Interactions between insulin like growth factor 1, thyroid hormones and blood energy metabolites in cattle with postpartum inactive ovaries

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Abstract: The relationship among insulin-like growth factor-1, thyroid hormones, energy metabolites and ovarian activity was investigated in cattle with postpartum inactive ovaries. The study was conducted on two groups of cows. The first group consisted of 10 cows with postpartum inactive ovaries (non-cyclic cows) based on rectal and ultrasonographic examination. The second group consisted of 8 cows in estrus (cyclic cows). The evaluated parameters included serum concentrations of variables of energy metabolites such as glucose (GLU), total lipids (TL), triglycerides (TG) and total cholesterol (TCH). Serum concentrations of total proteins (TP) were also measured. The hormones evaluated in this study included metabolic hormones such as insulin like growth factor-1 (IGF-1), thyroxine (T4), and triiodothyronine (T3) in addition to hormonal indicators of ovarian activity as progesterone (PRO) and estradiol (E2). The results revealed that serum levels of E2 was significantly lower (P<0.05) in the non-cyclic cows compared with the cyclic group. Serum GLU concentrations showed a significant decrease (P<0.05) while TL and TCH were significantly increased (P<0.05). Metabolic hormones profile demonstrated a significant decrease (P<0.05) in IGF-1, T4 and T3 in cows with inactive ovaries compared to the cyclic cows. Correlations between the monitored variables indicated that there was a significant positive correlation (P<0.05) between GLU and E2 and a significant negative relationship between TL, TCH and E2 (P<0.05). We reported a significant positive correlation (P<0.05) between IGF-1 and E2, GLU, T4 and T3. T4 was positively correlated (P<0.05) with E2, GLU, IGF-1 and T3. A significant positive correlation between TL and TCH was found while serum glucose showed no strong correlations with other energy-related metabolites. These results suggest that energy influences ovarian activity in postpartum lactating cows possibly through changes in secretory patterns of metabolic hormones.

Keywords: IGF-1; thyroid hormones; inactive ovary; energy metabolites

1. Introduction

Inactive ovary, also called true anestrus is a condition in which the ovaries are quiescent without signs of cyclicity or cycle related ovarian structures (Zulu et al., 2000). The condition is most frequently observed in high yielding dairy cows, first calf heifers or in postpartum lactating cows. From an economic point of view, poor reproductive efficiency caused by ovarian inactivity is the most important obstacle for increasing an animal productivity that can result in considerable economic losses in Egypt and all over the world (El-Khadrawy et al., 2008). Many factors can predispose to and exacerbate the problem such as nutritional intake, high production, energy deficiency, parasites, adverse climatic conditions, management stress and diseases. These factors compounded with lactation can further extend the postpartum anestrus period (Ahmed, 2007). Nutrition has long been known to have a profound influence on reproductive performance of female cattle, but measures of postpartum ovarian activity have been more closely related to energy balance (EB) (Beam and Butler, 1999; Gong, 2002; Armstrong et al., 2003; Butler et al., 2006). Lack of energy has been proved to adversely affect the size and ovulatory fate of the dominant follicle (Lucy, 1992; Kruip et al., 1996; Diskin et al., 2003) but the underlying mechanism remains poorly understood.

While, many investigations focused on the modulatory effect of energy intake on hypothalamic-pituitary axis, recent studies have tested the hypothesis that energy balance influences ovarian follicle development in cattle possibly through changes in some metabolic hormones that act as nutritional signals exert a direct effect at the ovarian level (Gong, 2002; Zulu et al., 2002; Spicer and Aad,
Insulin-like growth factor (IGF-I) was documented to be one of the most important potential hormonal mediators of ovarian function and has been reported to play a critical endocrine role controlling nutrient metabolism in cattle (Spicer et al., 1991). The hormone has been reported to act in synergy with FSH and LH in stimulating bovine granulosa and luteal cell steroidogenesis (Ahmad et al., 1996; Stewart et al., 1996). Also other factors such as thyroid hormones have been evaluated as potential regulators in ovarian steroidogenesis (Spicer et al., 2001). Very little data about the relationship between these metabolic hormones and postpartum ovarian activity in cows under our environmental conditions are available. Therefore, the aim of the current investigation was first to identify the patterns of secretion of IGF-I and free thyroid hormones in cows with postpartum inactive ovaries. A second objective was to evaluate associations of serum concentrations of these metabolic hormones with some blood energy metabolites and indicators of ovarian activity to throw light on the role of these hormones on the ovarian activity through establishing the effect of energy on secretory profiles of these hormones.

2. Material and Methods

Cattle:
A total of 18 lactating cows were used in this study. The cows were divided into two groups; the first group (non-cyclic cows) consisted of 10 cows aged from 5-8 years with inactive ovaries that were not observer in postpartum oestrus for over than 4 months. Inactive ovaries were diagnosed by two consecutive rectal examinations which revealed bilateral small ovaries which were flat and smooth. The diagnosis was confirmed by ultrasonographic examination. Ultrasonography was done by linear array scanner which produces a real time B-mode image (scanner 480-Vet-scan, Pie medical Co.). Scanner was equilibrated a five MHz transducer designed for intrarectal insertion in cow. The second group (cyclic cows) was consisted of 8 cows in estrus and was considered as control. The cows were apparently healthy, in good body condition and had no history of other reproductive disorders. No treatments with hormones were administered during the last 2 months.

Blood samples:
Blood samples were collected from both groups and serum harvested from blood samples was kept at -20 °C until assayed for the following parameters:

Hormonal indicators of ovarian activity:
Serum progesterone (PRO) and estradiol (E2) levels were determined by Enzyme-linked Immunosorbent assay (ELISA) micro-well technique using kits supplied by Hellabio biokits company (USA) and following the manufacturer's instructions.

Blood energy metabolites:
The serum values of variables of energy metabolism including glucose (GLU), total lipids (TL), triglycerides (TG) and total cholesterol (TCH), in addition to total protein (TP) were assessed by spectrophotometric method using commercial diagnostic kits of Spinreact S.A.Co (Spain).

Metabolic hormones:
Serum concentrations of IGF-1, thyroxin (T4) and triiodothyronine (T3) were measured by radioimmunoassay (RIA) using commercial kits supplied by Synbiotics Corporation, 11011 via Frontera, San Diego, according to the manufacturer’s instructions.

Statistical analysis:
All results were expressed as mean ± standard error (SE). Statistical differences between the two groups were compared using Student's t-test at 0.05 level of probability. Correlation between two specific monitored variables was determined with the Pearson's simple correlation method. A difference was considered significant at $P < 0.05$.

3. Results

Hormonal indicators of ovarian activity:
The results shown in (Table 1) reveals that serum concentrations of estradiol were significantly decreased in the non-cyclic cows ($P < 0.05$) compared to the cyclic group. No significant differences in serum levels of progesterone between the two groups were observed.

Blood energy metabolites & total proteins:
Cows with inactive ovaries demonstrated a significant decrease in the mean values of blood glucose levels ($P < 0.05$) in comparison to the control cyclic group (Table 2). The mean values of serum total lipids and total cholesterol were significantly higher ($P < 0.05$) in the non-cyclic cows. The average values of triglycerides and total protein did not show significant differences between the two groups.

Table 1. Serum concentrations of PRO and E2 in cows with postpartum inactive ovaries compared to the control cyclic group (Values are means ± SE).
Correlation between TL and TCH while serum energy metabolism, we found significant positive correlation between T3 and E2, GLU, IGF-1, and T4. (Table 4). There was also a significant positive correlation between T4 and both TL and TCH was reported (Table 4). A significant positive correlation was found between E2 and the low levels of serum PRO (0.44±0.01ng/dl) (Ahmed et al., 2010). Serum concentrations of PRO in both cyclic and non-cyclic cows were almost similar. In this study, the cyclic cows were in estrus phase (follicular phase) which is usually characterized by its low levels of PRO (Stabenfeldt et al., 1969; Diaz, 1986; Jazayeri et al., 2010).

In the present study cows with inactive ovaries were lactating and were not observed in postpartum estrus for over than 4 months. Laboratorially inactive ovaries were indicated by the significant decrease in the serum concentrations of E2 and the low levels of serum PRO (0.44±0.01ng/dl) (Ahmed et al., 2010). Serum concentrations of PRO in both cyclic and non-cyclic cows were almost similar. In this study, the cyclic cows were in estrus phase (follicular phase) which is usually characterized by its low levels of PRO (Stabenfeldt et al., 1969; Diaz, 1986; Jazayeri et al., 2010).

Significant differences were indicated by * P < 0.05.

**Table 2. Serum values of some energy metabolites and total protein in non-cyclic cows compared to the control cyclic group. (Values are means ± SE).**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cyclic</th>
<th>Non-cyclic</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLU (mg/dl)</td>
<td>110.00±5.20</td>
<td>73.40±1.71*</td>
</tr>
<tr>
<td>TL (mg/dl)</td>
<td>404.43±1.94</td>
<td>414.00±1.73*</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>80.46±0.31</td>
<td>79.70±0.35</td>
</tr>
<tr>
<td>TCH (mg/dl)</td>
<td>162.13±1.15</td>
<td>174.64±3.51*</td>
</tr>
<tr>
<td>TP (g/dl)</td>
<td>7.66±0.03</td>
<td>7.06±0.07</td>
</tr>
</tbody>
</table>

Significant differences in the values between the two groups were indicated by * P < 0.05.

Metabolic hormones:

When compared to the cyclic group, non-cyclic cows showed a significant decrease (P <0.05) in the mean values of IGF-1, T4 and T3 (Table 3).

**Table 3: Serum concentrations of IGF, T4 and T3 in cows with inactive ovaries compared to the control cyclic group (Values are means ± SE).**

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Cyclic</th>
<th>Non-cyclic</th>
</tr>
</thead>
<tbody>
<tr>
<td>IGF-1 (ng/ml)</td>
<td>53.16±1.41</td>
<td>38.46±1.58*</td>
</tr>
<tr>
<td>T4 (µg/ml)</td>
<td>5.12±0.08</td>
<td>3.23±0.12*</td>
</tr>
<tr>
<td>T3 (ng/dl)</td>
<td>108.17±0.57</td>
<td>87.86±2.38*</td>
</tr>
</tbody>
</table>

Correlations between the monitored variables:

By assessment of correlations of changes in means between the monitored hormones and variables of energy metabolism, we recorded a significant positive correlation (P <0.05) between GLU and E2 and a significant negative relationship between TL, TCH and E2 (P <0.05) (Table 4). A significant positive correlation (P <0.05) was found between IGF-1 and E2, GLU, T4 and T3. T4 was positively correlated (P <0.05) with E2, GLU, IGF-1 and T3, while a significant negative relationship between T4 and both TL and TCH was reported (Table 4). There was also a significant positive correlation between T3 and E2, GLU, IGF-1, and T4. By evaluation of correlation between variables of energy metabolism we found significant positive correlation between TL and TCH while serum glucose showed no strong correlations with other energy-related metabolites (Table 4).

4. Discussions

Cattle play an important role in the agricultural economy in many countries in the world. It is well known that the reproductive disturbances in cows are the main cause of their infertility, or even sterility, and reduced their productive efficiency (El-Khadrawy et al., 2008). Inactive ovary is a major and important reproductive hindrance in which, the ovarian follicles fail to reach mature size and ovulate (Zulu et al., 2000). The reason of this condition may be insufficient release or production of gonadotropins to induce follicular development or it may reflect the failure of ovaries to respond to gonadotropins (Ahmed, 2007).

In the present study cows with inactive ovaries were lactating and were not observed in postpartum estrus for over than 4 months. Laboratorially inactive ovaries were indicated by the significant decrease in the serum concentrations of E2 and the low levels of serum PRO (0.44±0.01ng/dl) (Ahmed et al., 2010). Serum concentrations of PRO in both cyclic and non-cyclic cows were almost similar. In this study, the cyclic cows were in estrus phase (follicular phase) which is usually characterized by its low levels of PRO (Stabenfeldt et al., 1969; Diaz, 1986; Jazayeri et al., 2010).

Resumption of postpartum ovarian function is controlled by numerous systemic and intraovarian factors but previous reports have found that the ability of postpartum lactating cows to resume estrus is dependent upon their EB (Kendrick, 1997).

In this regard, the results of the present study revealed a significant decrease in serum concentrations of glucose in cows with inactive ovaries which may indicate lack of energy resulting from NEB due to increasing demands of the mammary glands for glucose for milk synthesis (Seifi et al., 2007; Gabriel Koč et al., 2009). Glucose is the principal source of energy for the life processes of the mammalian cell. The mammary gland is a major glucose utilizing tissue, principally for biosynthesis of lactose, the predominant molecular species in milk (Kaneko et al., 1997). The only one precursor of lactose is plasma glucose (Kaneko et al., 1997). The two main sources of plasma glucose are absorption from the gut and gluconeogenesis (Kaneko et al., 1997). In ruminants, little glucose is absorbed from the gut, so the overwhelming bulk of it is synthesized through the process of gluconeogenesis that occurs mainly in liver by utilizing other substrates such as ruminal volatile fatty acids that result from fermentation of dietary glucose in the rumen (Kaneko et al., 1997).
Table 4. The correlation between the selected hormones and variables of energy metabolism in the non-cyclic group (Pearson's correlation test).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>IGF-1</th>
<th>T4</th>
<th>T3</th>
<th>PRO</th>
<th>E2</th>
<th>GLU</th>
<th>TL</th>
<th>TG</th>
<th>TCH</th>
</tr>
</thead>
<tbody>
<tr>
<td>IGF-1</td>
<td>1</td>
<td>0.950*</td>
<td>0.968*</td>
<td>0.608</td>
<td>0.943*</td>
<td>0.985*</td>
<td>-0.737</td>
<td>0.628</td>
<td>-0.620</td>
</tr>
<tr>
<td>T4</td>
<td>0.950*</td>
<td>1</td>
<td>0.947*</td>
<td>0.778</td>
<td>0.987*</td>
<td>0.961*</td>
<td>-0.827*</td>
<td>0.557</td>
<td>-0.814*</td>
</tr>
<tr>
<td>T3</td>
<td>0.968*</td>
<td>0.947*</td>
<td>1</td>
<td>0.590</td>
<td>0.963*</td>
<td>0.927*</td>
<td>-0.811</td>
<td>0.770</td>
<td>-0.644</td>
</tr>
<tr>
<td>PRO</td>
<td>0.608</td>
<td>0.778</td>
<td>0.590</td>
<td>1</td>
<td>0.705</td>
<td>0.699</td>
<td>-0.460</td>
<td>0.142</td>
<td>-0.653</td>
</tr>
<tr>
<td>E2</td>
<td>0.943*</td>
<td>0.987*</td>
<td>0.963*</td>
<td>0.705</td>
<td>1</td>
<td>0.933*</td>
<td>-0.885*</td>
<td>0.608</td>
<td>-0.812*</td>
</tr>
<tr>
<td>GLU</td>
<td>0.985*</td>
<td>0.961*</td>
<td>0.927*</td>
<td>0.699</td>
<td>0.933*</td>
<td>1</td>
<td>-0.704</td>
<td>0.506</td>
<td>-0.659</td>
</tr>
<tr>
<td>TL</td>
<td>-0.737</td>
<td>-0.827</td>
<td>-0.811</td>
<td>-0.460</td>
<td>-0.885*</td>
<td>-0.704</td>
<td>1</td>
<td>-0.561</td>
<td>0.905*</td>
</tr>
<tr>
<td>TG</td>
<td>0.628</td>
<td>0.557</td>
<td>0.770</td>
<td>0.142</td>
<td>0.608</td>
<td>0.506</td>
<td>-0.561</td>
<td>1</td>
<td>-0.216</td>
</tr>
<tr>
<td>TCH</td>
<td>-0.620</td>
<td>-0.814*</td>
<td>-0.644</td>
<td>-0.653</td>
<td>-0.812*</td>
<td>-0.659</td>
<td>0.905*</td>
<td>-0.216</td>
<td>1</td>
</tr>
</tbody>
</table>

* Correlation is significant at the 0.05 level.

In lactating cows, if there is a mismatch between mammary drain of glucose for lactose synthesis and gluconeogenesis via inadequate energy intake, the cow will experience NEB and hypoglycemia will result (Kaneko et al., 1997). On the other hand, the present results demonstrated significant higher values of total cholesterol in the non-cyclic cows. Increased lipolysis due to hypoglycemia will result in increased serum levels of low density lipoproteins (LDLs). The rate of cholesterol synthesis is increased with the associated increase in plasma LDLs concentrations and thus hypercholesterolemia results (Meyer and Harvey, 1998; Anna et al., 2004).

Hypothyroidism seen in the non-cyclic cows may be another strong reason for this hypercholesterolemia. Thyroid hormones stimulates LDLs receptors and promotes uptake of cholesterol, therefore lack of thyroid hormones results in decreased LDLs receptors and decreased cholesterol uptake (Anna et al., 2004). This approach is further supported by the significant negative relationship seen between T4 and TCH (Table 4). Total lipids significantly increased in the non-cyclic cows probably due to hypercholesterolemia.

In the present work, we found significant positive correlations between the mean values of glucose and estradiol suggesting a role for glucose in the regulation and resumption of estrous cycling after parturition. Glucose appeared to play a role in the nutritional regulation of GnRH release and in turn pulsatile LH secretion (Diskin et al., 2003). The same was mentioned by (Kruip et al., 1996) who suggested that lack of energy leads to a lower glucose concentration, inducing lipolysis and resulting in a lower LH release. Low glucose levels also could reduce follicular responsiveness to LH and ultimately shut down follicular estradiol production (Diskin et al., 2003). Decreased dietary energy intake in cyclic heifers was associated with decreased concentrations of progesterone in follicular fluid of small and medium follicles, and decreased size of large follicles (Spicer et al., 1991) while increasing EB was proportional to the number of large follicles in postpartum dairy cows with significant differences in follicular development related to body condition (Lucy et al., 1991). Poor expression of estrus has been reported to be associated with reduced plasma levels of LH and estradiol and inconsistent growth and development of ovarian follicles caused by NEB (Grimard et al., 1995).

The underlying mechanism of the effect of EB on reproductive activity remains poorly understood. However, several reports have implicated involvement of various metabolic hormones such as IGF-1 in relation to the energy balance in the dynamic growth of ovarian follicles. These studies suggested that the changes in ovarian activity due to energy...
shortage are a result of direct actions of these metabolic hormones on the ovary (Beam and Butler, 1999; Gong, 2002; Armstrong et al., 2003) but such reports are very little in Egypt.

IGF-1 has been postulated as a potent activator of ovarian follicular growth. Specifically, IGF-1 in conjunction with gonadotropins have been reported as an important stimulators of mitosis and ovarian steroid production by granulosa and theca cells, which are required for normal oocyte development and hormonal feedback signalling to the hypothalamus and pituitary (Spicer and Aad, 2007; Tosca et al., 2008; Grado-Ahuir et al., 2009; Kolesarova1 et al., 2009).

Although many of the previous studies tended to monitor the levels of IGF-1 in the follicular fluid, there is strong evidence that direct nutritional effects on ovarian function appear to operate through hepatic rather than follicular regulation of IGF-1, and on systemic concentrations of IGF-1 (Diskin et al., 2003).

Based on these reports, we evaluated the serum secretory pattern of IGF-1 in relation to the variables of energy metabolites and elements of reproductive activity. The data demonstrated that serum concentrations of IGF-1 were significantly lower in cows with inactive ovaries (Table3). Similar findings were reported by Zulu et al., (2002) who found that IGF-1 levels were higher and rose sharply in cows that cycled normally than in cows with inactive ovaries.

Moreover, significant positive relationship was found between IGF-1 and serum concentrations of E2 supporting the identification of IGF-1 as an important metabolic modulator of postpartum ovarian activity in cows.

In cattle, increases in the population of small ovarian follicles were associated with increases in circulating concentrations of IGF-1 (Gong, 2002). Stimulatory effects of IGF-1 on estradiol production by mammalian granulosa cells were also well documented. Some studies reported that follicular fluid IGF-1 levels increased as follicular fluid estradiol and follicle size increased (Echternkamp et al., 1994). These effects were due in part to its ability to enhance the action of gonadotropins on ovarian follicular steroidogenesis by increasing theca-interstitial cell LH binding affinity and/or binding capacity (Cara et al., 1990).

Since many studies have suggested that the influence of IGF-1 on ovarian activity is related to EB, statistical correlations between IGF-1, estradiol and some variables of energy metabolites were performed (Table 4). The results indicated strong positive relationships between changes in blood glucose, peripheral IGF-1 and estradiol. Thus, it appears that IGF-1 is likely acts as a mediator of energy induced alterations in ovarian function in the postpartum period in cows. Rhoads et al., (2008) stated that in liver, growth hormone receptor and IGF-1 production are dynamically regulated by lactation and energy balance. Reduced IGF-1 secretion caused by NEB could alter ovarian follicular estradiol production, thereby suppressing the expression of estrus (Spicer et al., 1990).

At the molecular level, dietary energy intake has been proved to affect the expression of mRNA encoding components of the ovarian IGF system and these changes can directly increase the bioavailability of intrafollicular IGF-1. This, in turn, can increase the sensitivity or response of follicles to FSH and is one mechanism through which nutrition can directly affect follicle recruitment (Armstrong et al., 2003).

In regard to the thyroid hormones, the present results revealed that significant lower values of T4 and T3 were observed in cows with inactive ovaries with a strong positive relationship was detected between the two hormones and E2. An association between thyroid hormones and reproductive efficiency was plausible. Thyroid hormone receptors and for their mRNA have been detected in porcine (Maruo et al., 1992) and human (Zhang et al., 1997). Others demonstrated an augmentation of progesterone secretion and, to a lesser extent, estradiol secretion into the human granulosa cells medium by the addition of thyroid hormone to the medium of human granulosa cells in vitro (Wakim et al., 1995).

In buffalo cows hypothyroidism was associated with cessation of behavioral signs of estrus as well as low plasma progesterone levels (Ahmed and Ezzo, 1998).

In cattle studies demonstrated that follicular fluid contains the free fractions of thyroid hormones suggesting that thyroid hormones are required for bovine ovarian follicular function (Blaszczyk et al., 2006).

The exact mechanism by which the thyroid hormones regulate steroidogenesis is not well known. It was supported that thyroid hormones may have direct stimulatory effects on ovarian function via synergistic action with follicular stimulating hormone (FSH) to induce the differentiation of granulosa cells (Spicer et al., 2001). Although T3 and T4 had little or no effect on aromatase activity, they could provide important estrogen precursors to granulosa cells and thus indirectly increase estradiol production which is the primary hormone stimulating estrous behavior (Spicer et al., 2001). Interestingly, Blaszczyk et al (2006) found a significant negative correlation between levels of T4 and T3 and cholesterol in the follicular fluid of bovine follicles suggesting that the
modulatory influence of thyroid hormones on steroidogenesis in bovine follicles may consist in free thyroid hormones participating in intra-follicular metabolism of cholesterol which is the primary substrate of steroid synthesis. This hypothesis is further confirmed in this study by the significant negative relationship found between T4 and TCH (Table 4). Additionally, a significant positive relationship was reported between T4 and T3 and GLU. Hypoglycemia has been reported to be associated with a decrease in hypothalamic thyrotropin-releasing hormone and pituitary thyroid stimulating hormone (Leung et al., 1975). Also an increase in serum T3 was observed following glucose ingestion (Koh et al., 1994) or during a short term glucose infusion (Langer and Gschwendtova, 1999). These findings suggest that thyroid hormones are important modulators of bovine ovarian function partly via alterations that involve energy metabolism.

5. Conclusions
The results presented above can provide new supportive evidence about the importance of energy balance in the resumption of postpartum reproductive efficiency in the lactating cows. Our results also demonstrate that the incidence of low reproductive performance in the postpartum lactating cows is associated with a decrease of some metabolic hormones such as IGF-1, T4 and T3 which are equally important metabolic modulators of postpartum ovarian activity. The results suggest, however, a necessity to carry out further studies in order to profile the secretory patterns of these hormones in peripheral blood together with the pattern of their concentrations in the follicular fluid in the same animal. Additionally, the positive relationships between changes in some energy metabolites, peripheral IGF-1, thyroid hormones and blood indicators of ovarian activity support the identification that the effect of alterations in energy balance on postpartum ovarian function could be mediated through the effect of these alterations on secretory patterns of these metabolic hormones. Finally these data may improve our understanding of some factors associated with the ovarian activity in the postpartum lactating cows and how these factors are interacted which may lead to better methods for reproductive management.

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