Sex Steroid Levels throughout Gestation in Cows Carrying Normal and Malformed Fetuses

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ABSTRACT. Birth of malformed/stillborn calves is a liability to farmers and diagnosis of the condition early in gestation would be of immense economic benefit. We report on peripheral plasma progesterone (P₄), estrone (E₁) and estradiol (E₂) concentrations quantified by radioimmunoassay throughout gestation in twin embryo recipient cows carrying normal (cow N), freemartin (cow F) and schistosomus reflexus fetuses (cow S). The undulating plasma P₄ profiles were identical in all three cows throughout gestation apart from that the concentration in cow F dramatically declined on day 254 and it subsequently gave birth to stillborn calves. The plasma E₁ concentration progressively increased in cow N to peak at parturition and then rapidly declined a day after parturition. E₁ levels were lower in cow F than in cow N and exhibited a sudden increase in concentration at day 254 of gestation followed by a dramatic decline. Cow S had lower E₁ levels throughout gestation than cow N and showed an undulating profile. The plasma E₂ profile paralleled the plasma E₁ profile in all the cows but the E₂ concentration throughout gestation was lower than the E₁ levels. Plasma E₁ and E₂ levels declined to < 20 pg/ml in cow N a day after parturition as opposed to > 150 pg/ml E₁ and > 20 pg/ml E₂ levels, respectively, in cows F and S. Our results indicate that E₁ and E₂ are better than P₄ as prognostic indicators of fetal in-utero status as well as the number of fetuses a cow is gestating.—**KEY WORDS:** cattle, estrogen, malformed pregnancy, progesterone.

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Abnormal births account for approximately 0.5% of all pregnancies in cattle [11, 16]. Among these, freemartin and schistosomus reflexus are the two most common conditions encountered [11, 12, 16]. The etiology of freemartinism in cattle is well described elsewhere [11, 12, 16] and this is one of the many setbacks to the establishment of superfetation in cattle. Schistosomus reflexus has also been observed in other species [16] but its etiology remains unknown.

Early diagnosis of abnormal pregnancies would be of immense economic benefit to the farmer since in most cases dystocia is the norm; birth is usually via assisted delivery or cesarean section and the majority of the dams later develop breeding problems [11, 12, 16]. In humans, cytogenetics via amniocentesis to check for chromosomal aberrations [18] or ultrasonography to check for fetal gross abnormalities is frequently employed during gestation in high-risk pregnancies [13]. At present, the cost of these techniques, as well as the compact anatomical positioning of the reproductive organs within the pelvic/abdominal cavity of domestic animals, currently excludes its commercial application in animal industry.

The concentration of estrogens, primarily of fetoplacental origin, is substantially elevated during gestation in a number of species [19]. Consequently, in humans, monitoring of maternal estrogen profile allows prognosis of fetal well being and advance planning of neonatal care [5]. In domestic animals abnormal levels of hormones other than those of ovarian source have been reported in prolonged gestation [11, 12, 16]. To date, there are no

reports in the literature on steroid levels of ovarian and/or fetoplacental source in cows carrying congenitally malformed fetuses, although their concentration during the normal cycle, pregnancy and postpartum period have been well defined [1–4, 7]. Therefore, we report on steroid levels of ovarian and fetoplacental source throughout gestation in cows carrying normal, freemartin and schistosomus reflexus fetuses.

MATERIALS AND METHODS

The Holstein cows were part of a herd housed and managed in the National Institute of Animal Industry that served as recipients in an investigation to establish multiple pregnancies by using *in vitro* fertilized embryos. In order to induce multiple corpora lutea (C.L.) prior to the embryo transfer the cows were treated commencing in the mid-luteal phase (Day 10 to 14; day 0 was defined as the first day of standing estrus) of the preceding cycle with 2,000 I.U. of pregnant mare serum gonadotropin (PMSG, Serotropin, Teikoku Zoki Pharmaceuticals Co., Tokyo, Japan) and 30 mg of Prostaglandin F_{2α} (Panacelan, Daiichi Seiyaku Co., Tokyo, Japan) in split injections two days later.

The Japanese Black cow embryos were generated by *in vitro* fertilization [10] and non-surgically transferred on day 7 of the estrous cycle. A twin pregnancy was established by transfer of individual embryos into each uterine horn. Pregnancy was confirmed at day 30 by ultrasonography.

Blood was collected via jugular venipuncture into a 50 ml heparinized polypropylene tube, placed immediately on ice and centrifuged (1,800 × g at 4°C for 1 hr) within 1

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hr of collection. The harvested plasma was stored at -20°C until assayed. Blood was collected about every third day from day 0, then daily for the last 10 days of gestation and sampling was stopped one day post-partum.

Plasma ($20\ \mu\text{l}$) progesterone (P_4) was extracted and quantified by radioimmunoassay as described elsewhere [3]. Assay sensitivity was $< 0.5\ \text{ng/ml}$ and intra- and inter-assay coefficients of variation (C.V.) were 7 and 11%, respectively.

Plasma ($2.5\ \text{ml}$) estrone (E_1) and estradiol (E_2) were

extracted by diethyl ether, separated by column chromatography (Sephadex LH-20, Pharmacia, Uppsala, Sweden) and their concentrations were quantified by radioimmunoassay [3]. Assay sensitivities for E_1 and E_2 were $< 0.5\ \text{pg/ml}$. Intra-assay C.V.s for estrone and estradiol were 10.3 and 10.7%, respectively. Inter-assay C.V.s for E_1 and E_2 were 16.3 and 15.1%, respectively.

RESULTS

Cow 281 (Cow S) received twin embryos and twin pregnancy was confirmed at day 30 by ultrasonography, however, it gave birth to a single schistosomus reflexus calf weighing 67.5 kg at 281 days of gestation. Cow 284 (Cow F) gave birth prematurely to a set of stillborn calves, a male weighing 27.0 kg and a freemartin weighing 18.5 kg, at 254 days of gestation. In addition, it had a grossly discoloured placenta, too. Coincidentally, cow F in a preceding pregnancy gave birth to a live set of twins and among these one was again a freemartin (the male was 42.5 kg and the female was 37.0 kg in weight at birth). Cow 315 (Cow N) gave birth to two live male calves weighing 42.0 and 32.0 kg, respectively, at day 279 of gestation. Freemartin and schistosomus reflexus condition were diagnosed by gross examination only.

The plasma P_4 profiles were identical in the MSG treated cows as shown in Fig. 1. In cow F the P_4 concentration abruptly declined around day 254 to $< 1\ \text{ng/ml}$. On the other hand, the P_4 concentration in the preceding pregnancy of this cow remained elevated up to about day 280 but its plasma P_4 concentration was much lower than the MSG treated cows, as shown in Fig. 1. In

Fig. 1. Plasma progesterone concentration throughout gestation in cows N($\square$), F($\blacktriangle$), S($\circ$), and F's preceding pregnancy (---), (Day 0=day of embryo transfer).

Fig. 2. Plasma E_1 ($\triangle$) and E_2 ($\blacklozenge$) concentrations throughout gestation in (a) Cow N and (b) Cow S. ($\blacktriangledown$) indicates day of parturition (Day 0=day of embryo transfer).

cows N and S, the plasma P_4 concentration rapidly declined as parturition approached and reached a concentration of < 1 ng/ml on the day of parturition.

The plasma E_1 concentration increased rapidly from about days 60 to 100 in cow N compared to cows F and S as seen in Figs. 2a, 2b and 3a. Cows F and N exhibited a linear increase in plasma E_1 concentration between days 100 to 250, whereas, cow S showed an undulating profile during the same period and its plasma E_1 concentration was lower than cow N (Figs. 2 and 3). Cow F showed a sudden upsurge in E_1 concentration at day 254 (parturition day) and then it declined dramatically on the subsequent day (Fig. 3a). However, the peak concentration attained on the day of parturition in this cow was less than that seen at parturition in cows N and S. On the other hand, cow F in the preceding pregnancy had a comparable profile to cow N between days 100 to parturition (Fig. 3b). Plasma E_1 concentration was markedly lower in cow S from days 250 to parturition than in cow N (Fig. 2).

The plasma E_2 profile paralleled the plasma E_1 profile in all the cows as shown in Figs. 2 and 3 but the plasma E_2 concentration throughout gestation was lower than the E_1 levels.

Plasma E_1 and E_2 levels declined to < 20 pg/ml in cow N one day post-partum as opposed to > 150 pg/ml E_1 and > 20 pg/ml E_2 levels, respectively, in cows F and S (Figs. 2a, 2b and 3a).

DISCUSSION

The corpus luteum is the main source of P_4 and is independant of fetal regulation throughout gestation in

the cow although other organs have been implicated in contributing to the P_4 pool [2, 4, 7]. Furthermore, there is no evidence in the cow of a placental shift in P_4 production as has been reported in other species [19]. This is additionally substantiated by the similar plasma P_4 profiles of singleton and twin-bearing cows [2]. In the cow, P_4 concentration is commonly monitored to diagnose pregnancy but its accuracy is limited [6]. It has also been reported as a poor indicator of embryonic or fetal death in the buffalo [8], cow [2, 17] and pig [10]. In addition, the results of the present investigation further indicate (Fig. 1) that monitoring peripheral P_4 levels is of little pre-natal prognostic value in cattle and is of limited use in predicting the number of fetuses a cow is gestating after E.T. as illustrated by the similar plasma P_4 profiles of cows N and S.

The gradual increase in estrogen concentration throughout gestation to peak at parturition is characteristic of calving cows [1, 2, 4]. This progressive increase probably reflects the maturation of the placenta with the concomitant growth of the fetus. In humans, the fetus supplies 90% of the precursors for placental estrogen synthesis [5], as a result, monitoring of maternal estrogen profile allows indirect prediction of fetal well-being. Currently there is no evidence of fetal contribution to placental steroidogenesis in cattle though the genotype of the fetus has been reported to influence the amount of estrogen synthesized by the placental unit [15]. Adalakoun *et al.* [1] reported that low levels of unconjugated estrogen were characteristic of aborting cattle. Interestingly, similar findings have also been observed in aborting buffaloes [8]. In the pig, a relationship between the litter size and

Fig. 3. Plasma E_1 (Δ) and E_2 ($\blacklozenge$) concentrations throughout gestation in (a) Cow F and (b) Cow F's preceding pregnancy. ($\downarrow$) indicates day of parturition (Day 0=day of embryo transfer).

estrogen levels has been established [9]. Sreenan *et al.* [20] reported high levels of total estrogens in twin-bearing cows compared to singleton cows, whereas, Adelakoun *et al.* [1] only found significant differences in E_2 levels between twin-bearing and singleton cows. Our findings indicate that E_1 and E_2 show parallel changes, although E_2 concentrations were markedly lower throughout gestation than E_1 . Therefore, it is tempting to suggest that the low levels of plasma E_1 and E_2 in cow S throughout gestation after twin E.T. compared to cow N would indicate the possibility of differentiating singleton from twin-bearing cows and/or diagnosing fetal loss by the sequential monitoring of peripheral free estrogen concentrations. However, our results are based on individual cases, therefore there is a need for a more comprehensive study employing a larger number of animals to verify this. The cause of death of the fetuses in cow F was not exhaustively investigated except for the noting of the grossly discoloured placenta but interestingly, Echternkamp [4] reported that estrogens secreted by the placenta were correlated to the birth weight of the calf and that low peripheral levels were suggestive of a patho-physiological condition of the placenta. Apparently, the birth weights of the calves in cow F in the present study were approximately half at birth compared to cow N's calves. However, in cow F's preceding pregnancy the calf birth weights were comparable to cow N. Therefore, the possibility of placental compromise as a prime cause of low E_1 and E_2 levels and eventual death of the fetuses leading to premature birth rather than the abnormal genotype can not be ruled out in cow F. Furthermore, Echternkamp [4] in an investigation of hormonal levels in multiple pregnancies of cows, noted no difference in the estrone sulphate levels between cows gestating normal and freemartin calves. On the other hand, our results indicate that the trend of the E_1 and E_2 plasma profile could be used for prognostic prenatal diagnosis of the in-utero fetus in cattle as substantiated by the disparate profiles of cows N and S. In addition, high E_1 and E_2 concentrations a day after parturition in cows F and S could be attributed to the retained placenta as high estrogen concentrations have been observed for several days in aborting cows with retained placenta [14, 17]. Therefore, characterization of E_1 and E_2 profiles could be useful in identifying cows gestating fetuses with multiple defects rather than just genotypic aberrations with little or no obvious phenotypic malformations.

To conclude, present results indicate that both E_1 and E_2 are better prognostic indicators than progesterone for gauging fetal in-utero status as well as the number of fetuses a cow is carrying. Further studies are needed to exploit the potential of monitoring estrogens in cows, as in humans, for prognostic prenatal diagnosis as it is essential to develop diagnostic procedures to identify dams with abnormal fetus(es) so as to induce selective abortion early in gestation. Furthermore, estrogens are secreted in vast amounts in milk and urine thus there is potential for developing simpler methods of monitoring their concen-

tration for commercial application.

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