—Mini Review—

Elasticity Measurement of Zona Pellucida Using a Micro Tactile Sensor to Evaluate Embryo Quality

Yoshinobu Murayama[1], Mitsutoshi Yoshida[2], Jinji Mizuno[3], Hiroko Nakamura[3], Sumiko Inoue[1], Yuri Watanabe[1], Kazuyuki Akaishi[3], Hiroaki Inui[3], Christos E. Constantinou[4] and Sadao Omata[1]*

[1]*College of Engineering, Nihon University, 1 Nakagawara, Tokusada, Tamuramachi, Koriyama, Fukushima 963-8642, Japan*
[2]*Faculty of Agriculture, Kagoshima University, 1-21-24 Gunmoto, Kagoshima, Kagoshima 890-0065, Japan*
[3]*Inui Maternity Clinic, 3-5-18 Namiki, Koriyama, Fukushima 963-8025, Japan*
[4]*Stanford University, School of Medicine, Stanford, CA 94305, USA*

Abstract: *To enable improved success rates of IVF, we developed the technology to optimize embryo selection with the highest implantation potential while ensuring no damage to embryos using zona elasticity as the selection criterion. In this communication we outline the biomechanics and safety of zona pellucida (ZP) elasticity measurement using the micro tactile sensor (MTS) system and demonstrate the specific changes in ZP elasticity during oocyte maturation, fertilization, and early embryo development. Zona hardening was demonstrated mechanically following fertilization at the pronuclear (PN) stage, and Young's modulus decreased gradually as the embryo developed. Evaluation of the quality of expanded blastocysts (EPB) showed that the quality of EPBs could also be evaluated from elasticity parameters. Furthermore, the observations indicated that ZPs of embryos generated in vivo were significantly harder than those of embryos generated in vitro at each stage. Preliminary results also indicated that denuded oocytes matured in vitro did not show zona hardening following parthenogenetic activation by strontium chloride, suggesting that sufficient maturation and consequent oocyte activation may be evaluated by increases in ZP elasticity. We conclude that MTS-elective single embryo transfer can be applied to human assisted reproductive technology to enable embryo quality evaluation in both early embryos and EPBs.*

Key words: *Zona Pellucida, Elasticity, Quality, SET, MTS*

Received: January 30, 2008
Accepted: February 22, 2008
*To whom correspondence should be addressed.
e-mail: murayama@ee.ce.nihon-u.ac.jp

Introduction

Recent advances in biomedical engineering have made it possible to manipulate cells or tissues *in vitro* for therapy, and have therefore provided great benefits to assisted reproductive technology (ART), such as *in vitro* fertilization (IVF). The goal of IVF treatment in humans is to achieve a viable singleton pregnancy followed by vaginal delivery of a healthy child. However, it is difficult to maintain the natural quality of the embryo *in vitro*, so the IVF success rate is still quite low. There is also general agreement that twin pregnancy is the most severe complication of IVF, resulting in considerable medical risks for both mother and infants, as well as increased obstetric and neonatal costs. Thus, the importance and effectiveness of elective single embryo transfer (eSET) with top quality embryos are widely accepted as reducing the number of multiple births for patients undergoing ART. However, decreasing the number of transferred embryos can reduce the chance of achieving pregnancy. Therefore, it is necessary to develop an appropriate culture device, culture medium, smart cell manipulation system, and a method to evaluate the quality of embryos to select one embryo for eSET that will improve the maintenance of embryo quality *in vitro* and thus achieve a higher IVF

success rate. To do this, it is of paramount importance to develop a combination of improved selection criteria and improved culture conditions to optimize selection of the embryo with the highest implantation potential. Several studies have suggested different types of criteria for the selection of embryos with the highest implantation potential [1, 2]. However, these criteria are qualitative evaluations of morphology and may differ among physicians and embryologists. Thus, it is necessary to develop a novel sensing technology to estimate embryo quality with quantitative criteria ensuring that the method is strictly non-invasive and results in no damage to embryos. Against this background, new technologies have been developed for eSET application. For example, scanning electrochemical microscopy non-invasively measures respiration activity of individual embryos [3]. As it provides important information about metabolic activity, respiration is correlated with embryo quality, and embryos with higher oxygen consumption are better candidates for further development into good quality embryos, which have the potential for higher pregnancy rates after embryo transfer.

In the present study, we measured the hardening and softening of the zona pellucida (ZP) during oocyte maturation, fertilization, and early embryo development using a novel Micro Tactile Sensor (MTS) [4–6]. We demonstrated that the dramatic changes in oocyte/embryo zona elasticity are most likely due to oocyte/embryo activation. Furthermore, zona elasticity may play an important role in oocyte protection, fertilization, polyspermy block, hatching, and oviductal transport. Therefore, the measurement of zona elasticity using the MTS should benefit quantitative quality assessment if significant relations between zona elasticity changes and embryo quality exist.

Here, we first overview the history of biomechanics in an attempt to measure the ZP elasticity. Second, we describe the MTS and its application for ZP elasticity measurement. Third, we summarize the data demonstrating the changes in ZP elasticity during oocyte maturation, fertilization, and early embryo development in mice, pigs, and humans. Finally, we review the usefulness of ZP elasticity as an indication of embryo quality and for use in human ART.

Elasticity of the Zona Pellucida

The elasticity changes of the mammalian egg pose a unique challenge in determining the sequence of fertilization. The zona pellucida, the ovum's extracellular layer of glycoprotein, which is approximately 15 μm in thickness, has been reported to be altered following fertilization in a process described as the zona reaction [7]. In the zona reaction, increased resistance to dissolution by various biochemical agents, a phenomenon known as zona hardening [8], has been evaluated in many species [9, 10]. However, although the term zona hardening implies a mechanical change, the physical hardness of the zona pellucida is largely unknown because of a lack of appropriate measurement methods.

The first attempt to measure zona hardness was made by Green in 1987, who used a quartz fiber method to measure zona hardness hamster ova, and concluded that sperm cannot penetrate the ZP solely by force [11]. The second attempt to measure zona hardness was made by Drobnis *et al.* in 1988, who developed a ZP capillary suction apparatus [12]. Hamster and mouse oocytes/embryos were deformed by decreasing the fluid pressure in a suction pipette, and the ZP hardness was estimated from the ZP deformation measured on video recordings of microscope images of very high magnification. The authors concluded that the mouse ZP hardened following fertilization; however, there were still several unresolved theoretical issues in interpreting the results obtained. Tension at the surface is only an estimate of the stiffness of ZP, and the theory was based on the concepts of plates and shell deformation [13, 14], which do not apply directly in cases where there is a large degree of deformation, such as in soft tissue. In general, temporal changes of mechanical properties during cell activity are quite small, necessitating the use of relatively high sensitivity instrumentation for their measurement. A third attempt to measure zona hardness was made by Sun et al. in 2003 with the benefit of recent developments in microelectromechanical system (MEMS) technology [15]. They developed an MEMS-based two-axis cellular force sensor to measure forces applied to embryos. The sensor had spring widths of less than 5 μm, and were capable of resolving forces up to 25 μN with a resolution as low as 0.01 μN. Nevertheless, the experiments caused severe deformation of up to 60 μm of the oocytes/embryos. Consequently, there were theoretical difficulties in calculating the elastic modulus of the ZP as the results are also related to cellular pressure, ZP thickness, total cell size, *etc*. To overcome these problems, a very small contact deformation in the measurement with the benefit of high sensitivity is required to realize a local stiffness measurement in which the elastic modulus can be considered constant and viscosity can be neglected. Therefore, we

Fig. 1. Micro tactile sensor.

developed an MTS in accordance with the above characteristics.

Micro Tactile Sensor (MTS)

The MTS was fabricated using a cylindrical piezoelectric transducer (PZT) that generates ultrasound (Fuji Ceramics, Shizuoka, Japan) and a glass needle with a 20-μm tip spherical point which made contact with the ZP surface (Fig. 1). The ultrasound evanescence in the ZP from the contact point depends on the elasticity of the ZP. The operation of the MTS is based on the principle of ultrasonic contact impedance measurement [16] and the phase shift method [4, 17–21]. The formulation and comprehensive presentation of the related equations are described in detail in these previous papers. As can be seen in Fig. 1, the MTS was made similar to a microinjection needle so that it could be mounted easily on a standard intracytoplasmic sperm injection (ICSI) system with a smart manipulator (P and M Co., Ltd., Fukushima, Japan) with a resolution of 10 nm movement. Although the ultrasonic contact impedance measurement was developed almost 40 years ago for hardness testing of metals, the method had not been previously applied to soft materials, such as living tissues. This was because the ultrasonic hardness tester does not have sufficiently high sensitivity. The phase shift operation made it possible to increase the signal to noise ratio of the resonator sensor remarkably, and the sensor performs with ultra-high sensitivity (almost 1,000 times higher than previous resonator sensors [22–24]), sufficient to measure the elasticity of a single cell with almost no deformation. If the displacement during measurement is very small, we can apply Hertz's theory to the contact between MTS and the specimen by considering the specimen as a completely elastic tissue. Consequently, Young's modulus can be determined from the slope of the change in resonance frequency *vs.* the tip displacement (Hz/μm). Young's modulus in kPa is the natural international system unit (SI unit) of elasticity.

Application of the MTS to ZP Elasticity Measurement

The schematic diagram of the MTS set-up used to measure Young's modulus of ZP is shown in Fig. 2. The oocyte or the embryo was transferred to a drop of medium on a slide under a glass cover. The space between the slide and the glass cover was about 4 mm, and the ends of the glass cover were supported by silicone blocks (Fig. 3). The suction pipette was introduced through one end of this chamber, and the oocyte/embryo was held with the pipette using gentle suction. The MTS was introduced through the other end of the preparation uniaxial to the suction pipette. At the moment the MTS enters the solution, its resonance frequency changes and continues to change as it penetrates further into the solution. To avoid this artifact of change in resonance frequency during measurement, the MTS was kept fixed and the oocyte/embryo was gradually pressed against the MTS up to an indentation of approximately 10 μm at a constant velocity of 10 μm/s under computer control. The change in resonance frequency from 0 to 5 μm of tip displacement was measured and the slope values (Hz/μm) were then calculated. The absolute value of Young's modulus can be calculated directly from the change in resonance frequency of the MTS. However, in general, surface contact area is not exactly equal to the theoretical value due to the complex effects of surface tension and adhesion. Furthermore, it is very difficult to measure the surface contact area accurately. Accordingly, if the surface is mostly uniformly flat at the point of measurement, and if the modulus remains constant during the measurement, it is more accurate to evaluate Young's modulus indirectly by comparing the value obtained from the MTS with that of a standard material under the same conditions. Before each

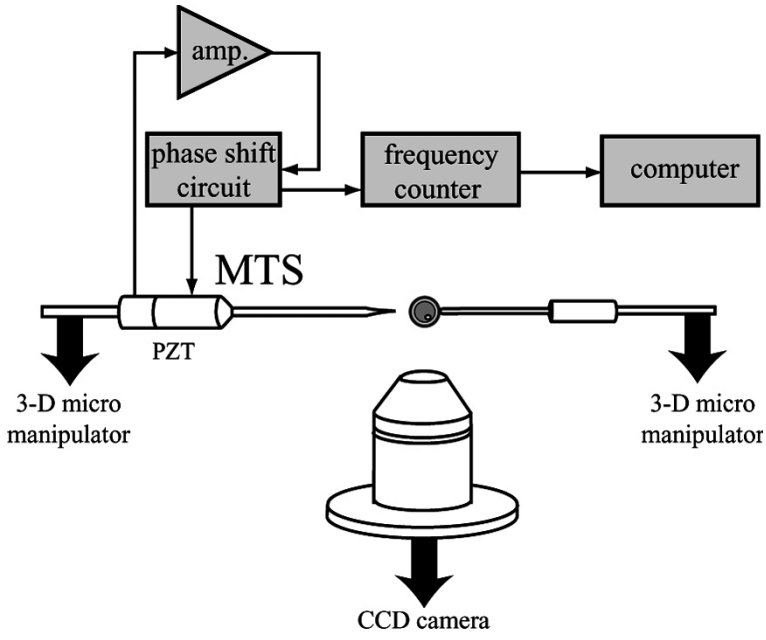

Fig. 2. Schematic diagram of micro mechanical sensing platform. Contact of the MTS with the embryo was observed on a monitor and recorded on video via CCD camera.

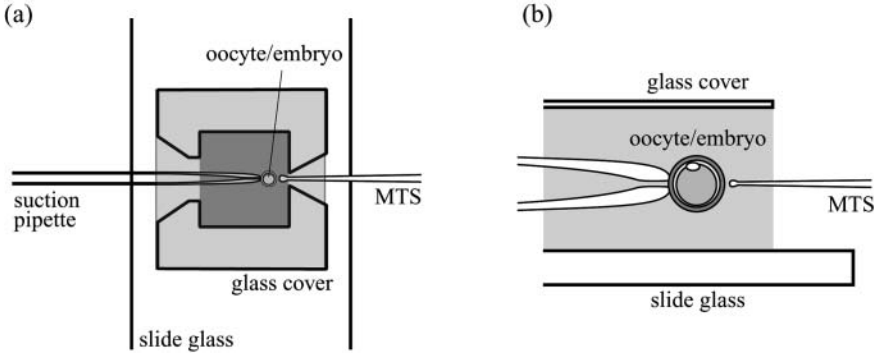

Fig. 3. The measurement chamber and the method of measuring the elasticity of oocytes/embryos. (a) Top view and (b) side view. Under microscopic control, a suction pipette supported the oocyte/embryo in the same horizontal axis as the micro tactile sensor (MTS).

measurement, the MTS was calibrated using gelatin (Lot26H0347; Sigma) as a known elasticity standard as described previously [4]. The elasticity of the gelatin gel was modified by varying the water content to 4, 6, and 8%. Gelatin is widely used as a tissue-mimicking elastic model as it provides a wide range of degrees of stiffness approximating those of soft tissue. A great deal of experimental work with the force-deformation method has shown that the modulus of gelatin is linear, elastic [25], and independent of probe radius [26] for a narrow range of deformation less than a depth of 10% of the sample's thickness. Resonance frequency changed as the gelatin was pressed against the MTS and was plotted *vs.* tip displacement. As described above, the slope values (Hz/μm) of the embryos were plotted against Young's moduli of the gelatin samples, which were 21.8, 42.3, and 63.8 kPa for the 4, 6, and 8% samples, respectively. These Young's modulus values of the gelatin samples were determined by the force-deformation method using a metal rod 1 mm in

diameter, which was pressed against the gelatin blocks, each 5 cm thick.

Safety of the MTS Measurement

It is important to ensure the safety of the MTS measurement of embryos. The detection principle of the MTS is based on the phase shift of ultrasonic resonance and ultrasound is radiated from the tip of the MTS. Although powerful ultrasound may damage cells, the ultrasonic vibrations of the MTS are too weak (not detectable by any method) to cause microstreaming inside a cell, or to have any thermal or cavitation effects. Consequently, it can be theoretically concluded that the MTS does not damage cells. In addition, the tip of the MTS is glass which is non-toxic. Furthermore, we experimentally examined the safety of the MTS measurement [5]. First, blastocysts were tested using the MTS and transferred to recipient mice. Forty blastocysts were tested and transferred into 6 recipients (MTS group). Twenty blastocysts were also transferred into 3 recipients without testing (control group). In total, 19 pups (47.5%) were born in the MTS group and 9 pups (45.0%) were born in the control group, so the birth rate was slightly but not significantly higher in the former than in the latter. In addition, all of the pups in the MTS group were found to be morphologically normal and showed normal growth. Second, no abnormalities were found in a chromosome test performed on 8 mice selected at random from the MTS group. Finally, female mice were mated with males from the MTS group and 58 pups were born. This procedure was repeated 5 times and eventually 322 pups were born normally at the fifth generation. Consequently, we concluded that both theoretically and experimentally, the MTS test does not cause any damage to embryos.

Changes in ZP Elasticity during Oocyte Maturation, Fertilization, and Early Embryo Development

Drobnis *et al.* and Sun *et al.* concluded that the ZP of embryos was 1.8 times and 2.4 times harder than that of the oocytes, respectively [12, 15]. Although both studies demonstrated zona hardening, the experiments caused severe deformation of up to 60 μm of the oocytes/embryos. Consequently, it was theoretically difficult to calculate the elastic modulus of the ZP as the results are affected by cellular pressure, ZP thickness, total cell size, *etc*. Furthermore, for oocytes, especially at the metaphase II (MII) stage, cortical granules may be released by physical stimulation and severe deformation may cause the zona reaction. Therefore, additional experiments to measure the elasticity of the oocyte/embryo ZP are important.

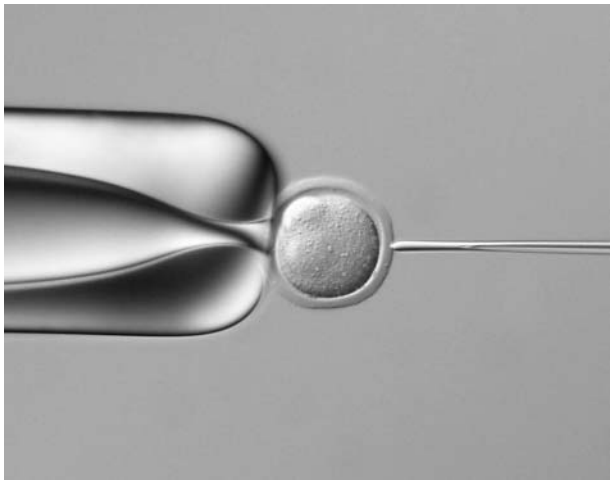

Fig. 4. Under microscopic control, the egg was pressed against the MTS until deformation of 5 μm. Magnification is ×400.

We measured the changes in physical hardness of the ZP of porcine ova during maturation and after fertilization *in vitro* using the MTS [6]. Immature oocytes (GV) immediately after collection, mature oocytes (MII) at 42 h of *in vitro* maturation (IVM), pronuclear (PN) stage embryos at 12 h post-insemination, and blastocysts (BL) at 168 h post-insemination were used for the measurements of ZP elasticity, which was evaluated as gelatin concentration by comparison with a standard curve. The gelatin concentrations corresponding to each stage were as follows: 3.9% (GV), 3.6% (MII), 3.7% (PN), and 2.6% (BL). The results indicate modulation of the physical ZP hardness in porcine ova during maturation and after fertilization *in vitro*.

In addition, we calculated the Young's modulus of ZP in mice and evaluated the sequence of changes in elasticity during oocyte maturation, fertilization, and early embryo development [5]. With MTS tip indentation of 5 μm, deformation was very small and the cell retained its original spherical shape (Fig. 4). In each measurement, resonance frequency increased linearly as deformation increased. The results of the Young's modulus measurement of mouse zona pellucida at GV, MII, PN, 2-cell, 4-cell, 8-cell, M, and early blastocyst stages are summarized in Table 1. Young's modulus of ZP at the PN stage was significantly higher than that at

Table 1. Young's modulus of zona pellucida

Cell stage	Number	Young's modulus (kPa)
GV-stage oocytes	30	22.8 ± 10.4^{b}
Metaphase-II oocytes	74	8.26 ± 5.22^{a}
PN-stage embryos	66	22.3 ± 10.5^{b}
2-cell embryos	41	13.8 ± 3.54^{a}
4-cell embryos	19	12.6 ± 3.34^{a}
8-cell embryos	6	5.97 ± 4.97^{a}
Morulae	8	1.88 ± 1.34^{a}
Early blastocysts	4	3.39 ± 1.86^{a}

Values are mean ± standard deviation. a versus b (P<0.01). GV, germinal vesicle; PN, pronuclear.

the MII stage (P<0.01). Consequently, zona hardening was demonstrated mechanically. Interestingly, once the ZP hardened following fertilization at the PN stage, Young's modulus decreased gradually as the embryo developed. Moreover, the ZP at the GV stage was significantly higher than that at the MII stage, indicating that the ZP became softer as the oocyte matured.

Quality Evaluation of Expanded Blastocysts (EPB)

During embryonic development toward hatching, the diameter of blastocysts increases and the thickness of the ZP decreases as the blastocyst repeatedly contracts and re-expands. Niimura inferred that re-expansion of contracted blastocysts occurs by active transport and accumulation of Na^+ from trophectoderm cells into the blastocoelic fluid as a result of the action of Na^+/K^+-ATPase activated in the membrane of trophectoderm cells [27, 28]. In addition, Na+ necessary to re-expand of blastocysts was thought to be transported into trophectoderm cells through Na^+ channels and Na^+/H^+ exchangers from outside the blastocysts. Furthermore, Niimura *et al.* demonstrated that cytochalasin B (CB)-treated mouse blastocysts had a larger number of strong contractions indicating that the actin filament-mediated movements of trophectoderm cells are related to contractions of blastocysts [28]. Also strong contractions and subsequent lesser expansion resulted in low embryo quality, and Niimura *et al.* reported that strong contractions occurred more in blastocysts that failed to complete hatching and that the low ability of hatching in CB-treated blastocysts was closely related to strong contractions at high frequency.

Based on the expansion of blastocysts and decrease in thickness of the ZP, it can be inferred that the elasticity of ZP increases as the tension on ZP increases and the ZP structure becomes dense. Consequently, analysis of morphology and mechanical properties of EPB can be useful for evaluating their quality. It is clear that the quality of blastocysts varies depending on a number of criteria, such as the number of cells, trophectoderm to inner cell mass ratio, blastocoele expansion, overall appearance, *etc*. (IETS Manual). The value of the elasticity constant of EPB measured using the MTS is not due solely to the elasticity of ZP as the ZP becomes thinner and local deformation is no longer achieved. Consequently, it becomes difficult to calculate the elastic modulus of ZP and the deformation depends on the overall structure and inner pressure. In addition, ultrasound radiated from the tip of the MTS transmits deeper into cells adjacent to the inner surface of ZP. Therefore, the elastic constant of EPB measured using the MTS represents a function of ZP elasticity, inner pressure, *i.e.*, the tension applied on ZP and cell number of the inner cell mass. For this reason, it would be better to use the term "elastic constant" for EPB rather than the term "Young's modulus," which indicates the mechanical attributes of a material.

We examined the physical hardness of ZP in bovine embryos generated both *in vivo* and *in vitro* using the MTS [29]. Embryos generated *in vivo* were recovered from 6 Japanese Black cows superovulated with multiple injections of FSH. On day 7 after insemination, morulas, early blastocysts, and blastocysts were recovered by non-surgical flushing of uterine horns. Morulas, early blastocysts, and blastocysts at days 5 to 7 post-insemination *in vitro* were used for measurement of ZP hardness. The ZP hardness values, converted into gelatin concentration equivalents, of bovine embryos generated *in vivo* at each stage were 3.95% (n=9), 4.14% (n=32), and 3.92% (n=14) for morulas, early blastocysts, and blastocysts, respectively. On the other hand, ZP hardness values of bovine embryos generated *in vitro* were 3.42% (n=56), 3.32% (n=36), and 3.23% (n=23), respectively.

We examined the relationship between the elasticity and inner cell mass (ICM) cell number in cultured mouse EPBs in which the quality was graded by removing half the number of cells at the 8-cell stage [30]. The results indicated that the elasticity at the ICM side was 22.4 ± 9.1 kPa and 16.3 ± 4.2 kPa for control and four-cell removed EPBs, respectively, showing softer stiffness with lower embryo quality based on the ICM cell numbers of 22.4 ± 2.3 and 18.2 ± 1.6, respectively. In addition, the numbers of pups born were 61% (44/72) and 39% (14/36) for control and four-

cell removed EPBs, respectively. Therefore, we suggested that the quality of EPBs could be evaluated by their elasticity. Together, these observations indicate that ZPs of embryos generated *in vivo* are significantly harder than those of embryos generated *in vitro* at each stage.

Quality Evaluation of Embryos at the Early Stage

Following fertilization, cortical granules (CG) release their contents into the perivitelline space in an event known as the cortical reaction. CG exudates alter the properties of the ZP, which is know as the zona reaction, and thus block polyspermic penetration. CG release is dependent on Ca^{2+} rise and calcium-dependent proteins. A relatively low Ca^{2+} rise is sufficient to induce a partial cortical reaction, while a higher level of Ca^{2+} is required to complete the cortical reaction [31]. A previous study suggested that CG exocytosis in mature oocytes is dependent on calcium-dependent proteins [32].

We examined the correlation between mechanical ZP hardening and the amount of CG release in mouse eggs [33]. MII oocytes immediately after collection (MII group), MII oocytes after vitrification/thawing (MII-vitrification group), MII oocytes activated with 8% ethanol for 5 min (MII ethanol group), and PN embryos at 16 h after mating (PN group) were used for measurement of ZP elasticity as experimental models of intact (CG not released), partially activated (CG partially released), parthenogenetically activated (CG fully but artificially released), and naturally activated (CG fully and naturally released) ova, respectively. The results showed that Young's moduli of the MII, MII-vitrification, MII ethanol, and PN groups were 7.10 ± 4.26 kPa (n=26), 9.70 ± 5.13 kPa (n=22), 10.8 ± 3.45 kPa (n=16), and 22.3 ± 7.33 kPa (n=26), respectively. The ZP in the PN group was significantly hardened following fertilization (3.1-fold) as described above. Ethanol activation resulted in significant ZP hardening (1.5-fold), but the effect was less than that seen in natural fertilization, indicating that the artificial CG release following ethanol activation was not sufficient to harden the ZP to the same degree as natural fertilization although Guyas and Yuan suggested that activation of mouse oocytes with 8% ethanol may resemble the normal CG release with some CG remaining intact in the deeper portions of the oocyte cortical region [34]. Vitrification/melting caused ZP hardening, but the effect was not significant, indicating that the partial CG release slightly hardened the ZP. That is, the mechanical ZP hardening was shown to be dependent on the amount of CG released into the perivitelline space. In mammalian oocytes, CGs are still present in penetrated oocytes at the GV stage and immature oocytes do not have the ability to block polyspermic penetration [35–37]. The ability of oocytes to release CGs after sperm penetration develops after GVBD, and is not fully developed until the MII stage or near the time of ovulation. Our preliminary data showed that denuded oocytes matured *in vitro* did not have zona hardening following parthenogenetic activation by strontium chloride, indicating that maturation and consequent oocyte activation may be evaluated by increases in ZP elasticity.

Application of the MTS-eSET in Human Assisted Reproductive Technology

As described in the previous sections, changes of ZP elasticity were demonstrated in pigs and mice, and analysis of ZP elasticity could be used to evaluate the embryo quality in both early embryos and expanded blastocysts.

We examined the correlations between subsequent ZP elasticity changes after IVF treatment and quality of embryos in humans with the approval of our institutional review board (IRB) and the Japan Society of Obstetrics and Gynecology, and with informed consent from the subject couple [38]. Eight oocytes were collected and Young's modulus of ZP was measured at MII, one day (D1), and five days (D5) after insemination. Each oocyte was fertilized in the conventional IVF manner. The results are summarized in Fig. 5 with all the eggs numbered. The eggs could be classified into three groups. Egg number 5 showed relatively high Young's modulus with ideal zona hardening and subsequent zona softening, and the Young's modulus of ZP at D5 was the highest among all the eggs even after softening of ZP. The ZPs of egg numbers 1, 3, 4, 6, and 7 also hardened after IVF treatment but showed incomplete zona softening, indicating that the eggs failed to sufficiently harden at D1. The ZP of egg numbers 2 and 8 did not harden after fertilization. The quality of all eggs was also scored according to Gardner's scoring scheme, and only egg number 5 showed 4AA quality, indicating consistency between mechanical and morphological quality evaluation. The patient chose to undergo eSET with the top quality embryo elected by both MTS and morphological evaluation, and delivered a healthy infant in 2006. Between November 2005 and

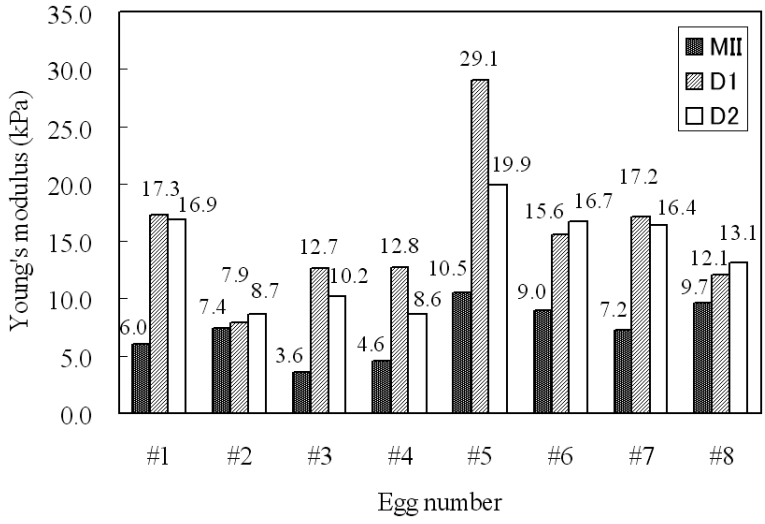

Fig. 5. Changes in ZP elasticity in human eggs.

March 2007, a total of 22 patients in 23 cycles of IVF were studied to evaluate the impact of MTS-eSET. The Young's modulus of ZP was analyzed retrospectively, and the results showed that Young's moduli of ZP of implanted and non-implanted EPBs were 17.9 ± 6.6 kPa and 13.5 ± 5.6 kPa, respectively, indicating that good quality EPBs with high implantation potential were more elastic. The pregnancy rate after MTS-eSET was 33.3% (7/21), while that after double-embryo transfer (DET) examined during the same period was 30.8% (24/78). There were no multiple pregnancies after MTS-eSET, whereas the twin rate after DET was 29.2% (7/24).

Conclusion

To enable improved success rates of IVF, we developed technology to optimize embryo selection with the highest implantation potential while ensuring no damage to embryos using zona elasticity as the selection criterion. In this communication we have outlined the biomechanics and safety of the ZP elasticity measurement using the MTS system and demonstrated the specific changes in ZP elasticity during oocyte maturation, fertilization, and early embryo development. The results show that zona hardening was demonstrated mechanically following fertilization at the PN stage, and that Young's modulus decreases gradually as embryos develop. It was shown that the quality of EPBs could also be evaluated from elasticity parameters. Furthermore observations indicated that ZPs were significantly harder in embryos generated *in vivo* than in those generated *in vitro* at each stage. Preliminary results also indicate that denuded oocytes matured *in vitro* do not show zona hardening following parthenogenetic activation by strontium chloride, suggesting that degree of maturation and consequent oocyte activation may be evaluated by increases in ZP elasticity. We conclude that the MTS-eSET can be applied in human ART to enable embryo quality evaluation in both early embryos and EPBs.

References

1) Farin, P.W., Britt, J.H., Shaw, D.W. and Slenning, B.W. (1995): Agreement amonb evaluators of bovine embryos produced in vivo or in vitro. Theriogenology, 44, 339–349.
2) Boiso, I., Veiga, A. and Edward, R.G. (2002): Fundamentals of human embryonic growth in vitro and the selection of high-quality embryos for transfer. Reprod. Biomed. Online, 5, 328–350.
3) Abe, H. (2007): A non-invasive and sensitive method for measuring cellular respiration with a scanning electrochemical microscopy to evaluate embryo quality. J. Mamm. Ova. Res., 24, 70–78.
4) Murayama, Y., Constantinou, C.E. and Omata, S. (2004): Micro-mechanical sensing platform for the characterization of the elastic properties of the ovum via uniaxial measurement. J. Biomechanics, 37, 67–72.
5) Murayama, Y., Mizuno, J., Kamakura, H., Fueta, Y., Nakamura, H., Akaishi, K., Anzai, K., Watanabe, A., Inui, H. and Omata, S. (2007): Mouse zona pellucida dynamically changes its elasticity during oocyte maturation, fertilization and early embryo development. Human Cell, 19, 119–125.
6) Yoshida, M., Imura, H., Murayama, Y., Sakuma, I., Tuji, T.

and Omata, S. (2000): Use of a piezo-electric ceramic tactile sensor to evaluate zona pellucida hardness at different stages of pig oocyte and embryo generated in vitro. Biol. Reprod., 66, 518.
7) Braden, A.W., Austin, C.R. and David, H.A. (1954): The reaction of zona pellucida to sperm penetration. Aust. J. Biol. Sci., 7, 391–409.
8) Schmell, E.D., Gulyas, B.J. and Hedrick, J.L. (1983): Egg surface changes during fertilization and the molecular mechanism of the block to polyspermy, Academic Press, New York.
9) Hatanaka, Y., Nagai, T., Tobita, T. and Nakano, M. (1992): Changes in the properties and composition of zona pellucida of pigs during fertilization in vitro. J. Reprod. Fertil., 95, 431–440.
10) Iwamoto, K., Ikeda, K., Yonezawa, N., Noguchi, S., Kudo, K., Hamano, S., Kuwayama, M. and Nakano, M. (1999): Disulfide formation in bovine zona pellucida glycoproteins during fertilization: Evidence for the involvement of cystine cross-linkages in hardening of the zona pellucida. J. Reprod. Fertil., 117, 395–402.
11) Green, D.P. (1987): Mammalian sperm cannot penetrate the zona pellucida solely by force. Exp. Cell Res., 169, 31–38.
12) Drobnis, E.Z., Andrew, J.B. and Katz, D.F. (1988): Biophysical properties of the zona pellucida measured by capillary suction: Is zona hardening a mechanical phenomenon? J. Exp. Zool., 245, 206–219.
13) Timoshenko, S. and Goodier, J.N. (1951): Theory of elasticity, 2nd ed., McGraw-Hill, New York.
14) Hiramoto, Y. (1970): Rheological properties of sea urchin eggs. Biorheology, 6, 201–234.
15) Sun, Y., Wan, K., Roverts, K.P., Bischof, J.C. and Nelson, B.J. (2003): Mechanical property characterization of mouse zona pellucida. IEEE Trans. Nanobio., 2, 279–286.
16) Kleesattel, C. and Gladwell, G.M.L. (1968): The contact—impedance meter-1. Ultrasonics, 6, 175–180.
17) Murayama, Y., Constantinou, C.E. and Omata, S. (2005): Development of Tactile Mapping system for the stiffness characterization of tissue slice using novel tactile sensing technology. Sensors and Actuators A: Physical, 120, 543.
18) Murayama, Y. and Omata, S. (2004): Fabrication of micro tactile sensor for the measurement of micro-scale local elasticity. Sensors and Actuators A: Physical, 109, 202–207.
19) Murayama, Y. and Omata, S. (2005): Considerations in design and sensitivity optimization of the Micro Tactile Sensor. IEEE Transactions on Ultrasonics, Ferroelectrics, and Frequency Control (in press).
20) Omata, S., Murayama, Y. and Constantinou, C.E. (2004): Real time robotic tactile sensor system for the determination of the physical properties of biomaterials. Sensors and Actuators A: Physical, 112, 278–285.
21) Omata, S. and Terunuma, Y. (1992): New tactile sensor like the human hand and its applications. Sensors and Actuators A: Physical, 35, 9–15.
22) Harb, S.M. and Vidic, M. (1995): Resonator-based touch-sensitive probe. Sensors and Actuators A: Physical, 50, 23–29.
23) Kanda, T., Morita, T., Kurosawa, M.K. and Higuchi, T. (1999): A rod-shaped vibro touch sensor using PZT thin film. IEEE Trans. UFFC, 46, 875–882.
24) Woody, S.C. and Smith, S.T. (2003): Resonance-based vector touch sensors. Precision Engineering, 27, 221–233.
25) Hall, T.J., Bilgen, M., Insana, M.F. and Krouskop, T.A. (1997): Phantom materials for elastography. IEEE Transactions on Ultrasonics, Ferroelectrics and Frequency Control, 44, 1355–1365.
26) Gregson, C.M., Hill, S.E., Mitchell, J.R. and Smewing, J. (1999): Measurement of the Rheology of Polysaccharide Gels by Penetration. Carbohydrate Polymers, 38, 255–259.
27) Niimura, S. (2003): Time-lapse videomicrographic analyses of contractions in mouse blastocysts. J. Reprod. Dev., 49, 413–423.
28) Niimura, S. and Wakasa, R. (2001): Hatching and contraction in mouse blastocysts treated with cytochalasin B. J. Reprod. Dev., 47, 317–323.
29) Itoi, F., Himaki, T., Kubota, C., Hirose, J., Miyamura, M., Hamano, S., Murayama, Y., Omata, S. and Yoshida, M. (2007): Differences exist in zona pellucida hardness between in vivo- and in vitro-generated bovine embryos. Reprod. Fertil. Dev., 19, 207.
30) Akaishi, K., Nakamura, H., Mizuno, J., Fueta, Y., Kamakura, H., Murayama, Y., Inui, H. and Omata, S. (2006): Development of ovum evaluation system for human assisted reproductive technology: verification of the utility and the safety. J. Mamm. Ova Res., 23, 144.
31) Raz, T., Ben-Yosef, D. and Shalgi, R. (1998): Segregation of the pathways leading to cortical reaction and cell cycle activation in the rat egg. Biol. Reprod., 58, 94–102.
32) Abbott, A.L. and Ducibella, T. (2001): Calcium and the control of mammalian cortical granule exocytosis. Front. Biosci., 6, D792–D806.
33) Murayama, Y., Fueta, Y., Kamakura, H., Nakamura, H., Mizuno, J., Akaishi, K., Inui, H. and Omata, S. (2006): Is cortical granule release a sufficient condition for zona reaction? J. Mamm. Ova Res., 23, 135.
34) Gulyas, B.J. and Yuan, L.C. (1985): Cortical reaction and zona hardening in mouse oocytes following exposure to ehanol. J. Exp. Zool., 233, 269–276.
35) Abbott, A.L., Fissore, R.A. and Ducibella, T. (2001): Identification of a translocation deficiency in cortical granule secretion in preovulatory mouse oocytes. Biol. Reprod., 65, 1640–1647.
36) Ducibella, T. (1996): The cortical reaction and development of activation competence in mammalian oocytes. Hum. Reprod. Update, 2, 29–42.
37) Wang, W., Hosoe, M., Li, R. and Shioya, Y. (1997): Development of the competence of bovine oocytes to release cortical granules and block polyspermy after meiotic maturation. Dev. Growth. Differ., 39, 607–615.
38) Inui, H., Jinji, M., Hiroko, N., Kazuyuki, A., Yoshinobu, M. and Sadao, O. (2007): Reliable ovum evaluation system by engineering technology, Micro Tactile Sensor (MTS) system for Elective Single embryo Transfer (ESET) on human ART. Fertil. Steril., 88, S101–S102.