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研究種目：基盤研究（Ｓ）

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研究課題名（和文）　卵子の細胞分化・死滅調節系の解明による次世代型動物発生工学技術の基盤形成

研究課題名（英文）　Development of fundamental basis for embryo-biotechnology in next generation by clarifying the regulatory mechanisms of cell differentiation and apoptosis of oocytes

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研究成果の概要：

１個体からの受精可能卵子の大量生産を目標として卵子の細胞分化・死滅の調節系の解明を行い、新規調節因子を同定するとともに、これを踏まえ受精能・体細胞初期化能高発現卵子生産などの技術を開発した。さらに、直径 70μｍ未満のマウス卵胞卵子由来の産子や家畜ブタ体外成熟卵子をレシピエントとする体細胞ミニブタ作出に成功した。

交付額

（金額単位：円）

	直接経費	間接経費	合　計
2004 年度	19,100,000	5,730,000	24,830,000
2005 年度	15,300,000	4,590,000	19,890,000
2006 年度	15,300,000	4,590,000	19,890,000
2007 年度	15,300,000	4,590,000	19,890,000
2008 年度	15,400,000	4,620,000	20,020,000
総　計	80,400,000	24,120,000	104,520,000

研究分野：家畜繁殖学

科研費の分科・細目：畜産学・獣医学 基礎獣医学・基礎畜産学

キーワード：受精、体細胞初期化、卵巣特異的遺伝子、卵母細胞の死滅、卵母細胞の生存促進

１．研究開始当初の背景

次世代の発生工学の基盤形成には、１個体からの卵子の大量生産、受精能・体細胞初期化能の高い卵子の生産、受精能の改良に係わる現象の解明や関連技術の開発が重要である。

２．研究の目的

次の４点に集中して研究を行う。（1）受精能・体細胞初期化獲得の分子メカニズムの解明、（２）卵母細胞の死滅のメカニズムの解明、（３）卵胞におけるシグナルの生成・伝搬のメカニズムの解明、（４）受精能・体細胞初期化能の高い卵子の大量生産及びミトコンドリア置換技術の開発

３．研究の方法

研究の目的ごとの方法は次の通りである。

（１）受精能・体細胞初期化獲得の分子メカニズムの解明：受精能・体細胞初期化能発現の調節系は成熟の調節系のカスケードの下流とリンクすると予想されることから卵母細胞の成熟に係わる因子の解析を通して研究を進める。

（２）卵母細胞の死滅のメカニズムの解明：卵胞顆粒膜細胞にのみ局在する細胞死受容体とそのデコイ受容体及び卵母細胞の生存を促進するヒアルロン酸合成酵素３を同定しているが、これを踏まえ研究を深化させる。（３）卵胞におけるシグナルの生成・伝搬のメカニズムの解明：卵母細胞内で発現するコネクシン分子やその発現調節因子を同定する。（４）受精能・体細胞初期化能の高い卵子の大量生産及びミトコンドリア置換技術の開発：上記の（１）～（３）の研究をもとに卵巣卵子の高度利用につながる技術開発を行う。

４．研究成果

（１）卵子の細胞分化・死滅の調節系の解明：

①Protein kinase B やアクチンフィラメントが減数分裂完了や発生開始能に関与し、紡錘体形成・配列が卵子の細胞分化の最終フェーズに関与することを明らかにした〈図１〉。

②細胞死阻害因子の同定に取り組み、２種類の新規細胞死阻害因子を同定した。③卵胞発育・生存促進にはマクロファージによる閉鎖卵胞の速やかな除去が必要であることや、polylactosamine で修飾された CD44 がマクロファージの活性化に関与することを明らかにした。④ヒアルロン酸・CD44 が卵胞内細胞（顆粒膜細胞）のアポトーシス抑制に関与することを明らかにした。⑤卵子特異的な新規コネクシン(Cx60)を同定した。Cx43 のリン酸化に FSH 及び PKA が関与することを明らかにした。⑥甲状腺ホルモンに卵巣血管網増殖促進作用があり、かつ閉鎖卵胞救助作用のあることを明らかにした。

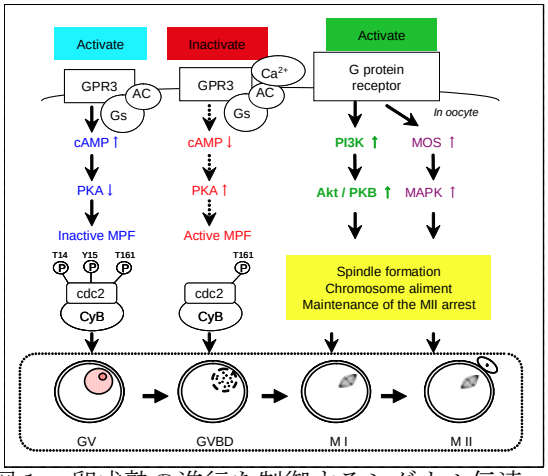

図１．卵成熟の進行を制御するシグナル伝達

（２）卵子の細胞分化・死滅の調節系の解明を基盤に開発した技術：①ブタ、マウス及びラットにおいて受精能・体細胞初期化能を強く発現する体外成熟培養法を開発した。②TAP発現ベクターを用いてGDF-9遺伝子を卵巣に導入し、初期卵胞のアポトーシス抑制・発育促進を可能にした。さらにラットにおいてGDF-9とVEGF遺伝子を組み合わせることにより、より強力な卵胞のアポトーシス抑制・発育促進法を開発した。③ES細胞由来の二次卵胞様構造体の形成に成功した。また、この構造体における性染色体型依存インプリンティングを確認した。④直径70μm未満のマウス初期卵胞卵子由来の産子を世界で初めて誕生させた〈図２〉。⑤体外培養によって得た家畜ブタ成熟卵子をレシピエントとしてミニブタ体細胞クローン作出に成功した〈図３〉。⑥体外成熟ブタ卵子をレシピエントとしてイヌES様細胞を樹立した〈図４〉。

図２．Tap-GDF-9遺伝子断片導入体外培養システムによる獲得産子

図３．体細胞ミニブタクローン

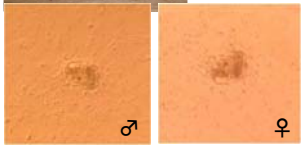

図４．ブタ卵細胞質をレシピエントとしたイヌ異種SCNT胚盤胞のアウトグロース像

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〔その他〕

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