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The production of pig preimplantation embryos *in vitro*: Current progress and future prospects

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ABSTRACT

Human assisted reproductive technology procedures are routinely performed in clinics globally, and some of these approaches are now common in other mammals such as cattle. This is currently not the case in pigs. Given that the global population is expected to increase by over two billion people between now and 2050, the demand for meat will also undoubtedly increase. With this in mind, a more sustainable way to produce livestock; increasing productivity and implementing methods that will lead to faster genetic selection, is imperative. The establishment of routine and production scale pig embryo *in vitro* production could be a solution to this problem. Producers would be able to increase the overall number of offspring born, animal transportation would be more straightforward and *in vitro* produced embryos could be produced from the gametes of selected elite. Here we review the most recent developments in pig embryology, outline the current barriers and key challenges that exist, and outline research priorities to surmount these difficulties.

1. Introduction

Human assisted reproductive technology (ART) procedures such as *in vitro* fertilisation (IVF), preimplantation genetic diagnosis (PGD) and gamete and embryo cryopreservation are well established and implemented in clinics worldwide; in fact in 2016 in the UK alone over 68,000 IVF treatment cycles were performed, resulting in 20,028 births [1]. Similarly, the mouse is widely used as a model for human ART procedures. In domestic farm animals, the motivation for performing IVF, and possibly PGD, is quite different. By 2050 the world population is predicted to increase from 7.6 to 9.8 billion [2], and the *per capita* increase in consumption of meat and milk is expected to increase by 20% [2]. Livestock production is also a significant contributor to global warming [3]. Solving these problems means that more meat needs to be produced from fewer animals in less time. This could potentially place an untenable demand, both on the environment and on food producers without sufficient innovation. This could potentially place an untenable demand, both on the environment and on food producers without sufficient innovation.

IVP in pigs is an attractive option for research fields such as reproductive biotechnology, transgenesis and biomedicine. Moreover, taking into account the genetic, anatomical and physiological similarities between pigs and humans, transgenic pigs may represent suitable donors of tissues and organs for xenotransplantation, regenerative medicine, as animal models of human hereditary diseases, or as animal bioreactors of recombinant human proteins/biopharmaceuticals [4–14].

The strategies of IVP that are commonly applied to generate porcine embryos encompass three crucial steps: 1) *in vitro* maturation (IVM); 2) IVF or somatic cell nuclear transfer (SCNT); and 3) *in vitro* culture (IVC) of fertilised or cloned embryos [15–26]. Although multiple methods have been used to create *in vitro* fertilised or nuclear transferred pig embryos, their developmental potential and quality are low in comparison both to their *in vivo* produced counterparts and to IVP embryos from other livestock species [27–36]. Therefore, more work is needed to achieve the efficient generation of high quality IVP derived pig embryos for the purposes of biotechnological and biomedical research [37–46].

As pigs account for c.40% of global meat consumption [4] a sustainable supply of pork to both developed and developing countries also requires increased productivity through rapid selection for greater feed conversion efficiency, improved disease resistance and enhanced

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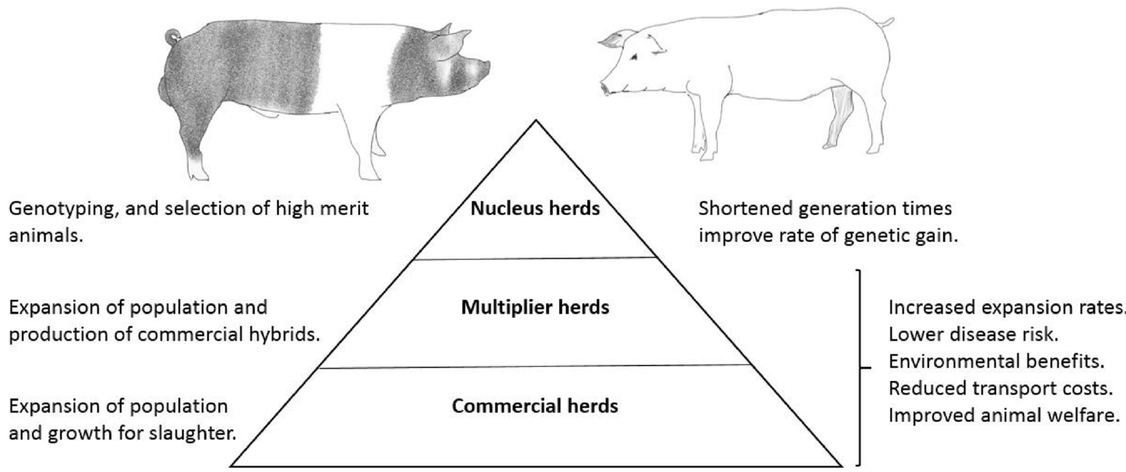

Fig. 1. Schematic representation of selection and production herds in pig production indicating where *in vitro* production can achieve production gains.

fertility. With this in mind, IVF, or more specifically, IVP could be greatly beneficial in the following ways.

1.1. Accelerating genetic progress

IVP embryos produced from the gametes of selected elite parents represent an excellent resource for improving food production. In recent years, food producers have made use of high-throughput genomic platforms, primarily single nucleotide polymorphism (SNP) chips [5], to determine genetic merit in new-borns. The speed and efficiency at which genetic improvement for such traits can be introduced is however constrained by the delay between conception and birth. Use of IVP embryos would theoretically have the potential to increase selection intensity as the first selection step would occur before the embryo is implanted, thereby immediately removing the requirement to gestate lower genetic merit animals and hence ensuring uterine resource is focused only on the genetically superior candidates.

1.2. Movement of genetics across international borders

As artificial insemination (AI) is widely used in animals of agricultural importance, semen samples (male genetics) are routinely distributed both nationally and internationally. For female genetics however, currently the only option in pigs is to transport live animals for establishing nucleus farms overseas. Use of vitrified IVP embryos on the other hand would facilitate the global transport of genetically superior stock in way that delivered higher animal-welfare, a lower-cost and increased bio-security. Moreover, if the embryos are sexed beforehand, the drawbacks of the waste from genetically unwanted males that have to be reared to market weight, including ammonia, methane and nitrous oxide [7], is eliminated.

1.3. Animal health and welfare

Farm animals carry a considerable number of endemic diseases and often it is necessary to move infected, and potentially infected, pigs into a “clean” farm. Current practice involves a pregnant female receiving a hysterectomy with foetuses *in utero*, followed by sacrificing the mother. In contrast, embryos produced in a lab have the potential to be “clean” and could be implanted into recipients on the farm significantly reduced disease risk (explored later). Similarly, when re-stocking a farm, it is imperative to have one supply at a time as mixing multiple populations risks transmission of disease. Embryos on the other hand, could be implanted into existing sows (following improvements in transfer techniques) which means that subsequent live births would receive the endemic immunological challenge of the farm at birth, and thereby would not introduce new disease to the existing population.

1.4. Further benefits

By producing IVP pig embryos, a resource for future work on genome editing, which could be used to improve livestock, is created. IVP embryos are also a useful resource for bio-banking, in particular, maintaining biodiversity by preserving rare breeds or lines. Finally, both pig and cattle embryos are an excellent model system for fundamental research into human IVF. Being large mammals, like ourselves, pigs and cattle (and, by extension, aspects of the cell biology of their embryos) have much closer similarity to humans than the classical mouse model for fundamental biological studies. As such, and in addition to other sources of embryos, these could be used to improve media, culture conditions and standard operating procedures when ethical issues preclude direct experimentation on human embryos.

Cattle IVP is now comparatively well established, thus enabling vast improvements to both beef and dairy production [8,15]; for example, the first use of Karyomapping, (a universal means of detecting chromosome disorders) for non-human purposes has recently been reported in cattle [47]. In pigs however, much work is still to be done and the received wisdom is that pig IVP is notoriously difficult to achieve. Given that challenges previously faced in human embryology have now been overcome, it seems that, with sufficient time and resources, a re-invention of the pig IVP process could be accomplished. Furthermore, with working protocols for embryo biopsy, genetic screening, sexing and possibly genome sequencing there is great potential for success. These benefits are summarised in Fig. 1, there however remain a number of significant challenges to the implementation of pig IVP. The purpose of this review is to summarise the state of the art in pig IVP, to outline the key challenges and to provide a road map for research priorities to surmount these.

2. The challenge of pig embryology and the importance of using chemically defined culture medium

The strived for, but not yet accomplished, 100% success rate in human IVF procedures is less important in agricultural animal embryological procedures where the key drivers are embryo quantity and cost. Given the comparatively high number of embryos required, the processes of IVM, IVF and IVC in these species is generally referred to as *in vitro* production (IVP). Whilst the first successful pig IVP was reported in 1986, IVP still has a relatively low success rate [16,19,20,48]. More recently, several pig IVP approaches have been developed that successfully generate embryos [34,43,49], however upscaling the process to the levels required for production and commercial