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研究課題名（和文）Progesterone fluctuated signals in females induced by reproductive aging

研究課題名（英文）Progesterone fluctuated signals in females induced by reproductive aging

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研究成果の概要（和文）：年を取ると、女性は不妊になりやすく、妊娠に関連する問題が増えます。これには、出産数の減少、出生先天異常、および分娩時の手術の必要性が含まれます。年配のマウスの研究では、ホルモンと遺伝子が妊娠に影響することが示された。エストロゲンは正常だが、プロゲステロンが高い。プロゲステロンの変化は、妊娠段階や子宮収縮に必要な遺伝子のトリガーを妨げる。年配の子宮細胞には、ホルモンバランスへの反応がないエピジェネティック変化があり、代替のリセット方法が必要。TCF23は新しい分娩開始ターゲットで、子宮筋の反応が不十分なTCF23ノックアウトマウスで明らかになった。

研究成果の学術的意義や社会的意義
研究は、マウスの生殖加齢に関する重要な洞察を提供し、特に妊娠と分娩時の合併症に焦点を当てます。目的は、脱落膜化や長期妊娠などの原因を最小限に抑えることです。若いおよび高齢マウスの妊娠中のホルモンおよび分子メカニズムを調査し、加齢雌性の主要な生理学的変化を明らかにします。さらに、若いが高齢の子宮筋層における遺伝子発現パターンと分娩の始まりを特定しました。新しい標的遺伝子が脱落膜化と分娩の開始に重要であることも明らかになりました。これらの発見は、加齢マウスで観察される子宮筋層の反応性の低下に対する治療介入の潜在的な標的を提供します。

研究成果の概要（英文）：Women become less fertile and experience more pregnancy problems as they get older. This includes fewer births, birth defects, and a higher need for surgery during childbirth. Here, we studied older pregnant mice to understand how hormones and genes affect pregnancy. We found that older mice have normal estrogen but higher levels of progesterone. The altered levels of progesterone disrupt the necessary progesterone signaling for various pregnancy stages, such as the withdrawal of progesterone at late pregnancy, essential for triggering myometrial contraction genes. Moreover, aged uterine cells exhibit epigenetic alterations that do not respond to hormone-balancing strategies, necessitating alternative epigenetic resetting methods. We also identified a novel target protein related to labor initiation, TCF23, which is involved in obstructed labor in mice. TCF23 knockout mice revealed poor responsiveness of the myometrium to remodeling and contraction.

研究分野：reproduction and development

キーワード：Aging　Uterus　Myometrium　TCF23

１．研究開始当初の背景

This research investigates reproductive aging in female mice, focusing on declining fertility and increased pregnancy complications. Key issues include decreased birth rates and embryonic malformation due to imperfect decidualization early in pregnancy, and a higher likelihood of operative delivery due to prolonged gestation. Both stages are influenced by hormonal levels. Comparing young and aged pregnant mice helps explain these complications. Initial findings suggest the protein TCF23 may impact uterine health and labor. Understanding these mechanisms could lead to treatments for reproductive aging complications.

２．研究の目的

The current research purpose to understand: ①Reproductive aging dynamics in mice and its correlation with pregnancy complications, focusing on the significance of progesterone withdrawal in late pregnancy and its impact on the onset of labor. ②To explore the intricate molecular mechanisms underlying hormonal shifts throughout aging. ③Comprehensive analysis of gene expression profiles across various stages of pregnancy and identifying novel genes associated with labor onset.

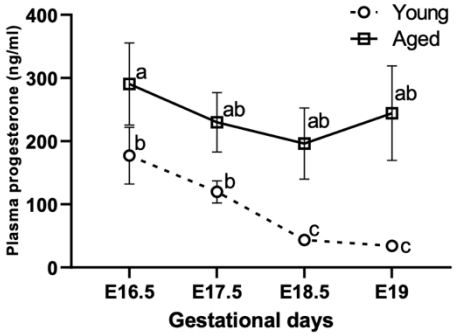
３．研究の方法

①Examine hormonal variability between young and aged mice using ELISA. ②Compare gestational outcomes in young and aged mice, including length, litter size, and pup viability. ③Conduct morphological, histological, and molecular analyses on ovaries from young and aged pregnant mice at different gestational stages to elucidate reasons behind altered hormonal levels. ④Assess myometrial responsiveness by analyzing progesterone receptors and contractility genes. ⑤Evaluate effects of oxytocin or progesterone receptor antagonist treatments on parturition in aged mice. ⑥Conduct differential gene expression analysis on myometrial samples collected at different gestational time points from both young and aged mice to identify genes associated with labor onset and duration.

４．研究成果

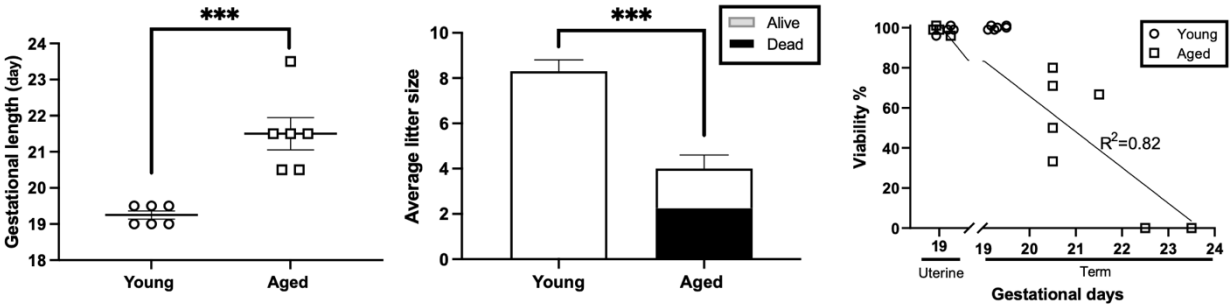
① Hormonal variability in aged female mice were observed. A minor alteration in estrogen levels, but higher levels of progesterone compared to young mice during pregnancy, with no evidence of progesterone withdrawal during late pregnancy (**Figure 1**). This suggests a possible defective corpus luteum, the main source of progesterone during pregnancy.

Figure 1



②Aged mice have significantly longer gestation, smaller litter size, and higher fetal demise rates ($P<0.001$) as seen in **Figure 2**. There is a strong inverse relationship between gestation length and pup viability in aged mice ($r^2=0.80$, $P<0.01$), not seen in young mice ($r^2=0.006$, $P>0.05$). Intra

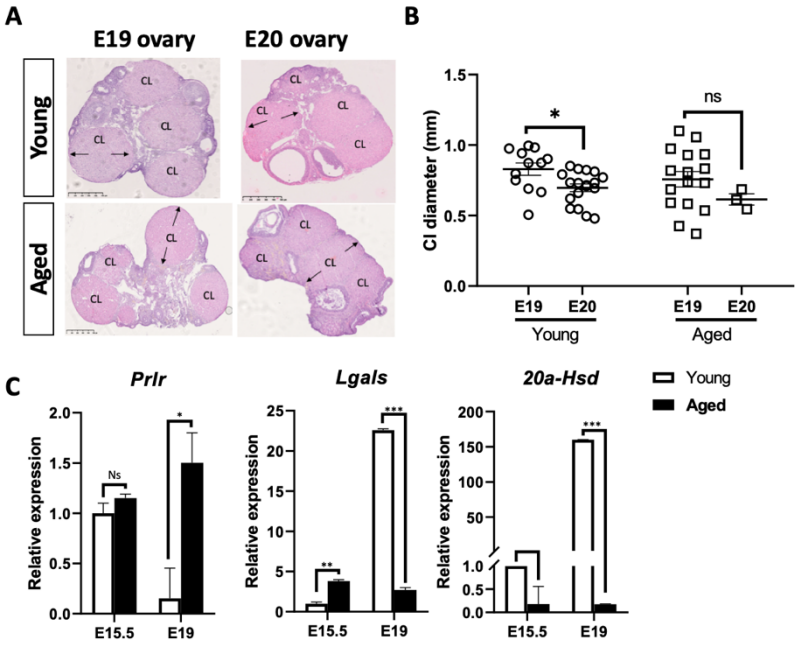
Figure 2



uterine analysis revealed unexpelled dead embryos and stillborn pups at late pregnancy. Inadequate progesterone withdrawal during late pregnancy potentially contribute to these adverse outcomes.

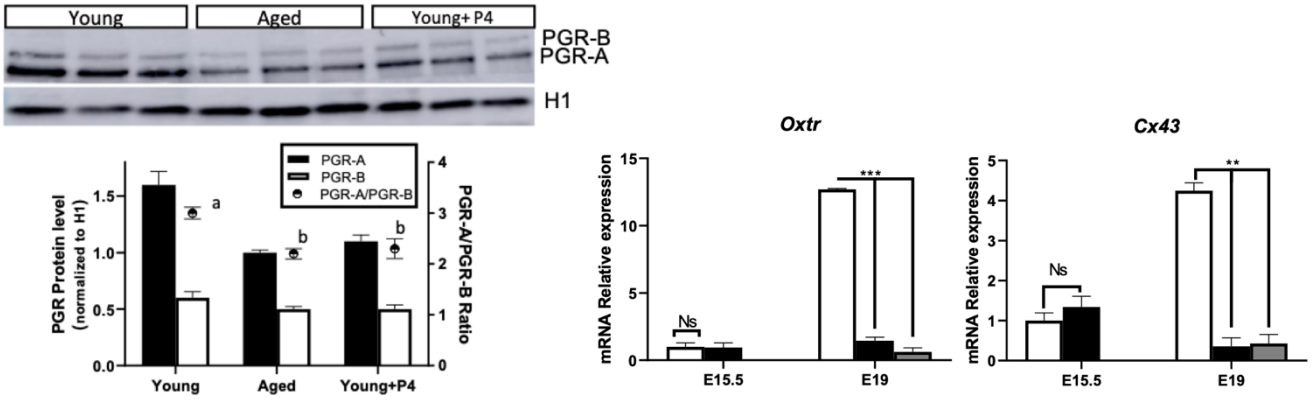
③The contribution of impaired ovarian luteolysis of corpus luteum and its effect on progesterone withdrawal in aged mice during elongated gestation was an evidence. As shown in **Figure 3**, the morphological, histological, and molecular analysis were performed for young and aged pregnant mice ovaries at E19 and E20. The results show that the diameter of the corpus luteum in aged mice ovaries is larger than that of young mice, indicating impaired structural luteolysis in aged mice. The relative mRNA levels of genes in corpora lutea at E15.5 and E19 indicated the impaired functional luteolysis-related genes in aged mice.

Figure 3



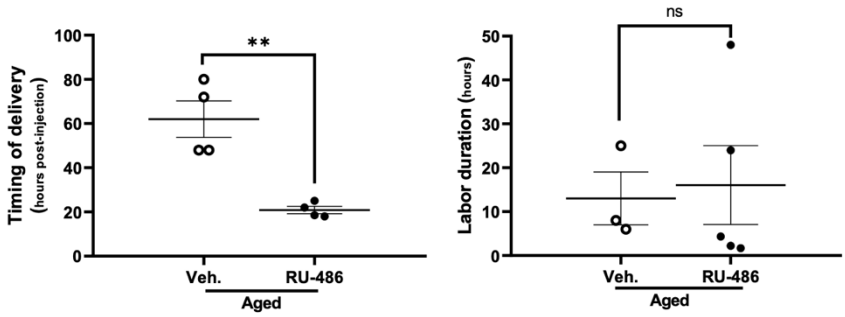
④The effects of impaired luteolysis and inadequate progesterone withdrawal in aged mice at late pregnancy impacted liganded PgrA dissociation in the myometrium. **Figure 4** shows that aged mice had significantly lower mRNA and protein levels of PgrA and a lower PgrA/PgrB ratio compared to young mice. These findings suggest aged mice have reduced PgrA dissociation, contributing to lower myometrial expression of contractility genes (Oxtr, Cx43) near term phenomena.

Figure 4

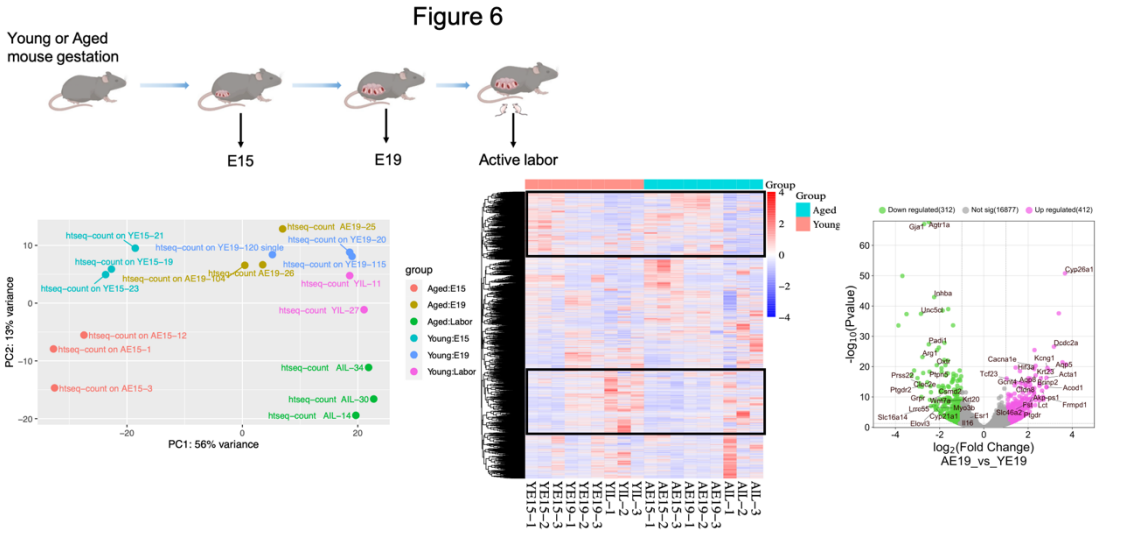


⑤Exogenous P4 antagonist, RU486, rescue delivery timing (**Figure 5**), but prolonged labor presist. Aged mice were able to deliver a living first pup, and the parturition onset was induced compared to vehicle-injected aged mice. However, aged mice remained in labor for more than 24 hours, and all of the pups that experienced prolonged labor died.

Figure 5

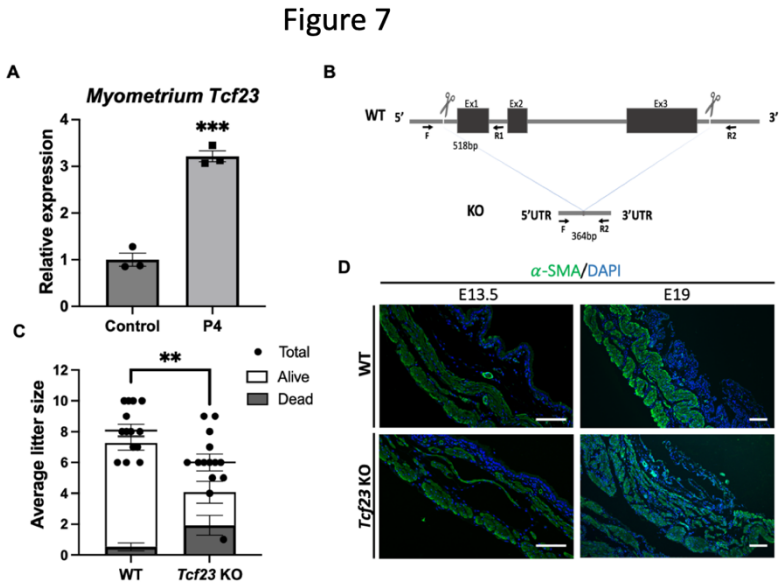


⑥ Differential gene expression analysis of myometrium reveals significant differences in gene expression between aged and young mice myometrium. RNA was isolated at mid-gestation, late gestation, and during active labor as elucidated in **Figure 6**. PCA plot identified a road map in young but not in aged myometrium along with the labor onset. A total of 1488, 1650, and 499 genes were differentially expressed in aged E15 vs. young E15, aged E19 vs. young E19, and aged in labor vs. young in labor, respectively. Our analysis of differential gene expression based on exon read counts exhibited a significant increase in prominent labor-associated players such as Gja1, Ptgs2, and Oxtr, as well as adhesion molecules and proteins Vcan, Thbs1, and Ceacam1, which are known to exhibit elevated levels at term in young mice while being significantly downregulated in aged myometrium due labor. Conversely, genes encoding proteins responsible for cell-extracellular matrix interactions, calcium signaling and proteins regulating myometrium response to low oxygen tension were found to be significantly up-regulated in aged myometrium compared to young. Based on these RNA-seq data, we observed a clustering region within each time point of collection, as expected, that was resistant to altering its expression with the onset of



labor, similar to young myometrium. Furthermore, we identified a new target gene related to onset in labor and labor duration based on the comparison between young and aged myometrium.

⑦ TCF23, a helix-loop-helix transcription factor, was identified as a downstream target of P4, predominantly expressed in female reproductive organs, especially in uterine stromal and smooth muscle cells (**Figure 7**). Its expression peaks during midgestation and is specifically regulated by P4, not estrogen. Analysis of knockout mice aged 4-6 months show subfertility, reduced litter size, and defective parturition, with disrupted myometrial structure and altered collagen organization. RNA-Seq analysis of KO myometrium indicates dysregulation of genes linked to cell adhesion, extracellular matrix organization, and myometrium contraction. TCF23 deficiency results in impaired myometrial remodeling, causing parturition delay and fetal demise, highlighting its critical role as a downstream mediator of progesterone in uterine remodeling.



In summary, we describe a study on the reproductive aging of mice and the complications that can arise during pregnancy and parturition. We found that aged pregnant mice showed defective parturition, prolonged labor, and frequent fetal demise due to inadequate progesterone withdrawal at late pregnancy, which is required for myometrium PgrA dissociation and translocation to the nucleus, triggering myometrial contraction genes. Moreover, looking at the myometrial transcriptomes at mid-gestation, late gestation, and during active labor, we found a road map for the gene expression pattern in young but not in aged myometrium along with the labor onset. Furthermore, a new target gene, TCF23, related to onset in labor and labor duration were identified.

５．主な発表論文等

〔雑誌論文〕　計1件（うち査読付論文　0件／うち国際共著　0件／うちオープンアクセス　0件）

１．著者名 Ammar Asmaa Y.、Minisy Fatma M.、Shawki Hossam H.、Mansour Mohamed、Hemeda Shabaan A.、El Nahas Abeer F.、Sherif Ahmed H.、Oishi Hisashi	４．巻 12
２．論文標題 Exposure to a Low-Oxygen Environment Causes Implantation Failure and Transcriptomic Shifts in Mouse Uteruses and Ovaries	５．発行年 2024年
３．雑誌名 Biomedicines	６．最初と最後の頁 1016～1016
掲載論文のDOI（デジタルオブジェクト識別子） 10.3390/biomedicines12051016	査読の有無 無
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 －

〔学会発表〕　計5件（うち招待講演　0件／うち国際学会　1件）

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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６．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考

７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関