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EFFECT OF PARITY ON ENDOMETRIAL GLANDS IN GRAVID RABBITS

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SUMMARY

The uterus undergoes intense remodeling in pregnancy and subsequent involution in the postpartum period. One of the anatomical changes that occur in the gravid endometrium is increased glandular density. Parity has been shown to be protective against certain endometrial pathologies probably as a result of retained changes that take place during pregnancy. The findings of the current study may help provide the anatomical basis for different traits noted as the parity rises. Nine rabbits**,** California white breed (*oryctolagus cuniculus*), were obtained from a private farmer. The subjects were grouped as follows; primiparous rabbits in group 1, Para 1 and Para 2-3 in group 2, and Para >4 in group 3. Once successfully mated, they were housed in pens, fed, and given adequate supply of water. On day 18 of pregnancy, the rabbits were sacrificed and uterus harvested en bloc. Five-millimeter specimens were obtained from the uterus and processed for light microscopy. Quantification of endometrial glands and their size was done using the computer program Image J. Endometrial gland density was noted to decrease with a rise in parity such that the percentage proportion in the primigravid rabbit was 45% compared to that of 34% and 37.5% in the biparous and multiparous groups respectively. The endometrial gland duct circumference also increased as the parity increased. The present study reveals that a high parity is characterized by fewer, wider endometrial glands.

Key words: Endometrial glands, Parity, Gravidity

INTRODUCTION

The endometrium comprises an epithelium (glandular and luminal), and the underlying endometrial stroma (Williams et al., 1995). Its main function is to support the nascent embryo via its glandular secretions (Lessey, 2000). Endometrial glands have been shown to increase in number during pregnancy and regress gradually at the end of pregnancy (Hempstock et al., 2004). Parity has been shown to be an independent prognostic factor in endometrial pathologies such as malignant mixed mesordermal tumors (Marth et al., 1997). There is evidence that grand-multiparity (5 or more pregnancies) is protective against endometrial cancer; likely as a result of the repeated protective role of progesterone (Hinkula et al., 2002). Since endometrial carcinoma has been shown to have a higher incidence in the nulliparous and low parity females, we postulated that repeated pregnancies have got an effect on the distribution of endometrial glands. Reports on the effect of parity on the endometrium are scarce.

METHODOLOGY

Nine rabbits**,** california white breed (*oryctolagus cuniculus*), were obtained from a private farmer. The subjects were grouped as follows; primiparous rabbits in group 1, Para 1 and Para 2-3 in group 2, and Para >4 in group 3. These rabbits were mated. Successful mating was evidenced by presence of a vaginal plug. Following mating, they were housed in a pen in the animal house, at the Department of Medical Physiology, University of Nairobi. They were provided with a constant food and water supply. On day 18 of pregnancy, which was a standardized day for late pregnancy, the rabbits were euthanized by injection of 20 mg/kg of phenorbarbitone. The abdominal cavity was accessed and uterus obtained en bloc. Tissue slices were obtained from different regions of the uterus by systematic sampling. These specimens were processed for paraffin embedding. The sections were stained with the Haematoxylin and Eosin stain to demonstrate general tissue architecture.

The sections were examined using a Zeiss® photomicroscope at magnification X 40 and X 100 and X 400. At X100, the proportion of glands/ducts within the endometrial stroma was used to represent the "density of glands/ducts."

This was determined using the point intercept method by dropping a grid in ImageJ software (Fig. 1) as described by Pang *et al*., (2009). The number of glands in each box of the grid was counted and the total area where the counting was done was also obtained. In each group of the pregnant rabbits five slides of the endometrium were selected randomly. Counting was done at 5 random points for each slide. An average was obtained for each group subsequently.

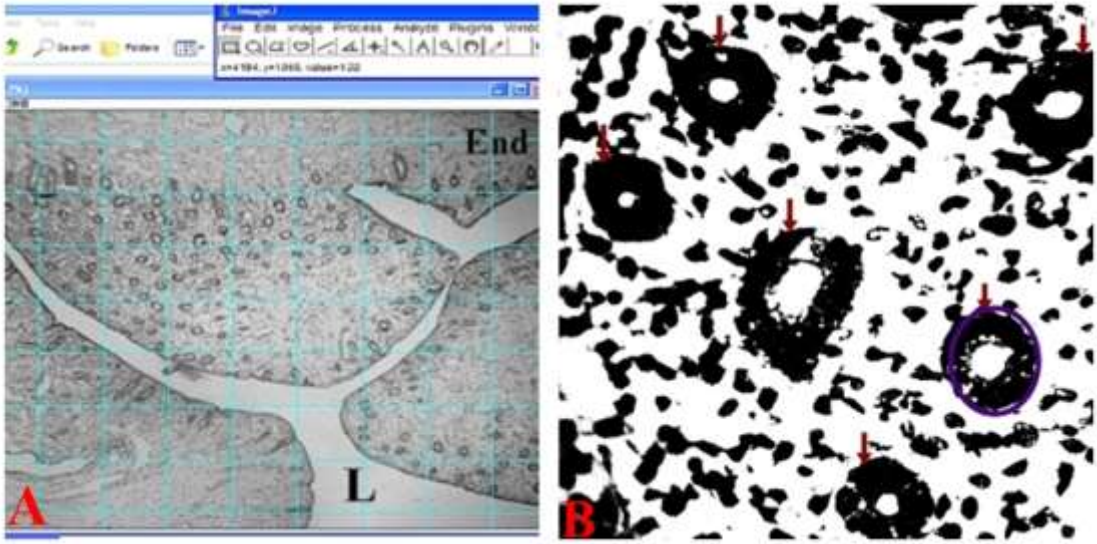

Fig. 1: **A:** A black and white photomicrograph transformed by the Image J program, with a superimposed grid from the same program. It is a section of a primiparous rabbit uterus from the mid-cornual part, stained with H and E, at X 100 magnification. The numbers of endometrial glands within each box were counted. L is the lumen of the uterus. **B:** A binary/black and white photomicrograph of the endometrial stroma of a primigravid uterus at X400, following H and E staining and subsequent transformation using the program image J. A tool was used to draw around the gland as shown by the purple line to get the circumference. The glands are as shown by the red arrows.

The proportion of the area of the endometrium occupied by endometrial glands was presented as a percentage as follows:

$$\frac{100 \times \text{Total pixels occupied by endometrial glands}}{\text{Total pixels occupied by a given area of the endometrium}}$$

In each group of rabbits, the circumferences of 15 endometrial glands were measured at X400 and their average obtained per field. Five sections were studied per group. This was done using the program Image J. A tool on this program measured the circumference of the glands in pixels. The flexible tool was taken round the inner circumference of a gland and the measurement obtained was recorded in pixels. The equivalent of 1mm in pixels was done by marking a distance of 1mm between two points on a piece of paper that was mounted on a slide. This slide was examined at X400 and photographed (Fig.1A). The distance between these two points was used to calibrate the grid of image J to give its equivalent in pixels. The images were converted to black and white and their binary form to remove color as a confounder in measuring pixels. For each groups of rabbits 15 glands were measured and an average was obtained. The figure was the converted from pixels to millimeters.

RESULTS

The endometrium of the uteri studied comprises an epithelial lining and the endometrial stroma. In all cases the epithelium was of the simple columnar type. Simple tubular glands lined by a columnar epithelium ran from the lining epithelium into the endometrial stroma. Found within the endometrial stroma were blood vessels and loose connective tissue. The mucosa of the primiparous endometrium (group 1) was noted to have a predominance of simple tubular endometrial glands. Simple columnar cells lined these glands. These cells contained hyperchromatic nuclei. In the second group (biparous-quadriparous), the endometrial glands were fewer. The multiparous endometrium also had fewer endometrial glands compared to the primiparous. The cells lining these glands displayed columnar morphology, with hyperchromatic nuclei. With a rise in parity it was also noted that the glands had a wider lumen. The glandular epithelium in the multiparous endometrium also exhibited papillary-like projection (Fig. 4C). Endometrial gland density of the uterine mucosa was determined as described in above. The proportion of the endometrial glands/ducts within the endometrial stroma was 45% in the primigravid group, 34% in the biparous-quadriparous rabbits and 37.5% in the multiparous rabbits. The results are as shown in the bar chart (Fig. 2) below. The luminal circumference of the endometrial glands/ducts was noted to increase with a rise in parity as shown in Table 1.

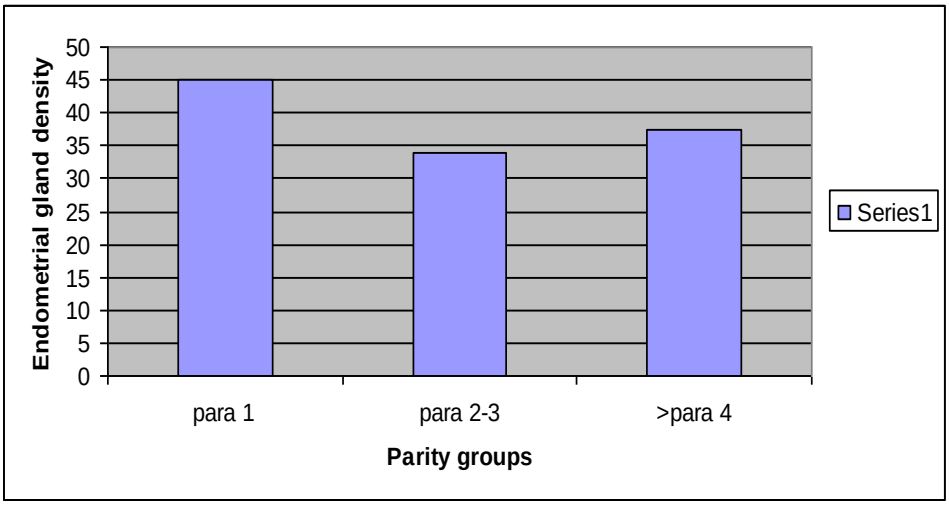

Fig. 2: Bar graph showing the distribution of endometrial glands in the different parity groups

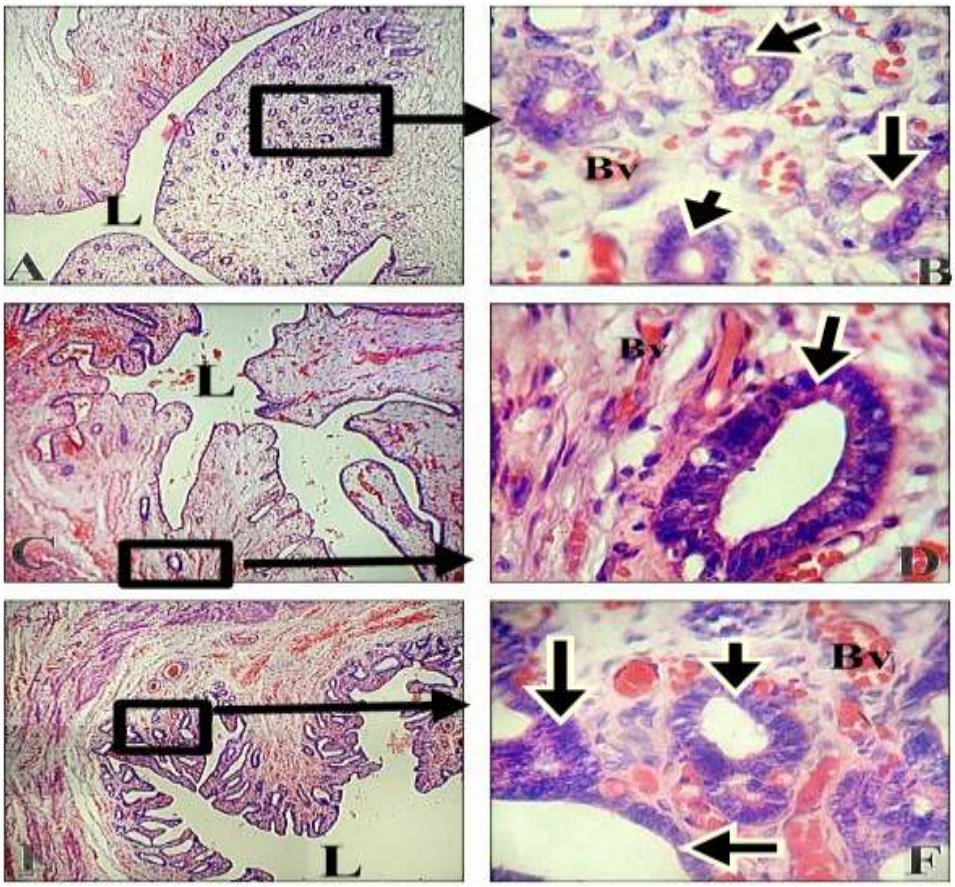

Fig.3: Endometrial glands in the different parity groups stained with H and E
A: The endometrium of a primiparous uterus taken at the mid-cornual section, at X100. Note the numerous endometrial glands (arrows). **B:** B is inset from the rectangle in A at a higher magnification, X400, Note the

hyperchromatic nuclei of the simple columnar cell lining the gland as shown by the arrow and the narrow glandular lumen. Bv is for blood vessels **C:** The endometrium of biparous-quadriparous uterus at the mid-cornual section, at X100. Note that the endometrial glands (arrows) are fewer compared to A above. **D:** The endometrium of biparous-quadriparous uterus at the mid-cornual section, at a higher magnification, X400, the lumen of the glands (shown by arrows) is larger. Bv is for the blood vessels. **E:** The endometrium of multiparous uterus at the mid-cornual section, at X100. Note that the endometrial glands are fewer compared to A above. Papillary-like projections of the mucosa project into the lumen L. **F**: The endometrium of multiparous uterus at the mid-cornual section, at a higher magnification, X400, reveals a wider glandular lumen and the hyperchromatic nuclei of the simple columnar cell lining the gland as shown by the arrow.

Table 1: Circumferences of lumen of endometrial glands in the different parity groups

Parity of the Rabbits	**Luminal circumference of endometrial glands in micrometers (µm)**
Primigravida (Group 1)	247.13 $\pm$ 0.02
Biparous-Quadriparous (Group 2)	771.86 $\pm$ 0.01
Multiparous (Group 3)	797.31 $\pm$ 0.01

P=0.01. The values are given as mean $\pm$ SD

DISCUSSION

In the present study, there was a decrease in the glandular density within the endometrium with a rise in parity in pregnant rabbits, such that the percentage proportion within the endometrial stroma was 45%, 34% and 37.5% in the primiparous group, biparous-quadriparous and multiparous groups respectively. To the best of our knowledge, this is the first study correlating endometrial glands with parity. The findings of this study could provide some explanation of observations of previous studies. For example, reduced endometrial glands characterize endometriosis also known as chronic degenerative endometrial disease. This condition has been shown to correlate positively with a rise in parity in mares as opposed to age (Bracher et al., 1996). It is therefore possible that the progressive loss if endometrial glands in higher parity groups as observed in this study is part of a physiological degeneration.

It is known that endometrial carcinoma is more common in nulliparous and low parity females (Albrektesen, 2009). Repeated strong exposure to progesterone has been thought to play a protective role. While studying the effect of progesterone on non-pregnant bovine endometria, Wang et al., (2007), proposed that the increased endometrial gland density after estrogen exposure may be driven by changes in total endometrial area rather than by proliferation and regression of glandular cells. According to these authors, a smaller endometrial area gives an impression of higher glandular density. Future studies can look at the effect of parity on total endometrial size/density; perhaps this may help explaining the findings of this study.

In thoroughbred mares, surface endometrial gland density has been found to correlate positively with the greater surface density of placental cotyledons observed in these animals (Lefranc and Allen, 2007). In a separate

study, multiparous mares were noted to have the lowest microcotyledons surface density (Wilsher and Allen, 2003). The lower density of placental cotyledons observed in multiparous females by these authors may be explained by the reduced endometrial gland density in the higher parity group as seen in the current study.

During pregnancy endometrial glands undergo extensive hypertrophy and hyperplasia to provide increasing histotrophic support for the conceptus (Finn and Martin 1976). The secretory products of endometrial glands are essential for the establishment of uterine receptivity and conceptus implantation (Gray et al., 2001a, b). Inhibition of postnatal growth of these glands via gene knock out resulted in infertility (Spencer and Gray 2006). Ewes that lack uterine glands and histotroph fail to exhibit normal estrous cycle or maintain pregnancy beyond day 14. Development of uterine glands (adenogenesis) in mammals typically begins in the postnatal period and involves budding of nascent luminal epithelium and extensive proliferation as they grow into the surrounding stroma, elongate and mature (Cooke et al., 2012). In rabbit, sheep and pig a servomechanism is proposed to regulate endometrial gland development and differentiated function during pregnancy. It involves sequential actions of ovarian steroid hormones, pregnancy recognition signals and lactogenic hormones from the pituitary and placenta (Gray et al., 2001a). In the postpartum period, the endometrial glands undergo involution (Gray et al., 2003). A complete decline of progesterone shortly before parturition is what is responsible for the postpartum endometrial degeneration (Degafa et al., 2006). In the equine endometrium rapid degeneration of the uterine glands occurs after pregnancy such that by day 7 postpartum the endometrial histology is similar to that observed during normal proestrous (Gray et al., 2001a, b). It is therefore plausible that the intensity of endometrial gland proliferation reduces as parity increases.

The current study observed that endometrial gland duct size increases with a rise in parity. While studying non pregnant bovine uteri, Wang et al., (2007), established that whereas high progesterone levels was associated with an increase in endometrial gland density, it was accompanied by a decrease in endometrial gland size. These findings are in agreement with what was observed in the pregnant rabbits used in this study. The primiparous rabbits had the highest endometrial gland density as well as the smallest gland duct circumference of 217 + 0.02 μm compared to a duct circumference of 797 $\pm$ 0.01 μm in the multiparous females.

CONCLUSION

Parity has an effect on endometrial gland density and luminal circumference in gravid rabbits. Future studies can correlate the findings of this study and those observed in the clinical setting regarding parity and endometrial gland pathologies.

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