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The Role of Veterinarians in Human *In Vitro* Embryo Production

Daniela Paes de Almeida Ferreira Braga & Edson Borges Jr.

ABSTRACT

***Background*:** Infertility affects approximately 15% of couples of childbearing age, and there is evidence that fertility has been declining at an accelerated pace in recent years. Recent advances in assisted reproductive technologies (ART) have made biological parenthood possible for couples who previously had no hope of childbearing. On the other hand, the primary use of embryo transfer in cattle has been amplification of the reproductive rates of valuable females. In 2007, 245,257 bovine embryos were produced globally; Brazil accounted for 86.6% of this output. The popularity of this technology has contributed to the growing knowledge of embryology among veterinarians. Because of ethical concerns regarding the use of human subjects, bovines have been used as a model for the study of reproductive biology in women. Therefore, this manuscript will discuss approaches for oocyte selection and sperm selection that were developed in animal models and adapted for ART in humans.

***Review*:** One of the most important factors determining the outcome of embryo development is gamete quality. Numerous attempts have been made to identify prognostic factors for oocyte development potential. One promising approach uses spindle imaging as a predictor of oocyte quality. The development of a polarised light microscope that evaluates the birefringence of living cells enabled the evaluation of highly birefringent oocyte spindles without damage to the cell. Polarised light microscopy also enables the evaluation of other birefringent, sub-cellular oocytesstructures, such as the zona pellucida. The birefringence of the meiotic spindle and the zona pellucida are valuable tools in predicting the developmental potential of oocytes and embryos. The post-genomic era has facilitated the growth of non-invasive approaches to the study of embryonic physiology and to predicting oocyte developmental competence and viability. These approaches include the assessment of proteomic profiling and, most recently, of the oocyte and embryonic metabolome. Although intracytoplasmic sperm injection ICSI outcomes are apparently unrelated to basic sperm parameters, recent reports have suggested a paternal contribution to the success of embryonic development and implantation, and a new method for sperm evaluation has been proposed. It is now possible to examine the nuclear morphology of spermatozoa at 6600x magnification. This technique has allowed the introduction of a modified ICSI procedure called “intracytoplasmic morphologically selected sperm injection” (IMSI). As a consequence, real-time, detailed morphological sperm examination using motile sperm organelle morphology examination (MSOME) enables the best available spermatozoa to be selected prior to oocyte injection. Recent reports have suggested that normal nuclear morphology, as viewed under high magnification, may be useful for selecting spermatozoa with higher developmental capacity.

***Conclusion*:** Studies in animal reproduction have allowed the development of non-invasive methods of evaluating sperm and oocytes prior to their use in ART. Gamete quality biomarkers, once identified, may allow embryos with the highest implantation potential to be selected with greater accuracy. The use of such biomarkers may thus yield an increased chance of pregnancy, which is the primary objective of ART.

Keywords: assisted reproduction, non-invasive biomarkers, oocyte quality, sperm morphology.

Fertility - Assisted Fertilization Center, São Paulo, SP, Brazil. CORRESPONDENCE: D.P.A.F. Braga [dani@webbraga.com/dbraga@fertility.com.br - FAX: +55 (11) 3885-9858]. Av. Brigadeiro Luis Antonio, n. 4545, CEP:01401-002, São Paulo, SP, Brazil.

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I. INTRODUCTION

Human infertility is defined as the failure to conceive after one year of unprotected intercourse [4] and affects approximately 15% of couples at childbearing age, with substantial clinical and social impacts [7].

There is some evidence that fertility has been decreasing at an accelerated rate in recent years. One prominent cause of reduced fertility is age-related decline in the number and quality of eggs; women now wait longer to have their first child than they did in previous decades [35]. In the so-called natural fertility populations in which no birth control is practised, fertility starts to decline at age 30 or earlier [21]. Based on such historical cohorts, it is known that the time required to conceive a child increases with age; the risk of infertility is also higher for older women [40].

Moreover, lifestyle factors and nutritional status are known to be critical determinants of normal reproductive function. A combination of reduced exercise, changes in dietary composition and social habits have contributed to a growing worldwide epidemic of infertility [45].

Recent advances in assisted reproductive technologies (ART) have made biological parenthood possible for couples who previously had no hope of childbearing. Since the first healthy child was conceived via *in vitro* fertilisation (IVF) [54], the use of ART has increased dramatically; as many as 1% of all children are now conceived *in vitro* [2]. More recently, intracytoplasmic sperm injection (ICSI) has become the treatment of choice for severe male factor infertility [48].

On the other hand, the primary application of embryo transfer in cattle has been to amplify the reproductive rates of valuable females. Ideally, embryo transfer can be used to enhance stock improvements and to increase marketing opportunities for purebred cattle. After transferable embryos are collected from a donor cow, it is decided which of the available recipients should receive embryos, with the goal being to achieve the greatest number of pregnancies [61]. In 2007, 245,257 bovine embryos were produced globally; Brazil accounted for 86.6% of this output [52]. The popularity of these technologies has contributed to the growing knowledge of embryology among veterinarians.

Moreover, follicular development in cattle has been characterised in detail over the last two decades [26], and the bovine model [1] was the foundation for the recent discovery of follicular wave development in women [6,7]. Indeed, the development of ovarian follicular waves in women is comparable to the follicular wave phenomenon described in cattle. In both species, the menstrual [7] and oestrous [16,26] cycles are characterised by two or three follicular waves.

The main challenge for successful IVF is the production of viable embryos with high implantation potential. Although high-quality human embryos may be available for transfer, only a small proportion of patients undergoing IVF will ever achieve a pregnancy. In fact, previous studies most of the embryos transferred into the uterus fail to implant [19]. Oocyte quality influences the implantation potential of IVF-derived embryos [18,60] and recent reports have also suggested a paternal contribution to the success of embryonic development and implantation [31,47].

Because of ethical concerns regarding the use of human subjects, and human embryos in particular, bovines have been used as a model for the study of reproductive biology in women [16,38]. Therefore, this manuscript will describe approaches for oocyte selection and sperm selection that were developed in animal models and adapted for human ART.

II. EVALUATION OF HUMAN OOCYTES

Advances in reproductive medicine have made clear that one of the most important factors determining the outcome of embryonic development is oocyte quality; in particular, success depends on optimal nuclear and cytoplasmic oocyte maturity [15,17].

There are many published reports on the impact of oocyte morphology on embryonic development [18,60]. Numerous attempts have been made to identify the characteristics of oocyte morphology that can be used to predict oocyte quality, fertilisation rates and embryonic development; however, the predictive value of the criteria used in these studies is still controversial.

2.1 Meiotic spindle image

During very early transition from Metaphase I to Metaphase II of meiosis, a highly dynamic microtubule structure called the meiotic spindle is formed. The meiotic spindle controls the movement of chromosomes throughout meiosis; the spindle is also involved in various functions that are essential for fertilisation and in early post-fertilisation events, such as chromosome segregation and genomic stability after oocyte activation [20,51].

Over the years, transmission electron microscopy and immunofluorescence have been used to study oocytes from both humans and experimental animals; these techniques are used to detect spindle aberrations and to identify conditions that adversely affect spindle function and chromosome segregation, for instance, at IVF. Errors in meiotic division may be one of the most common causes of aneuploidy [15].

One promising approach uses spindle imaging as a predictor of oocyte quality [15,59]. The development of a polarised light microscope that evaluates the birefringence of living cells enabled the evaluation of the highly birefringent oocyte spindles (Figure 1) without damage to the cell [33,46].

Previous reports have demonstrated that oocyte developmental competence is lower when meiotic spindles are not detected under polarised light microscopy. It has been suggested that the absence of meiotic spindles detection may compromise not only fertilisation but also embryonic development [30,33,59]. Moreover, a previous study presented by our group demonstrates that when only embryos derived from oocytes with detectable spindles were used, higher pregnancy and implantation rates were achieved [36]. These data suggest that spindle visualisation can be an important tool in predicting oocyte and embryo competence.

2.2 Zona Pellucida Birefringence

Polarised light microscopy also enables the evaluation of other birefringent sub-cellular oocyte structures, such as the zona pellucida [29,49]. The zona pellucida is a unique extracellular coat that surrounds the maturing oocyte during ovulation, fertilisation, and early embryonic development [22].

One possible role of zona birefringence in predicting embryonic implantation potential has been previously discussed [50]. Shen *et al.* [29] reported a higher zona retardance in oocytes contributing to conception cycles when compared to oocytes in non-conception cycles. Embryonic development was also reported to be better in embryos derived from oocytes with high zona birefringence [41,50]. Higher rates of implantation, pregnancy and live birth have also been reported when transferred embryos were derived from oocytes with high birefringence zona pellucida [37,41]. Moreover, when only embryos derived from

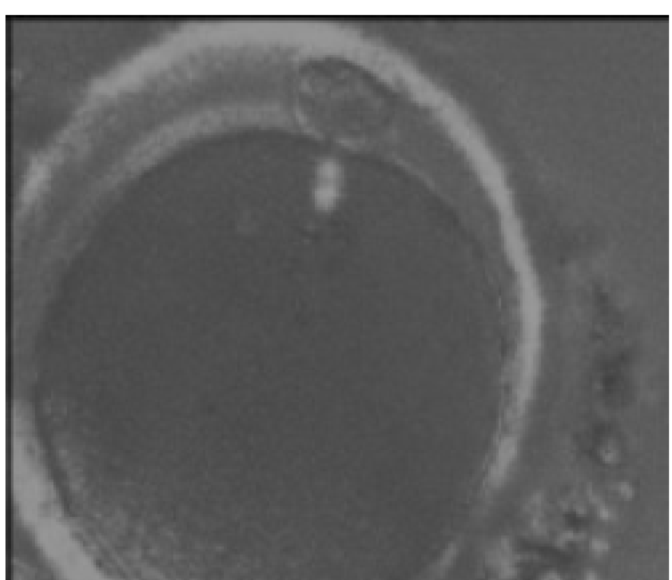

Figure 1. Meiotic spindle in a human oocyte under polarised light microscopy.

oocytes with high zona birefringence were transferred, the miscarriage rate was considerably lower [37].

2.3 Assessment of metabolites and proteins in the oocyte culture media

In the *post*-genomic era, research efforts have deepened our understanding of the relationships among genomes, DNA transcripts, proteins, metabolites and the phenotypes expressed in cells and organisms. Microarray studies have provided a great deal of information regarding gene expression and the changing transcriptome. Nonetheless, gene expression is only one aspect of the complex regulatory network that allows cells to respond to intracellular and extracellular signals [24].

Although transcript analysis of granulosa or cumulus cells can provide information regarding oocyte quality, oocyte or embryo transcriptomics is invasive [14,27]. This limitation has prompted the development of non-invasive adjunctive technologies for use in the study of embryonic physiology and for predicting oocyte and embryonic developmental competence and viability [27]. These approaches include the assessment of glucose, lactate, pyruvate, or amino acid levels in the embryonic culture media, evaluation of oxygen consumption, proteomic profiling, and most recently, examination of the oocyte and embryo metabolome [5,12].

Proteomics alone involves several sophisticated techniques, including imaging, mass spectrometry and bioinformatics to identify, quantify and characterise a proteome. It has been suggested, that both metabolomic and proteomic tests may be strong predictor for implantation potential of human oocytes [43,53].

III. EVALUATION OF HUMAN SPERMATOZOA

Although ICSI outcomes are apparently not related to basic sperm parameters [32,55], recent reports have suggested a paternal contribution to successful embryonic development and implantation [31,47].

Intracytoplasmic sperm injection is usually performed under an optical magnification of 400x. This magnification makes it possible to detect, in living cells, most of the sperm anomalies that are recognised in fixed and stained sperm samples in conventional basic sperm analyses. This system, however, has severe limitations, as only major morphological defects are detectable. The more minor morphologic defects related to ICSI outcome [11] are often overlooked.

One new technique, "motile sperm organelle morphology examination" (MSOME), uses unstained, real-time observation of spermatozoa. It is now possible to examine the nuclear morphology of spermatozoa at a magnification of 6600x (Figure 2) using Nomarski differential interference contrast [13].

Because MSOME is an unstained cytological technique, its incorporation, together with a micromanipulation system, has allowed the

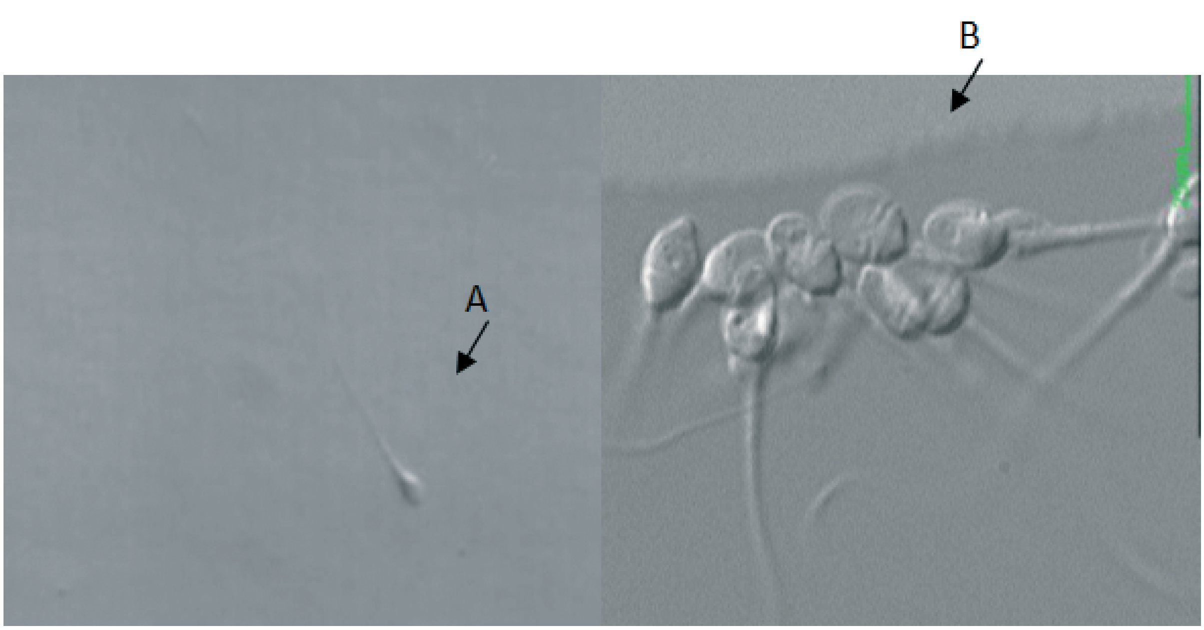

Figure 2. A: A human sperm under 400X magnification. B: Human sperm under 6600X magnification.

introduction of a modified ICSI procedure called "intracytoplasmic morphologically selected sperm injection" (IMSI). In this procedure, real-time, detailed morphological sperm examination at magnifications ranging from 6600x to 13000x [25] enables the selection of the best available spermatozoa prior to oocyte injection.

A positive correlation between sperm morphological normality as determined through MSOME and the fertilisation rate has previously been demonstrated [13]. Moreover, it has been suggested that high magnification sperm morphology has a major impact on the percentage of high quality embryos [9,10,13], as well as on the implantation rate [10,13,60], pregnancy rate [8,10,13] and miscarriage rate [8,10,28]. Recently, Figueira *et al.* [23] demonstrated that morphological normality of the sperm nucleus also has a significant impact on the occurrence of aneuploidy in the developing embryo.

In summary, these data suggest that the selection of sperm for intracytoplasmic injection based on normal nuclear morphology as seen under high magnification may be a useful tool in selecting euploid spermatozoa with higher developmental capacity.

IV. CONCLUSION

Ethical concerns surrounding experiments on human embryos and gametes have largely restricted studies on the molecular biology of human gametes to non-human animal experiments.

Studies in non-human animal reproduction have allowed the development of non-invasive methods of evaluating sperm quality and oocyte quality prior to their use in ART.

The evaluation of human gamete quality has important benefits when applied to ART. These benefits are especially important in countries with legal restrictions, where oocyte selection prior to IVF has particular importance. Moreover, the identification of gamete quality biomarkers may allow more accuracy in the selection of the best embryo for transfer with the highest implantation potential. The use of such biomarkers may also yield an increased chance of pregnancy, which is the primary objective of ART.

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