al., 1981). In contrast, $PGF_{2\alpha}$ concentration in whole semen collected from water buffalo did seem to be positively correlated to total and live sperm concentration, but this relationship did not exist for PGE_2 (Reddy et al., 1982). In the current study, these data were based on a single sample obtained from a limited number of animals, and the Relative Fertility Rating

was based on numerous years of non-return data. Additionally, all samples were obtained from bulls utilized by a commercial AI organization and these companies typically utilize animals with high fertility. If animal fertility, season of year, age of animal, or frequency of semen collection affects seminal $PGF_{2\alpha}$ concentrations, the current data could be misinterpreted. Therefore, further examination of seminal $PGF_{2\alpha}$ concentrations is warranted and caution should be used to account for fertility, breed of animal, season of sample collection, time since previous collection, and animal age.

In contrast, PGE_2 concentration in extended bovine semen was not correlated to the Relative Fertility Rating. In the human, PGE_2 was elevated only in men with persisting varicocele, low sperm counts, and severely impaired motility (Schlegel et al., 1981) and either lower or higher than normal PGE_2 seminal concentrations are associated with decreased sperm concentration and motility (Isodori, et al., 1980). In addition, men with idiopathic infertility have lower seminal PGE_2 concentrations compared to fertile men (Bygdeman et al., 1970; Collier et al., 1975). If PGE_2 has similar effects on sperm function and fertility in the bull, it is likely that animals displaying inconsistent PGE_2 concentrations would not be utilized by the company where these samples were obtained, and therefore, based on the current data it would appear that PGE_2 is not correlated to fertility.

The ratio of PGE_2 to $PGF_{2\alpha}$ was also not correlated to the Relative Fertility Rating, but the slope of the line appeared to suggest the as the ratio of these prostaglandins increased, the Relative Fertility Rating increased. Perhaps, the ratio of these two opposing prostaglandins is more important than the individual concentration

of either $PGF_{2\alpha}$ or PGE_2. When $PGF_{2\alpha}$ or PGE_2 were administered individually or in combination to cows during diestrus, the greatest increase in intrauterine pressure occurred when these prostaglandins were administered in combination (Hirsbrunner et al., 2003). Prostaglandin $F_{2\alpha}$ has been reported to elicit contractions in myometrium collected from sows (Yu et al., 1993), and cows during the proestrus (Patil et al., 1980) and diestrus (Hirsbrunner et al., 2003) phases. Prostaglandin $F_{2\alpha}$ added to boar semen resulted in increased myometrial contractions in sows inseminated with this semen (Cheng et al., 2001). Willenburg and coworkers (2003) also reported increased number, but not amplitude or duration, of myometrial contractions in sows following insemination with $PGF_{2\alpha}$-supplemented semen. This increased number of uterine contractions has been reported to cause higher sperm numbers to reach the upper portions of the reproductive tract in rabbits (Spilman et al., 1973), ewes (Edqvist et al., 1975), and sows (Willenburg et al., 2003). Kelly (1978) also speculated that the ratio of these opposing prostaglandins may be important to human fertilization rate, in that $PGF_{2\alpha}$ may promote the movement of an increased number of spermatozoa to the site of fertilization by stimulating uterine contractions and PGE_2 may cause relaxation of the utero-tubal junction to allow an increased passage rate of spermatozoa. However, the same limitations of animal number, single sample obtained on a specific day of the year and animal age apply to these data; additional samples should be examined for seminal $PGF_{2\alpha}$ and PGE_2 concentrations to validate these data and conclusions. Other components of bovine seminal plasma, including concentrations of prostaglandin D-synthase (Fouchécourt et al., 2002), interleukin-10 (Denison et al., 1999), and relaxin

(Kohsaka et al., 2003), and sperm with greater binding affinity for heparin-binding proteins (Bellin et al., 1994), have been identified that appear to be indicators of bull fertility. However, few if any of these methods are utilized on a broad-scale or frequent basis. Possibly, further evaluation of endogenous prostaglandin concentrations, as compared to fertility, will result in the validation of a method to accurately predict bull fertility.

In contrast to a previous report, when final $PGF_{2\alpha}$ concentrations in extended bovine $PGF_{2\alpha}$-supplemented semen were reported to be either 75, 225 or 675 μg/ml (Abbitt et al., 1977), addition of $PGF_{2\alpha}$, at either 500 or 5,000 pg/ml of $PGF_{2\alpha}$ to extended bovine semen did not affect post-thaw motility immediately after thawing, after 180 min of incubation, or *in vitro* fertilization rate. Similarly, addition of increasing amounts of $PGF_{2\alpha}$ to fresh boar semen, resulting in final concentrations of either 25, 50 or 100 μg/ml $PGF_{2\alpha}$, caused no change in sperm motility (Maes et al., 2003). Likewise, addition of $PGF_{2\alpha}$ to ram semen had no post-thaw effect and improved conception of ewes inseminated with this $PGF_{2\alpha}$-enhanced semen (Gustafsson et al., 1975). Abbitt and coworkers (1977) final concentrations of $PGF_{2\alpha}$ in extended bovine semen was nearly a million-fold greater than endogenous concentration of $PGF_{2\alpha}$ previously reported for bovine semen; it was determined that this superfluous quantity of $PGF_{2\alpha}$ was detrimental to sperm function. An excessive quantity of $PGF_{2\alpha}$ added to human semen also resulted in significantly decreased motility (Cohen et al., 1977; Didolkar and Roychowdhury 1980). Likewise, when 2,500 ng/ml of $PGF_{2\alpha}$ was added to semen collected from subfertile men, sperm

motility was improved; however, when that amount was increased 10-fold (25,000 ng/ml) and was added to similar semen this effect was reversed, resulting in no net improvement in sperm motility (Grunberger et al., 1981). However, a threshold quantity of $PGF_{2\alpha}$ does appear to be necessary for normal sperm function. Cyclooxygenase inhibitors added to mouse semen had no effect on motility, but caused decreased *in vitro* fertilization, and reduced the acrosome reaction when added to guinea pig semen (Joyce et al., 1987); however, these negative effects were overcome by the addition of a mixture of $PGF_{2\alpha}$ and PGE_2. In addition, treatment of mouse sperm with indomethacin, an inhibitor of prostaglandin synthesis, decreased sperm motility and *in vitro* fertilization rate that could also be overcome by the addition of $PGF_{2\alpha}$ (Hayashi et al., 1988). Therefore, it appears that $PGF_{2\alpha}$ may have paradoxical effects on sperm function, in that the reduction of seminal $PGF_{2\alpha}$ concentration may decrease sperm function, but addition of prostaglandins at levels only slightly above endogenous levels may either have no effect or a beneficial influence on sperm function, and addition at significantly higher levels may be detrimental to sperm function. A dose-response experiment should be conducted, which includes the previous supplementary levels, to elucidate the quantity of supplementary $PGF_{2\alpha}$ that could be added to extended bovine semen without causing a reduction in post-thaw motility or fertilization capability.

Analysis of first service conception rates of cows inseminated with either 0, 500, or 5000 pg/ml supplementary $PGF_{2\alpha}$ revealed a treatment x technician interaction. Further examination of these data revealed that conception rates for the

two experienced technicians appeared to increase slightly with increasing semen $PGF_{2\alpha}$ concentrations. However, the less experienced technician attained the highest conception rates for semen containing 0 and 5000 pg/ml of added $PGF_{2\alpha}$, and the lowest conception rate for semen containing 500 pg/ml added $PGF_{2\alpha}$. In contrast to previous reports when $PGF_{2\alpha}$ was added to semen and pregnancy rates were improved in ewes (Gustafsson et al., 1975) and swine (Peña et al., 2000), semen utilized in the current study containing 500 or 5000 pg/ml supplementary $PGF_{2\alpha}$ did not significantly improve first service conception rate and resulted in a treatment x technician interaction. Because exogenous $PGF_{2\alpha}$ was not detrimental to the proportion of normal sperm or sperm motility after thawing and a slight increase in conception rate with $PGF_{2\alpha}$-enhanced semen was obtained by the experienced technicians, further research should be conducted to elucidate the quantity of $PGF_{2\alpha}$ that could be added to extended bovine semen without impairing post-thaw characteristics and potentially improve conception to AI.

Conception rate of heifers receiving an i.m. injection of $PGF_{2\alpha}$ immediately after AI with sub-fertile (30% post-thaw motility) semen was dramatically improved compared to control heifers that did not receive a post-breeding treatment. Similarly, but using semen of normal fertility, $PGF_{2\alpha}$ administered i.m. at the time of AI or added to diluted semen just before AI increased the number of sperm that could be recovered from the uterus and oviducts compared to ewes that did not receive exogenous $PGF_{2\alpha}$ (Edqvist et al., 1975). Increased numbers of sperm in the upper segments of the reproductive tract following $PGF_{2\alpha}$ administration near the time of

insemination have also been reported for the rabbit (Spilman et al., 1973; Hawk et al., 1982), and sow (Willenburg et al., 2003). These reports of increased number of sperm in the upper segments of the reproductive tract are most likely the result of increased myometrial contractions. Prostaglandin $F_{2\alpha}$ has been reported to increase myometrial contractility in sows (Yu et al., 1993; Willenburg et al., 2003), cows during the proestrus (Patil et al., 1980) and diestrus (Hirsbrunner et al., 2003) phases, and women (Coutinho and Maia, 1971). By inducing myometrial contractions, it appears that more sperm were transported to the site of fertilization in the current study. However, to date there have been no reports of the effect of $PGF_{2\alpha}$ administration on the transport of sperm in the cow.

Dairy cows receiving an i.m. injection of $PGF_{2\alpha}$ at the time of insemination of displayed a 2-fold increase in conception rate compared to cows that did not receive a $PGF_{2\alpha}$ injection at this time. Similarly, cows receiving an i.v. injection of $PGF_{2\alpha}$ immediately after AI displayed a significantly higher conception rate compared to cows receiving a 2 ml i.v. injection of bi-distilled water after AI (Prinzen et al., 1991). Additionally, a similar increase in fertility was observed after injection of $PGF_{2\alpha}$ at the time of AI in rabbits (Hawk et al., 1982), ewes (Edqvist et al., 1975), and sows (Peña et al., 1998; Horvat and Bilkei, 2003) Similarly, although administered via the semen, $PGF_{2\alpha}$-supplemented semen increased fertility in rabbits (Spilman et al., 1973), ewes (Edqvist et al., 1975; Gustafsson et al., 1975), and sows (Peña et al., 2000; Horvat and Bilki, 2003; Kos and Bilkei, 2004).

Collectively, these data suggest an important role for seminal $PGF_{2\alpha}$ in reproduction. Although animals in the current studies were estrous synchronized with the Cosynch+CIDR and Ovsynch systems, further research should be conducted to determine if type of estrous synchronization system utilized will affect the effectiveness of $PGF_{2\alpha}$ treatment at the time of insemination. In addition, only limited numbers of animals were available for the experiments examining the effectiveness of $PGF_{2\alpha}$ injection at the time of insemination. Further research should be conducted to validate the apparent improvement in fertility following post-AI $PGF_{2\alpha}$ administration.

BIBLIOGRAPHY

Abbitt, B., G.E. Seidel, Jr., and W.E. Berndtson. 1977. Effect of tris (hydroxymethyl) aminomethane salt of prostaglandin $F_{2\alpha}$ on post-thaw motility of bovine spermatozoa. J. Dairy Sci. 60:1991-1993.

Aitken, R.J., and R.W. Kelly. 1985. Analysis of the direct effects of prostaglandins on human sperm function. J. Reprod. Fertil. 73:139-146.

Anderson, M.W., and T.E. Eling. 1976. Prostaglandin removal and metabolism by isolated perfused rat lung. Prostaglandins 11:645-677.

Armstrong, D.T., and D.L. Grinwich. 1972. Blockade of spontaneous and LH-induced ovulation in rats by indomethacin, an inhibition of prostaglandin biosynthesis. Prostaglandins 1:21-28.

Armstrong, D.T., D.L. Grinwich, Y.S. Moon, and J. Zamecnik. 1974. Inhibition of ovulation in rabbits by intrafollicular injection of indomethacin and prostaglandin F antiserum. Life Sci. 14:129-140.

Aron, C.L., G. Asch, and J. Roos. 1966. Triggering of ovulation by coitus in the rat. Int. Rev. Cytol. 20:139-172.

Arosh, J.A., S.K. Banu, S. Kimmins, P. Chapdelaine, L.A. McLaren, and M.A. Fortier. 2004. Effect of interferon-τ on prostaglandin biosynthesis, transport, and signaling at the time of maternal recognition of pregnancy in cattle: evidence of polycrine actions of prostaglandin E_2. Endocrinology 145:5280-5293.

Bagna, B., C. Schwabe, and L.L. Anderson. 1991. Effect of relaxin on facilitation of parturition in dairy heifers. J. Reprod. Fertil. 91:1991-1993.

Barcikowski, B., J.C. Carlson, L. Wilson, and J.A. McCracken. 1974. The effect of endogenous and exogenous estradiol-17β on the release of prostaglandin $F_{2\alpha}$ from the ovine uterus. Endocrinology 95:1340-1349.

Barrios, B., R. Pérez-Pé, M. Gallego, A. Tato, J. Osada, T. Muiño-Blanco. 2000. Seminal plasma proteins revert cold-shock damamge on ram sperm membrane. Biol. Reprod. 63:1531-1537.

Bass, J.W., P.C. Molan, and P. Shannon. 1983. Factors in seminal plasma that affect the viability and motility of spermatozoa. Reprod. Fertil. 68:275-280.

Bellin, M.E., H.E. Hawkins, and R.L. Ax. 1994. Fertility of range beef bulls grouped according to presence or absence of heparin-binging proteins in sperm membranes and seminal fluid. J. Anim. Sci. 72:2441-2448.

Bergström, S., H. Danielsson, and B. Samuelsson. 1964. The enzymatic formation of prostaglandin E_2 from arachidonic acid prostaglandins and related factors 32. Biochim. Biophys. Acta 90:207-210.

Bergström, S., and J. Sjövall. 1960a. The isolation of prostaglandin E from sheep prostate glands. Acta Chem. Scand. 14:1701-1705.

Bergström, S., and J. Sjövall. 1960a. The isolation of prostaglandin F from sheep prostate glands. Acta Chem. Scand. 14: 1693-1700.

Berridge, M.J., and R.F. Irvine. 1984. Inositol trisphosphate, a novel second messenger in cellular signal transduction. Nature 321:315-321.

Bilkei, G. 1995. Herd health strategy for improving the reproductive performance of pigs. Hung. Vet. J. 10:766-768

Bjersing, L., and S. Cajander. 1975. Ovulation and the role of the ovarian surface epithelium. Experientia 31:605-608.

Blandau, R.J., and P. Gaddum-Rosse. 1974. Mechanism of sperm transport in pig oviducts. Fertil. Steril. 25:61-67.

Bonnin, P., L. Huynh, R. L'Haridon, N Chene, and J. Martal. 1999. Transport of uterine $PGF_{2\alpha}$ to the ovaries by systemic circulation and local lymphovenous-arterial diffusion during luteolysis in sheep. J. Reprod. Fertil. 116:199-210.

Brännström, M., R.D. Koos, W.J. LeMaire, and P.O. Janson. 1987. Cyclic adenosine 3',5'-monophosphate-induced ovulation in the perfused rat ovary and its mediation by prostaglandins. Biol. Reprod. 37:1047-1053.

Breitbart, H., J. Lax, R. Rotem, and Z. Naor. 1992. Role of protein kinase C in the acrosome reaction of mammalian spermatozoa. Biochem. J. 281:473-476.

Bronson, R.A. 1999. Antisperm antibodies: a critical evaluation and clinical guidelines. J. Reprod. Ummunol. 45:159-183.

Bygdeman, M. 1969. Prostaglandins in human seminal fluid and their correlation to fertility. Int. J. Fertil. 14:228-231.

Bygdeman, M., B. Fredricsson, K. Svonbirg, and B. Samuelsson. 1970. The relationship between fertility and prostaglandin content of seminal fluid in men. Fertil. Steril. 21:622-629.

Bygdeman, M., and O. Holmberg. 1966. Isolation and identification of prostaglandins from ram seminal plasma. Acta Chem. Scand. 20:2308-2310.

Cajander, S., and L. Bjersing. 1976. Further studies on the epithelium covering preovulatory rabbit follicles with special reference to lysosomal alterations. Cell and Tissue Research 169:129-141.

Castellini, C., P. Lattaioli, M. Moroni, and A. Minelli. 2000. Effect of seminal plasma on the characteristics and fertility of rabbit spermatozoa. Anim. Reprod. Sci. 63:275-282.

Chan, B.S., J.A. Satrinano, M. Pucci, and V.L. Schuster. 1998. Mechanism of prostaglandin E_2 transport across the plasma membrane of HeLa cells and Xenopus oocytes expressing the prostaglandin transporter "PGT". J. Biol. Chem. 273:6689-6697.

Chaudhuri, G. 1971. Intrauterine device: possible role of prostaglandins. Lancet 1:480.

Cheng, H., G.C. Althouse, and W.H. Hsu. 2001. Prostaglandin $F_{2\alpha}$ added to extended boar semen at processing elicits *in vitro* myometrial contractility after 72 hours of storage. Theriogenology. 55:1901-1906.

Cheng, H., G.C. Althouse, and W.H. Hsu. 2003. Concentrations of endogenous prostaglandin $F_{2\alpha}$ in boar semen and effect of 72-h incubation period on exogenous prostaglandin $F_{2\alpha}$ concentration in extended boar semen. Prostaglandins Other Lipid Mediat. 70:285-290.

Chenoweth, P.J., F.M. Hopkins, J.C. Spitzer, and R.E. Larsen. 1993. Guidelines for using the bull breeding soundness evaluation form. Pages 1-5 in Theriogenology Handbook B-10. Society for Theriogenology, Hastings, NE.

Clarke, G.N. 1988. Immunoglobulin class and regional specificity of antispermatozoal autoantibodies blocking cervical mucus penetration by human spermatozoa. Am. J. Reprod. Immunol. Microbiol. 16:135-138.

Cohen, M.S., M.J. Colin, M. Colimbu, and R.S. Hotchkiss. 1977. The effects of prostaglandins on sperm motility. Fertil. Steril. 28:78-85.

Collier, J.G., R.J. Flower, and S.L. Stanton. 1975. Seminal prostaglandins in infertile men. Fertil. Steril. 26:868-871.

Corner GW. 1919. On the origin of the corpus luteum of the sow from both granulosa and theca interna. Am J Anat. 26:117-183.

Correa, J.R., M.M. Pace, and P.M. Zavos. 1997. Relationships among frozen-thawed sperm characteristics assessed via the routine analysis, sperm functional tests and fertility of bulls in an artificial insemination program. Theriogenology 48:721-731.

Coutinho, E.M., and H.S. Maia. 1971. The contractile response of the human uterus, fallopian tubes, and ovary to prostaglandins in vivo. Fertil. Steril. 22:539-543.

Cox, R.I., P.E. Mattner, and G.D. Thorburn. 1971. Changes in ovarian secretion of oestradiol-17β around oestrus in sheep. J. Endocrinol. 49:145-146.

Csepli, J., and A.I. Csapo. 1975. The effect of the prostaglandin $F_{2\alpha}$ analogue ICI 81008 on uterine small arteries and on blood pressure. Prostaglandins 10:689-697.

Davis, J.S., L.L. Weakland, D.A. Weiland, R.V. Farese, and L.A. West. 1987. Prostaglandin $F_{2\alpha}$ stimulates phosphatidylinositol 4,5-bisphosphate hydrolysis and mobilizes intracellular Ca^{2+} in bovine luteal cells. Proc. Nat. Acad. Sci. 84:3728-3732.

DeCooman, S., P. Gilliaux, and K. Thomas. 1983. Immunoreactive relaxin-like substance in human split ejaculates. Fertil. Steril. 39:111-113.

DeJarnette, J.M., M.L. Day, R.B. House, R.A. Wallace, and C.E. Marshall. 2001. Effect of GnRH pretreatment on reproductive performance of postpartum suckled beef cows following synchronization of estrus using GnRH and $PGF_{2\alpha}$. J. Anim. Sci. 79:1675-1682.

Del Vecchio, R.P., W.D. Sutherland, and R.G. Sasser. 1995. Prostaglandin $F_{2\alpha}$, progesterone, and oxytocin production by cultured bovine luteal cells treated with prostaglandin E_2 and pregnancy-specific protein B. Prostaglandins 50:137-150.

Denison, F.C., V.E. Grant, A.A. Calder, and R.W. Kelly. 1999. Seminal plasma components stimulate interleukin-8 and interleukin-10 release. Mol. Hum. Reprod. 5:220-226.

Didolkar, A.K., and D. Roychowdhury. 1980. Effects of prostaglandins E-1, E-2, F-1a and $F\text{-}_{2\alpha}$ on human sperm motility. Andrologia 12:135-140.

Diekman, A.B., and E. Goldberg. 1995. Sperm antigens: where are the private specifities? Pages 1-21 in Immunology of human reproduction. M. Kurpisz and N. Fernandez, eds. Oxford: BIOS Scientific.

Dousset, B., F. Hussenet, M. Daudin, L. Bujan, B. Foliguet, and P. Nabet. 1997. Seminal cytokine concentrations (11-1β, IL-2, IL-6, sRIL-2, SRIL-6), semen parameters and blood hormone status in male infertility. Hum. Reprod. 12:1476-1479.

Drobnis, E.Z. and J.W. Overstreet. 1992. Natural history of mammalizn spermatozoa in the female reproductive tract. Oxf. Rev. Reprod. Biol. 14:1-45.

Edqvist, S., S. Einarsson, and B. Gustafsson. 1975. Effect of prostaglandin $F_{2\alpha}$ on sperm transport in the reproductive tract of the ewe. Acta Vet. Scand. 16:149-151.

Eliasson, R. 1959. Studies on prostaglandin; occurrence, formation and biological actions. Acta Physiol. Scand. 46(Suppl. 158):1-73.

Evans J.H., D.M. Spencer, A. Zweifach, C.C. Leslie. 2001. Intracellular calcium signals regulating cytosolic phospholipase A2 translocation to internal membranes. J. Biol. Chem. 276:30150-30160.

Ferreira, S.H., and J.R. Vane. 1967. Prostaglandins: their disappearance from and release into the circulation. Nature 216:868-873.

Fields, M.J., and P.J. Fields. 1986. Luteal neurophysin in the non-pregnant cow and ewe: immunocytochemical localization in membrane bound secretory granules of the large luteal cells. Endocrinology 118:1723-1725.

Fitz, T.A., E.J. Mock, M.H. Mayan, and G.D. Niswender. 1984. Interactions of prostaglandins with subpopulations of ovine luteal cells. II. Inhibitory effects of $PGF_{2\alpha}$ and protection by PGE_2. Prostaglandins 28:127-138.

Flint, A.P.F. 1995. Interferon, the oxytocin receptor and the maternal recognition of pregnancy in ruminants and non-ruminants: a comparative approach. Reprod. Fertil. Dev. 7:313-318.

Flint, A.P.F., and E.L. Sheldrick. 1982. Ovarian secretion of oxytocin is stimulated by prostaglandin. Nature 297:587-588.

Flint, A.P.F, E.L. Sheldrick, T.J. McCann, and D.S.C. Jones DSC. 1990. Luteal oxytocin: characteristics and control of synchronous episodes of oxytocin and $PGF_{2\alpha}$ secretion at luteolysis in ruminants. Dom. Anim. Endocrinol. 7:111-124.

Foote, R.H., C.C. Brockett, and M.T. Kaproth. 2002. Motility and fertility of bull sperm in whole milk extender containing antioxidants. Anim. Reprod. Sci. 71:13-23.

Foote, R.H., and M.T. Kaproth. 2002. Large batch freezing of bull semen: effect of time of freezing and fructose on fertility. J. Dairy Sci. 85:453-456.

Fouchécourt, S., G. Charpigny, P. Reinaud, P. Dumont, and J. Dacheux. 2002. Mammalian lipocalin-type prostaglandin D2 synthase in the fluids of the male genital tract: putative biochemical and physiological functions. Biol. Reprod. 66:458-467.

Fouchécourt, S., F. Dacheux, and J.L. Dacheux. 1999. Glutatione-independent prostaglandin D2 synthase in ram and stallion epididymal fluids: origin and regulation. Biol. Reprod. 60:558-566.

Fowden, A.L., R. Harding, and M.M. Ralp. 1987. The nutritional regulation of prostaglandin E concentration in the fetus and pregnant ewe during gestation. J. Physiol. (Lond.) 394:1-12.

Funk, C.D. 2001. Prostaglandins and Leukotrienes: Advances in Eicosanoid Biology. Science. 294:1871-1875.

Garbers, D.L., W.D. Lust, N.L. First, and H.A. Lardy. 1971. Effects of phosphodiesterase inhibitors and cyclic nucleotides on sperm respiration and motility. Biochemistry 10:1825-1831.

Garner, D.L., C.A. Thomas, C.G. Gravance, C.E. Marshall, J.M. DeJarnette, and C.H. Allen. 2001. Seminal plasma addition attenuates the dilution effect in bovine sperm. Theriogenology 56:31-40.

Gerena, R.L., D. Irikura, N. Eguchi, Y. Urade, and G.J. Killian. 2000. Immunocytochemical localization of lipocalin-type prostaglandin D synthase in the bull testis and epididymis and on ejaculated sperm. Biol. Reprod. 62:547-556.

Gerena, R.L., D. Irikura, Y. Urade, N. Eguchi, D.A. Chapman, and G.J. Killian. 1998. Identification of a fertility-associated protein in bull seminal plasma as lipocalin-type prostaglandin D synthase. Biol. Reprod. 58:826-833.

Gilbert, C.L., P.J. Cripps, and D.C. Wathes. 1992. Effect of oxytocin on the pattern of electromyographic activity in the oviduct and uterus of the ewe around oestrus. Reprod. Fertil. Dev. 4:193-203.

Graham, E.F., M.K.L. Schmehl, B.K. Evenson, and D.S. Nelson. 1980. Problems with laboratory assays. Pages 1-8 in Proc. 8th Tech. Conference on Artificial Insemination and Reprod., National Assoc. Anim. Breeders, WI

Green, K., M. Bygdeman, M. Toppozada, and N. Wiqvist. 1974. The role of prostaglandin $F_{2\alpha}$ in human parturition. Am. J. Obstet. Gynecol. 120:25-31.

Grunberger, W., U. Maier, and G. Lunglmayr. 1981. Effect on prostaglandin F 2 alpha on sperm motility in vitro. Reproduccion 5:141-144.

Gu, W., G.E. Rice, and G.D. Thornburn. 1990. Prostaglandin E_2 and $F_{2\alpha}$ in mid-pregnant rat uterus and at parturition. Prostaglandins Leukot. Essent. Fatty Acids 40:27-30.

Gusovsky, F. 1991. Prostaglandin receptors in NIH 3T3 cells: coupling of one receptor to adenylate cyclase and of a second receptor to phospholipase C. Mol. Pharmacol. 40:633-638.

Gustafsson, B., S. Edqvist, and S. Einarsson. 1975. The fertility of deep-frozen ram semen supplemented with $PGF_{2\alpha}$. Acta Vet. Scand. 16:468-470.

Harkema, W., B. Colenbrander, B. Engel, and H. Woelders. 2004. Effects of exposure of epididymal boar spermatozoa to seminal plasma on the binding of zona pellucida proteins during in vitro capacitation. Theriogenology 61:215-226.

Harper, M.J.K. 1973. Stimulation of sperm movement from the isthmus to the site of fertilization in the rabbit oviduct. Biol. Reprod. 8:369-377.

Hawk, H.W., B.S. Cooper, and H.H. Conley. 1982. Effect of acetylcholine, prostaglandin $F_{2\alpha}$ and estradiol on number of sperm in the reproductive tract of inseminated rabbits. J. Anim. Sci. 55:891-900.

Hawk, H.W., and S.E. Echternkamp. 1973. Uterine contractions in the ewe during prostaglandin-regulated oestrus. J. Reprod. Fertil. 34:347-349.

Hawkins, D.F. 1968. Relevance of prostaglandins to problems of human subfertility. Page 1 in Prostaglandins Symposium of the Worchester Foundation for Experimental Biology P.W Ramwell, and J.E. Shaw eds. John Wiley, New York.

Hawkins, D.F., and A.H. Labrum. 1961. Semen prostaglandin levels in fifty patients attending a fertility clinic. J. Reprod. Fertil. 2:1-10.

Hayashi, S., Y. Noda, and T. Mori. 1988. Analysis of the role of prostaglandins in the fertilization process. Eur. Obstet. Gynecol. Reprod. Biol. 29:287-297.

Henault, M.A., G.J. Killian, J.F. Kavanaugh, and L.C. Griel, Jr. 1995. Effect of accessory sex gland fluid from bulls of differing fertilities on ability of cauda epididymal sperm to penetrate zona-free bovine oocytes. Biol. Reprod. 52:390-397.

Hinkovska, V.T., A.B. Momchilova, D.H. Petkova, and K.S. Koumanov. 1987. Phospholipase A_2 activity in ram spermatozoa plasma membranes. Int. J. Biochem. 19:569-572.

Hirai, H., K. Tanaka, O. Yoshie, K. Ogawa, K. Kenmotsu, Y. Takamori, M. Ichimasa, K. Sugamura, M. Nakamura, S. Takano, and K. Nagata. 2001. Prostaglandin D2 selectively induces chemotaxis in T helper type 2 cells, eosinophils, and basophils via seven-transmembrane receptor CRTH2. J. Exp. Med. 193:255-261.

Hirsbrunner, G., B. Knutti, U. Küpfer, H. Burkhardt, and A. Steiner. 2003. Effect of prostaglandin E_2, DL-cloprostenol, and prostaglandin E_2 in combination with D-cloprostenol on uterine motility during diestrus in experimental cows. Anim. Reprod. Sci. 79:17-32.

Hoedmaker, M., L.A. Lund, and W.C. Wagner. 1992. Influence of arachidonic acid metabolites and steroids on function of bovine polymorphonuclear neutrophils. Am. J. Vet. Res. 53:1534-1539.

Hoffmann, A., D. Bähner, N. Betat, J. Lauber, and G. Gross. 1996. Developmental expression of murine β-trace in embryos and adult animals suggest a function in maturation and maintenance of blood-tissue barriers. Dev. Dynam. 207:332-343.

Holmes, P.V., P.O. Janson, J. Sogn, B. Kallfelt, W.J. LeMaire, K.B. Ahren, S. Cajander, and L. Bjersing. 1983. Effects of $PGF_{2\alpha}$ and indomethacin on ovulation and steroid production in the isolated perfused rabbit ovary. Acta Endocrinol. (Copenh.) 104:233-239.

Horvat, G., and G. Bilkei. 2003. Exogenous prostaglandin $F_{2\alpha}$ at time of ovulation improves reproductive efficiency in repeat breeder sows. Theriogenology 59:1479-1484.

Hunter, R.H.F. 1977. Function and malfunction of the fallopian tubes in relation to gametes, embryos and hormones. Europ. J. Obstet. Gynec. Reprod. Biol. 7:267-283.

Hunter, R.H.F., B. Cook, and N.L. Poyser. 1983. Regulation of oviduct function in pigs by local transfer of ovarian steroids and prostaglandins: a mechanism to influence sperm transport. Europ. J. Obstet. Gynec. Reprod. Biol. 14:225-232.

Islam, F., Y. Watanabe, H. Morii, and O. Hayaishi. 1991. Inhibition of rat brain prostaglandin D synthase by inorganic selenocompounds. Arch. Biochem. Biophys. 289:161-166.

Isidori, A., D. Conte, G. Laguzzi, P. Giovenco, and F. Dondero. 1980. Role of seminal prostaglandins in male fertility. I. Relationship of prostaglandin E and 19-OH prostaglandin E with seminal parameters. J. Endocrinol. Invest. 3:1-4.

Itoh, S., R. Lu, Y. Bao, J.D. Morrow, L.J. Roberts, and V.L. Schuster. 1996. Structural determinates of substrates for the prostaglandin transporter PGT. Mol. Pharmacol. 50:736-742.

Jaeger, J.R., J.C. Whittier, L.R. Corah, J.C. Meiske, K.C. Olson, and D.J. Patterson. 1992. Reproductive response of yearling beef heifers to a melengestrol acetate-prostaglandin $F_{2\alpha}$ estrus synchronization system. J. Anim. Sci. 70:2622-2627.

Jakobsson, P.J., S. Thorén, R. Morgenstern, and B. Samuelsson. 1999. Identification of human prostaglandin E synthase: a microsomal, glutathione-dependent, inducible enzyme, constituting a potential novel drug target. Proc. Natl. Acad. Sci. 96:7220-7225

Januskauskas, A., and H. Rodrigues-Martinez. 1995. Assessment of sperm viability by measurement of ATP, membrane integrity and motility in frozen/thawed bull semen. Acta Vet. Scand. 36:571-574.

Januskauskas, A., L. Söderquist, H.G. Häärd, M.C. Häärd, N. Lundeheim, and H. Rodrigues-Martinez. 1996a. Estimation of sperm viability in frozen-thawed semen from Swedish A.I. bulls. Zentralbl Veterinarmed A. 43:281-287.

Januskauskas, A., L. Söderquist, H.G. Häärd, M.C. Häärd, N. Lundeheim, and H. Rodrigues-Martinez. 1996b. Influence of sperm number per straw on the post-thaw sperm viability and fertility of Swedish Red and White AI bulls. Acta Vet. Scand. 37:461-470.

Johnson, M.M., and B.P. Setchell. 1968. Protein and immunoglobin content of rete testis fluid of rams. J. Reprod. Fertil. 17:403-406.

Joyce, C.L., N.A. Nuzzo, L. Wilson, Jr., and L.J.D. Zaneveld. 1987. Evidence for a role of cyclooxygenase (prostaglandin synthetase) and prostaglandins in the sperm acrosome reaction and fertilization. J. Androl. 8:74-82.

Juang, H.H., A.I. Musah, C. Schwab, and L.L. Anderson. 1990. Immunoreactive relaxin in boar seminal plasma and its correlation with sperm motility. Anim. Reprod. Sci. 22:47-53.

Kalloo, F.A., N.A. Wani, M.Z. Khan, and G.M. Wani. 2003. Effects of partial to complete removal of seminal plasma on cryopreservation of bovine semen. Indian J. Anim. Sci. 73:63-65.

Kanai, N., R. Lu, J.A. Satriano, Y. Bao, A.W. Wolkoff, and V.L. Schuster. 1995. Identification and characterization of a prostaglandin transporter. Science 268:866-869.

Karim, S.M.M. 1972. Physiological role of prostaglandins in the control of parturition and menstruation. J. Reprod. Fertil. 16(Suppl.):105-119.

Karmazyn, M. 1989. Synthesis and relevance of cardiac eicosanoids with particular emphasis on ischemia and reperfusion. Can. J. Physiol. Pharmacol. 67:912-921.

Kelly, R.W. 1977. Effect of seminal prostaglandins on the metabolism of human spermatozoa. J. Reprod. Fertil. 50:217-222.

Kelly, R.W. 1978. Prostaglandins in semen: Their occurrence and possible physiological significance. International J. Andrology. 1:188-200.

Kelly, R.W. 1995. Immunosupressive mechanisms in semen: implications for contraception. Hum. Reprod. 10:1686-1693.

Kelly, R.W., G. Skibinski, and K. James. 1994. The immunosuppressive contribution of prostaglandin components of human semen and their ability to elevate cyclic adenosine monophosphate levels in peripheral blood mononuclear cells. J. Reprod. Immunol. 26:31-40.

Keskintepe, L., and B.G. Brackett. 1996. In vitro development competence of in vitro-matured bovine oocytes fertilized and cultured in completely defined media. Biol. Reprod. 55:333-339.

Killian, G.J., D.A. Chapman, and L.A. Rogowski. 1993. Fertility-associated proteins in Holstein bull seminal plasma. Biol. Reprod. 49:1202-1207.

Kim, L., Y.S. Weems, P.J. Bridges, B.R. LeaMaster, L. Ching, D.L. Vincent, and C.W. Weems. 2001. Effects of indomethacin, luteinizing hormone (LH), prostaglandin E2 (PGE2), trilostane, mifepristone, ethamoxytriphetol (MER-25) on secretion of prostaglandin E (PGE), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and progesterone by ovine corpora lutea of pregnancy or the estrous cycle. Prostaglandins Other Lipid Mediat. 63:189-203.

King, W.G., and S. Rittenhouse. 1989. Inhibition of protein C by staurosporine promotes elevated accumulation of inositol triphosphates and tetrabiphosphate in human platelets exposed to thrombin. J. Biol. Chem. 264:6070-6074.

Kjæstad, H., E. Ropstad, and K. Andersen Berg. 1993. Evaluation of spermatological parameters used to predict the fertility of frozen bull semen. Acta Vet. Scand. 34:299-303.

Kohsaka, T., K. Hamano, H. Sasada, S. Watanabe, T. Ogine, E. Suzuki, S. Nishida, H. Takahara, and E. Sato. 2003. Seminal immunoreactive relaxin in domestic animals and its relationship to sperm motility as a possible index for predicting the fertilizing ability of sires. Int. J. Androl. 26:115-120.

Kohsaka, T., H. Sasada, H. Takahara, E. Sato, K. Bamba, and O.D. Sherwood. 2001. The presence of specific binding sites on boar spermatozoa for porcine relaxin and its action on their motility characteristics. J. Reprod. Devel. 47:197-204.

Kohsaka, T., H. Takahara, H. Sasada, T. Kawarasaki, K. Bamba, J. Masaki, and S. Tagami. 1992. Evidence for immunoreactive relaxin in boar seminal vesicles using combined light and electron microscope immunocytochemistry. J. Reprod. Fertil. 95:397-408.

Koos, R.D., M.R. Clark, P.O. Janson, K.E. Ahren, and W.J. LeMaire. 1983. Prostaglandin levels in preovulatory follicles from rabbit ovaries perfused in vitro. Prostaglandins 25:715-724.

Kos, M., and G. Bilkei. 2004. Prostaglandin $F_{2\alpha}$ supplemented semen improves reproductive performance in artificially inseminated sows. Anim. Reprod. Sci. 80:113-120.

Kunapuli, P., J.A. Lawson, J.A. Rokach, J.L. Meinkoth, and G.A. FitzGerald. 1998. Prostaglandin F2α and the isoprostane, 8,12-iso-isoprostane $F_{2\alpha}$-III, induce cardiomyocyte hypertrophy. Differential activation of downstream signaling pathways. J. Biol. Chem. 273:22442-22452.

Kurzrok, R., and C.C. Lieb. 1930. Biochemical studies of human semen. II. The action of semen on the human uterus. Proc. Soc. Exp. Biol. Med. 28:268-272.

Lamb, G.C., J.S. Stevenson, D.J. Kesler, H.A. Garverick, D.R. Brown, and B.E. Salfen. 2001. Inclusion of an intravaginal progesterone insert plus GnRH and prostaglandin $F_{2\alpha}$ for ovulation control in postpartum suckled beef cows. J. Anim. Sci. 79:2253-2259.

Larssen, B., and H. Rodríguez-Martínez. 2000. Can we use in vitro fertilization tests to predict semen quality? Anim. Reprod. Sci. 60-61:327-336.

Lau, I.F., S.K. Saksena, and M.C. Chang. 1974. Prostaglandin F in the uterine horns of mice with intrauterine devices. J. Reprod. Fertil. 37:429-432.

Ledwozyw, A., R. Pruszkowska, and A. Kadziolka. 1986. Isolation and identification of prostaglandins of the E and F series in testes and semen of lowland-black-white breed of bulls. Acta Physiologica Hungarica. 68:11-17.

Lee, C.N., and R.L. Ax. 1984. Concentrations and composition of glycosaminoglycans in the female bovine reproductive tract. J. Dairy Sci. 67:2006-2009.

Lenz, R.W., R.L. Ax, H.J. Grimek, and N.L. First. 1982. Proteoglycan from bovine follicular fluid enhances an acrosome reaction in bovine spermatozoa. Biochem. Biophys. Res. Commun. 106:1092-1098.

Lenz, R.W., G.B. Ball, M.L. Liebfried, R.L. Ax, and N.L. First. 1983a. Chondroitin sulfate facilitates an acrosome reaction in bovine spermatozoa as evidenced by light microscopy, electron microscopy and in vitro fertilization. Biol. Reprod. 28:683-690.

Lenz, R.W., G.B. Ball, M.L. Liebfried, R.L. Ax, and N.L. First. 1983b. In vitro maturation and fertilization of bovine oocytes are temperature dependent processes. Biol. Reprod. 29:173-179.

Leung, P.C., T. Minegishi, F.Z. Zhou, and B. Ho-Yuen. 1986. Induction of polyphosphoinositide breakdown in rat corpus luteum by prostaglandin $F_{2\alpha}$. Endocrinology 119:12-18.

Lewis, G.S. 2003. Role of ovarian progesterone and potential role of prostaglandin $F_{2\alpha}$ and prostaglandin E_2 in modulating the uterine response to infectious bacteria in postpartum ewes. J. Anim. Sci. 81:285-293.

Liggins, G.C., S. Grieves, D.R. Bangham, D.H. Calan, J.A. Parsons, and C.J. Robinson. 1971. Possible role for prostaglandin $F_{2\alpha}$ in parturition in sheep. Nature 232:629-631.

Liggins, G.C., R.J. Fairclough, S.A. Grieves, J.Z. Kendall, and S.J. Knox. 1973. The mechanism of initiation of parturition in the ewe. Recent Prog. Horm. Res. 29:111-149.

Linford, E., F.A. Glover, C. Bishop, and D.L. Stewart. 1976. The relationship between semen evaluation methods and fertility in the bull. J. Reprod. Fertil. 47:283-291.

Loumaye, E. S. DeCooman, and K. Thomas. 1980. Immuno reactive relaxin-like substance in human seminal plasma. J. Clinical Endocrinology and Metabolism 50:1142-1143.

Lu, R., and V.L. Schuster. 1996. Cloning, in vitro expression, and tissue distribution of a human prostaglandin transporter cDNA (hPGT). J. Clin. Invest. 98:1142-1149.

Lucy, M.C., H.J. Billings, W.R. Butler, L.R. Ehnis, M.J. Fields, D.J. Kesler, J.E. Kinder, R.C. Mattos, R.E. Short, W.W. Thatcher, R.P. Wettemann, J.V. Yelich, and H.D. Hafs. 2001. Efficacy of an intravaginal progesterone insert and an injection of $PGF_{2\alpha}$ for synchronizing estrus and shortening the interval to pregnancy in postpartum beef cows, peripubertal beef heifers, and dairy heifers. J. Anim. Sci. 79:982-995.

MacDonald, P.C. S. Koga, and M.L. Casey. 1991. Decidual activation in parturition; examination of amniotic fluid for mediators of the inflammatory response. Ann. NY Acad. Sci. 622:315-330.

Madore, E., N. Harvey, J. Parent, P. Chapdelaine, J.A. Arosh, and M.A. Fortier. 2003. An aldose reductase with 20α-hydroxysteroid dehydrogenase activity is most likely the enzyme responsible for the production of prostaglandin $F_{2\alpha}$ in the bovine endometrium. J. Biol. Chem. 278:11205-11212.

Maes, D.G.D., B. Mateusen, T. Rijsselaere, S. De Vliegher, A. Van Soon, and A. de Kruif. 2003. Motility characteristics of boar spermatozoa after addition of prostaglandin $F_{2\alpha}$. Theriogenology 60:1435-1443.

Magness, R.R., J.M. Huie, G.L. Hoyer, T.P. Huecksteadt, L.P. Reynolds, G.J. Seperich, G. Whysong, and C.W. Weems. 1981. Effect of chronic ipsilateral or contralateral intrauterine infusion of prostaglandin E_2 (PGE_2) on luteal function of unilaterally ovariectomized ewes. Prostaglandins Med. 6:389-401.

Mah, J., J.E. Tilton, G.L. Williams, J.N. Johnson, and M.J. Marchello. 1985. The effect of repeated mating at short intervals on reproductive performance of gilts. J. Anim. Sci. 60:1052-1054.

Mai, J., and J.E. Kinsella. 1980. Prostaglandin E1 and E2 in bovine semen: quantification by gas chromatography. Prostaglandins 20:187-197.

Marks, J.L., and R.L Ax. 1985. Relationship of non return rates of dairy bulls to binding affinity of heparin to sperm. J. Dairy Sci. 68:2078-2082.

Marsh, J.M., and W.J. LeMaire. 1974. Cyclic AMP accumulation and steroidogenesis in the human corpus luteum: effect of gonadotropins and prostaglandins. J. Clin. Endocrinol. Metab. 38:99-106.

Mathews, C.K., K.E. van Holde, and K.G. Ahern. 2000. Biochemistry 3rd ed. Benjamin/Cummings, San Francisco, CA.

Matson, P.L., S.M. Junk, J.W. Spittle, and J.L. Yovich. 1988. Effect of antispermatozoal antibodies in seminal plasma upon spermatozoal function. . Int. J. Androl. 11:101-106.

Maxwell, W.M.C., G. Evans, S.T. Mortimer, L. Gillan, E.S. Gellatly, and C.A. McPhie. 1999. Normal fertility in ewes after cervical inseminiation with frozen-thawed spermatozoa supplemented with seminal plasma. Reprod. Fertil. Dev. 11:123-126.

Mazumdar, S., and A.S. Levine. 1998. Antisperm antibodies: etiology, pathogenesis, diagnosis, and treatment. Fertil. Steril. 70:799-810.

McCracken, J.A., D.T. Baird, and J.R. Goding. 1971. Factors affecting the secretion of steroids from the transplanted ovary in the sheep. Recent Progr. Horm. Res. 27:527-582.

McCracken, J.A., E.E. Custer, J.A. Eldering, and A.G. Robinson. 1996. The central oxytocin pulse generator: a pacemaker for the ovarian cycle. Acta Neurobiol. Exp. 56:819-32.

Miller, D.J., M.A. Winer, and R.L. Ax. 1990. Heparin-binding proteins from seminal plasma bind to bovine spermatozoa and modulate capacitation by heparin. Biol. Reprod. 42:899-915.

Mitchell, M.D., A.P. Flint, J. Bibby, J. Brunt, J.M. Arnold, A.B. Anderson, and A.C. Turnbull. 1978. Plasma concentrations of prostaglandins during late human pregnancy: influence of normal and preterm labor. J. Clin. Endocrinol. Metab. 46:947-951.

Monneret, G., S. Gravel, M. Diamond, J. Rokach, and W.S. Powell. 2001. Prostaglandin D_2 is a potent chemoattractant for human eosinophils that acts via a novel DP receptor. Blood 98:1942-1948.

Morita, I., M. Schindler, M.K. Regier, J.C. Otto, T. Hori, D.L. DeWitt, and W.L. Smith. 1995. Different intracellular locations for prostaglandin endoperoxide H synthase-1 and -2. J. Biol. Chem. 270:10902-10908.

Myatt, L., M.A. Bray, D. Gordon, and J. Morley. 1975. Macrophages on intrauterine contraceptive devices produce prostaglandins. Nature 257:227-228.

Nakao, A. T. Watanabe, S. Taniguchi, M. Nakamura, Z. Honda, T. Shimizu, and K. Kurokawa. 1993. Characterization of prostaglandin F2α receptor of mouse 3T3 fibroblasts and its functional expression in Xenopus laevis oocytes. J. Cell Physiol. 155:257-264.

Naoaki, R.L., K. Y. Bao, and V.L. Schuster. 1996. Cloning, in vitro expression, and tissue distribution of a human prostaglandin transporter cDNA (hPGT). J. Clin. Invest. 98:1142-1149.

National Animal Health Monitoring System. 1997. Part 1: reference of 1997 beef cow-calf management practices. USDA-APHIS, Washington, DC.

National Animal Health Monitoring System. 1998. Part III: reference of 1997 beef cow-calf production management and disease control. USDA-APHIS, Washington, DC.

Nass, S.J., D.J. Miller, M.A. Winer, and R.L. Ax. 1990. Male accessory sex glands produce heparin-binding proteins that bind to cauda epididymal spermatozoa and are testosterone dependent. Mol. Reprod. Dev. 25:237-246.

Narumiya, S., and G.A. FitzGerald. 2001. Genetic and pharmacological analysis of prostanoid receptor function. J. Clin. Invest. 108:25-30.

Narumiya, S., Y. Sugimoto, and F. Ushikubi. 1999. Prostanoid receptors: structures, properties, and functions. Physiol. Rev. 79:1193-1226.

Nebbia, C., C. Brando, E. Burdino, R. Rasero, F. Valenza, R. Arisio, and G. Ugazio. 1987. Effects of chronic administration of sodium selenite on rat testes. Res. Commun. Chem. Pathol. Pharmacol. 58:183-197.

Newcomer, M.E. 1993. Structure of the epididymal retinoic acid binding protein at 2.1 A resolution. Structure 1:7-18.

Niswender, G.D., L.E. Reichert Jr., and D.R. Zimmerman. 1970. Radioimmunoassay of serum levels of luteinizing hormone throughout the oestrous cycle in pigs. Endocrinology 87:576-580.

Norman, A.W., and G. Litwack. 1997. Hormones. 2nd ed. Academic Press, San Diego, CA.

Okamura, H., A. Takenaka, Y. Yajima, and T. Nishimura. 1980. Ovulatory changes in the wall at the apex of the human Graafian follicle. J. Reprod. Fertil. 58:153-155.

Ollero, M., J.A. Cebrian-Perez, and T. Muiño-Blanco. 1997. Improvement of cryopreserved ram sperm heterogeneity and viability by addition of seminal plasma. J. Androl. 18:732-739.

Orczyk, G.P. and H.R. Behrman. 1972. Ovulation blockade by asprin or indomethacin; in vivo evidence for a role of prostaglandin in gonadotropin secretion. Prostaglandins 1:3-21.

Orwig, K.E., J.E. Bertrand, B.R. Ou, N.E. Forsberg, and F. Stormshak. 1994. Involvement of protein kinase-C, calpains, and calpastatin in prostaglandin $F_{2\alpha}$-induced oxytocin secretion from the bovine corpus luteum. Endocrinology134:78-82.

O'Shea, J.D., and R.E. Phillips. 1974. Contractility in vitro of ovarian follicles from sheep and the effects of drugs. Biol. Reprod. 10:370-379.

Otto, J.C., and W.L. Smith. 1994. The orientation of prostaglandin endoperoxide synthases-1 and -2 in the endoplasmic reticulum. J. Biol. Chem. 269:19868-19875.

Parkinson, T.J. 2004. Evaluation of fertility and infertility in natural service bulls. Vet. J. 168:215-229.

Parrish, J.J., J.L. Susko-Parrish, M.L. Leibfried-Rutledge, E.S. Crister, W.H. Eyestone, and N.L. First. 1986. Bovine *in vitro* fertilization with frozen-thawed sperm. Theriogenology 25:591-600.

Parsons, S.D., G.L. Hunter, and A.A. Rayner. 1967. Use of probit analysis in a study of the effect of the ram on time of ovulation in the ewe. J. Reprod. Fertil. 14:71-80.

Patil, R.K., S.N. Sinha, S. Einarsson, and I. Settergren. 1980. The effect of prostaglandin $F_{2\alpha}$ and oxytocin on bovine myometrium in vitro. Nord. Vet. Med. 32:474-479.

Peña, F.J., J.C. Domínguez, B. Alegre, and J. Peláez. 1998. Effect of vulvomucosal injection of $PGF_{2\alpha}$ at insemination on subsequent fertility and litter size in pigs under field conditions. Anim. Reprod. Sci. 52:63-69.

Peña, F.J., J.C. Domínguez, J. Peláez, and B. Alegre. 2000. Intrauterine infusion of $PGF_{2\alpha}$ at insemination enhances reproductive performance of sows during the low fertility season. The Vet. J. 159:259-261.

Pento, J.T., R.J. Cenedella, and E.K. Inskeep. 1970. Effects of prostaglandins E_1 and $F_{2\alpha}$ upon carbohydrate metabolism of ejaculated and epididymal spermatozoa in vitro. J. Anim. Sci. 30:409-411.

Piper, P.J., J.R. Vane, and J.H. Wyllie. 1970. Inactivation of prostaglandins by the lungs. Nature 225:600-604.

Post, N.M., D.L. Kreider, and R.W. Rorie. 2005. Case study: timed insemination in beef heifers after synchronization of estrus with controlled intravaginal drug releasing device and melengestrol acetate. Prof. Anim. Sci. 21:107-113.

Poyser, N.L. 1974. Some aspects of prostaglandins in reproduction. Biochem. Soc. Trans. 2:1196-1200.

Prinzen, R., F. Allgayer, U. Bartz, and E. Huber. 1991. Effects of prostaglandin $F_{2\alpha}$ on conception results of heifers and cows. Tierärtzliche Umschau 1:27-35.

Pursel, V.G., and E.F. Graham. 1967. Phospholipids of bovine spermatozoa and seminal plasma. J. Reprod. Fert. 14:203-211.

Pursley, J. R., M. O. Mee, and M. C. Wiltbank. 1995. Synchronization of ovulation in dairy cows using $PGF_{2\alpha}$ and GnRH. Theriogenology 44:915.

Quarles, L.D., D.M. Haupt, G. Davidia, and J.P. Middleton. 1993. Prostaglandin $F_{2\alpha}$-induced mitogenesis in MC3T3-E1 osteoblasts: role of protein kinase-C-mediated tyrosine phosphorylation. Endocrionology 132:1505-1513.

Ramwell, P., and J.E Shaw. 1971. The biological significance of the prostaglandins. Ann. NY Acad. Sci. 180:10-13.

Randel, R.D., R.E. Short, D.S. Christensen, and R.A. Bellows. 1973. Effects of various mating stimuli on the LH surge and ovulation time following synchronization of estrus in the bovine. J. Anim. Sci. 37:128-130.

Reddy, B.B., A.S.N. Murty, V.S.N. Rao, P.N. Rao, and K.S. Rao. 1982. Seminal prostaglandins E2 and F2 alpha in Murrah buffalo bulls. J. Nuclear Agri. Biol. 11:126-127.

Renfree, M.B., G. Shaw, and T.P. Fletcher. 1994. Evidence for the essential role of prostaglandins for parturition in a marsupial, Macropus eugenii. J. Reprod. Fertil. 102:433-446.

Reyes-Moreno, C., M. Boilard, R. Sullivan, and M.-A. Sirard. 2002. Characterization and indentification of epididymal factors that protect ejaculated bovine sperm during in vitro storage. Biol. Reprod. 66:159-166.

Roberts, J.S., and J.A. McCracken. 1976. Does prostaglandin F2α released from the uterus by oxytocin mediate the oxytocic action of oxytocin? Biol. Reprod. 15: 457-463.

Roberts, J.S., J.A. McCracken, J.E. Gavagon, and M.S. Soloff. 1976. Oxytocin-stimulated release of prostaglandin $F_{2\alpha}$ from ovine endometrium in vitro: correlation with estrous cycle and oxytocin receptor binding. Endocrinology 99:1107-1114.

Saari, T., K. Jahnukainen, and P. Pollanen. 1996. Auto-antigenicity outside the blood-testis barrier in the rat testis. J. Reprod. Immunol. 31:65-80.

Sako, T., A.I. Tauber, A.Y. Jeng, S.H. Yupsa, and P.M. Blumberg. 1988. Contrasting actions of staurosporine, a protein kinase C inhibitor, on human neutrophils and primary mouse epidermal cells. Cancer Res. 48:4646-4650.

Saksena, S.K., and M.J. Harper. 1974. Prostaglandin-mediated action of intrauterine devices: F-prostaglandins in the uterine horns of pregnant rabbits with intrauterine devices. Fertil. Steril. 25:121-126.

Saksena, S.K., I.F. Lau, and V.D. Castracane. 1974. Prostaglandin mediated action of IUDs. II. F-prostaglandins (PGF) in the uterine horn of pregnant rats and hamsters with intra-uterine devices. Prostaglandins 5:97-106.

Salli, U., S. Supancic, and F. Stormshak. 2000. Phosporylation of myristoylated alanine-rich C kinase substrate (MARCKS) protein is associated with bovine luteal cell exocytosis. Biol. Reprod. 63:12-20.

Sandberg, F., A. Ingelman-Sundberg, and G. Rydén. 1963. The effect of prostaglandin E1 on the human uterus and the fallopian tubes in vitro. Acta Obstet. Gynecol. Scand. 42:269-278.

Sandberg, F., A. Ingelman-Sundberg, and G. Rydén. 1964. The effect of prostaglandin E_2 and E_3 on the human uterus and fallopian tubes in vitro. Acta Obstet. Gynecol. Scand. 43:95-102.

Sasaki, Y., T. Kohsaka, T. Kawarasaki, H. Sasada, T. Ogine, K. Bamba, and H. Takahara. 2001. Immunoreactive relaxin in seminal plasma of fertile boars and its correlation with sperm motility characteristics determined by computer-assisted digital image analysis. Int. J. Androl. 24:24-30.

Schallenberger, E., D. Schams, B. Bullermann, and D.L. Walters. 1984. Pulsatile secretion of gonadotrophins, ovarian steroids and ovarian oxytocin during prostaglandin-induced regression of the corpus luteum in the cow. J. Reprod. Fertil. 71:493-501.

Schams, D., A. Lahlou-Kassi, and P. Glatzel. 1982. Oxytocin concentrations in peripheral blood during the oestrous cycle and after ovariectomy in two breeds of sheep with low and high fecundity. J. Endocrinol. 92:9-13.

Schlegel, W., S. Rotermund, G. Farber, and E. Nieschlag. 1981. The influence of prostaglandins on sperm motility. Prostaglandins 21:87-99.

Schmidt, G., P.V. Holmes, C.H. Owman, N.O. Sjöberg, and B. Walles. 1986. The influence of prostaglandin E_2 and indomethacin on progesterone production and ovulation in the rabbit ovary perfused in vitro. Biol. Reprod. 35:815-821.

Schuster, V.L. 1998. Molecular mechanisms of prostaglandin transport. Ann. Rev. Physiol. 60:221-242.

Seals, R.C., I. Metamoros, and G.S. Lewis. 2002a. Relationship between postpartum changes in 13, 14-dihydro-15-keto-$PGF_{2\alpha}$ concentrations in Holstein cows and their susceptibility to endometritis. J. Anim. Sci. 80:1068-1073.

Seals, R.C., M.C. Wulster-Radcliffe, and G.S. Lewis. 2002b. Modulation of the uterine response to infection bacteria in postpartum ewes. Am. J. Reprod. Immunol. 47:57-63.

Sellers, S., M.D. Mitchell, J. Bibby, A.B.M. Anderson, and A.C. Turnbull. 1981. Comparison of plasma concentrations of prostaglandin levels in term and preterm labour. Br. J.Obstet. Gynaecol. 88:362-366.

Shalev, Y., M. Shemesh, T. Levinshal, S. Marcus, and H. Breitbart. 1994. Localization of cyclooxygenase and production of prostaglandins in bovine spermatozoa. J. Reprod. Fertil. 101:405-413.

Sharma, R.C., and R.J. Fitzpatrick. 1974. Effect of oestradiol 17β and oxytocin treatment on prostaglandin F alpha release in the anoestrous ewe. Prostaglandins 6:97-105.

Sherwood, O.D., C.C. Chang, G.W. Bevier, and P.J. Dziuk. 1975. Radioimmunoassay of plasma relaxin levels throughout pregnancy and at parturition in the pig. Theriogenology 97:843-837.

Shier, W.T. 1979. Activation of high levels of endogenous phospholipase A_2 in cultured cells. Proc. Natl. Acad. Sci. 76:195-199.

Shulman, S. 1995. Auto and isoimmunological reactions in the reproductive tract of mammals. Pages 53-78 in Immunology of human reproduction. M. Kurpisz and N. Fernandez, eds. Oxford: BIOS Scientific.

Signoret, J.P., F. du Mesnil du Buisson, and P. Maulén. 1972. Effect of mating on the onset and duration of ovulation in the sow. J. Reprod. Fertil. 31:327-330.

Singh, L.P., A. Sadiku, and O.P. Verma. 1979. Prostaglandin $F_{2\alpha}$-induced response in the bovine ovary, oviduct (uterine tube), and uterus. Am. J. Vet. Res. 40:1789-1791.

Skau, P.A., and I. Folstad. 2004. Does immunity regulate ejaculate quality and fertility in humans? Behav. Ecol. 16:410-416.

Smith, W.L., D.L. DeWitt, and R.M. Garavito. 2000. Cyclooxygenases: structural, cellular, and molecular biology. Annu. Rev. Biochem. 69:145-182.

Soderquist, L., L. Janson, K. Larsson, and S. Einarsson. 1991. Sperm morphology and fertility in A.I. bulls. Zentralbl Veterinarmed A. 38:534-543.

Spat, A., P.G. Bradford, J.S. McKinney, R.P. Rubin, and J.W. Putney Jr. 1986. A saturable receptor for ^{32}P-inostitol-1,4,5-triphosphate in hepatocytes and neutrophils. Nature 319:514-516.

Spencer, A.G., J.W. Woods, T. Arakawa, I.I. Singer, and W.L. Smith. 1998. Subcellular localization of prostaglandin endoperoxide H synthases-1 and -2 by immunoelectron microscopy. J Biol Chem. 273:9886-9893.

Spilman, C.H., and R.T. Duby. 1972. Prostaglandin mediated luteolytic effect of an intrauterine device in sheep. Prostaglandins 2:159-168.

Spilman, C.H., A.E. Finn, and J.F. Norland. 1973. Effect of prostaglandins on sperm transport and fertilization in the rabbit. Prostaglandins. 4:57-64.

Spilman, C.H., and M.J.K. Harper. 1973. Effect of prostaglandins on oviduct motility in estrous rabbits. Biol. Reprod. 9: 36-45.

Stevenson, J.S., G.C. Lamb, S.K. Johnson, M.A. Medina-Britos, D.M. Grieger, K.R. Harmoney. J.A. Cartmill, S.Z. El-Zarkouny, C.R. Dahlen, and T.J. Marple. 2003. Supplemental norgestomet, progesterone, or melengestrol acetate increases pregnancy rates in suckled beef cows after timed insemination. J. Anim. Sci. 81:571-586.

Stolla, R., and G. Schmid. 1990. Effects of natural and synthetic PGF2 alpha preparations on the uterine contractility of cattle. Berl. Munch. Tierarztl. Wochenschr. 103:198-202.

Sugimoto, Y., A. Yamasaki, E. Segi, K. Tsuboi, Y. Aze, T. Nishimura, H. Oida, N. Yoshida, T. Tanaka, M. Katsuyama, K. Hasumoto, T. Murata, M. Hirata, F. Ushikubi, M. Negishi, A. Ichikawa, and S. Narumiya. 1997. Failure of parturition in mice lacking the prostaglandin F receptor. Science 277:681-683.

Swann, R.W., P.J. O'Shaughnessy, S.D. Birkett, D.C. Wathes, D.G. Porter, and B.T. Pickering. 1984. Biosynthesis of oxytocin in the corpus luteum. FEBS Lett 174:262-266.

Tanaka, T., Y. Urade, H. Kimura, N. Eguchi, A. Nishikawa, and O. Hayaishi. 1997. Lipocalin-type prostaglandin D synthase (β-trace) is a newly recognized type of retinoid transporter. J. Biol. Chem. 272:15789-15795.

Tash, J.S., and F.R.S. Mann. 1973. Adenosine 3':5'-cyclic monophosphated in relation to motility and sequence of spermatozoa. Proc. R. Soc. Lond. Biol. 184:109-114.

Tatum, R.B. 1972. Intrauterine contraception. Am. J. Obstet. Gynecol. 112 :1000-1023.

Theodosis, D.T., F.B.P. Wooding, E.L. Sheldrick, and A.P.F. Flint APF. 1986. Ultrastructural localization of oxytocin and neurophysin in the ovine corpus luteum. Cell Tissue Res. 243:129-135.

Thomas, W.K., and G.E. Seidel, Jr. 1993. Effects of cumulus cells on culture of bovine embryos derived from oocytes matured and fertilized in vitro. J. Anim. Sci. 71:2506-2510.

Thorburn, G.D. 1991. The placenta, prostaglandins and parturition; a review. Reprod. Fertil. Devel. 2:277-294.

Thorburn, G.D., S.A. Hollingworth, and S.B. Hooper. 1991. The trigger for parturition in sheep: fetal hypothalamus or placenta? J. Devel. Physiol. 15:71-79.

Thun, R., M. Hurtado, and F. Janett. 2002. Comparison of Biociphos-Plus® and TRIS-egg yolk extender for cryoperservation of bull semen. Theriogenology 57:1087-1094.

Tokugawa, Y., I. Kunishige, Y. Kubota, K. Shimoya, T. Nobunaga, T. Kimura, F. Saji, Y. Murata, N. Eguchi, H Oda, Y. Urade, and O. Hayaishi. 1998. Lipocalin-type prostaglandin D synthase in human male reproductive organs and seminal plasma. Biol. Reprod. 58:600-607.

Turek, P.J. 1999. Infections, immunology, and male infertility. Infertil. Reprod. Med. Clin. North Am. 10:435-470.

USDA. 1998. Reproductive technology in beef cow-calf herds. Available: http://www.aphis.usda.gov/vs/ceah/ncahs/nahms/beefcowcalf/beef97/bf97repro.pdf. Accessed Sept. 8, 2005.

USDA. 2005. Cattle. National Agricultural Statistics Service. Available: http://usda.mannlib.cornell.edu/reports/nassr/livestock/pct-bb/catl0705.pdf. Accessed Sept. 7, 2005.

Van Dorp, D.A., R.K. Beerthuis, D.H. Nugteren, and H. Vonkeman. 1964. The biosynthesis of prostaglandins. Biochim. Biophys. Acta 90:204-207.

Ventura, W. P., and M. Freund. 1973. Evidence of a new class of uterine stimulants in rat semen and male accessory gland secretions. J. Reprod.d Fertil. 33:507-511.

Vera, O., L.A. Vásqucz, and M.G. Muñoz. 2003. Semen quality and presence of cytokines in seminal fluid of bull ejaculates. Theriogenology 60:553-558.

Vezza, R. J. Rokach, and G.A. Fitzgerald. 2001. Prostaglandin $F_{2\alpha}$ receptor-dependent regulation of prostaglandin transport. Mol. Pharm. 59:1506-1513.

Virutamasen, P., K.H. Wright, and E.E. Wallach. 1972. Effects of prostaglandins E2 and F2 on ovarian contractility in the rabbit. Fertil. Steril. 23:675-682.

Vlachos, K., A. Kovatsis, E. Tsoukali, and V. Kotsaki. 1973. Isolation, identification, and biological effect of bull semen prostaglandins. Dtsch Tierarztl Wochenschr. 80:100-102.

Voglmayr, J.K. 1973. Prostaglandin $F_{2\alpha}$ concentration in genital tract secretions of dairy bulls. Prostaglandins 4:673-678.

von Euler, U.S. 1936. On the specific vaso-dilating and plain muscle stimulation substances from accessory genital glands in man and certain animals (prostaglandin and vesiglandin). J. Physiol. (Lond.) 88:213-234.

von Euler, U.S. 1939. Further studies of prostaglandin, the physically active substance of certain genital glands. Skand Arch Physiol. 81:65-80.

Watanabe, K., R. Yoshida, T. Shimizu, and O. Hayaishi. 1985. Enzymatic formation of prostaglandin F2 alpha from prostaglandin H2 and D2. Purification and properties of prostaglandin F synthetase from bovine lung. J. Biol. Chem. 260:7035-7041.

Wathes D.C., and R.W. Swann. 1982. Is oxytocin an ovarian hormone? Nature 297:225-227.

Wathes, D.C., R.W. Swann, S.D. Birkett, D.G. Porter, and B.T. Pickering. 1983. Characterization of oxytocin, vasopressin, and neurophysin from the bovine corpus luteum. Endocrinology 113:693-698.

Watson, A.J., P. De Sousa, A. Caveney, L.C. Barcroft, D. Natale, J. Urquhart, and M.E. Westhusin. 2000. Impact of bovine oocyte maturation media on oocyte transcript levels, blastocyst development, cell number, and apoptosis. Biol. Reprod. 62:355-364.

Watson, A.J., P.H. Watson, D. Warnes, S.K. Walker, D.T. Armstrong, and R.F. Seamark. 1994. Preimplantation development of in vitro-matured and in vitro-fertilized ovine zygotes: comparison between coculture on oviduct epithelial cell monolayers and culture under low oxygen atmosphere. Biol. Reprod. 50:715-724.

Watson, E.D. 1988. Eicosanoids and phagocytic and bactericidial activity of equine neutrophils. Equine Vet. J. 20:373-375.

Weber, J.A., D.A. Freeman, D.K. Vanderwall, and G.L. Woods. 1991. Prostaglandin E_2 hastens oviductal transport of equine embryos. Biol. Reprod. 45:544-546.

Weems, Y.S., P.J. Bridges, R.G. Sasser, L. Ching, B.R. LeaMaster, D.L. Vincent, and C.W. Weems. 2002. Effect of mifepristone on pregnancy, pregnancy-specific protein B (PSPB), progesterone, estradiol-17β, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and prostaglandin E (PGE) in ovariectomized 90-day pregnant ewes. Prostaglandins Other Lipid Mediat. 70:195-208.

Werth, L.A., J.C. Whittier, S.M. Azzam, G.H. Deutscher, and J.E. Kinder. 1996. Relationship between circulating progesterone and conception at the first postpartum estrus in young primiparous beef cows. J. Anim. Sci. 74:616-619.

Wiemer, K.E., A.J. Watson, V. Polanski, A.I. McKenna, G.H. Fick, and G.A. Schultz. 1991. Effects of maturation and coculture treatments on the developmental capacity of early bovine embryos. Mol. Reprod. Dev. 30:330-338.

Willenburg, K.L., G.M. Miller, S.L. Rodriguez-Zas, and R.V. Knox. 2003. Influence of hormone supplementation to extended semen on artificial insemination, uterine contractions, establishment of a sperm reservoir, and fertility in swine. J. Anim. Sci. 81:821-829.

Woelders, H., A. Mathiijs, and B. Engel 1997. Effects of trehalose, and sucrose, osmolarity of the freezing medium, and cooling rate on viability and intactness of bull sperm after freezing and thawing. Cryobiology 35:93-105.

Wood, P.D., J.A. Foulkes, R.C. Shaw, and D.R. Melrose. 1986. Semen assessment, fertility and the selection of Hereford bulls for use in AI. J. Reprod. Fertil. 76:783-795.

Yates, J.H., J.E. Chandler, A.L. Canal, and J.B. Paul.2003. The effect of nocturnal sampling on semen quality and the efficiency of collection in bovine species. Theriogenology 60:1665-1667.

Yoon, T.H., and I.H. Kim. 2002. Phosphatidylcholine isolation from egg yolk phospholipids by high-performance liquid chromatography. J. Chromatogr. A. 949:209-216.

Yu, H., D.-H. Shin, and W.H. Hsu. 1993. Effects of oxytocin (OT), vasopressin (VP), and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) on porcine myometrial contractility in vitro and their dependence on Ca^{2+} influx. Biol. Reprod. 48(Suppl.):162. (Abstr.)

Yule, T.D., G.D. Montoya, L.D. Russel, T.M. Williams, and K.S.K. Tung. 1988. Auto-antigenic germ cells exist outside the blood-testis barrier. J. Immunol. 141:1161-1167.

Zarrow, M.X., and J.H. Clark. 1968. Ovulation following vaginal stimulation in a spontaneous ovulatory and its implications. J. Endocrinol. 40:343-352.

Zeyneloglu, H.B., and H. Yarali. 2002. Antisperm antibodies: fact or fiction? Immunol. Allergy Clin. North Am. 22:471-501.

Zhang, B.R., B. Larsson, N. Lundeheim, and H. Rodriguez-Martinez. 1998. Sperm characteristics and zona pellucida binding in relation to field fertility of frozen-thawed semen from dairy AI bulls. Int. J. Androl. 21:207-216.

Zhang, B.R., B. Larsson, N. Lundeheim, M.G.H. Håård, and H. Rodriguez-Martinez. 1999. Prediction of bull fertility by combining in vitro assessments of frozen-thawed semen from young dairy bulls entering an AI-programme. Int. J. Androl. 22:253-260.

Zingg, H.H., E. Grazzini, C. Breton, A. Larcher, F. Rozen, C. Russo, G. Guillon, and B. Mouillac. 1998. Genomic and non-genomic mechanisms of oxytocin receptor regulation. Pages 287-295 in Vasopressin and Oxytocin. Molecular, Cellular, and Clinical. Adv. Exp. Med. Biol. H.H. Zingg, C.W. Bourque, and D.G. Bichet, ed. Plenum Press, NY.

Zoller, L.C., and J. Weisz. 1980. A demonstration of regional differences in lysosomal membrane permeability in the membrane granulosa of Graafian follicles in cryostat sections of the rat ovary: a quantitative cytochemical study. Endocrinology 106:871-877.

Zurrier, R.B., G. Weissmann, S. Hoffstien, S. Kammerman, and H.H. Tai. 1974. Mechanism of lysosomal enzyme release from human leukocytes. II. Effects of cAMP and cGMP, autonomic agonists, and agents which affect microtubule function. J. Clin. Invest. 53:297-309.

APPENDIX TABLE 1

ENDOGENOUS PROSTAGLANDIN $F_{2\alpha}$ CONCENTRATION IN BOVINE WHOLE SEMEN, SEMINAL PLASMA AND EXTENDED SEMEN, AND THEIR POTENTIAL EFFECT ON FEMALE FERTILITY – Experiment 1 raw data

Bull	Breed	Source	PGF
AN 178	Beef	Whole	233.0
AN 178	Beef	Extended	267.0
AN 213	Beef	Whole	147.0
AN 213	Beef	Extended	203.0
AN 226	Beef	Whole	173.0
AN 226	Beef	Extended	231.0
AN 234	Beef	Whole	174.0
AN 234	Beef	Extended	691.0
AN 76	Beef	Whole	340.0
AN 76	Beef	Extended	214.0
H 2344	Dairy	Whole	142.0
H 2344	Dairy	Extended	144.0
H 2393	Dairy	Whole	237.0
H 2393	Dairy	Extended	575.0
H 2448	Dairy	Whole	194.0
H 2448	Dairy	Extended	184.0
H 2517	Dairy	Whole	231.0
H 2517	Dairy	Extended	189.0
H 4364	Dairy	Whole	52.2
H 4364	Dairy	Extended	175.0
H 4637	Dairy	Whole	259.0
H 4637	Dairy	Extended	144.0
H 4937	Dairy	Whole	62.6
H 4937	Dairy	Extended	169.0
H 5099	Dairy	Whole	194.0
H 5099	Dairy	Extended	179.0
H 5157	Dairy	Whole	154.0
H 5157	Dairy	Extended	134.0
H 5206	Dairy	Whole	117.0
H 5206	Dairy	Extended	155.0
H 5211	Dairy	Whole	229.0
H 5211	Dairy	Extended	245.0
H 5653	Dairy	Whole	71.1

Bull	Breed	Source	PGF
H 5653	Dairy	Extended	159.0
H 5710	Dairy	Whole	220.0
H 5710	Dairy	Extended	512.0
H 5742	Dairy	Whole	459.0
H 5742	Dairy	Extended	210.0
H 5785	Dairy	Whole	165.0
H 5785	Dairy	Extended	180.0
H 5801	Dairy	Whole	215.0
H 5801	Dairy	Extended	165.0
H 5841	Dairy	Whole	293.0
H 5841	Dairy	Extended	124.0
H 5851	Dairy	Whole	194.0
H 5851	Dairy	Extended	143.0
H 6250	Dairy	Whole	154.0
H 6250	Dairy	Extended	408.0
H 8416	Dairy	Whole	120.0
H 8416	Dairy	Extended	455.0
SM 42	Beef	Whole	226.0
SM 42	Beef	Extended	387.0

APPENDIX TABLE 2

ENDOGENOUS PROSTAGLANDIN $F_{2\alpha}$ CONCENTRATION IN BOVINE WHOLE SEMEN, SEMINAL PLASMA AND EXTENDED SEMEN, AND THEIR POTENTIAL EFFECT ON FEMALE FERTILITY – Experiment 2 raw data

Bull	source	dilution	initial	Step	PGF
H2344	semen	1.10	521.0	1	138.6
H2344	semen	1.10	521.0	2	217.1
H2344	semen	1.10	521.0	3	141.0
H2344	semen	1.10	521.0	4	358.9
J195	semen	1.10	565.0	1	295.8
J195	semen	1.10	565.0	2	125.8
J195	semen	1.10	565.0	3	262.7
J195	semen	1.10	565.0	4	179.5
H7858	semen	1.10	332.0	1	420.4
H7858	semen	1.10	332.0	2	277.5
H7858	semen	1.10	332.0	3	262.7
H7858	semen	1.10	332.0	4	423.8
H5605	semen	1.10	386.0	1	.
H5605	semen	1.10	386.0	2	.
H5605	semen	1.10	386.0	3	333.2
H5605	semen	1.10	386.0	4	229.4
H6474	semen	1.10	399.0	1	551.0
H6474	semen	1.10	399.0	2	282.5
H6474	semen	1.10	399.0	3	273.6
H6474	semen	1.10	399.0	4	294.1
H7664	semen	1.10	499.0	1	706.0
H7664	semen	1.10	499.0	2	354.4
H7664	semen	1.10	499.0	3	316.6
H7664	semen	1.10	499.0	4	278.1
H5581	semen	1.10	311.0	1	363.6
H5581	semen	1.10	311.0	2	260.7
H5581	semen	1.10	311.0	3	201.2
H5581	semen	1.10	311.0	4	182.9
H2344	semen	1.15	521.0	1	.
H2344	semen	1.15	521.0	2	.
H2344	semen	1.15	521.0	3	.
H2344	semen	1.15	521.0	4	139.5
J195	semen	1.15	565.0	1	266.9

Bull	source	dilution	initial	Step	PGF
J195	semen	1.15	565.0	2	-3.8
J195	semen	1.15	565.0	3	165.2
J195	semen	1.15	565.0	4	101.6
H7858	semen	1.15	332.0	1	465.0
H7858	semen	1.15	332.0	2	293.6
H7858	semen	1.15	332.0	3	235.9
H7858	semen	1.15	332.0	4	202.1
H5605	semen	1.15	386.0	1	260.8
H5605	semen	1.15	386.0	2	191.1
H5605	semen	1.15	386.0	3	215.6
H5605	semen	1.15	386.0	4	172.5
H6474	semen	1.15	399.0	1	479.1
H6474	semen	1.15	399.0	2	244.8
H6474	semen	1.15	399.0	3	224.5
H6474	semen	1.15	399.0	4	250.7
H7664	semen	1.15	499.0	1	637.7
H7664	semen	1.15	499.0	2	399.8
H7664	semen	1.15	499.0	3	412.5
H7664	semen	1.15	499.0	4	375.0
H5581	semen	1.15	311.0	1	287.8
H5581	semen	1.15	311.0	2	193.7
H5581	semen	1.15	311.0	3	126.6
H5581	semen	1.15	311.0	4	152.5
H2344	semen	1.20	521.0	1	153.4
H2344	semen	1.20	521.0	2	113.3
H2344	semen	1.20	521.0	3	98.4
H2344	semen	1.20	521.0	4	79.3
J195	semen	1.20	565.0	1	175.0
J195	semen	1.20	565.0	2	126.0
J195	semen	1.20	565.0	3	128.5
J195	semen	1.20	565.0	4	167.1
H7858	semen	1.20	332.0	1	230.3
H7858	semen	1.20	332.0	2	187.6
H7858	semen	1.20	332.0	3	185.5
H7858	semen	1.20	332.0	4	226.8
H5605	semen	1.20	386.0	1	529.9
H5605	semen	1.20	386.0	2	263.5
H5605	semen	1.20	386.0	3	144.3

Bull	source	dilution	initial	Step	PGF
H5605	semen	1.20	386.0	4	168.1
H6474	semen	1.20	399.0	1	370.6
H6474	semen	1.20	399.0	2	223.5
H6474	semen	1.20	399.0	3	219.5
H6474	semen	1.20	399.0	4	319.4
H7664	semen	1.20	499.0	1	512.6
H7664	semen	1.20	499.0	2	306.0
H7664	semen	1.20	499.0	3	254.5
H7664	semen	1.20	499.0	4	264.4
H5581	semen	1.20	311.0	1	490.4
H5581	semen	1.20	311.0	2	292.1
H5581	semen	1.20	311.0	3	298.8
H5581	semen	1.20	311.0	4	126.9
H2344	semen	1.25	521.0	1	11.7
H2344	semen	1.25	521.0	2	107.4
H2344	semen	1.25	521.0	3	136.8
H2344	semen	1.25	521.0	4	.
J195	semen	1.25	565.0	1	120.2
J195	semen	1.25	565.0	2	.
J195	semen	1.25	565.0	3	.
J195	semen	1.25	565.0	4	144.3
H7858	semen	1.25	332.0	1	257.9
H7858	semen	1.25	332.0	2	190.8
H7858	semen	1.25	332.0	3	166.9
H7858	semen	1.25	332.0	4	170.6
H5605	semen	1.25	386.0	1	425.5
H5605	semen	1.25	386.0	2	170.5
H5605	semen	1.25	386.0	3	169.3
H5605	semen	1.25	386.0	4	200.4
H6474	semen	1.25	399.0	1	426.5
H6474	semen	1.25	399.0	2	305.7
H6474	semen	1.25	399.0	3	231.6
H6474	semen	1.25	399.0	4	216.9
H7664	semen	1.25	499.0	1	449.5
H7664	semen	1.25	499.0	2	196.7
H7664	semen	1.25	499.0	3	308.8
H7664	semen	1.25	499.0	4	190.9
H5581	semen	1.25	311.0	1	301.5

Bull	source	dilution	initial	Step	PGF
H5581	semen	1.25	311.0	2	154.0
H5581	semen	1.25	311.0	3	132.9
H5581	semen	1.25	311.0	4	220.4
H2344	semen	1.30	521.0	1	.
H2344	semen	1.30	521.0	2	162.1
H2344	semen	1.30	521.0	3	143.5
H2344	semen	1.30	521.0	4	135.1
J195	semen	1.30	565.0	1	.
J195	semen	1.30	565.0	2	.
J195	semen	1.30	565.0	3	91.7
J195	semen	1.30	565.0	4	.
H7858	semen	1.30	332.0	1	261.6
H7858	semen	1.30	332.0	2	187.6
H7858	semen	1.30	332.0	3	225.1
H7858	semen	1.30	332.0	4	207.5
H5605	semen	1.30	386.0	1	309.0
H5605	semen	1.30	386.0	2	163.8
H5605	semen	1.30	386.0	3	155.9
H5605	semen	1.30	386.0	4	148.7
H6474	semen	1.30	399.0	1	312.1
H6474	semen	1.30	399.0	2	223.2
H6474	semen	1.30	399.0	3	184.4
H6474	semen	1.30	399.0	4	177.2
H7664	semen	1.30	499.0	1	309.4
H7664	semen	1.30	499.0	2	233.2
H7664	semen	1.30	499.0	3	129.4
H7664	semen	1.30	499.0	4	103.9
H5581	semen	1.30	311.0	1	349.0
H5581	semen	1.30	311.0	2	265.6
H5581	semen	1.30	311.0	3	212.9
H5581	semen	1.30	311.0	4	165.2
J195	semen	1.35	565.0	1	.
J195	semen	1.35	565.0	2	120.6
J195	semen	1.35	565.0	3	106.7
J195	semen	1.35	565.0	4	.
H7858	semen	1.35	332.0	1	271.3
H7858	semen	1.35	332.0	2	192.6
H7858	semen	1.35	332.0	3	213.7

Bull	source	dilution	initial	Step	PGF
H7858	semen	1.35	332.0	4	196.8
H5605	semen	1.35	386.0	1	299.2
H5605	semen	1.35	386.0	2	172.2
H5605	semen	1.35	386.0	3	148.9
H5605	semen	1.35	386.0	4	104.3
H6474	semen	1.35	399.0	1	644.4
H6474	semen	1.35	399.0	2	107.7
H6474	semen	1.35	399.0	3	150.4
H6474	semen	1.35	399.0	4	172.9
H7664	semen	1.35	499.0	1	334.7
H7664	semen	1.35	499.0	2	233.4
H7664	semen	1.35	499.0	3	251.0
H7664	semen	1.35	499.0	4	89.0
H5581	semen	1.35	311.0	1	308.5
H5581	semen	1.35	311.0	2	251.5
H5581	semen	1.35	311.0	3	166.4
H5581	semen	1.35	311.0	4	147.4
J195	semen	1.40	565.0	1	.
J195	semen	1.40	565.0	2	.
J195	semen	1.40	565.0	3	.
J195	semen	1.40	565.0	4	190.0
H7858	semen	1.40	332.0	1	305.2
H7858	semen	1.40	332.0	2	217.1
H7858	semen	1.40	332.0	3	175.9
H7858	semen	1.40	332.0	4	174.8
H5605	semen	1.40	386.0	1	202.5
H5605	semen	1.40	386.0	2	107.0
H5605	semen	1.40	386.0	3	99.3
H5605	semen	1.40	386.0	4	78.4
H6474	semen	1.40	399.0	1	417.9
H6474	semen	1.40	399.0	2	206.5
H6474	semen	1.40	399.0	3	154.9
H6474	semen	1.40	399.0	4	140.1
H7664	semen	1.40	499.0	1	341.9
H7664	semen	1.40	499.0	2	130.8
H7664	semen	1.40	499.0	3	132.9
H7664	semen	1.40	499.0	4	190.6
H5581	semen	1.40	311.0	1	279.3

Bull	source	dilution	initial	Step	PGF
H5581	semen	1.40	311.0	2	194.9
H5581	semen	1.40	311.0	3	152.5
H5581	semen	1.40	311.0	4	166.3
J195	semen	1.80	565.0	1	51.4
J195	semen	1.80	565.0	2	42.9
J195	semen	1.80	565.0	3	43.6
J195	semen	1.80	565.0	4	50.4
H7858	semen	1.80	332.0	1	239.2
H7858	semen	1.80	332.0	2	138.3
H7858	semen	1.80	332.0	3	120.1
H7858	semen	1.80	332.0	4	94.3
H5605	semen	1.80	386.0	1	145.9
H5605	semen	1.80	386.0	2	85.3
H5605	semen	1.80	386.0	3	59.3
H5605	semen	1.80	386.0	4	52.6
H6474	semen	1.80	399.0	1	116.5
H6474	semen	1.80	399.0	2	112.1
H6474	semen	1.80	399.0	3	87.1
H6474	semen	1.80	399.0	4	.
H7664	semen	1.80	499.0	1	142.0
H7664	semen	1.80	499.0	2	91.2
H7664	semen	1.80	499.0	3	98.6
H7664	semen	1.80	499.0	4	87.2
H5581	semen	1.80	311.0	1	218.7
H5581	semen	1.80	311.0	2	149.7
H5581	semen	1.80	311.0	3	145.4
H5581	semen	1.80	311.0	4	123.6

APPENDIX TABLE 3

ENDOGENOUS PROSTAGLANDIN $F_{2\alpha}$ CONCENTRATION IN BOVINE WHOLE SEMEN, SEMINAL PLASMA AND EXTENDED SEMEN, AND THEIR POTENTIAL EFFECT ON FEMALE FERTILITY – Experiment 3 raw data

Bull	source	dilution	initial	Step	PGF
H2344	semen	1.15	521.0	1	.
H2344	semen	1.15	521.0	2	.
H2344	semen	1.15	521.0	3	.
H2344	semen	1.15	521.0	4	139.5
H2344	semen	1.15	521.0	5	.
J195	semen	1.15	565.0	1	266.9
J195	semen	1.15	565.0	2	-3.8
J195	semen	1.15	565.0	3	165.2
J195	semen	1.15	565.0	4	101.6
J195	semen	1.15	565.0	5	135.6
H7858	semen	1.15	332.0	1	465.0
H7858	semen	1.15	332.0	2	293.6
H7858	semen	1.15	332.0	3	235.9
H7858	semen	1.15	332.0	4	202.1
H7858	semen	1.15	332.0	5	199.1
H5605	semen	1.15	386.0	1	260.8
H5605	semen	1.15	386.0	2	191.1
H5605	semen	1.15	386.0	3	215.6
H5605	semen	1.15	386.0	4	172.5
H5605	semen	1.15	386.0	5	238.5
H6474	semen	1.15	399.0	1	479.1
H6474	semen	1.15	399.0	2	244.8
H6474	semen	1.15	399.0	3	224.5
H6474	semen	1.15	399.0	4	250.7
H6474	semen	1.15	399.0	5	201.7
H7664	semen	1.15	499.0	1	637.7
H7664	semen	1.15	499.0	2	399.8
H7664	semen	1.15	499.0	3	412.5
H7664	semen	1.15	499.0	4	375.0
H7664	semen	1.15	499.0	5	277.0
H5581	semen	1.15	311.0	1	287.8
H5581	semen	1.15	311.0	2	193.7
H5581	semen	1.15	311.0	3	126.6

Bull	source	dilution	initial	Step	PGF
H5581	semen	1.15	311.0	4	152.5
H5581	semen	1.15	311.0	5	-87.5
H2344	semen	1.20	521.0	1	153.4
H2344	semen	1.20	521.0	2	113.3
H2344	semen	1.20	521.0	3	98.4
H2344	semen	1.20	521.0	4	79.3
H2344	semen	1.20	521.0	5	.
J195	semen	1.20	565.0	1	175.0
J195	semen	1.20	565.0	2	126.0
J195	semen	1.20	565.0	3	128.5
J195	semen	1.20	565.0	4	167.1
J195	semen	1.20	565.0	5	143.1
H7858	semen	1.20	332.0	1	230.3
H7858	semen	1.20	332.0	2	187.6
H7858	semen	1.20	332.0	3	185.5
H7858	semen	1.20	332.0	4	226.8
H7858	semen	1.20	332.0	5	190.8
H5605	semen	1.20	386.0	1	529.9
H5605	semen	1.20	386.0	2	263.5
H5605	semen	1.20	386.0	3	144.3
H5605	semen	1.20	386.0	4	168.1
H5605	semen	1.20	386.0	5	207.1
H6474	semen	1.20	399.0	1	370.6
H6474	semen	1.20	399.0	2	223.5
H6474	semen	1.20	399.0	3	219.5
H6474	semen	1.20	399.0	4	319.4
H6474	semen	1.20	399.0	5	200.4
H7664	semen	1.20	499.0	1	512.6
H7664	semen	1.20	499.0	2	306.0
H7664	semen	1.20	499.0	3	254.5
H7664	semen	1.20	499.0	4	264.4
H7664	semen	1.20	499.0	5	224.4
H5581	semen	1.20	311.0	1	490.4
H5581	semen	1.20	311.0	2	292.1
H5581	semen	1.20	311.0	3	298.8
H5581	semen	1.20	311.0	4	126.9
H5581	semen	1.20	311.0	5	154.9
H2344	semen	1.25	521.0	1	11.7

Bull	source	dilution	initial	Step	PGF
H2344	semen	1.25	521.0	2	107.4
H2344	semen	1.25	521.0	3	136.8
H2344	semen	1.25	521.0	4	.
H2344	semen	1.25	521.0	5	.
J195	semen	1.25	565.0	1	120.2
J195	semen	1.25	565.0	2	.
J195	semen	1.25	565.0	3	.
J195	semen	1.25	565.0	4	144.3
J195	semen	1.25	565.0	5	169.3
H7858	semen	1.25	332.0	1	257.9
H7858	semen	1.25	332.0	2	190.8
H7858	semen	1.25	332.0	3	166.9
H7858	semen	1.25	332.0	4	170.6
H7858	semen	1.25	332.0	5	148.6
H5605	semen	1.25	386.0	1	425.5
H5605	semen	1.25	386.0	2	170.5
H5605	semen	1.25	386.0	3	169.3
H5605	semen	1.25	386.0	4	200.4
H5605	semen	1.25	386.0	5	180.4
H6474	semen	1.25	399.0	1	426.5
H6474	semen	1.25	399.0	2	305.7
H6474	semen	1.25	399.0	3	231.6
H6474	semen	1.25	399.0	4	216.9
H6474	semen	1.25	399.0	5	194.9
H7664	semen	1.25	499.0	1	449.5
H7664	semen	1.25	499.0	2	196.7
H7664	semen	1.25	499.0	3	308.8
H7664	semen	1.25	499.0	4	190.9
H7664	semen	1.25	499.0	5	238.9
H5581	semen	1.25	311.0	1	301.5
H5581	semen	1.25	311.0	2	154.0
H5581	semen	1.25	311.0	3	132.9
H5581	semen	1.25	311.0	4	220.4
H5581	semen	1.25	311.0	5	217.4
H7870	seminal	1.15	554.0	1	235.4
H7870	seminal	1.15	554.0	2	165.3
H7870	seminal	1.15	554.0	3	93.1
H7870	seminal	1.15	554.0	4	89.3

Bull	source	dilution	initial	Step	PGF
H7870	seminal	1.15	554.0	5	73.3
H2344	seminal	1.15	131.0	1	304.8
H2344	seminal	1.15	131.0	2	421.7
H2344	seminal	1.15	131.0	3	215.0
H2344	seminal	1.15	131.0	4	199.6
H2344	seminal	1.15	131.0	5	169.6
J195	seminal	1.15	81.5	1	408.4
J195	seminal	1.15	81.5	2	240.7
J195	seminal	1.15	81.5	3	207.0
J195	seminal	1.15	81.5	4	190.9
J195	seminal	1.15	81.5	5	.
H7551	seminal	1.15	156.0	1	584.4
H7551	seminal	1.15	156.0	2	241.2
H7551	seminal	1.15	156.0	3	213.0
H7551	seminal	1.15	156.0	4	193.9
H7551	seminal	1.15	156.0	5	171.9
H7858	seminal	1.15	85.1	1	596.9
H7858	seminal	1.15	85.1	2	269.3
H7858	seminal	1.15	85.1	3	224.7
H7858	seminal	1.15	85.1	4	175.6
H7858	seminal	1.15	85.1	5	229.6
H5605	seminal	1.15	26.0	1	371.8
H5605	seminal	1.15	26.0	2	225.3
H5605	seminal	1.15	26.0	3	171.5
H5605	seminal	1.15	26.0	4	180.6
H5605	seminal	1.15	26.0	5	162.6
H7664	seminal	1.15	35.3	1	263.5
H7664	seminal	1.15	35.3	2	182.3
H7664	seminal	1.15	35.3	3	229.7
H7664	seminal	1.15	35.3	4	223.0
H7664	seminal	1.15	35.3	5	158.0
H7870	seminal	1.20	554.0	1	202.1
H7870	seminal	1.20	554.0	2	140.8
H7870	seminal	1.20	554.0	3	136.1
H7870	seminal	1.20	554.0	4	57.7
H7870	seminal	1.20	554.0	5	53.7
H2344	seminal	1.20	131.0	1	282.4
H2344	seminal	1.20	131.0	2	216.7

Bull	source	dilution	initial	Step	PGF
H2344	seminal	1.20	131.0	3	181.6
H2344	seminal	1.20	131.0	4	162.9
H2344	seminal	1.20	131.0	5	150.9
J195	seminal	1.20	81.5	1	351.3
J195	seminal	1.20	81.5	2	209.4
J195	seminal	1.20	81.5	3	201.6
J195	seminal	1.20	81.5	4	146.4
J195	seminal	1.20	81.5	5	.
H7551	seminal	1.20	156.0	1	449.9
H7551	seminal	1.20	156.0	2	203.8
H7551	seminal	1.20	156.0	3	203.1
H7551	seminal	1.20	156.0	4	166.6
H7551	seminal	1.20	156.0	5	125.6
H7858	seminal	1.20	85.1	1	543.0
H7858	seminal	1.20	85.1	2	255.1
H7858	seminal	1.20	85.1	3	184.4
H7858	seminal	1.20	85.1	4	192.2
H7858	seminal	1.20	85.1	5	206.2
H5581	seminal	1.20	101.0	1	198.4
H5581	seminal	1.20	101.0	2	269.9
H5581	seminal	1.20	101.0	3	133.4
H5581	seminal	1.20	101.0	4	101.4
H5581	seminal	1.20	101.0	5	249.4
H7664	seminal	1.20	35.3	1	159.0
H7664	seminal	1.20	35.3	2	45.9
H7664	seminal	1.20	35.3	3	165.4
H7664	seminal	1.20	35.3	4	174.7
H7664	seminal	1.20	35.3	5	148.7
H7870	seminal	1.25	554.0	1	144.1
H7870	seminal	1.25	554.0	2	39.4
H7870	seminal	1.25	554.0	3	88.2
H7870	seminal	1.25	554.0	4	57.7
H7870	seminal	1.25	554.0	5	38.7
H2344	seminal	1.25	131.0	1	321.9
H2344	seminal	1.25	131.0	2	191.9
H2344	seminal	1.25	131.0	3	155.5
H2344	seminal	1.25	131.0	4	137.7
H2344	seminal	1.25	131.0	5	151.7

Bull	source	dilution	initial	Step	PGF
J195	seminal	1.25	81.5	1	343.9
J195	seminal	1.25	81.5	2	194.8
J195	seminal	1.25	81.5	3	191.9
J195	seminal	1.25	81.5	4	159.6
J195	seminal	1.25	81.5	5	.
H7551	seminal	1.25	156.0	1	327.9
H7551	seminal	1.25	156.0	2	178.4
H7551	seminal	1.25	156.0	3	129.3
H7551	seminal	1.25	156.0	4	122.7
H7551	seminal	1.25	156.0	5	138.7
H7664	seminal	1.25	35.3	1	251.6
H7664	seminal	1.25	35.3	2	163.6
H7664	seminal	1.25	35.3	3	.
H7664	seminal	1.25	35.3	4	127.5
H7664	seminal	1.25	35.3	5	113.5

APPENDIX TABLE 4

ENDOGENOUS PROSTAGLANDIN $F_{2\alpha}$ CONCENTRATION IN BOVINE WHOLE SEMEN, SEMINAL PLASMA AND EXTENDED SEMEN, AND THEIR POTENTIAL EFFECT ON FEMALE FERTILITY – Experiment 4 raw data

Bull Code	Average Deviation	Relative fertility	$PGF_{2\alpha}$ pg/ml	PGE_2 pg/ml	Ratio PGE:PGF
H5841	-4.947	1.00	159.7	195.5	1.224425887
H5922	-3.807	1.25	161.5	259.5	1.606811146
H4937	-3.627	1.25	551.0	563.0	1.021778584
H5862	-3.000	1.50	253.0	189.0	0.747035573
H2544	-2.103	1.50	185.0	261.0	1.410810811
H5992	-1.826	1.75	162.5	317.0	1.950769231
H5099	-0.822	2.00	303.0	396.0	1.306930693
H5605	-0.762	2.00	220.0	179.0	0.813636364
H5630	0.372	2.25	261.5	392.5	1.500956023
H5211	0.816	2.25	131.0	367.0	2.801526718
H5801	1.454	2.50	162.0	475.0	2.932098765
H4637	1.621	2.50	173.0	240.5	1.39017341
H5760	1.912	2.50	174.5	229.5	1.315186246
H6302	2.133	2.75	174.0	333.5	1.916666667
H5491	2.422	2.75	142.7	225.0	1.577102804
H6155	2.814	2.75	177.0	244.5	1.381355932

APPENDIX TABLE 5

IMPROVEMENT OF CONCEPTION RATE IN CATTLE RECEIVING EXOGENOUS PROSTAGLANDIN $F_{2\alpha}$ ADMINISTERED AT THE TIME OF INSEMINATION – Experiment 1 *in vitro* raw data

Bull	Trt	Fertilization rate	Embryo cleavage rate
Bull A	0	9.68	3.00
Bull A	0	20.59	1.57
Bull A	0	16.00	2.00
Bull B	0	22.22	2.17
Bull B	0	21.43	2.00
Bull B	0	15.38	1.75
Bull A	500	12.12	2.00
Bull A	500	.	.
Bull A	500	.	.
Bull B	500	12.50	3.00
Bull B	500	18.18	2.17
Bull B	500	10.71	3.00
Bull A	5000	20.83	2.20
Bull A	5000	22.22	1.50
Bull A	5000	10.71	2.30
Bull B	5000	9.09	1.50
Bull B	5000	17.86	2.00
Bull B	5000	9.52	1.00

APPENDIX TABLE 6

IMPROVEMENT OF CONCEPTION RATE IN CATTLE RECEIVING EXOGENOUS PROSTAGLANDIN $F_{2\alpha}$ ADMINISTERED AT THE TIME OF INSEMINATION – Experiment 1 *in vivo* raw data

Cow #	Loc.	Cow Age	PPI to brd	Bull Code	PGF Trt	Tech.	Calving interval	Concep	Preg
2116	Union	1	.	Bull A	5000	1	.	1	1
2020	Union	1	.	Bull B	500	1	.	0	0
2219	Union	1	.	Bull A	0	1	.	0	1
2230	Union	1	.	Bull B	0	1	.	1	1
2105	Union	1	.	Bull A	0	1	.	1	1
2154	Union	1	.	Bull B	500	1	.	1	1
2006	Union	1	.	Bull B	0	1	.	1	1
2207	Union	1	.	Bull B	5000	1	.	1	1
2151	Union	1	.	Bull A	5000	1	.	1	1
8017	Union	5	64	Bull A	5000	3	348	1	1
6186	Union	7	88	Bull A	500	3	391	0	1
9211	Union	4	65	Bull A	500	3	349	1	1
9223	Union	4	81	Bull B	0	3	388	0	1
9165	Union	4	86	Bull B	0	3	362	1	1
4004	Union	9	68	Bull A	5000	3	.	0	0
0216	Union	3	74	Bull A	0	3	372	0	1
8052	Union	5	76	Bull A	5000	3	356	1	1
5009	Union	8	54	Bull B	500	3	337	1	1
8220	Union	5	75	Bull A	500	3	362	1	1
5030	Union	8	77	Bull B	0	3	380	0	1
6216	Union	7	81	Bull A	500	3	397	0	1
5004	Union	8	79	Bull A	5000	3	380	0	1
3041	Union	10	73	Bull B	5000	3	383	0	1
9269	Union	4	57	Bull B	5000	3	337	1	1
6165	Union	7	73	Bull A	500	3	381	0	1
6141	Union	7	76	Bull A	0	3	364	1	1
5197	Union	8	63	Bull A	500	3	441	0	1
4135	Union	9	78	Bull B	0	3	362	1	1
5132	Union	8	56	Bull B	500	3	382	0	1
5207	Union	8	73	Bull B	500	3	.	0	0
6185	Union	7	81	Bull A	5000	3	385	0	1

Cow #	Loc.	Cow Age	PPI to brd	Bull Code	PGF Trt	Tech.	Calving interval	Concep	Preg
009	Union	3	58	Bull A	5000	3	361	0	1
5027	Union	8	73	Bull B	5000	3	353	1	1
0143	Union	.	.	Bull B	5000	3	.	1	1
4007	Union	9	80	Bull B	500	3	.	0	0
8122	Union	5	81	Bull A	500	3	366	1	1
6145	Union	7	72	Bull A	500	3	361	1	1
5204	Union	8	71	Bull B	0	3	354	1	1
9215	Union	4	78	Bull B	5000	3	378	0	1
9152	Union	4	64	Bull B	5000	3	341	1	1
5214	Union	8	76	Bull A	0	3	363	1	1
1116	Union	12	78	Bull A	500	3	379	0	1
9217	Union	4	58	Bull A	0	3	381	0	1
6177	Union	7	77	Bull B	0	3	384	0	1
9019	Union	4	57	Bull B	500	3	350	1	1
4039	Union	9	68	Bull B	500	3	.	0	0
8046	Union	5	80	Bull A	500	3	360	1	1
6187	Union	7	88	Bull A	500	3	373	1	1
2061	Union	11	57	Bull B	0	3	367	0	1
9116	Union	4	69	Bull B	500	3	.	0	0
8037	Union	5	56	Bull B	0	3	359	0	1
6126	Union	7	59	Bull A	500	3	337	1	1
5002	Union	8	60	Bull A	0	3	365	0	1
9046	Union	4	79	Bull B	0	3	392	0	1
7106	Union	6	86	Bull B	500	3	411	0	1
8016	Union	5	77	Bull B	5000	3	360	1	1
8031	Union	5	65	Bull A	500	3	.	0	0
9173	Union	4	57	Bull A	500	3	382	0	1
0125	Union	3	54	Bull B	0	3	338	1	1
5127	Union	8	60	Bull B	500	3	363	0	1
7110	Union	6	80	Bull B	0	3	355	1	1
9123	Union	4	74	Bull A	0	3	396	0	1
0103	Union	3	73	Bull A	0	3	.	0	0
5195	Union	8	77	Bull B	5000	3	359	1	1
8226	Union	5	63	Bull B	5000	3	338	1	1
6137	Union	7	87	Bull B	500	3	390	0	1
1176	Union	12	75	Bull A	0	3	359	1	1
3154	Union	10	71	Bull A	5000	3	.	0	0

Cow #	Loc.	Cow Age	PPI to brd	Bull Code	PGF Trt	Tech.	Calving interval	Concep	Preg
9147	Union	4	75	Bull B	0	3	357	1	1
5003	Union	8	59	Bull B	500	3	339	1	1
0101	Union	3	61	Bull B	0	3	337	1	1
7140	Union	6	90	Bull A	0	3	373	1	1
5007	Union	8	69	Bull A	500	3	352	1	1
6128	Union	7	81	Bull B	0	3	383	0	1
8237	Union	5	80	Bull B	5000	3	363	1	1
1075	Union	12	76	Bull B	5000	3	410	0	1
7152	Union	6	60	Bull A	500	3	.	0	0
8027	Union	5	85	Bull A	0	3	361	1	1
6020	Union	7	62	Bull B	5000	3	345	1	1
0145	Union	3	55	Bull A	0	1	369	0	1
0136	Union	3	69	Bull A	500	1	393	0	1
0102	Union	3	56	Bull A	5000	1	332	1	1
0193	Union	3	51	Bull A	500	1	356	0	1
7123	Union	6	49	Bull A	5000	1	330	1	1
025	Union	3	54	Bull A	500	1	355	0	1
0165	Union	3	50	Bull A	0	1	331	1	1
9239	Union	4	60	Bull A	5000	1	345	1	1
8101	Union	5	58	Bull B	500	1	377	0	1
9101	Union	4	60	Bull B	5000	1	.	0	0
5249	Union	8	37	Bull A	0	1	322	1	1
8243	Union	5	53	Bull A	5000	1	353	0	1
0142	Union	3	58	Bull A	500	1	345	1	1
9025	Union	4	59	Bull B	0	1	356	0	1
1098	Union	12	43	Bull B	0	1	325	1	1
014	Union	3	74	Bull A	0	1	.	0	0
5160	Union	8	60	Bull A	500	1	377	0	1
007	Union	3	37	Bull A	500	1	337	0	1
8218	Union	5	57	Bull B	0	1	364	0	1
0214	Union	3	89	Bull B	5000	1	368	1	1
9001	Union	4	54	Bull B	500	1	374	0	1
1105	Union	2	100	Bull A	500	1	404	0	1
1242	Union	2	115	Bull B	500	1	.	0	0
1189	Union	2	86	Bull B	5000	1	384	0	1
1141	Union	2	82	Bull A	0	1	362	1	1
1024	Union	2	108	Bull A	5000	1	415	0	1

Cow #	Loc.	Cow Age	PPI to brd	Bull Code	PGF Trt	Tech.	Calving interval	Concep	Preg
1119	Union	2	115	Bull A	500	1	390	1	1
1007	Union	2	87	Bull B	500	1		0	0
1112	Union	2	85	Bull B	0	1	358	1	1
1196	Union	2	91	Bull A	5000	1	369	1	1
1046	Union	2	58	Bull A	0	1	341	1	1
1148	Union	2	84	Bull A	0	1	383	0	1
1241	Union	2	104	Bull B	500	1	406	0	1
1019	Union	2	106	Bull B	5000	1	378	1	1
1222	Union	2	113	Bull A	5000	1	386	1	1
1137	Union	2	114	Bull A	5000	1	.	0	0
1238	Union	2	106	Bull A	500	1	407	0	1
1133	Union	2	64	Bull B	500	1	.	0	0
1005	Union	2	96	Bull B	0	1	373	1	1
1004	Union	2	96	Bull A	0	1	376	1	1
1154	Union	2	108	Bull A	5000	1	397	1	1
1251	Union	2	89	Bull A	0	1	373	1	1
1139	Union	2	111	Bull B	5000	1	411	0	1
9130	Union	4	37	Bull B	500	3	314	1	1
7128	Union	6	60	Bull B	0	3	364	0	1
6140	Union	7	93	Bull B	500	3	372	1	1
9255	Union	4	59	Bull B	5000	3	343	1	1
033	Union	3	56	Bull A	5000	3	342	1	1
9238	Union	4	39	Bull A	500	3	325	1	1
016	Union	3	56	Bull B	0	3	353	0	1
2013	Union	11	60	Bull B	0	3	340	1	1
0181	Union	3	49	Bull B	500	3	350	0	1
9208	Union	4	60	Bull A	500	3	368	0	1
8211	Union	5	53	Bull A	0	3	.	0	0
0110	Union	3	48	Bull B	500	3	328	1	1
8234	Union	5	45	Bull B	5000	3	328	1	1
0166	Union	3	50	Bull B	5000	3	354	0	1
9144	Union	4	58	Bull A	5000	3	337	1	1
9191	Union	4	56	Bull A	0	3	339	1	1
0222	Union	3	45	Bull B	5000	3	332	1	1
0224	Union	3	47	Bull B	500	3	372	0	1
0210	Union	3	36	Bull B	500	3	357	0	1
004	Union	3	57	Bull A	5000	3	334	1	1

Cow #	Loc.	Cow Age	PPI to brd	Bull Code	PGF Trt	Tech.	Calving interval	Concep	Preg
0155	Union	3	47	Bull A	0	3	327	1	1
1002	Union	2	87	Bull A	5000	3	365	1	1
1190	Union	2	87	Bull B	0	3	391	0	1
1144	Union	2	93	Bull B	0	3	389	0	1
1172	Union	2	89	Bull B	500	3	369	1	1
1018	Union	2	105	Bull A	0	3	387	1	1
1206	Union	2	69	Bull B	5000	3	342	1	1
1109	Union	2	103	Bull B	5000	3	426	0	1
1013	Union	2	82	Bull B	500	3	364	1	1
1028	Union	2	86	Bull A	500	3	380	1	1
1120	Union	2	91	Bull A	500	3	366	1	1
1150	Union	2	108	Bull B	0	3	389	1	1
1132	Union	2	106	Bull B	0	3	408	0	
1134	Union	2	76	Bull B	500	3	383	0	1
1123	Union	2	81	Bull A	500	3	360	1	1
1181	Union	2	111	Bull A	0	3	394	1	1
1145	Union	2	94	Bull B	0	3	376	1	1
1117	Union	2	110	Bull B	5000	3	391	1	1
1228	Union	2	112	Bull B	500	3	393	1	1
1186	Union	2	104	Bull A	500	3	389	1	1
1181	Union	12	.	Bull A	500	3	.	0	0
1129	Union	2	106	Bull B	500	3	379	1	1
2109	Burns	1	.	Bull B	0	2	.	1	1
2068	Burns	1	.	Bull B	500	2	.	1	1
2085	Burns	1	.	Bull A	5000	2	.	1	1
2135	Burns	1	.	Bull A	5000	2	.	0	1
2048	Burns	1	.	Bull A	500	2	.	1	1
2098	Burns	1	.	Bull B	5000	2	.	0	1
2168	Burns	1	.	Bull B	5000	2	.	0	1
2063	Burns	1	.	Bull A	5000	2	.	1	1
2126	Burns	1	.	Bull A	500	2	.	0	1
2194	Burns	1	.	Bull A	5000	2	.	0	1
2124	Burns	1	.	Bull B	5000	2	.	1	1
2032	Burns	1	.	Bull B	0	2	.	0	0
2065	Burns	1	.	Bull A	0	2	.	1	1
2155	Burns	1	.	Bull A	500	2	.	0	1
2111	Burns	1	.	Bull A	500	2	.	0	1

Cow #	Loc.	Cow Age	PPI to brd	Bull Code	PGF Trt	Tech.	Calving interval	Concep	Preg
2106	Burns	1	.	Bull B	5000	2	.	1	1
2011	Burns	1	.	Bull B	500	2	.	0	1
2002	Burns	1	.	Bull A	0	2	.	0	1
2010	Burns	1	.	Bull A	500	2	.	0	1
2185	Burns	1	.	Bull A	500	2	.	0	1
2169	Burns	1	.	Bull B	500	2	.	1	1
2171	Burns	1	.	Bull B	500	2	.	0	1
2014	Burns	1	.	Bull A	500	2	.	0	1
2224	Burns	1	.	Bull A	500	2	.	1	1
2004	Burns	1	.	Bull A	0	2	.	1	1
2154	Burns	1	.	Bull B	0	2	.	1	1
2005	Burns	1	.	Bull B	5000	2	.	0	1
2039	Burns	1	.	Bull B	500	2	.	1	1
2166	Burns	1	.	Bull A	5000	2	.	0	1
2137	Burns	1	.	Bull A	0	2	.	1	1
2073	Burns	1	.	Bull A	0	2	.	1	1
2118	Burns	1	.	Bull B	500	2	.	1	1
2133	Burns	1	.	Bull B	500	2	.	1	1
2017	Burns	1	.	Bull B	0	3	.	0	1
2123	Burns	1	.	Bull B	500	3	.	0	1
2188	Burns	1	.	Bull B	5000	3	.	1	1
2093	Burns	1	.	Bull A	0	3	.	1	1
2038	Burns	1	.	Bull A	500	3	.	1	1
2107	Burns	1	.	Bull B	500	3	.	1	1
2082	Burns	1	.	Bull B	0	3	.	0	1
2183	Burns	1	.	Bull B	500	3	.	1	1
2225	Burns	1	.	Bull A	500	3	.	1	1
2129	Burns	1	.	Bull A	0	3	.	0	1
2092	Burns	1	.	Bull B	0	3	.	0	1
2167	Burns	1	.	Bull B	500	3	.	1	1
2007	Burns	1	.	Bull B	5000	3	.	0	1
2200	Burns	1	.	Bull A	5000	3	.	0	1
2020	Burns	1	.	Bull A	5000	3	.	0	1
2090	Burns	1	.	Bull B	5000	3	.	0	1
2009	Burns	1	.	Bull B	500	3	.	1	1
2152	Burns	1	.	Bull B	0	3	.	1	1
2021	Burns	1	.	Bull A	0	3	.	0	1

Cow #	Loc.	Cow Age	PPI to brd	Bull Code	PGF Trt	Tech.	Calving interval	Concep	Preg
2190	Burns	1	.	Bull A	5000	3	.	1	1
2003	Burns	1	.	Bull B	0	3	.	0	1
2041	Burns	1	.	Bull B	5000	3	.	1	1
2132	Burns	1	.	Bull B	0	3	.	1	1
2059	Burns	1	.	Bull A	0	3	.	1	1
2205	Burns	1	.	Bull A	5000	3	.	1	1
2006	Burns	1	.	Bull B	5000	3	.	1	1
2054	Burns	1	.	Bull B	500	3	.	0	1
2070	Burns	1	.	Bull B	0	3	.	1	1
2144	Burns	1	.	Bull A	500	3	.	0	1
2069	Burns	1	.	Bull B	5000	3	.	0	0
2157	Burns	1	.	Bull B	0	3	.	0	1
9028	Burns	4	100	Bull B	5000	2	394	1	1
7020	Burns	6	97	Bull B	5000	2	412	0	1
0182	Burns	3	83	Bull A	5000	2	363	1	1
8033	Burns	5	70	Bull A	0	2	347	1	1
0045	Burns	3	58	Bull A	5000	2	335	1	1
7045	Burns	6	93	Bull B	5000	2	.	0	0
0019	Burns	3	78	Bull B	500	2	382	0	1
0007	Burns	3	83	Bull A	500	2	387	0	1
7058	Burns	6	95	Bull A	5000	2	380	1	1
8011	Burns	5	80	Bull A	0	2	355	1	1
9108	Burns	4	50	Bull B	5000	2	359	0	1
0088	Burns	3	86	Bull B	500	2	381	0	1
9011	Burns	4	80	Bull A	0	2	376	0	1
0050	Burns	3	95	Bull A	5000	2	371	1	1
9212	Burns	4	90	Bull A	500	2	.	0	0
9077	Burns	4	74	Bull B	0	2	354	1	1
0002	Burns	3	61	Bull B	5000	2	344	1	1
176	Burns	10	50	Bull A	5000	2	.	0	0
0006	Burns	3	93	Bull B	5000	2	408	0	1
7063	Burns	6	74	Bull A	0	2	381	0	1
0068	Burns	3	97	Bull B	500	2	409	0	1
0179	Burns	3	78	Bull B	0	2	.	0	0
7008	Burns	6	59	Bull A	500	2	340	1	1
7060	Burns	6	80	Bull A	5000	2	360	1	1
0030	Burns	3	82	Bull A	5000	2	387	0	1

Cow #	Loc.	Cow Age	PPI to brd	Bull Code	PGF Trt	Tech.	Calving interval	Concep	Preg
0133	Burns	3	91	Bull B	0	2	409	0	1
7002	Burns	6	36	Bull B	500	2	354	0	1
0001	Burns	3	90	Bull A	0	2	.	0	0
0065	Burns	3	52	Bull A	500	2	331	1	1
7024	Burns	6	84	Bull A	5000	2	367	1	1
8027	Burns	5	82	Bull B	0	2	398	0	1
0023	Burns	3	84	Bull B	5000	2	388	0	1
9216	Burns	4	79	Bull A	0	2	380	0	1
8017	Burns	5	81	Bull A	500	2	.	0	0
0008	Burns	3	84	Bull B	5000	2	378	1	1
0143	Burns	3	60	Bull B	0	2	381	0	1
0016	Burns	3	92	Bull B	0	2	401	0	1
0155	Burns	3	66	Bull A	500	2	349	1	1
0055	Burns	3	92	Bull A	5000	2	401	0	1
0067	Burns	3	64	Bull B	500	2	342	1	1
7036	Burns	6	86	Bull B	0	2	362	1	1
7007	Burns	6	87	Bull B	0	2	389	0	1
8015	Burns	5	83	Bull A	5000	2	389	0	1
406	Burns	9	51	Bull A	0	2	337	1	1
0012	Burns	3	77	Bull A	0	2	386	0	1
7050	Burns	6	87	Bull B	0	2	360	1	1
0015	Burns	3	83	Bull B	5000	2	404	0	1
0056	Burns	3	89	Bull B	5000	2	364	1	1
7041	Burns	6	78	Bull A	500	2	.	0	0
0078	Burns	3	92	Bull A	500	2	419	0	1
0004	Burns	3	99	Bull A	5000	2	403	0	1
9158	Burns	4	80	Bull B	5000	2	396	0	1
7001	Burns	6	79	Bull B	0	2	378	0	1
0185	Burns	3	54	Bull A	0	2	355	0	1
8010	Burns	5	77	Bull A	5000	2	360	1	1
0039	Burns	3	91	Bull A	0	2	370	1	1
9047	Burns	4	96	Bull B	500	2	.	0	0
8026	Burns	5	81	Bull B	0	2	359	1	1
8032	Burns	5	70	Bull A	500	2	366	0	1
9080	Burns	4	59	Bull B	0	2	366	0	1
9174	Burns	4	55	Bull B	500	2	331	1	1
9200	Burns	4	84	Bull B	500	3	361	1	1

Cow #	Loc.	Cow Age	PPI to brd	Bull Code	PGF Trt	Tech.	Calving interval	Concep	Preg
9003	Burns	4	101	Bull B	0	3	376	1	1
0119	Burns	3	102	Bull B	500	3	372	1	1
0010	Burns	3	76	Bull A	0	3	373	0	1
0052	Burns	3	64	Bull A	500	3	341	1	1
9106	Burns	4	96	Bull B	0	3	371	1	1
9206	Burns	4	95	Bull B	500	3	392	0	1
7039	Burns	6	73	Bull B	5000	3	370	0	1
7026	Burns	6	75	Bull A	0	3	362	1	1
9222	Burns	4	90	Bull A	500	3	385	0	1
0149	Burns	3	80	Bull B	500	3	.	0	0
8009	Burns	5	80	Bull B	5000	3	.	0	0
9016	Burns	4	89	Bull B	0	3	365	1	1
9095	Burns	4	83	Bull A	5000	3	.	0	0
9134	Burns	4	90	Bull A	0	3	372	1	1
9024	Burns	4	76	Bull B	5000	3	355	1	1
9043	Burns	4	88	Bull B	500	3	405	0	1
7025	Burns	6	79	Bull B	500	3	361	1	1
0136	Burns	3	85	Bull A	500	3	360	1	1
9090	Burns	4	54	Bull A	500	3	327	1	1
7011	Burns	6	87	Bull A	5000	3	365	1	1
0009	Burns	3	95	Bull B	0	3	.	0	0
9175	Burns	4	93	Bull B	500	3	368	1	1
0073	Burns	3	86	Bull A	0	3	361	1	1
0038	Burns	3	55	Bull A	500	3	.	0	0
8038	Burns	5	84	Bull B	500	3	381	0	1
9131	Burns	4	84	Bull B	0	3	393	0	1
8001	Burns	5	80	Bull B	5000	3	403	0	1
8029	Burns	5	72	Bull A	5000	3	388	0	1
8023	Burns	5	76	Bull A	500	3	363	1	1
9202	Burns	4	90	Bull B	0	3	391	0	1
0046	Burns	3	67	Bull B	500	3	358	1	1
0170	Burns	3	61	Bull B	5000	3	342	1	1
0003	Burns	3	87	Bull A	5000	3	364	1	1
0154	Burns	3	90	Bull A	5000	3	.	0	0
0081	Burns	3	89	Bull B	5000	3	367	1	1
8005	Burns	5	85	Bull B	500	3	390	0	1
9079	Burns	4	91	Bull A	0	3	367	1	1

Cow #	Loc.	Cow Age	PPI to brd	Bull Code	PGF Trt	Tech.	Calving interval	Concep	Preg
0117	Burns	3	49	Bull A	0	3	371	0	1
7053	Burns	6	91	Bull A	5000	3	.	0	0
0014	Burns	3	99	Bull B	5000	3	.	0	0
0063	Burns	3	85	Bull B	5000	3	364	1	1
8043	Burns	5	83	Bull A	0	3	.	0	0
7017	Burns	6	58	Bull A	5000	3	344	1	1
283	Burns	8	45	Bull A	0	3	.	0	0
0212	Burns	3	54	Bull B	500	3	359	0	1
7057	Burns	6	83	Bull B	0	3	361	1	1
0230	Burns	3	82	Bull A	500	3	357	1	1
9114	Burns	4	95	Bull A	5000	3	369	1	1
8040	Burns	5	72	Bull B	5000	3	372	0	1
6013	Burns	7	38	Bull B	0	3	339	0	1
9147	Burns	4	84	Bull B	500	3	358	1	1
8025	Burns	5	98	Bull A	0	3	376	1	1
8036	Burns	5	79	Bull A	500	3	374	0	1
8041	Burns	5	80	Bull A	5000	3	384	0	1
0070	Burns	3	94	Bull B	0	3	406	0	1
8035	Burns	5	59	Bull B	5000	3	.	0	0
7038	Burns	6	84	Bull A	0	3	416	0	1
7055	Burns	6	75	Bull B	500	3	383	0	1
411	Burns	9	88	Bull A	0	2	388	0	1
221	Burns	8	91	Bull A	0	2	399	0	1
6040	Burns	7	66	Bull A	5000	2	345	1	1
8002	Burns	5	70	Bull B	0	2	381	0	1
131	Burns	10	90	Bull B	500	2	.	0	0
440	Burns	9	74	Bull B	5000	2	405	0	1
9030	Burns	4	84	Bull B	500	2	365	1	1
212	Burns	8	74	Bull A	500	2	370	0	1
9159	Burns	4	81	Bull A	5000	2	.	0	0
7031	Burns	6	91	Bull A	0	2	368	1	1
246	Burns	8	73	Bull B	5000	2	.	0	0
8046	Burns	5	95	Bull B	0	2	371	1	1
9074	Burns	4	97	Bull A	500	2	373	1	1
247	Burns	8	86	Bull A	500	2	394	0	1
2	Burns	10	88	Bull A	5000	2	367	1	1
6019	Burns	7	96	Bull B	5000	2	395	0	1

Cow #	Loc.	Cow Age	PPI to brd	Bull Code	PGF Trt	Tech.	Calving interval	Concep	Preg
6034	Burns	7	92	Bull B	0	2	405	0	1
8016	Burns	5	69	Bull A	0	2	350	1	1
9051	Burns	4	88	Bull A	0	2	390	0	1
6049	Burns	7	83	Bull A	500	2	400	0	1
6009	Burns	7	81	Bull B	5000	2	360	1	1
6024	Burns	7	94	Bull B	500	2	372	1	1
375	Burns	9	83	Bull A	0	2	387	0	1
6046	Burns	7	91	Bull A	5000	2	395	0	1
166	Burns	10	80	Bull A	0	2	.	0	0
369	Burns	9	75	Bull A	500	2	356	1	1
7042	Burns	6	78	Bull B	500	2	357	1	1
8006	Burns	5	95	Bull B	5000	2	374	1	1
242	Burns	8	60	Bull A	500	2	366	0	1
9052	Burns	4	88	Bull A	0	2	414	0	1
7019	Burns	6	80	Bull B	500	2	397	0	1
8030	Burns	5	73	Bull B	0	2	.	0	0
6010	Burns	7	78	Bull A	0	2	361	1	1
100	Burns	10	57	Bull A	5000	2	.	0	0
6039	Burns	7	72	Bull A	5000	2	349	1	1
6029	Burns	7	77	Bull B	5000	2	360	1	1
6032	Burns	7	96	Bull B	0	2	370	1	1
6033	Burns	7	70	Bull B	0	2	343	1	1
230	Burns	8	80	Bull A	500	2	352	1	1
9115	Burns	4	87	Bull A	5000	2	404	0	1
468	Burns	8	55	Bull B	500	2	.	0	0
6051	Burns	7	81	Bull B	5000	2	385	0	1
8050	Burns	5	92	Bull B	5000	2	387	0	1
7046	Burns	6	71	Bull A	0	2	392	0	1
37	Burns	10	71	Bull A	0	2	375	0	1
232	Burns	8	73	Bull A	5000	3	377	0	1
9214	Burns	4	71	Bull A	500	3	366	0	1
9101	Burns	4	80	Bull A	0	3	365	1	1
6003	Burns	7	78	Bull A	500	3	355	1	1
6001	Burns	7	100	Bull B	5000	3	408	0	1
9070	Burns	4	92	Bull B	500	3	370	1	1
9146	Burns	4	95	Bull B	0	3	421	0	1
305	Burns	8	77	Bull B	5000	3	.	0	0

Cow #	Loc.	Cow Age	PPI to brd	Bull Code	PGF Trt	Tech.	Calving interval	Concep	Preg
45	Burns	10	77	Bull A	500	3	.	0	0
9116	Burns	4	88	Bull A	5000	3	413	0	1
155	Burns	10	64	Bull B	0	3	342	1	1
6005	Burns	7	82	Bull B	5000	3	.	0	0
7061	Burns	6	88	Bull B	500	3	.	0	0
279	Burns	8	81	Bull A	0	3	359	1	1
206	Burns	8	81	Bull A	5000	3	.	0	0
235	Burns	8	80	Bull B	500	3	376	0	1
26	Burns	10	92	Bull B	0	3	396	0	1
6053	Burns	7	90	Bull B	500	3	370	1	1
8013	Burns	5	80	Bull A	5000	3	362	1	1
6048	Burns	7	74	Bull A	5000	3	355	1	1
223	Burns	7	80	Bull B	0	3	383	0	1
6015	Burns	7	74	Bull B	0	3	351	1	1
9151	Burns	4	85	Bull B	5000	3	368	1	1
190	Burns	10	76	Bull A	5000	3	.	0	0
301	Burns	8	86	Bull B	500	3	.	0	0
9019	Burns	4	80	Bull B	5000	3	369	1	1
9036	Burns	4	84	Bull B	0	3	405	0	1
362	Burns	9	75	Bull A	0	3	.	0	0
288	Burns	8	80	Bull B	0	3	354	1	1
218	Burns	8	80	Bull B	500	3	384	0	1
9013	Burns	4	80	Bull A	500	3	360	1	1
427	Burns	9	79	Bull A	500	3	364	1	1
421	Burns	9	68	Bull A	5000	3	383	0	1
266	Burns	8	71	Bull A	500	3	375	0	1
308	Burns	8	69	Bull B	500	3	370	0	1
6018	Burns	7	86	Bull B	5000	3	403	0	1
6002	Burns	7	91	Bull A	5000	3	374	1	1
323	Burns	9	74	Bull A	5000	3	353	1	1
262	Burns	11	91	Bull A	0	3	370	1	1
229	Burns	7	85	Bull B	0	3	411	0	1
8021	Burns	5	81	Bull B	5000	3	361	1	1
412	Burns	9	77	Bull B	0	3	373	0	1
25	Burns	8	86	Bull A	500	3	382	0	1
296	Burns	8	87	Bull A	500	3	369	1	1

APPENDIX TABLE 7

IMPROVEMENT OF CONCEPTION RATE IN CATTLE RECEIVING EXOGENOUS PROSTAGLANDIN $F_{2\alpha}$ ADMINISTERED AT THE TIME OF INSEMINATION – Experiment 2 raw data

Heifer #	Trt at brd	1st service conception	Conception to AI	Preg.
3121	PGF	0	1	1
3127	PGF	0	0	0
3129	PGF	1	1	1
3136	PGF	1	1	1
3138	PGF	0	0	1
3140	Con	1	0	0
3141	Con	0	1	1
3145	Con	0	0	1
3153	Con	0	0	1
3157	Con	0	1	0
3158	PGF	0	0	1
3169	PGF	0	0	1
3173	Con	0	0	0
3174	Con	0	0	1
3178	PGF	1	1	1
3183	Con	0	0	0
3187	PGF	1	1	1
3196	Con	0	0	0

APPENDIX TABLE 8

IMPROVEMENT OF CONCEPTION RATE IN CATTLE RECEIVING EXOGENOUS PROSTAGLANDIN $F_{2\alpha}$ ADMINISTERED AT THE TIME OF INSEMINATION – Experiment 3 raw data

Cow #	Age class	Treatment	Days PP at brd	No. previous AI attempts	Synch. Method	Preg.
516	C	C	150	1	ovsynch	0
516	C	PGF	100	0	beaver	0
566	C	PGF	100	0	beaver	1
648	C	PGF	100	0	beaver	0
660	C	C	100	0	beaver	0
672	C	C	150	1	ovsynch	0
677	C	PGF	100	0	beaver	1
707	C	PGF	100	0	beaver	0
727	C	C	100	0	beaver	1
728	C	PGF	200	3	ovsynch	0
728	C	PGF	200	3	ns	1
741	C	PGF	100	0	beaver	0
750	C	PGF	200	3	ovsynch	1
759	C	C	200	1	ovsynch	0
759	C	C	200	2	ns	0
761	C	PGF	100	0	beaver	0
763	C	PGF	100	0	beaver	1
768	C	C	100	0	beaver	1
778	C	PGF	100	1	ns	0
778	C	PGF	150	2	ovsynch	0
799	C	C	100	0	beaver	1
802	C	C	200	3	ovsynch	0
806	C	C	100	0	beaver	0
807	C	PGF	200	3	ns	1
808	C	PGF	100	0	beaver	0
809	C	C	150	1	ovsynch	0
811	C	C	100	0	beaver	1
813	C	PGF	100	0	beaver	0
814	C	C	150	2	ovsynch	0
815	C	C	150	2	ovsynch	0
815	C	PGF	100	0	beaver	0

Cow #	Age class	Treatment	Days PP at brd	No. previous AI attempts	Synch. Method	Preg.
816	C	PGF	100	0	beaver	0
819	C	C	100	0	ns	1
823	C	C	100	1	ns	1
836	C	C	100	0	beaver	0
857	H	PGF	.	2	ns	0
859	H	C	.	2	ns	0
860	H	PGF	.	2	ns	1
865	H	PGF	.	0	ns	1
866	H	C	.	0	ns	1
1106	C	C	100	0	beaver	0
1121	C	PGF	150	1	ovsynch	1
1124	C	PGF	200	3	ns	1
1131	C	C	100	0	beaver	0
1132	C	C	100	0	ns	0
1132	C	C	150	1	ns	0
1135	C	PGF	100	0	beaver	1
1155	C	C	100	0	beaver	0
1175	C	PGF	200	3	ns	0
1175	C	PGF	200	3	ovsynch	0
1181	C	C	200	3	ovsynch	1
1181	C	PGF	150	1	ovsynch	0
1184	C	C	200	2	ovsynch	1
1185	C	C	100	0	beaver	0
1187	C	PGF	150	2	ovsynch	1
1188	C	C	100	0	beaver	1
1196	C	C	150	1	ovsynch	0
1199	C	C	150	1	ns	0
1200	C	C	100	0	ns	0
1202	C	C	150	1	ovsynch	0
1204	C	PGF	100	0	beaver	0
1205	C	C	100	0	beaver	0