Institution: Royal Veterinary College
Unit of Assessment: A 6 Agriculture, Veterinary and Food Science
Title of case study: Advances in Assisted Reproduction – agricultural, human and conservation applications

1. Summary of the impact (indicative maximum 100 words)

Different aspects of Professor Paul Watson's research on artificial insemination and semen preservation techniques, have delivered impact in agricultural, human healthcare and ecological spheres. They have contributed to commercial breeding practices, particularly in pigs, providing substantially improved efficiencies and reliability. Research on reducing transmission of infectious agents by semen during storage in liquid nitrogen has been applied to human AI, informing and driving changes in practice to protect against contamination leading to infection. In the field of conservation, the RVC's research has made a significant contribution to international efforts directed at the survival of highly endangered species, supporting preservation of biodiversity.

2. Underpinning research (indicative maximum 500 words)

From before 1993, to his retirement in 2008, Paul Watson, Professor of Reproductive Cryobiology, and his research team studied aspects of artificial insemination and in particular, cryopreservation of spermatozoa. Their work has demonstrated capacitation changes resulting from freezing damage, resulting in lowered fertility in use of cryopreserved semen. Through research into cell damage and its prevention, including the use of oviductal proteins, seminal plasma, antifreeze proteins and glycoproteins to enhance sperm survival and fertility, the RVC's research has contributed to new biological media formulations relating to protection against cooling damage, dilution, premature capacitation and ice crystal formation [1,2].

The cryopreservation research led to a programme supported by BBSRC and the commercial PIC International Group, which identified genetic variability in cryopreserved sperm viability in boars [3]. Furthermore, the research, in collaboration with Dr William Holt at the Institute of Zoology, led to their establishment of improved cryopreservation techniques applicable to different mammalian species. Holt, supported by Watson, led a European Framework III Concerted Action project in 1994, to develop formal systems and guidelines for establishing Genetic Resource Banks (GRBs) [4].

In 2002, RVC published the results of a substantial field trial, supported by a large pig farm and commercial suppliers of semen for AI and sows [5]. This demonstrated both that simple, safe and effective transcervical insemination of sows, rather than the conventional insemination into the posterior region of the cervix, could be undertaken by stockmen in a commercial setting, by use of the novel catheter design (the Deepgoldenpig) developed by IMV Technologies in collaboration with the RVC researchers, and that fertility was unaffected by a much reduced sperm dose, if delivered directly to the uterus.

The close involvement of industry partners from different parts of the production chain (pig farms, boar stud, and reproductive technologies company) enabled continued research into optimising fertility and fecundity through management of boar contact [6]. The team showed 'Segregated Service Management' (SSM) – isolating weaned sows from boar stimulation until day 4 post-weaning, followed by full boar contact to elicit oestrus before AI, resulted in significantly improved farrowing rate and litter size, in comparison with conventional continuous contact.

This was followed by research into AI of gilts, which was not as commonly practised, due to the smaller dimension of the cervix representing a barrier, and susceptibility to mucosal damage. The research team demonstrated the practical application, with minimal training, of a novel modified catheter with a narrow extension (the Goldengilt, now produced by IMV Technologies). This was used with reduced semen concentration and still resulted in farrowing and litter sizes comparable to or better than usually observed commercially by natural service [7].

In 1997, the group identified the potential for transmission of infectious agents through leakage and contamination of semen stored in liquid nitrogen [8]. This paper has contributed to good practice guidelines for human and animal applications.

Other Quality and Relevance Indicators
Defra and commercial funding:
P. Watson. Maximising semen performance by improving sperm assessment & survival during and after cryopreservation. Genus-ABS . 2002-5. £265,000
P. Watson. Identification of genetic indicators of semen freezing susceptibility in boars. Defra. 2002. £151,000.
P. Watson. The development of a modern long-life storage diluent for fresh ram spermatozoa. Defra LINK. 2005-8. £491,000
Watson's contributions to this field were recognised through the award of the Marshall Medal of the Society for the Study of Reproduction in 2005 and the inaugural Brian Setchell Medal of the British Andrology Society in 2007.

3. References to the research (indicative maximum of six references)

1. Prathalingham, NS, Holt, WV, Revell, SG, Jones, S, Watson, PF 2006 Dilution of spermatozoa results in an improved viability following a 24-hr storage period but decreased acrosome integrity following cryopreservation. Animal Reproduction Science; 91, 11-22. DOI:org/10.1016/j.anireprosci.2005.04.001
2. Fazeli, A, Elliott, RM, Duncan, AE, Moore, A, Watson, PF, Holt, WV 2003 In vitro maintenance of boar sperm viability by a soluble fraction obtained from oviductal apical plasma membrane preparations. Reproduction;125(4):509-17. DOI:10.1530/rep.0.1250509
3. Thurston, LM, Siggins, K, Mileham, AJ, Watson, PF, Holt, WV 2002 Identification of amplified restriction fragment length polymorphism (AFLP) markers linked to genes controlling boar sperm viability following cryopreservation. Biology of Reproduction; 66, 545-554. DOI: 10.1095/ biolreprod66.3.545
4. Watson, PF, Holt, WV (eds.) 2001 Cryobanking the Genetic Resource: Wildlife Conservation for the future? Taylor and Francis, London. ISBN 0-748-40814-2.
5. Watson, PF, Behan, JR 2002 Intra-uterine insemination of sows with reduced sperm numbers: results of a commercially based field trial. Theriogenology; 57: 1683-1693. DOI:org/10.1016/S0093-691X(02)00648-9
6. Behan, JR, Watson, PF 2005 The effect of managed boar contact in the post-weaning period on the subsequent fertility and fecundity of sows. Anim Reprod Sci; 88, 319-324. DOI:org/10.1016/j.anireprosci.2004.12.009
7. Behan, JR, Watson, PF 2006 A field investigation of intra-cervical insemination with reduced sperm numbers in gilts. Theriogenology; 66, 338-343. DOI:org/10.1016/j.theriogenology.2005.11.020
8. Russell, PH, Lyaruu, VH, Millar, JD, Curry, MR, Watson, PF 1997 The potential transmission of infectious agents by semen packaging during storage for artificial insemination. Animal Reproduction Science; 47; 337-342. DOI:org/10.1016/S0378-4320(97)00017-1

4. Details of the impact (indicative maximum 750 words)

Agriculture
Artificial insemination is used principally in pig breeding, to enable cost-effective and wider access to high genetic value gene pools, and avoid transport of live animals with inherent risks of disease transmission and the potential for stress and injury in transit. The overall value in agriculture is to improve animal productivity and increase the food supply. Up to 85% of swine are now bred using AI. Improvements in efficiency, to which the RVC has contributed [a], have resulted in lowering AI costs and expansion in its use.

Although bull sperm are readily recovered viably after freezing, and are produced in quantity, boar sperm has not been as amenable for use in commercial AI. Sperm quantity per inseminate must be sufficient to ensure maximum fecundity, (which has restricted usable 'doses' per ejaculate), as economic value is driven by number of piglets per litter contributing to total lean tonnage. Use of

frozen/thawed sperm remains a small fraction of the total, commercially valuable for accessing high genetic value boars which deliver higher lean content in offspring. The novel formulation of cryopreservation biological media that Watson's team developed, informed by their research into factors affecting fresh and frozen/thawed sperm competence, have become standard in pig production using frozen/thawed sperm. The Knowledge Transfer Manager at the British Pig Executive (BPEX) confirms: "*The RVC's research has contributed to its [AI] expansion within the sector, and the accompanying economic and commercial benefits. Watson and Behan's work on improving the sperm competency of frozen/ thawed boar semen, through new, and now standard, biological media formulations, has brought a number of benefits."* [b]. This, together with the team's identification of genetic markers which indicate the viability of boar sperm for cryopreservation and likely fresh longevity, has addressed sub-optimal performance of frozen/thawed boar semen, as well as helping to overcome the short period of usability for fresh ejaculates, which formerly restricted access to the highest genetic stock, by reasons of distance and transport logistics.

RVC's research also demonstrated that a significantly smaller sperm sample may be used for post cervical AI – leveraging gain from high genetic value boars - with appropriate biological media, without loss of reproductive success [b]. The expertise of the RVC team was sought by world leading agricultural breeding business IMV Technologies, leading to a collaboration including field trials in pigs, using 50% of the previously accepted industry standard of sperm numbers per insemination, enabling twice as many inseminations per boar [5].

The new Deepgoldenpig catheter, originally developed in collaboration with IMV Technologies for use with fresh semen, additionally facilitated use of reduced sperm concentration to enable a wider application of cryopreserved semen from superior boars. Field trials for a second new catheter, Goldengilt, demonstrated that intracervical AI of gilts as well as sows, with small sperm doses, could be undertaken safely and simply by stockmen with appropriate training. IMV states that more than 30% of all pig breeders worldwide now use the company's 'gold standard' insemination solutions [c]. The efficacy of these new catheters, as demonstrated by the RVC's field trials research, has led to their being supplied through or copied by many other AI technology companies internationally, including e.g. Schippers and Innovis [d,e].

Evidence that managing contact between boars and sows prior to breeding can maximise breeding performance, has established SSM, in a refined form, as standard practice internationally in commercial pig breeding operations using AI [f]. This is also confirmed by BPEX: *"… [RVC] work played a key role in understanding the importance of the management of contact time in providing optimal conditions for insemination … developing practices which have now become standard in many commercial breeding operations*." [b].

As pig breeding has become professionalised in recent years, the value of these developments has contributed to the expansion and economic value of AI in commercial pig breeding. (Around 20-30% of total global production before the REF period, to 60-80%+ in 2013).

Human assisted reproduction

In seeking to further minimise the spread of infection, Watson's research into the transmission of infectious agents by semen during storage in liquid nitrogen has led to good practice guidelines for aseptic preparation and well-sealed packaging. The findings have been taken up in human medical AI to minimise risk of HIV or other viruses contaminating shared storage of semen samples [g]. The Scientific Director of the Centre for Reproductive Medicine, University Hospital Coventry, comments: *"In our clinical protocol, we use a purpose designed commercial kit that includes a disposable spacer device that avoids the tip of the straw coming into contact with the semen. This spacer is discarded once the straw is filled. […] The concept of keeping the semen out of contact with the tip of the straw for hygiene purposes, as recommended in the paper [research reference 8], […] has been adopted into routine clinical practice*." [h]

Conservation

The extensive studies at RVC on sperm cryopreservation have played a central role in the global development of Genetic Resource Banks (GRBs), now recognised as one of the most effective

means by which threatened species can be preserved and managed [i]. As the Head of the Centre for Species Survival at the Smithsonian Conservation Biology Institute has commented: *"…the concepts developed and the research conducted at the RVC are contributing to the acceptance and advancement of GRBs and cryobiology to benefit managing and conserving endangered species ... [these tools] are rapidly becoming common to zoo breeding programs throughout North America and Europe*". The RVC's research has therefore enhanced the success of efforts to maintain and increase biodiversity of small breeding populations without the costs, risks and impracticality of live animal transportation. Successful examples recorded by the Smithsonian include development of protocols to produce offspring from the AI of frozen sperm from the scimitar-horned oryx and cheetah, and the preservation of sperm of the black-footed ferret, one of the rarest mammals in all of North America. The species was thought extinct before 1979, but its rediscovery and an ongoing major captive breeding programme from 18 animals has enabled re-introduction at 19 sites in the USA, Canada and Mexico. Ferret offspring have been produced from sperm cryopreserved for more than two decades [j].The wild population is still endangered, but is estimated to have expanded since 2008 from 700 to 1,000 [k].

In conclusion, the RVC's research has helped to increase the productivity of domestic livestock in all developed farming territories, providing real economic value. This programme of work has additionally contributed to safeguarding of human health in assisted reproduction and made a critical contribution to successful captive breeding and maintenance of genetic biodiversity for wildlife conservation and species survival.

5. Sources to corroborate the impact (indicative maximum of 10 references)

a. http://www.imv-technologies.com/publications.html (Research reference [1 and 5] in Publications list) [accessed 30 Jul 2013]

b. Statement from Knowledge Transfer Manager, British Pig Executive. Held by RVC.

c. http://www.imv-technologies.com/le-groupe.html Corporate brochure [accessed 30 Jul 2013]

d. http://www.msschippers.com/all-products/gilts- [accessed 30 Jul 2013]

e. http://www.innovis.org.uk/breedingproducts/pigproducts.asp?Product_category_ID=4 [accessed 30 Jul 2013

f. http://www.thepigsite.com/articles/3/feed-and-nutrition/1653/does-absence-make-the-heat-grow-stronger [accessed 30 Jul 2013]

g. Talwar. P. (ed.) (2012) *Manual of Assisted Reproductive Technologies and Clinical Embryology*. Jaypee Brothers Medical Publishers, New Delhi. ISBN 978-93-5025-506-3. Pages 195, 214 and 228

h. Statement from Scientific Director of the Centre for Reproductive Medicine, University Hospital Coventry. Held by RVC.

i.Watson, P.F. and Holt, W.V. (eds.) (2001) *Cryobanking the Genetic Resource: Wildlife Conservation for the future?* Taylor and Francis, London. ISBN 0-748-40814-2.

j. Statement from Head, Centre for Species Survival, Smithsonian Conservation Biology Institute, Washington. Held by RVC.

k. http://www.blackfootedferret.org/reintroduction [accessed 2 Aug 2013]