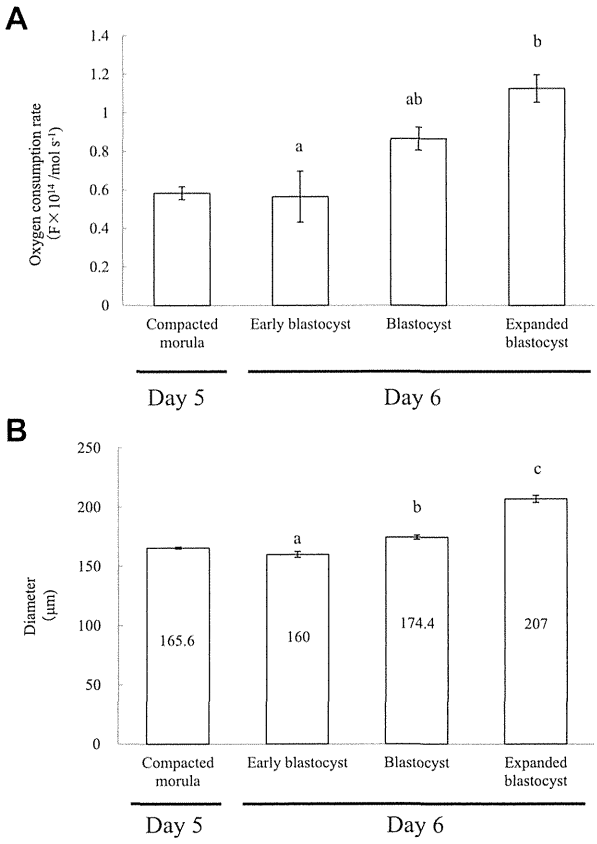

Fig. 1. Oxygen consumption rates (A) and diameter (B) of porcine embryos for each developmental stage. [a–c]Columns with different superscripts are significantly different ($P < 0.05$).

inverted fluorescent microscope through an ultraviolet filter to count the nuclei of the ICM and TE which were stained blue and pink, respectively.

2.5. Embryo transfer

Large White, Landrace, Yorkshire, and Berkshire sows (30.0 ± 3.0 [mean ± standard error of the mean] months of age) were used for recipients of non-surgical embryo transfer, which was performed according to the method previously described by Yoshioka et al. [24]. The recipients were treated with eCG (Peamex 1000 IU im) in the morning of the next day of weaning and hCG (Puberogen 500 IU im)

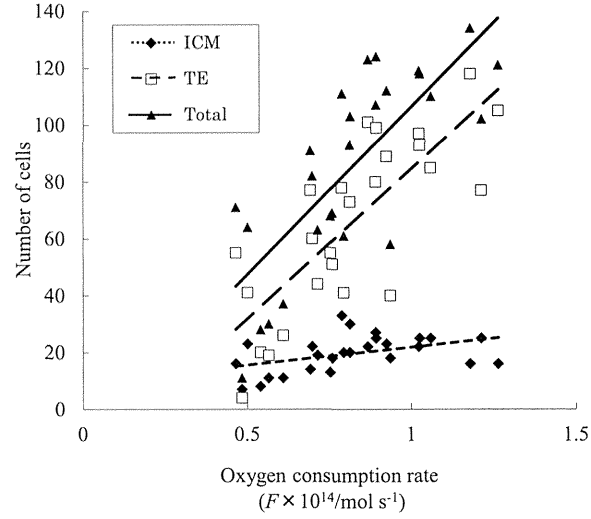

Fig. 2. Relationship between the oxygen consumption rate and the cell number in embryos collected on Day 6. Significant correlations were shown between the oxygen consumption rate and the number of inner cell mass (ICM) cells, trophectoderm (TE) cells, and total number of cells (Number of ICM cells: $P < 0.05$, $r = 0.429$; TE cells: $P < 0.0001$, $r = 0.783$; and total number of cells: $P < 0.0001$, $r = 0.769$).

at 72 hours after eCG treatment. The embryos were transferred 5 days after hCG treatment. Porcine intrauterine transfer catheters (Takumi; Fujihira Industry Co., Tokyo, Japan) were used to transfer the embryos. The inner tube filled with porcine blastocyst medium (PBM; IFP) [25] was inserted through the outer sheath into the uterine horn as deep as possible. A 0.25-mL straw filled with PBM contained 13 to 21 embryos was attached to the outside end of the tube. The cotton plug part of the straw was removed using a straw cutter, and a syringe was attached to the cut end, from which 1 mL of PBM was squeezed to inject the content of the straw into the uterine horn. Pregnancy was diagnosed by ultrasonography at 35 days after embryo transfer. All pregnant recipients were allowed to carry their litters to term, and the farrowing rates and litter sizes were recorded.

2.6. Experimental design

2.6.1. Experiment 1: relationships between the oxygen consumption rate, the developmental stage, and the number of cells in in vivo-derived porcine embryos

The oxygen consumption rates of embryos at the compacted morula stage on Day 5 (n = 29) and at the early

Table 1
Correlation between the oxygen consumption rate and the cell number in Day-6 blastocysts (n = 26).

	Diameter of embryo	Oxygen consumption rate	Number of ICM cells	Number of TE cells	Total cell number	Respiratory activity per 100 cells
Diameter of embryo	1					
Oxygen consumption rate	0.809***	1				
Number of ICM cells	0.506**	0.429*	1			
Number of TE cells	0.918***	0.783***	0.577**	1		
Total cell number	0.902***	0.769***	0.697***	0.988***	1	
Respiratory activity per 100 cells	−0.532**	−0.371	−0.631**	−0.681***	−0.718***	1

*P < 0.05, **P < 0.01, and ***P < 0.0001.
Abbreviations: ICM, inner cell mass; TE, trophectoderm.

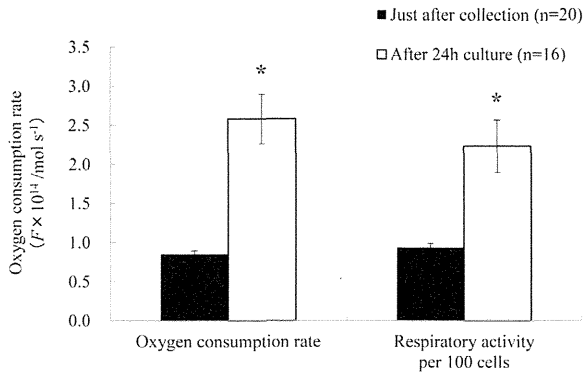

Fig. 3. Oxygen consumption rate and respiratory activity of embryos sampled on Day 6 just after collection and after 24 hours of culture. *Significantly different from Day-6 blastocysts ($P < 0.05$).

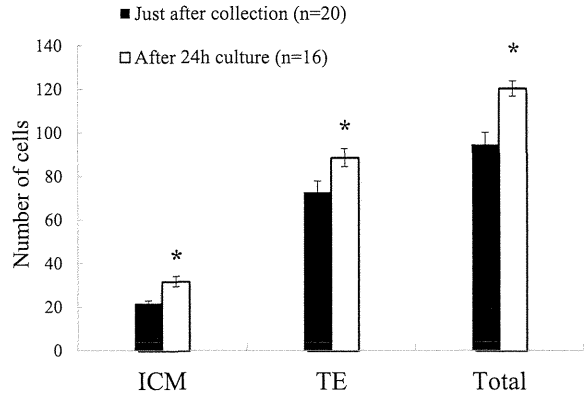

Fig. 4. Comparison of the cell number in embryos sampled on Day 6 just after collection and after 24 hours of culture. *Significantly different from freshly collected Day-6 blastocysts ($P < 0.05$). ICM, inner cell mass; TE, trophectoderm.

blastocyst (n = 8), blastocyst (n = 27), and the expanded blastocyst (n = 39) stages on Day 6 were measured and compared with the results of their morphologic evaluation. A total of 26 Day-6 embryos at the blastocyst stage were subjected to measure both of the oxygen consumption rate and cell numbers, from which the respiratory activity per 100 cells was calculated as the cellular respiratory activity of cells.

2.6.2. Experiment 2: oxygen consumption rate and cell numbers in in vivo–derived porcine embryos before and after in vitro culture

Day-6 embryos at the blastocyst stage (n = 36) were divided into two groups. Briefly, 20 blastocysts were evaluated for oxygen consumption rate, and then their cell numbers were counted immediately. The rest of the blastocysts (n = 16) were measured for oxygen consumption rate and then cultured in a PBM in a Reproplate (IFP) individually, for 24 hours under 5% CO_2, 5% O_2, and 90% N_2 at 38.5 °C. The number of cells in these blastocysts was also measured after culture.

2.6.3. Experiment 3: oxygen consumption rate of the in vivo–derived porcine embryos and their subsequent developmental potential

The Day-5 compacted morulae (n = 33) and the Day-6 blastocysts (n = 38) after measurement of the oxygen consumption rate were cultured in PBM in a Reproplate individually for 48 hours (Day-5 compacted morulae) or 24 hours (Day-6 blastocysts). After culture, the hatching from the zona pellucida was investigated.

2.6.4. Experiment 4: oxygen consumption of the in vivo–derived porcine embryo and conception rate

The compacted morulae and early blastocysts collected on Day 5 were measured for oxygen consumption rate and then transferred to recipients non-surgically to investigate the conception performance. Embryos were divided into two groups according to their oxygen consumption rates: average oxygen consumption of less than 0.58×10^{14}/mol s^{-1} (the lower oxygen consumption group) and more than 0.59×10^{14}/mol s^{-1} (the higher oxygen consumption group).

2.7. Statistical analysis

Data were statistically processed using a computer program for statistical processing SPSS (SPSS 16.0J. User's Guide; SPSS, Tokyo, Japan). ANOVA was performed followed by the Tukey HSD test. Linear relationships and correlation coefficients between the oxygen consumption rate and the cell numbers of embryos were determined by simple regression analysis and Pearson product–moment correlation coefficient analysis, respectively. The Fisher exact probability test was used to compare the conception rates, and a P value of less than 0.05 was considered statistically significant.

Embryos that hatched and those which did not hatch from the zona pellucida after culture were compared for their mean oxygen consumption rates at the time of collection as the explanatory variable, determining the difference from the mean for each embryo and calculating the discriminant function using the linear discriminant

Table 2
Correlation between the oxygen consumption rate and the cell number in Day-6 blastocysts after 24 hours of culture (n = 16).

	Oxygen consumption rate	Number of ICM cells	Number of TE cells	Total cell number	Respiratory activity per 100 cells
Oxygen consumption rate	1				
Number of ICM cells	0.232	1			
Number of TE cells	−0.506*	−0.536*	1		
Total cell number	−0.440	0.050	0.817***	1	
Respiratory activity per 100 cells	0.974***	0.220	−0.638**	−0.605*	1

*P < 0.05, **P < 0.01, and ***P < 0.001.
Abbreviations: ICM, inner cell mass; TE, trophectoderm.

Table 3
Oxygen consumption rates of Day-5 and -6 porcine embryos at collection and hatching from the zona pellucida after culture.[c]

Embryo collection	Hatching from the zona pellucida after culture	Number of embryos examined	Oxygen consumption rate ($F = 10^{14}$/mol s^{-1})
Day 5	Not hatched	17	0.50 ± 0.04
	Hatched	16	0.60 ± 0.04
	Total	33	0.55 ± 0.03
Day 6	Not hatched	16	0.77 ± 0.05[a]
	Hatched	22	1.05 ± 0.09[b]
	Total	38	0.93 ± 0.06

[a,b]Values in the same column with different superscripts are significantly different ($P < 0.05$).
[c] Day-5 and -6 embryos were checked for hatching after 48 and 24 hours, respectively, of culture in porcine blastocyst medium.

function. The probability of discrimination was then calculated from the determined discriminant function using the cross table of the discrimination probability.

3. Results

3.1. Experiment 1

The average oxygen consumption rate ($F \times 10^{14}$/mol s^{-1}) of the Day-5 embryos was 0.58 ± 0.03 for the compacted morula stage. Oxygen consumption rates of Day-6 embryos were 0.56 ± 0.13, 0.87 ± 0.6, and 1.13 ± 0.07 for the early blastocyst, blastocyst, and expanded blastocyst stages, respectively, and the value at the expanded blastocyst stage was significantly higher than that at the early blastocyst stage (Fig. 1A). On Day 6, the diameter of the embryos significantly increased with the progress of developmental stages (Fig. 1B).

Significant correlations were detected between the oxygen consumption rate of individual embryos and their diameter and cell numbers (in terms of ICM, TE, and total cells as well; Table 1). The oxygen consumption rate showed highly positive correlations with the total and TE cell numbers (Table 1 and Fig. 2).

3.2. Experiment 2

The oxygen consumption (2.58 ± 0.32) and respiration activity per 100 cells (2.23 ± 0.33) of embryos cultured for 24 hours from Day 6 were significantly higher than those of Day-6 blastocysts (0.85 ± 0.04 and 0.93 ± 0.05, respectively; Fig. 3). The total number of cells and the number of ICM and TE cells of Day-6 embryos after culture were also significantly greater than those of embryos before the culture (Fig. 4). The oxygen consumption rate of embryos (cultured from Day 6 for 24 hours) did not show significant correlation with the total cell numbers and the numbers of ICM cells of the embryos after 24 hours culture, but there was a significant correlation between the oxygen consumption rate and the respiratory activity after the culture (Table 2).

3.3. Experiment 3

The oxygen consumption rates of the porcine embryos collected on Day 5 or 6 and the culture outcome are shown in Table 3. In Day-5 embryos, the oxygen consumption rate before culture was tendentiously higher in those embryos that hatched after 48 hours of culture compared with those that did not hatch ($P = 0.08$). In Day-6 blastocysts, the oxygen consumption rates of those that hatched after 24 hours culture were higher than those of that did not hatch ($P < 0.05$). The discrimination probabilities of the Day-5 and -6 embryos, which were calculated using the values as the standards and the cross table of the discrimination probability, were 63.6% and 68.4%, respectively (Table 4).

3.4. Experiment 4

The embryos were transferred into each of 11 sows, of which three became pregnant (Table 5). Pregnancy was not obtained in any of the recipients in which embryos with low oxygen consumption rates were transferred. However, three of the seven recipients into which embryos with high oxygen consumption rates were transferred became pregnant. The three pregnant sows delivered 6.3 piglets on average (6, 3, and 11), and the mean gestation period was 116.3 ± 0.9 days.

4. Discussion

The present study clearly demonstrates that porcine embryos that could hatch after the subsequent culture

Table 4
Number of the Day-5 and -6 embryos resulted in false or true of whether hatched from the zona pellucida for each oxygen consumption class and the discrimination probabilities.

Embryo collection (number of embryos)	Prediction (number of embryos)	Number of embryos (%)		Discrimination probability (%)
		False	True	
Day 5 (33)	Not hatching (19)	7 (43.8)	12 (70.6)	63.6
	Hatched (14)	5 (29.4)	9 (56.3)	
Day 6 (38)	Not hatching (20)	8 (36.4)	12 (75.0)	68.4
	Hatched (18)	4 (25.0)	14 (63.6)	

Table 5
Conception rate of the embryos of each oxygen consumption class.

	Number of sows (number of embryos transferred)	Mean oxygen consumption rate ($F \times 10^{14}$/mol s^{-1})[c]	Number of pregnant sows (%)	Number of farrowed sows (%)	Number of piglets delivered (♂:♀)	Percentage of piglets born/embryo transferred (%)
High oxygen consumption	7 (118)	0.89 ± 0.03[a]	3 (42.9)	3 (42.9)	20[d] (12:8[d])	16.9
Low oxygen consumption	4 (58)	0.52 ± 0.02[b]	0 (0)	0 (0)	0	0
Total	11 (176)	0.77 ± 0.03	3 (27.3)	3 (27.3)	20[d] (12:8[d])	11.4

[a,b]Values in the same column with different superscripts are significantly different ($P < 0.05$).
[c] Mean ± standard error of the mean.
[d] Two piglets were stillborn.

showed higher oxygen consumption rates at the time of collection than those embryos that did not hatch. Oxygen consumption rate of the embryos at the blastocyst stage correlated with the number of cells. Moreover, piglets could be obtained by non-surgical transfer of embryos with high oxygen consumption rates.

These results corroborate with previous reports in bovine embryos, describing the correlation between their oxygen consumption rate and their viability [9–14] and conception rate [10,12,13]. Shiku et al. [9] examined the oxygen consumption of morula-stage bovine embryos and their subsequent hatching from the zona pellucida and reported that the hatching rate was higher in embryos showing oxygen consumption rates of at least 0.5 compared with those showing consumption rates lower than 0.5.

Our results revealing that Day-5 or -6 porcine embryos with high oxygen consumption rates are likely to show a high viability thereafter *in vitro* suggest that also in pigs, the oxygen consumption rate in freshly collected embryos may be a potent predictor for embryo viability.

The determining oxygen consumption values were calculated using the linear discriminant function and the oxygen consumption before the culture as the explanatory variable. The discrimination value for the hatched or not hatched in Day-5 and Day-6 embryos was 0.56 and 0.91, showing a discrimination probability of 63.6% and 68.4%, respectively. It suggests that both on Days 5 (at the compacted morula stage) and 6 (at the blastocyst stage), embryos could be discriminated for hatching ability by the measurement of their oxygen consumption rate. However, the oxygen consumption rate of Day-6 embryos at the expanded blastocyst stage was significantly higher than those at the early blastocyst stage, indicating that the oxygen consumption rate of porcine embryos on Day 6 increased greatly with the progress of development. In fact, there is a large variation in the developmental stage of embryos collected on Day 6, which should be considered when comparing oxygen consumption rates to assess embryo quality. Thus, the discrimination for the hatching ability by the measurement of oxygen consumption rate is may be appropriate 5 days after artificial insemination for the selection of transferable porcine embryos with high quality.

When we transferred Day-5 embryos (compacted morulae and early blastocysts) after the measurement of the oxygen consumption rate, 42.9% (3 of 7) of the recipients, to which embryos with high oxygen consumption rates were transferred, became pregnant and farrowed healthy piglets. In contrast, no pregnancy was obtained in the group to which the embryos with low oxygen consumption rates were transferred (0/4). Thus, embryos with high oxygen consumption rate may have superior conception ability compared with those with low oxygen consumption rate. Our overall result of pregnancy after non-surgical embryo transfer (27.3%) is similar to that of previous report (25% farrowing rate) on the transfer of *in vitro*–produced porcine embryos using the same deep intrauterine catheter instrument [25].

The oxygen consumption rate of blastocyst stage embryos on Day 6 showed a positive correlation with both the total cell numbers and the numbers of TE cells. Particularly, the embryos of higher oxygen consumption rate were found to have larger numbers of TE cells, suggesting the possibility of estimating the cell number from the oxygen consumption rate. In accordance with our results, Sugimura et al. [16] mentioned that anomalous low oxygen consumption rate in porcine SCNT blastocysts could be a sign of limited hatchability, which may be responsible for the low TE cell number and high apoptosis incidence. They also suggested that low oxygen consumption of Day-5 porcine SCNT blastocysts in the number of low TE cell may involve the high incidence of apoptosis in Day-7 blastocysts [16]. Furthermore, it has been reported that nitric oxide, which induces oxidative stress and apoptosis, and diamide-induced apoptosis may limit oxygen consumption at the blastocyst stage in the mouse embryo [26,27]. These findings suggest that the viability of TE cells may predominantly determine the oxygen consumption rate and greatly affect the porcine embryo quality.

The actual size of the embryo may also affect its oxygen consumption rate. It has been reported that *in vitro*–produced bovine blastocysts of larger diameter have higher oxygen consumption rate compared with smaller embryos [12]. Also, the positive correlation between the cell numbers in Day-7 bovine blastocyst and the length of their derivative conceptuses after transfer on Day 14 have been reported [28]. In pigs, asynchrony of TE elongation from Day 11 to 12 of pregnancy is evident in porcine concepti, and rapid progression through this phase has been associated with conceptus competency [29]. In the present study, the significant correlation was also observed between the oxygen consumption rate and the diameter of porcine embryo, suggesting that the diameter of the embryos was related to the quality of embryos.

Although, there was no significant correlation between the oxygen consumption rate and the respiratory activity in freshly collected Day-6 blastocysts, there was a significant correlation between the oxygen consumption rate and the respiratory activity of embryos after culture for 24 hours. These findings suggest that the oxygen consumption in freshly collected Day-6 embryos was more closely related to the actual number of cells rather than cellular respiratory activity which was correlated to oxygen consumption after additional culture.

In conclusion, the oxygen consumption rate of the *in vivo*–derived porcine embryos was significantly increased with the progress of development from the morula to the expanded blastocyst stage, and the embryos that hatched after additional culture showed higher rates of oxygen consumption than those that did not hatch. A significant correlation was observed in the Day-6 embryos between the oxygen consumption rate and the number of cells. After embryo transfer, successful pregnancies were only achieved with embryos showing high oxygen consumption rate, which, in turn, could develop to normal piglets. Therefore, the oxygen consumption by an embryo is likely to be related to its subsequent survival and conception, suggesting the possibility for the objective evaluation of the developmental capacity of an embryo based on its oxygen consumption rate.

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ARTにおける新技術

酸素消費と胚評価

阿部 宏之

- 細胞呼吸：細胞が外部から取り入れた酸素や酸素以外の酸化剤を用いて，養分を分解してエネルギーを発生させる生物現象．
- 電気化学計測：化学物質の性質を電気的に計測する方法．局所領域における生体反応を高感度・リアルタイムに計測できる．
- 走査型電気化学顕微鏡：マイクロ電極をプローブとして，目的試料の上部や近傍を走査し，プローブ電流を検出する装置．

はじめに

体外受精・胚移植（IVF-ET）において，移植前に質的に最も良好な胚を選択することは，妊娠率の向上，多胎妊娠の回避，流産率の低下のためにきわめて重要である．現在，胚の品質は割球の形態や数などの形態的特徴を基準に評価されているが，評価の基準となる胚の形態的特徴は定量性に欠けるため，判定結果が観察者の主観に左右される可能性がある．そこで筆者は，胚の品質を客観的に評価するための指標としてミトコンドリアの呼吸機能に着目し，細胞呼吸活性を指標とする新しい胚評価システムの開発に取り組んできた．

本稿では，電気化学計測技術を基盤とする酸素消費測定装置と，この装置を応用した新しい胚評価法を解説する．

形態観察および代謝物質測定による胚評価

形態的評価は，簡単・迅速で無侵襲的な方法であることから，最も有効な胚の品質評

あべ ひろゆき：山形大学大学院理工学研究科（〒992-8510　山形県米沢市城南4-3-16）

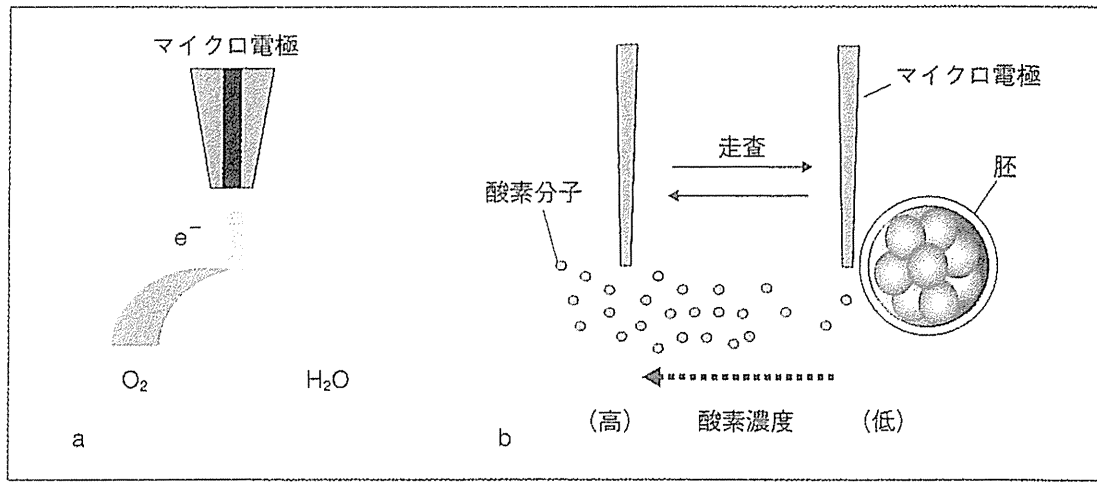

図1　マイクロ電極を用いた受精卵呼吸測定法

a：マイクロ電極は酸素の還元電位を検出する．
b：走査型電気化学顕微鏡による呼吸測定．呼吸により胚近傍の溶存酸素が減少するため，沖合との間に溶存酸素の濃度勾配が生じる．その酸素濃度差（電流値の差）から胚の酸素消費量を算出する．

価法として広く普及している．ヒトの初期分割期胚は，割球の形態とフラグメンテーションの割合を指標として評価するVeeckの分類法[1]や，Gardnerらが提案した胚盤胞の評価法[2]が広く用いられている．これら形態観察による胚評価は，簡便で非侵襲的な方法であり，その有効性も認められているが，評価の指標となる形態的特徴が定量性に欠けるという課題も指摘されている．

このため，客観的・定量的な指標として，胚の代謝産物や酸素消費に着目した胚評価が試みられている[3〜7]．特に，ミトコンドリアは酸化的リン酸化反応（呼吸）により細胞活動に必要なエネルギー（アデノシン三リン酸：ATP）を産生し，卵子や胚の代謝活動にも深く関与していることから，ミトコンドリア呼吸は胚の品質評価の有効な指標として注目されている．これまでに，蛍光発色法[8,9]や酸素センサー[10,11]を用いた細胞呼吸測定法が考案され，胚評価への応用が試みられているが，多くは測定感度や侵襲性などの面で課題があり，実用化には至っていない．

電気化学計測を応用した細胞呼吸測定装置の開発

電気化学計測法はプローブ電極による酸化還元反応を利用し，局所領域における生物反応を電気化学的に検出する技術であり[12]，この技術の有効な装置としてマイクロ電極をプローブとする走査型電気化学顕微鏡（scanning electrochemical microscopy：SECM）が注目されている．SECMの空間分解能はプローブであるマイクロ電極径に依存するため原子や分子レベルの解析は困難であるが，局所空間での化学反応の評価やイメージング，生体材料を用いたリアルタイム解析や化学反応誘起が可能であることから，局所領域の電気化学センシングなど種々の系で用いられている[13,14]．SECMは，酸素の還元電位を検出できるマイクロ電極をプローブとして用いることで，細胞の酸素消費量（呼吸）を高感度・非侵襲的に測定することができる（**図1**）．

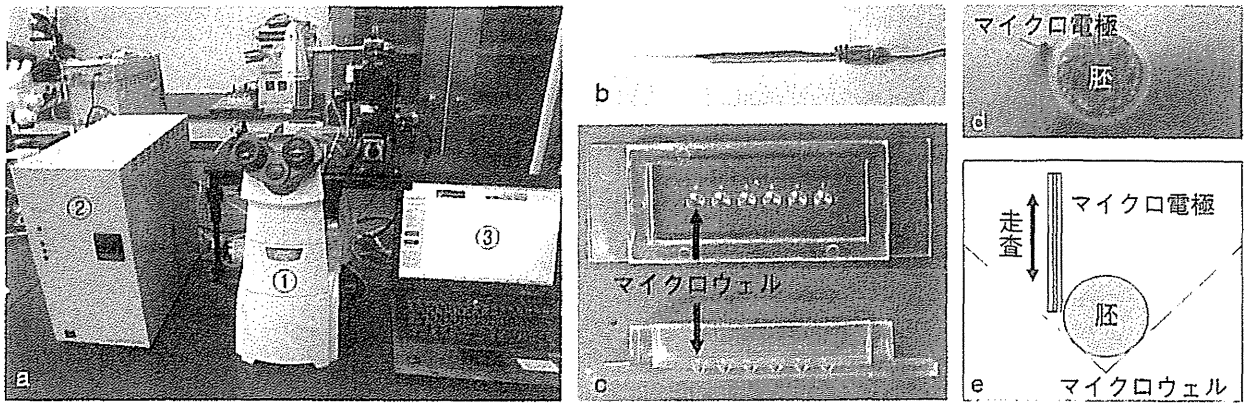

図2　走査型電気化学顕微鏡をベースに開発した「受精卵呼吸測定装置」

a：装置は，①倒立型顕微鏡，②ポテンショスタット，③ノートパソコン（呼吸能解析ソフトを内蔵）により構成されている．
b：呼吸測定用マイクロ電極：ディスク型白金マイクロ電極で，先端部が直径2～5 μm にエッチング加工された白金電極がガラスキャピラリーに熱封止されている．
c：多検体測定プレート：プレート底面には円錐形のマイクロウェルが6穴施されている．
d：マイクロウェル底部に静置したウシ胚．
e：マイクロ電極は胚近傍を鉛直方向に走査することで，胚の酸素消費量を測定する．

筆者らは，金属錆の検出装置として用いられてきたSECMを受精卵の呼吸計測に応用するための要素技術開発を行ってきた．その結果，SECMをベースに受精卵や微小組織などの酸素消費量を非侵襲的に測定できる「受精卵呼吸測定装置」の開発に成功した[15]．この呼吸測定システムは，倒立型顕微鏡，マイクロ電極の電位を一定に保持するポテンショスタット，酸素消費量算出のための専用解析ソフトを内蔵したノート型コンピュータにより構成されている（**図2a**）．倒立型顕微鏡のステージ上には，マイクロ電極の3次元走査を可能とするXYZステージが設置されており，生物試料の呼吸計測のために気相条件制御が可能な測定用チャンバーや保温プレートが設置できる．

単一胚の酸素消費測定

「受精卵呼吸測定装置」を用いた酸素消費（呼吸）測定には，超高感度マイクロ電極（**図2b**），多検体測定プレート（**図2c**）および専用の測定液を用いる．測定液を満たしたマイクロウェル内に試料（胚）を導入し，マイクロウェルの底部中心に静置させる（**図2d**）．酸素が還元可能な−0.6 V vs. Ag/AgClに電位を保持したのち，マイクロ電極を移動速度20～30 μm/s，走査距離150～300 μmの条件で透明帯近傍を鉛直方向に走査する（**図2e**）．通常，1回の呼吸測定では，マイクロ電極を3回走査したのちに球面拡散理論式[16,17]を基本とする解析ソフトを用いて胚の酸素消費量を算出する．

これまでに「受精卵呼吸測定装置」を用いて，ウシ，ブタ，マウスの単一胚の呼吸量測定に成功している．ほとんどの動物胚では，8細胞期までは酸素消費量は少なく，桑実胚から胚盤胞にかけて顕著に呼吸量が増加する（**図3**）．呼吸測定の有効性を検証するために呼吸活性の変化とミトコンドリアの発達との関係を電子顕微鏡により調べた結

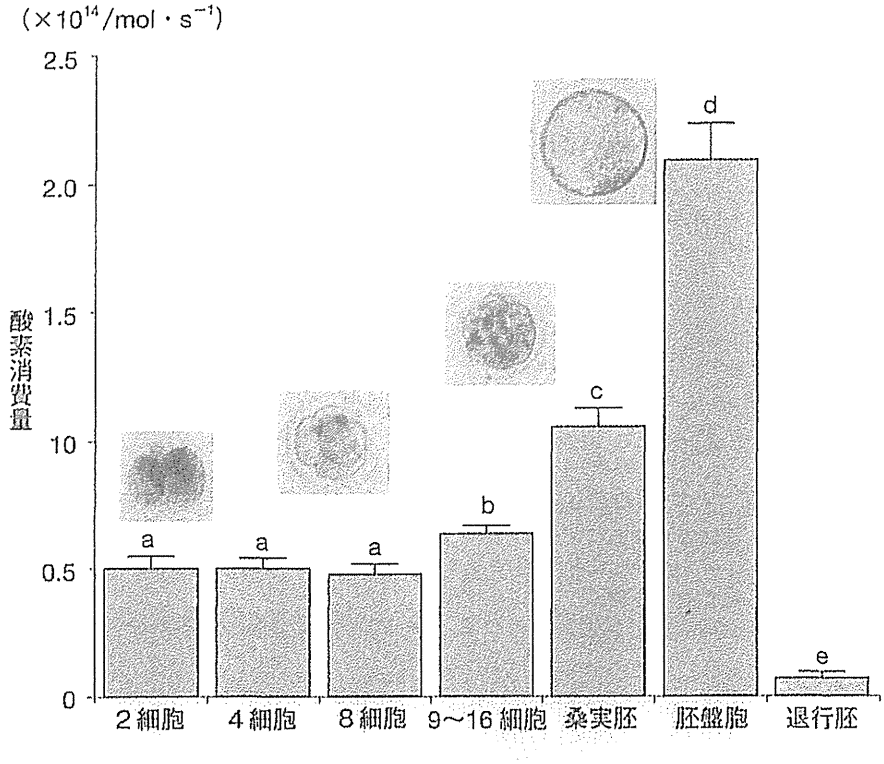

図3　ウシ体外受精胚の発生過程における呼吸量変化

桑実胚期から胚盤胞期にかけて呼吸量が増加する．退行胚ではほとんど酸素消費は検出されない．a～eの異符号間で有意差（$P<0.05$）がある．

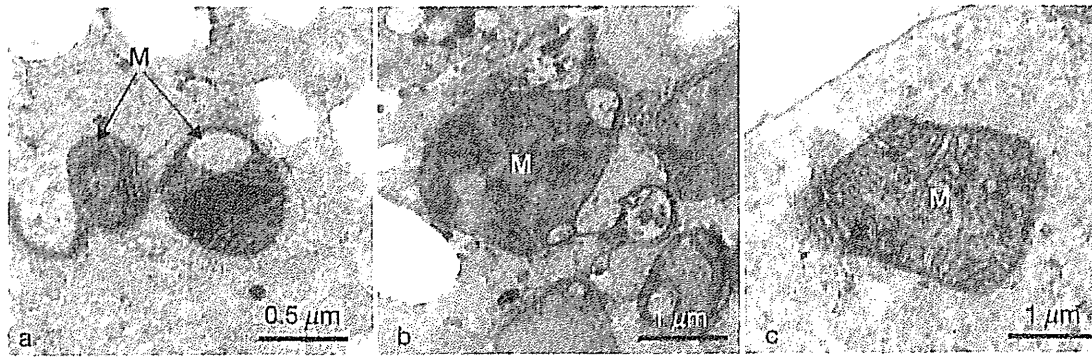

図4　ウシ体外受精胚の発生過程におけるミトコンドリアの微細形態変化

a：8細胞期胚，b：桑実胚，c：胚盤胞．M：ミトコンドリア

果，呼吸活性の低い8細胞期まではほとんどのミトコンドリアは未成熟であり，呼吸量が急激に増加する桑実胚から胚盤胞にかけてミトコンドリアの顕著な発達（クリステの拡張）が認められる（**図4**）[18]．このように，呼吸量の増加とミトコンドリアの発達は同じ発生ステージで起こることから，「受精卵呼吸測定装置」はミトコンドリアによる呼吸を高精度で検出していることがわかる．現在，胚評価における「受精卵呼吸測定装置」の有用性を検証するために，ミトコンドリアの細胞内局在，ミトコンドリア膜電

表1　ウシ胚の呼吸量と妊娠率の関係

移植時の発生ステージ	酸素消費量 ($F \times 10^{14}$/mol・s^{-1})	受胎胚数/移植胚数 (妊娠率%)
胚盤胞	$F \geq 1.0$	21/36 (58.3)
	$F < 1.0$	0/6 (0)
初期胚盤胞	$F \geq 0.8$	16/25 (64.0)
	$F < 0.8$	0/6 (0)
桑実胚	$F \geq 0.5$	17/28 (60.7)
	$F < 0.5$	1/12 (8.3)

位活性，エネルギー（ATP）産生および呼吸鎖複合体（シトクロームc酸化酵素：Cox）遺伝子発現の解析など，ミトコンドリア呼吸機能に関連する生物学的解析を総合的に展開している．

酸素消費測定による胚評価

酸素消費を指標とする胚評価法を確立するために，胚の品質と呼吸能の関係を詳細に調べた結果，これまでに多くの興味深い知見が得られている．ウシでは呼吸活性の高い桑実胚は，呼吸測定後に追加の培養を行うと多くの胚は品質良好な胚盤胞へと発生する[18]．また，凍結時に呼吸活性の高い胚盤胞は，融解したあとの生存率が良好であるという結果が得られている[19]．さらに，呼吸測定後の胚を借腹牛に移植し胚の呼吸活性と受胎率の関係を調べた結果，移植前の呼吸量が基準値以上（胚盤胞で 1.0×10^{14}/mol・s^{-1}，初期胚盤胞で 0.8×10^{14}/mol・s^{-1}，桑実胚で 0.5×10^{14}/mol・s^{-1}）の胚を移植した場合，60%前後の高い確率で妊娠するが，基準値以下の呼吸量の胚はほとんど受胎しない（**表1**）[20]．この結果は，ミトコンドリア呼吸（酸素消費）が胚評価の有力な指標になるとともに，「受精卵呼吸測定装置」が画期的な胚品質診断装置である可能性を示している．

「受精卵呼吸測定装置」を生殖医療分野で応用するためには，呼吸測定の非侵襲性および安全性の検証が不可欠である．電気化学的呼吸測定技術の安全性を検証するために，「受精卵呼吸測定装置」を用いて呼吸量を測定した胚を移植し，誕生した個体の正常性を生殖発生毒性試験により解析している．これまでの研究では，呼吸測定した胚の移植によって得られた産子に，通常の胚移植産子と比べて奇形発生率および染色体異常の増加，病理組織および行動の異常は全く確認されていない．このように「受精卵呼吸測定装置」による呼吸測定法は，胚に対して無侵襲・安全な計測方法であり，品質良好胚の効率的選別に有効であると考えている．