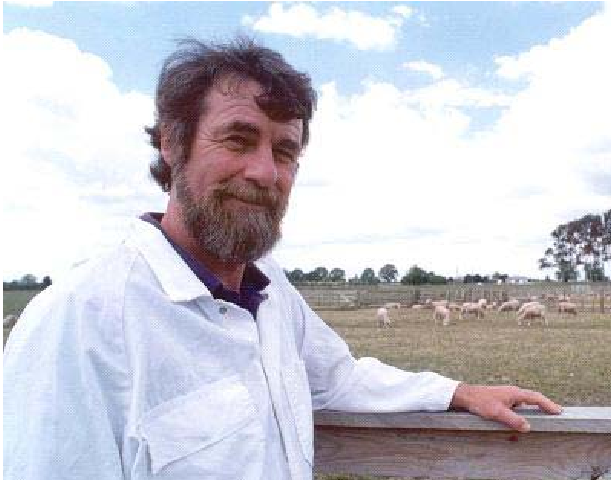

THE PROCEEDINGS OF THE
NEW ZEALAND SOCIETY
OF
ANIMAL PRODUCTION
VOLUME 62 2002

Andrew James (Jim) Peterson: A tribute to a far-reaching scientific career in reproductive biology



INTRODUCTION

The following is an extraordinary New Zealand Society of Animal Production publication summarising papers presented in March 2010 at a colloquium in honour of Dr. A.J. (Jim) Peterson (1945 – 2009), who worked as a scientist at AgResearch Ruakura, Hamilton. It is a unique collection that provides both a testament to his long involvement with the Society and also a "whistle-stop tour" through pioneering and groundbreaking work that has contributed to increased understanding of reproduction and the development of tools, such as oestrus synchronisation and calving induction protocols, that are now widely in use in the New Zealand and international dairy industry.

I would like to sincerely thank the authors for their contributions and for allowing us to print them here, to enable the information contained within to benefit a wider readership. True to form, there are also hints and suggestions for current researchers to think about, something that Jim would have wholeheartedly supported!

Truly- we stand on the shoulders of giants.

Penny Back
President

Hormones, hormones and more hormones

J.F. SMITH

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Our collaboration was both long and successful with a total of 39 publications as co-authors over a period of 32 years. Our first paper was in 1973 and our last in 2005. The collaboration was successful because it combined Jim's unique and pioneering ability to develop hormone assays with my knowledge and experience in reproductive physiology. It resulted in a number of seminal papers containing new knowledge that was then used by workers nationally and internationally to increase the understanding of hormones, their effects and interactions.

The topics covered in these papers were: hormone assays; oestrus; oestrous synchronisation; ovulation rate; luteolysis; conception and pregnancy; and anoestrous modelling. I intend to briefly touch on a few of the seminal papers and discuss the significance of the research results.

Hormone assays

Our collaboration commenced with the development of a range of hormone assays to enhance our studies of reproduction in sheep and cattle. The following is a list of the hormones that we published data on:- oestradiol 17β; oestrone; progesterone; provera; testosterone; androstenedione; 13,14-dihydro-15keto-prostaglandin F (PGFM); neurophysin I / II; follicle stimulating hormone (FSH) and luteinising hormone (LH).

The first important paper was by Peterson *et al.* (1975a) on the assay of oestradiol in cattle plasma.

Jim, Bob Fairclough and I had a flock of sheep that had antibodies to a whole spectrum of hormones. This was before the assay kit era, when you raised your own antibodies for assay work. This paper showed that the specificity of the oestradiol assay was influenced by the position of the steroid molecule to which the carrier protein was conjugated. Antisera produced against material conjugated at the C6 position were much more specific than that conjugated at the C17 position.

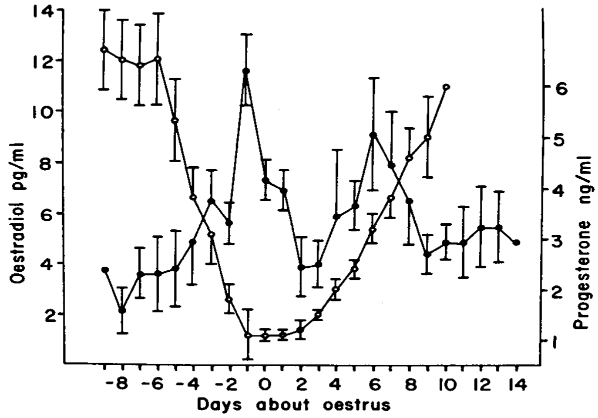
One problem we encountered was finding the presence of interference factors in cattle plasma that were not present in sheep plasma. These factors markedly altered the standard curve and resulted in spurious results (Figure 1). The only method to overcome this was a tedious procedure of chromatographic purification of the plasma before assay. Jim developed a new method to overcome this problem resulting in standard curves similar to ethanol standards and a rate of assay throughput five times that achieved using a chromatography purification step. The new method gave similar results to that using chromatography. The discovery of this interfering factor and development of a simple means of overcoming it revolutionised the adoption of steroid assays for cattle.

Jim and I believed there were two other major points raised by this publication that current researchers using assay kits, or even having their samples commercially assayed, should be alerted to. First, that the antisera being used are specific for the hormone being measured and secondly, that the assay procedure overcomes any factors that could be influencing the accuracy of the results.

Hormonal profiles about oestrus – cattle and sheep

There are two papers by Smith *et al.* in 1975 (Figure 2) and 1976 that contained classical figures of hormone profiles at oestrus in both species. Of note were the multiple peaks in oestradiol throughout the cycle. These data were published prior to the follicle wave theory.

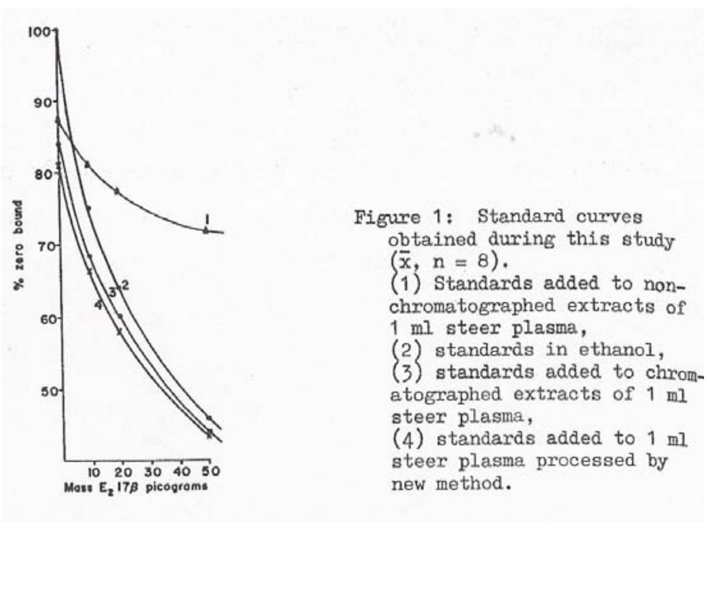
FIGURE 2: Plasma hormone levels in the cow. Changes in progesterone and oestrogen during the normal oestrous cycle (taken from Smith *et al.*, 1975).



— Changes in peripheral plasma levels (± s.e.) of oestradiol (●——●) and progesterone (○——○) during the normal bovine oestrous cycle (n = 12).

One of the advantages of having our own flock of sheep with antibodies was the availability of large quantities of antiserum that enabled us to examine the effect of free oestradiol levels on oestrus and ovulation (Fairclough *et al.,* 1976). Where there was no free oestradiol available as when it was blocked by the antisera, luteal regression occurred but there was no oestrus or ovulation (Figure 3). Fairclough *et al.* (1976) is one of a very few papers in which oestradiol values at the "fentogram" level has been accepted for publication, a reflection of the high specificity of the antisera used.

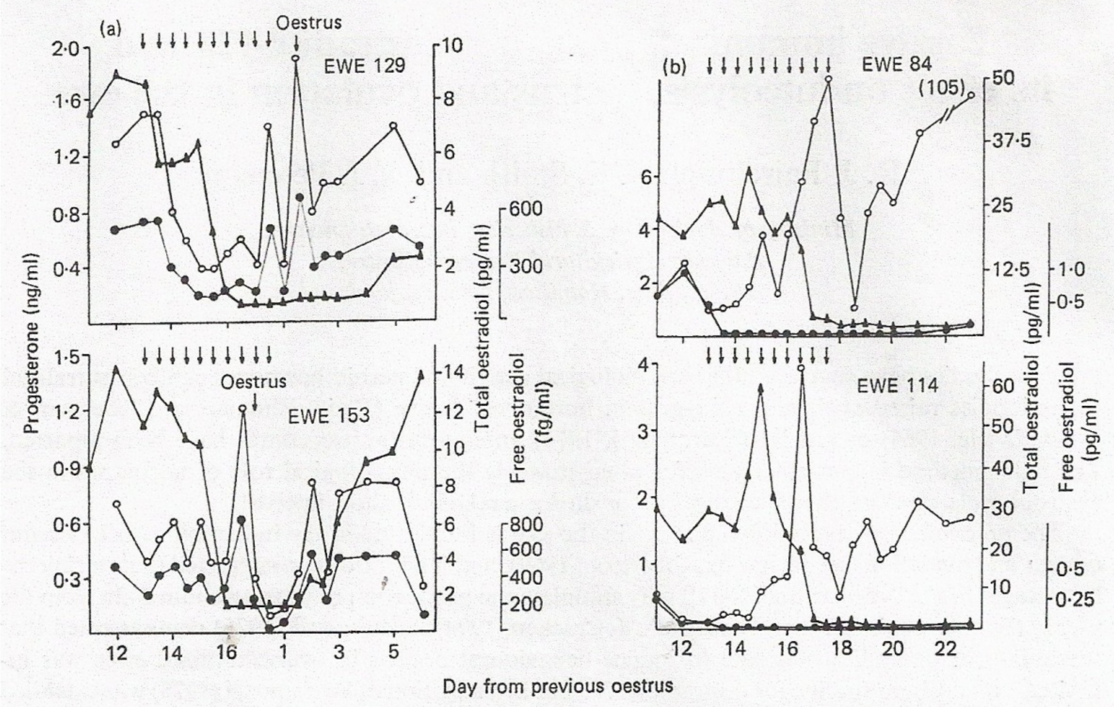
FIGURE 1: Radioimmunoassay of oestradiol-17 β in bovine peripheral plasma, with and without chromatography (taken from Peterson *et al.,* 1975a).



Luteolysis or conception

Luteolysis or conception is an either/or situation and the seminal paper here is by Peterson *et al.* (1975b). This paper shows the relationship between prostaglandin (PG) production, the decline in progesterone and the onset of oestrus. Because of the rapid metabolism in the body of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), Jim developed an assay that measured one of its more stable metabolites (13,14-dihydro-15keto-prostaglandin F (PGFM)). These were the days when science went 24/7; the days of mad scientists and even madder technicians bleeding animals every two hours around the clock for weeks on-end. Another paper (Peterson *et al.,* 1976) is very important as it shows the effect of conception, following embryo transfer, blocking the PGF release and thus maintaining the corpus luteum's progesterone production and pregnancy (Figure 4). These two papers had a major influence on the

FIGURE 3: Passive immunization against oestradiol-17β and its effect on luteolysis, oestrus and ovulation in the ewe (taken from Fairclough *et al.,* 1976).



Text-fig. 1. Changes in plasma concentrations of progesterone (▲) and free (●) and total (○) oestradiol-17β in (a) control ewes about the time of oestrus, and (b) ewes passively immunized against oestradiol-17β. The arrows indicate the times of injection of plasma from an ovariectomized ewe or of antiplasma to oestradiol-17β.

direction of international research in this area. The next logical step was showing the relationship between the release of oxytocin-induced neurophysin and prostaglandin levels (Fairclough *et al.,* 1980).

Oestrous synchronisation - progestagen sponges or prostaglandins

The work here (Smith *et al.,* 1979) pre-dated the synthetic prostaglandins and involved the intrauterine administration of PGF. The results were as anticipated with PG inducing luteolysis and reducing circulating progesterone levels. These studies then led onto the much larger field scale trials using the synthetic PGs, with much longer half lives, and the eventual development of commercial synchronisation technology for the New Zealand dairy industry.

Ovulation rate

Our studies in this area were mainly on the roles of LH and FSH in regulating ovulation rate. They centred on the differences in the levels of these hormones when factors that can influence ovulation rate such as breed nutrition, live weight and zearalenone, were altered. A paper by Smith *et al.* (1990) highlighted this by showing that different

FIGURE 4: Jugular levels of 13,14- dihydro-15-keto-prostaglandin F and progesterone around luteolysis and early pregnancy in the ewe (taken from Peterson *et al.,* 1976).

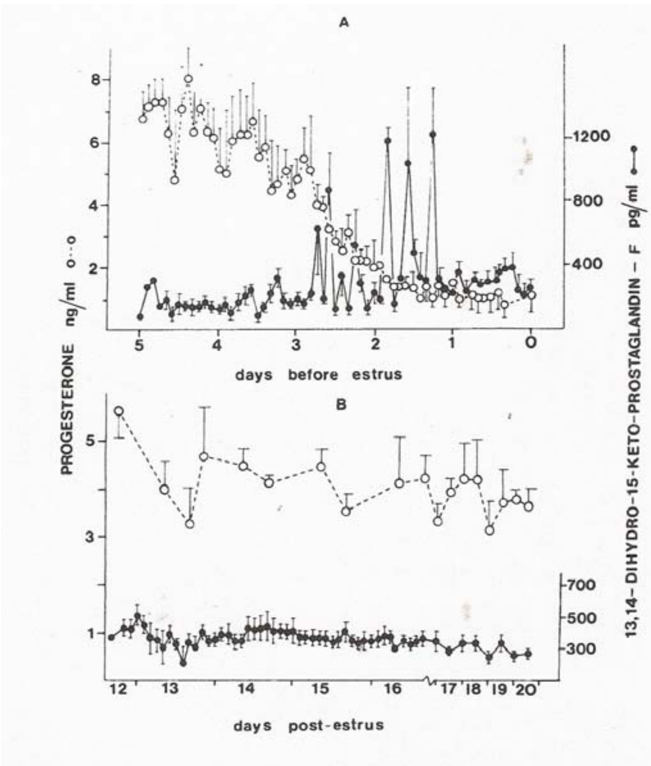


Figure 1. Periphal concentrations of progesterone and PGFM (± S.E.) in; (A) the 4 control ewes before estrus and (B) the 9 pregnant recipient ewes during days 12-20 after estrus.

factors that increase ovulation rate seem to influence the ovarian-pituitary-hypothalamic axis in different ways. Nutrition seems to increase FSH levels while Phenobarbital, which also increased ovulation rate, tended to have an opposite effect. This diversity of underlying hormonal changes was one of the instigators for Jim to embark into the mathematical modelling arena.

Conception rates and pregnancy

Reduced fertility of New Zealand dairy cows grazing pasture that is low in carbohydrate and high in rumen degradable protein has been attributed to the elevated levels of urea and ammonia in the systemic circulation. Our study (Smith *et al.,* 2000) used the intravenous infusion of these compounds to mimic changes in systemic levels under rotational strip grazing. Infusion of urea and ammonia resulted in marked increases in the plasma levels during the periods of infusion. Post- infusion ammonia levels fell rapidly and reached base levels within 30 minutes of infusion end, while urea levels were still elevated five hours later. Ammonia infusion resulted in an increase in follicular fluid ammonia levels while urea infusion did not. Both substances significantly lowered the pH of follicular fluid. These changes were sufficient to anticipate some detrimental effects on fertilisation and embryo development. However, when we examined the levels of bulk milk urea nitrogen (BMUN) in herds over the first seven weeks of mating, as a possible predictor of fertility problems, and their subsequent reproductive performance (Smith *et al.,* 2001), the conclusion was that BMUN were useless as a fertility predictor.

Anoestrus – modelling

This was the last area that Jim and I collaborated on (Smith *et al.,* 2005). Our modelling showed that LH pulse frequency and amplitude is influenced by oestradiol levels and that the length of time, or number of follicular cycles, needed for these to reach a threshold that enables ovulation to occur, governs the length of the anoestrus period.

The above is a very brief encapsulation of my collaboration with Jim. Jim was one of the best read scientists I have worked with. One of his few real aversions was of people who because of their lack of study of the relevant literature tried to “reinvent the wheel”. I hope that the list of references below, although dated, will contribute to a reduction in the incidence of Jim’s pet hate. Jim was not only a friend and colleague but in the Aussie vernacular; “He was a bloody good mate”.

Ruakura and radioimmumoassays

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I wish to pay tribute to Jim Peterson’s contribution to reproductive endocrinology by first outlining the platform that others created for him to do his work, and then by describing the excellent work that he did in pioneering the endocrinology of parturition in the cow.

A key figure in developing the science of animal production was Sir John Hammond of the University of Cambridge. A pioneering work of his was *The Physiology of Reproduction in the Cow* (Hammond, 1927). The tools that provided the information for this book were a pencil and a notebook, sharp knife and microscope. There was no chemistry and hormones were mentioned only in passing in an addendum. Nevertheless, the contribution of animal science to food production was recognized by the British government and the University of Cambridge, who established a laboratory to support Hammond’s work (Moor *et al.*, 2008). This laboratory pioneered much of the underlying technology for artificial insemination, carcass composition and nutrition of ruminants.

Of particular relevance for New Zealand was that the Department of Scientific and Industrial Research (DSIR) commissioned Hammond to write a report on how animal production research could be carried out here. This recommendation was taken up by the Department of Agriculture in 1938 and the Ruakura Animal Research Station was established (Scott, 1989). Not only were Hammond’s ideas important but his students were prominent in staffing and directing the research station:

- C.P. McMeekan, Foundation Director;
- L.R. Wallace, Nutritionist, Director;
- D.G. Edgar, Reproductive physiologist, Director;
- T.J. Robinson, Professor at University of Sydney, supervised J.F. Smith who became a reproductive physiologist at Ruakura;

FIGURE 5: Summary of foetal and maternal steroid hormone concentrations in the plasma of cattle around the time of parturition (taken from Hunter *et al.*, 1977).

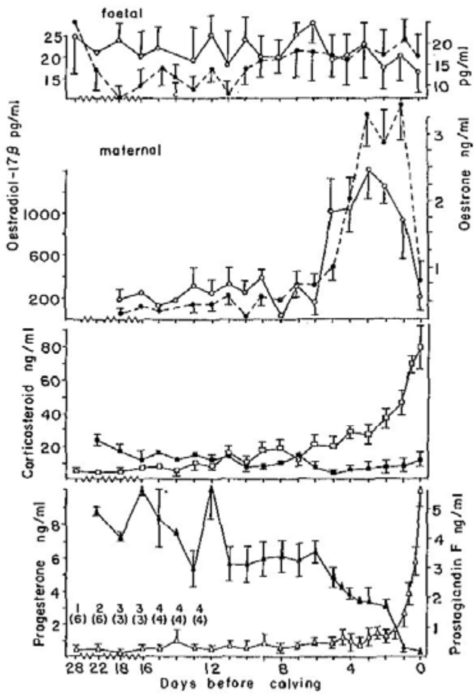

Fig. 1.

Mean changes in plasma concentrations of foetal and maternal utero-ovarian oestrone (●–●), oestradiol-17β (○–○), foetal (□–□) and maternal jugular (■–■) corticosteroids; maternal jugular progesterone (▲–▲) and utero-ovarian prostaglandins F (△–△) preceding parturition in the cow. Vertical lines indicate ± SEM with number of cows, and number of observations in brackets. From day 12 until term there was a single observation on each of five cows.

- D.S. Hart, Senior Lecturer, Lincoln University, supervised R.A.S. Welch who became a reproductive physiologist and Director of AgResearch;
- M.F. McDonald, Associate Professor, Massey University, supervised H.R. Tervit who became a reproductive physiologist and embryologist who headed the Reproductive Technologies Group at Ruakura.

The work of Edgar (1953) was particularly important in Hammond's laboratory. This showed that progesterone could be chemically detected in the venous blood from an ovary with an active corpus luteum. When he joined the Ruakura station, he showed the patterns of progesterone levels in the ovarian venous blood throughout the oestrous cycle and pregnancy (Edgar & Ronaldson, 1958). This was pioneering work and propelled Ruakura to the front line of endocrine studies in farm animals. The patterns and levels of hormones have never been challenged and this paper remains the key reference.

The importance of Jim Peterson's story is that by 1970, Edgar was Director of Ruakura. When radio-immunoassays were developed that could detect hormone levels in small amounts of peripheral blood, he could see the immediate importance of recruiting scientists with these skills and appointed Jim along with R. (Bob) J. Fairclough. They immediately set up a suite of assays, the pioneering nature of which is illustrated by the paper on their method for oestradiol (Peterson *et al.*, 1975a).

The application of these assays was vital in understanding the endocrinology of natural and induced parturition in the cow. Liggins (1968) demonstrated the primary role of the fetal pituitary-adrenal axis in initiating parturition in sheep. At Ruakura, we investigated the practical application of induced parturition by corticosteroids in dairy cows (Welch, 1971). The endocrine paper that underpinned this work (Hunter *et al.*, 1977) is a paper of extreme elegance and will remain the benchmark of this topic (Figure 5).

We now know for all time that:

- Corticosteroids in the fetal calf bloodstream rise over the two weeks before calving to levels several times that in the cow;
- There is a positive relationship between fetal cortisol levels and maternal oestrogen levels in the earlier phase of parturition;
- There is a negative relationship between fetal cortisol, maternal oestrogen and maternal progesterone just before parturition;
- There is a negative relationship between maternal prostaglandin and maternal progesterone.

All due to the work of Jim Peterson.

Thank you Jim.

P4+

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Publications

The title of this section as "P4+" may seem like a text message language, possibly with Jim as its recipient. It is texting short-hand for "Progesterone Plus" and it represents a significant area of common interest that Jim and I shared for about 20 years.

One of my first investigative responses to learning of this colloquium was to check out the search engine "Scopus'. The "Scopus" list of Jim's publications could be described as diverse, with most involving four or more co-authors. Jim did not subscribe to methods of merit marking for scientists that relied on prodigiously publishing peer-reviewed manuscripts. Most of his publications were sequels to extensive discussion and reflection. Many have "citation scores" of over 20. This is notable, given that the median citation score for manuscripts in the applied sciences, especially the animal sciences, is less than 5. For a manuscript to achieve a citation score of over 100 is definitely as significant as scoring a test century in cricket.

Jim has one manuscript with a current "citation score" of 113. It is the 1993 manuscript published in Animal Reproduction Science, Volume 33, pages 1 to 25 and entitled: "A new intravaginal progesterone releasing device for cattle (CIDR-B) for oestrous synchronisation, increasing pregnancy rates and the treatment of post-partum anoestrus". The authors were: K.L. Macmillan and A.J. Peterson.

CIDR Background

Although Macmillan and Peterson (1993) was not the first manuscript published on the CIDR-B, it has become the "bench-mark" one that authors make reference to when proving they understand all there is to understand about the CIDR (controlled internal drug releaser). Many fail to realise that Japan is the only country where this original form of the CIDR-B is still used. It contains 1.9 g of progesterone (P4), whereas the most widely used "optimised" form now contains only 1.32 g of P4. Both forms of the CIDR-B now have annual world-wide sales exceeding 4 million units per annum. It is of note that the CIDR-B was the third form to be moulded as a registered commercial product. The first form was the CIDR-S; and it is no longer moulded. The CIDR-G has had limited demand. This will likely increase significantly with the recent approval by the USA Department of Food and Agriculture, Food and Drug Administration that will allow Pfizer to market this product to sheep flock owners in the USA, but not to goat herd owners!

Some highlights of the reference manuscript

Jim's involvement in the keynote CIDR reference is apparent. No other study since has even remotely conducted an investigation in which nine ovariectomised cows each completed six or seven treatment cycles, each of 15 days with nine strategically timed samplings during each cycle; that is 550 samples! This is one of the few studies that has attempted to measure cow-to-cow and within-cow variation in plasma P4 concentrations. Day 1 P4 concentrations ranged from 4.5 to 9.0 ng/ml for individual cows with an overall mean of 6.7 ng/ml. The within cow coefficient of variation throughout the 15 days ranged from 25% to 66%.

A second trial involved 100 cycling Friesian heifers which each received a 15-day CIDR treatment with devices moulded to contain 0, 0.63, 1.25, 1.90 or 2.53 g P4 per insert. The resulting data were used to calculate the relationship between the initial P4 content and the residual content which was extracted from the used device with a protocol developed and verified by Jim. The result was a quadratic equation with an R^2 of 95.3%. A sequel to this trial involved treating cows with one or three CIDR inserts for 15 days. The multiple inserts produced a three-fold increase in plasma P4 concentrations but did not alter average residual P4 content/insert.

As frequently as this comprehensive manuscript may have been cited, many of these authors proceed to demonstrate a lack of understanding "P4 Technology". Other important elements of this manuscript included:

(i) demonstrating the impact of supplemental P4 during metoestrus on subsequent inter-oestrus interval with a key variable being duration of treatment and cycle day at insert removal;

(ii) demonstrating a stage of cycle at device insertion x interval to oestrus in heifers injected with PGF at insert removal;

(iii) confirming that treatment periods longer than the duration of an ovarian follicle wave of eight days produced concise synchrony associated with reduced fertility;

(iv) identifying the unreliability of administering 10 mg of oestradiol benzoate (ODB) in a gelatin capsule as a luteolytic agent, but preferably substituting it with a lower injectable dose to achieve follicle wave termination followed by new wave emergence;

(v) completing a series of three field trials involving 3,000 cows that demonstrated the potential to increase conception rates by up to 8%, with this effect being stage-of-cycle specific to the period from four to nine days post-insemination;
(vi) utilising a CIDR-treatment to successfully re-synchronise returns to synchronised first inseminations; and,
(vii) treatment of the post-partum anoestrus condition associated with ovarian hypoplasia in pasture-fed dairy cows in combination with eCG (then called PMSG (pregnant mare serum gonadotrophin)).

Collectively, these trials involved almost 5,000 cows, approximately 6,000 treatment cycles and over 8,000 plasma samples. The acknowledgements recognised the original concepts developed by Bob Welch and Doug Millar, the dedicated support of Viliami Taufa, Tony Day, Di Barnes and Harold Henderson, the provision of CIDR inserts through Graham Duirs, the PG by the Upjohn Company, and the PMSG by Pastoral Consultants, as well as access to herds of dairy heifers arranged by Adrian Rhodes through the Livestock Improvement Corporation (LIC).

It is fair to claim that this highly cited manuscript authored by Jim Peterson and myself can be described as "THE CIDR blockbuster". Given the ongoing nature of CIDR research, especially in the USA, the "citation score" will continue to increase.

Some CIDR reflections

Not surprisingly, the contents of this blockbuster manuscript stimulated a wave of further CIDR-based research. Type in the four letters: C-I-D-R into a science-based research engine and over 500 titles will be listed. Some of the more significant studies include:

(i) Burke *et al.* (2000) showed that the within-day variation in plasma P4 concentrations was influenced by feeding times as the post-feeding increase in metabolic rate was associated with lower concentrations of P4;
(ii) Burke *et al.* (1996) also demonstrated that: "Oestradiol potentiates a prolonged progesterone-induced suppression of LH release in ovariectomised cows"; a highly significant paper;
(iii) McDougall's (2001) novel observations that injected oestradiol benzoate could be used to synchronise wave-like patterns of ovarian follicle development in conjunction with supplemental P4 in the hypoplastic ovaries of anoestrous cows, and that the resulting dominant follicle could be ovulated again with injected ODB following CIDR insert removal. This indication for CIDR use has become THE major reason for current CIDR sales;
(iv) Lynch *et al.* (1999) imaginatively demonstrated that the negative effects of administering P4 during metoestrus on cycle length and fertility could be neutralised, and in the case of fertility, reversed by injecting GnRH to ovulate the dominant follicle in the first post-oestrus follicle wave;
(v) Day *et al.* (2000) combinied synchronisation of cycling cows with treatment for anoestrus in non-cycling cows, as well as re-synchronising returns to service to achieve average herd pregnancy rates of 55% to the first synchronised insemination, 75% following re-synchronisation and 87.5% within the first 45 days of the planned start of mating; and
(vi) Xu *et al.* (1997) showed that the inclusion of a CIDR insert in a double injection prostaglandin F program with 13 days between injections, could substantially overcome the negative effects on post-treatment submission rates and conception rates that are largely ignored by those recommending these double prostaglandin F programs.

These few selective observations are presented to indicate that the earlier trail-blazing research described in the "Macmillan-Peterson" manuscript has been a stimulus for a wealth of local and international research.

Concluding comments

Few fully appreciate the fact that the CIDR-based research that Jim and I conducted and co-ordinated used heifers that weighed about 300 kg and cows that weighed 450 kg. The heifers would have been consuming about 10 kg dry matter (DM)/day, the cows about 18 kg DM/day. Daily milk yields would have averaged about 22 litres. Little wonder that when the CIDR-insert developed for use in "Kiwi cattle" is used with Holstein cows weighing over 700 kg, consuming over 20 kg DM/day of high energy concentrate and maintaining daily yields of about 45 litres with 3 times per day milking, the treatment outcomes are rather different. Maybe we need to mould a new form of "Holstein CIDR".

I hope that you now agree that the title of "P4+" is an appropriate one to recognise Jim's contribution to the scientific establishment of the World's most widely used and recognised CIDR insert. He deserves to retain that infectious smile on his face.

Pre-implantation development in ruminants

R.S.F. LEE

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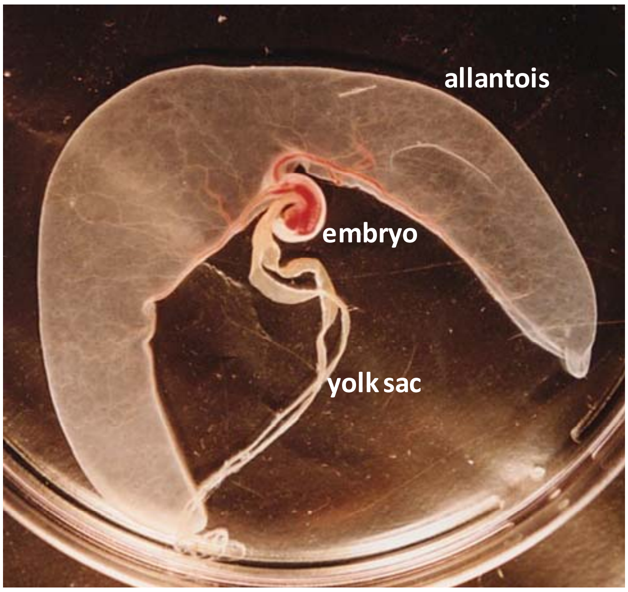
One of the later areas of research that Jim was involved with during his time in the Reproductive Technologies group at Ruakura was the identification of factors that are important in ruminant embryo development, from the time of conception, leading up to the formation of the placenta (Peterson & Lee, 2003). This is the period where most of the embryonic losses occur. During this time, the embryo must first "switch on" genes from its own DNA so that it can start producing the proteins and other molecules that will allow it to continue developing and growing. In addition, the embryo needs to send signals to the mother through the uterus to recognize its presence and adapt the maternal environment to support its development, the maternal recognition of pregnancy. Interferon-tau (IFN-τ), which is produced by the embryo, interacts with the uterine tissue and prevents the uterus from releasing prostaglandin pulses that will normally cause the corpus luteum (CL) of the oestrus cycle to regress, thereby permitting the ovary to start another cycle and shed more eggs (Roberts, 1989).

The extra-embryonic membranes that develop from the embryo play ever changing roles in embryo survival prior to the formation of the placenta. The first of these extra-embryonic membranes to form is the trophoblast, which is in close apposition to the epithelial cells of the uterine lumen. Proteins secreted by the trophoblast mediate the communication between the embryo and the uterus and these include those like IFN-τ that effect maternal recognition of pregnancy. Others include those that modify the connective tissues in the uterus so as to facilitate adhesion of the embryo to the uterine luminal surface and thus initiate the process of implantation. The trophoblast in these early stages absorbs the nutrients it requires through a range of mechanisms and in addition, synthesizes proteins that bind to small molecules, such as minerals, lipids and vitamins, that are essential for embryonic development. Hormones that modulate and adapt the maternal environment and metabolism to support the changing needs of the developing embryo are also produced by the trophoblast.

The next extra-embryonic membrane to form is the yolk sac (Figure 6). The membrane structure exists transiently but plays an important role in early embryo nutrition, formation of the primitive circulation of the embryo and the first blood cells. The yolk sac secretes most of the "serum" proteins, such as transferring and the apolipoproteins, that are normally produced later by the liver. These proteins transport nutrients to the embryo via the primitive pumping heart and the circulatory network. Of all the extraembryonic membranes of the pre-implantation conceptus, that is the embryo with its extraembryonic membranes, the yolk sac is the most metabolically active (Lee *et al*., 1998). In effect, the yolk sac performs the function of the placenta prior to implantation.

The last of the extra-embryonic membranes to form is the allantois, which emerges from the posterior end of the embryo from around Day 16 of pregnancy in sheep and Day 22 of pregnancy in cattle. The allantois rapidly fills with fluid, whilst at the same time, the blood cells and vessels develop to provide the circulatory network that eventually connects the fetus with the placenta. In fact, the allantois takes over the role of the yolk sac as the site for the production of the blood cells, which later migrate to and colonise the embryonic and fetal liver where they continue to proliferate and differentiate into the blood and immune cells. Thus the allantois, which later fuses with the trophoblast to form the chorioallantoic membrane, is pivotal in the development of the placenta, and ultimately pregnancy, in ruminants. In a series of experiments, where the reproductive tracts were collected from pregnant cows from Day 22 until about Day 124, it was observed that malformation of allantois, leading to the failed development of the placentomes was the one of the major causes of first trimester fetal losses (Peterson & Lee 2003). Further studies indicated that the development of the fetal cotyledons that later form part of the placentomes is

FIGURE 6: A Day 26 bovine conceptus with the overlying trophoblast removed.



dependent on the cross-talk between the chorioallantoic membrane and specialized areas in the endometrium, the caruncles. Hence, at the membrane-caruncular interface, the caruncles must receive the appropriate signals from the chorioallantoic membrane and respond with appropriate signals that promote placental development at these sites.

Recipient contribution to pregnancy success

Successful establishment of a pregnancy requires both an inherently good embryo and a recipient uterus that is capable of supporting the development of that embryo. Using a model developed by McMillan to distinguish superior and inferior recipients (McMillan, 1998), two herds were selected at AgResearch, Ruakura, with markedly different pregnancy rates after repeated rounds of embryo transfers. Despite the almost seven-fold difference in pregnancy rates between the herds, no differences could be found in ovarian-dependent parameters, such as the length of the oestrous cycles, follicular dynamics or circulating progesterone profiles. Greater than half of the pregnancy failures occurred before or around the time of maternal recognition of pregnancy suggesting that uterine rather than ovarian factors were greater contributors to the different rates of embryo survival between the groups. The effect of a sub-optimal uterine environment on embryo development was seen in the percentage of embryos that had elongated by Day 14, being 67% in the superior recipient versus 14% in the inferior recipients, showing that the uteri of the superior recipients were better at supporting embryonic development. The uteri in the superior recipients were better at stimulating IFN-τ secretion by the embryo and were also more responsive to the IFN-τ. The main role of IFN-τ in pregnancy establishment is to block the release of $PGF_{2\alpha}$ from the uterus. Using an oxytocin challenge that elicits the release of $PGF_{2\alpha}$ from the uterus, lower levels of $PGF_{2\alpha}$ were released into the peripheral circulation of the superior compared with the inferior recipients. Thus, the higher pregnancy rates in the superior recipients could be attributed to lower levels of $PGF_{2\alpha}$ being made and secreted by the uterus and higher levels of IFN-τ secretion by the embryo, thereby providing better conditions and opportunities for the establishment of a pregnancy.

Work characterizing the uterine environment of superior and inferior recipient cows is continuing after Jim's untimely death. Proteins secreted by the uterine glands or the uterine luminal epithelium have been studied by several groups for a long time and the identities of many are known. However, there are many more waiting to be identified and with modern technologies of protein identification, coupled with the completion of the bovine genome sequencing project, it is now feasible to do this. Work is underway to flush uterine luminal fluid proteins from the uteri of cows that are proven superior recipients and the protein composition will be compared with samples from inferior recipients. Gene expression analysis will also be carried out with uterine tissue samples collected from cows with a poor reproductive history and compared with samples from cows with normal fertility. This will allow better characterization of the uterine environment that is supportive of embryo survival.

The maternal-conceptus interactions

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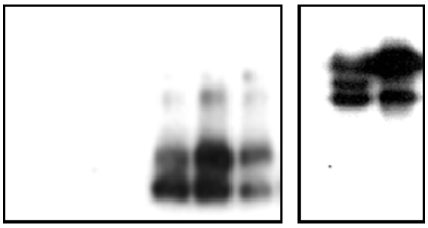
Establishment of pregnancy in a timely fashion is the goal of every dairy farmer. As described previously, this is only possible if the embryo secretes IFN-τ the pregnancy recognition signal, and the signal is received by the maternal endometrium to result in maintenance of the corpus luteum. The early work in the 1990s was carried out to determine the mechanisms and influence of IFN-τ produced by the embryo.

During the stage of IFN-τ production the conceptus undergoes rapid growth and expansion. Using the sheep as a model, it was discovered that the insulin-like growth factors I & II (IGF-I and IGF-II), potentially responsible for this growth, were present in the uterine luminal fluid (ULF) of pregnant ewes (Peterson *et al.,* 1992). The IGF binding proteins (IGFBPs) secreted by the embryo (Peterson *et al.,* 1991) modulate the bioactivities of IGF-I and IGF-II in the ruminant uterine environment. Specifically, proteolysis of IGFBP-3 may selectively increase the bioavailability of IGF-I in the uterine environment where there is a preponderance of IGF-II (Peterson *et al.,* 1998*a*). Regulation of IGFBP-3 expression was identified as

FIGURE 7: A representative Western ligand blot of IGFBPs in ovine ULF through days 3-15 of the oestrous cycle (oestrous d0), and days 17 and 19 in pregnant ewes. Each lane contained 30µg protein and the blot was probed with [125]I IGF-2 (taken from Peterson *et al.*, 1998a).



under oestrogen control (Peterson *et al.,* 1998*b*) and the proteolytic processing of IGFBP-3 under progesterone control (Peterson *et al.,* 1998*a*) (Figure 7). The importance of hormonal control in the preparation of the uterus and maintenance of pregnancy by alterations in endometrial gene expression has since been well documented (Spencer *et al.,* 2008).

Later studies, into early embryo development, were undertaken to assist in discovery of cattle management strategies to improve pregnancy rates. Embryonic loss in cattle was pinpointed to a critical window between Day 7 and Day 14 of gestation (McMillan *et al.,* 1996), when pregnancy recognition occurs. The relationship between embryo development and IFN-τ production was examined in cattle with a history of high, compared with low, pregnancy rates and identified slower conceptus growth rates in cows with a poor pregnancy history (Pearson *et al*., 1999). Although progesterone intervention has been linked to conceptus growth rate and survival (Spencer *et al*., 2008), progesterone concentrations were no different between the high and low pregnancy rate groups. Rather, they differed in the capacity of their uteri to secrete prostaglandin (Peterson & Lee, 2003). This has lead to current studies to discover markers of uterine responsiveness, and thus cows with superior pregnancy outcomes.

In addition, the cause of late gestation pregnancy losses following *in vitro* produced embryo transfer, which inhibited uptake of the technology by the industry, has been examined. The cause of these losses was identified as poor development of the allantois (Peterson & McMillan, 1998c), requiring modification to the culture medium for improved outcomes. Recent investigations to understand the poor pregnancy rate in somatic cell nuclear transfer produced embryos have concentrated on differences in gene expression in the placenta compared with AI pregnancy (Ledgard *et al*., 2009).

Naso-genetal relationship in sheep or Dr Vither and that after dinner speech….

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No story about Jim is complete without some mention of his sense of humour and the practical jokes he played. In 1981 John Smith, in his capacity as Secretary of the New Zealand Society of Animal Production, received an abstract for the annual conference to be held in Dunedin from some non-existent people in MAF media services, namely Toserjen and Vither (1982), on naso-genital relationships in sheep. It took John a number of weeks to work out that the names were anagrams for two of his co-workers, A.J. Peterson and H.R. Tervit. The Management Committee then accepted the abstract and challenged the authors to prepare a full 30 minute presentation at the conference dinner at the Glenfalloch Restaurant on the Otago Peninsula. The resulting presentation on how they managed to record those ram nasal temperatures is part of New Zealand Society of Animal Production history.

Ladies and Gentlemen,

Are your rams weak kneed or randy? I would like to share with you our experimental results which have lead to a world first. This is the ability to counteract the decrease in libido which occurs with increasing age in rams and, unfortunately also in males of other species.

This revolutionary discovery is the result of our pioneering research on the management implications of naso-genital relationships in animals. Now, naso-genital relationships play an important role in the life of a variety of mammals, including primates. This is most obvious in monkeys, baboons and apes. Baboons for example, show very obvious similarities between the nasal and genital regions. However, the relationships do occur to a greater or lesser degree in humans. For example, I am sure that we are all aware of the brown nose syndrome, influencing human interactions, especially job promotions.

Indeed, the human nose is a much underrated organ, capable of the most prodigious physical and physiological acts with a versatility unparalleled by the other appendages of the body. The relationship between sexual processes and noses is however inadequately understood in most mammals. We have attempted to rectify this situation in sheep, and as part of our on-going investigations on naso-genital relationships, and have developed a technique for measuring rams nasal temperatures.

The technique used is a modified Nasal Irrigation Treatment or NIT, using a rubber hose and thermometer. The hose is placed in the nasal cavity and the temperature is recorded. One problem with this method is that the temperature of the nasal passages fluctuates during respiration, being reduced during inspiration by evaporation, and increased on expiration by warm air from the lungs. To overcome these fluctuations, the rams were trained to hold their breath upon application of a scrotal twist, and a Ministry of Transport breathalyser bag used to check cessation of breathing. Testicular beads were present to remind the ram of past pleasures and to encourage him to comply with the experimental protocol since, should he fail to do so, he ran the risk of losing the organs concerned.

In our trial to relate nasal temperature to libido, young and old NIT trained rams had their temperatures measured and were then introduced to penned ewes in oestrus. A young ram quickly identified, by subtle markings on her back, an oestrous ewe and then proceeded to court her. Older rams were not so active and sometimes needed assistance. To check that every ewe was tupped, a new oestrus device, the Donnelly Dinger, developed by Ruakura engineers, as a spin-off from their work on protein extraction, was used.

Immediately after mating, the sexually replete rams were encouraged to rest. The effective marking ability of the new oestrus detection device was noticeable. The rams were helped from the pen to receive appropriate highly skilled medical care and attention, with the presence of the anaesthetic machine for treatment of cases of "orgasmic shock syndrome". The nasal temperature was again recorded and this ended the experiment. The complete NIT data set was then subjected to the most rigorous statistical analysis using the Duganzich P. Test. This incorporates the Jury factor, to solve the perennial problem of wayward data.

Results show that:

- As reported by us at last year's conference, post-mating temperatures are about 3ºC higher than pre-mating temperatures.
- There is a greater temperature rise in the left nostril as opposed to the right nostril.

This reflects the well-known scrotal asymmetry with the left testis of rams lying lower in the scrotum than the right. Thus, when the testes rise during mating, the left has to travel further than the right. This results in a greater temperature differential in the left nostril. Scrotal asymmetry is not unique to rams. Also, anatomical asymmetry is not confined to the scrotum, but occurs in other paired organs of the body.

The rise in temperature in the ram's upper respiratory tract during mating indicates marked vasodilation and greatly increases risk of pulmonary infection. Preliminary results indicate that after mating, there is a greater chance of *Pithomyces* spore inhalation exacerbating the onset of facial eczema in localities of high risk. Thus, during the mating season, farmers are strongly advised to take special post-mating care of their rams, as demonstrated in Figure 8. The care is especially critical during adversely hot or cold weather and at night. It is recognised that this type of care involves some effort but rams should at least be supplied with a stable and restful environment. Farmer reaction to this advice was gauged using the universally recognised Smith Rigid Digit scale. This scale is from 0 (low) to 10 (high). Their reaction ranged from 2 (mild) to 8 (extremely gratifying).

The most significant results are:

- Young rams have a much higher libido than old rams. This is reflected in the number of mating attempts, both successful and unsuccessful, over 10 minutes and is measured in the Jagusch Rickter or JR Scale.
- The older rams suffer a larger post-mating rise in temperature than young rams and should therefore be treated accordingly.

Fortuitously, our trials were conducted at the same time as Ruakurium was discovered. In a flash of insight, rarely seen in scientific circles, we postulated that the topical application of Ruakurium to the testes of aged rams should rejuvenate the rams. To test this hypothesis, an experiment was conducted at Hilldale Farm. Rams were constrained in a crush and the Ruakurium added to a glass beaker containing ice for one minute to allow topical uptake of Ruakurium. Care must be taken not to handle the testes as, as many of you are aware, any application of digits to the scrotum can cause an artificial elevation, not only of the testes, and therefore affect local uptake and excretion of substrates. This study showed conclusively that treatment of rams with Ruakurium overcomes the decrease in libido which occurs with increasing ram age. The mating of one treated 12 year old Suffolk ram with three ewes resulted in the birth of 20 lambs.

FIGURE 8: Post mating care of rams (taken from Toserjen & Vither, 1982).

In conclusion, we have shown inclusively that careful post-mating husbandry of rams is needed to maximise ewe fertility and that old rams can be effectively rejuvenated. We feel that the results presented here will have enormous application and significance in the New Zealand farming industry.

Thank you.

P.A. Toserjen and R.T. Vither

Jim Peterson's career achievements have had a huge influence in the field of ruminant reproductive biology. Those of us who have had the privilege to work with him will remember most his vast knowledge of just about anything, his keenness to "muck" in and help anyone and his ability to tell a good yarn, whilst leaving you wondering if he was pulling your leg! Mostly, we remember him for his friendship, his infectious smile and the cheeky twinkle in his eyes.

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